Project title:	Managing ornamental plants sustainably (MOPS)
Project number:	CP 124
Work package title:	The efficacy of soil setting for the control of Fusarium in soil
Work package leader:	Erika Wedgwood
Report:	Final report, December 2015
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Location of work:	ADAS Boxworth Battlegate Road Cambridge CB23 4NN
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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.

Erika Wedgwood
Plant Pathologist / Study Director
ADAS

Signature ..... Date 23 December 2015.

Report authorised by:

John Atwood
Project Leader
ADAS

Signature Date 26 January 2016

CONTENTS

GROWERS SUMMARY

Headline

Adding an organic product to soil and sealing it to encourage microbial activity and thence anaerobic disinfestation reduced *Fusarium* inoculum in summer, but was not effective in winter.

Background and expected deliverables

Soil-borne pathogens invariably build up in the soil as a result of intensive mono-cropping which is generally practiced for high-value speciality crops such as cut flowers. After the loss of methyl bromide and the restriction of Basamid (dazomet) use to once in every three years, soil disinfestation for cut flowers grown under protection now largely relies on steam sterilisation. This method, whilst usually effective, is not without risk and is costly, labour-intensive and not environmentally sustainable in the long-term. Alternative methods of soil disinfestation that are effective, sustainable and practical to apply are urgently required. The major soil-borne pathogen of ornamental crops in the UK is *Fusarium* (e.g. affecting lisianthus, column stocks), but results are likely to be relevant to other soil-borne diseases, such as *Verticillium* spp. and *Sclerotinia* spp.).

Anaerobic soil disinfestation (ASD), is a potential non-chemical alternative for glasshouse and field crops. It involves incorporation of specified organic matter (with a known C/N ratio and protein content) into soil at a high moisture content before covering it with an oxygen-impermeable film for four to six weeks. Efficacy is believed to arise from the production of low molecular weight fungi-toxic acids and other chemicals. There is strategic work on the technique, known as soil setting, by Wageningen University and applied research by a commercial company in the Netherlands (Thatchtec BV), seeking to understand the mechanisms of activity with a view to optimising efficacy and reducing treatment time to two weeks. The technique has been used in commercial organic tomato production in the UK and initial results look promising. Scientific assessment of the level of control of *Fusarium* by the use of organic fermentation products of high protein content of specific composition from Thatchtec (Herbie products) is required, as these could be utilised by soil-growing cut flower growers to reduce pathogen levels in the soil between crops.

The specific objectives of this work (2014 and 2015) are:

- To determine the efficacy of Herbie organic material products against *Fusarium oxysporum* in soil.
- To determine the effect of temperature on the efficacy of the Herbie treatment.

Summary of the work and main conclusions

Pot based experiments followed guidance provided by Thatchtec, the manufacturer of the Herbie soil setting products used in the trial, and aimed to simulate soil glasshouse anaerobic soil disinfestation (ASD).

In both 2014 and 2015, pots were filled with 8 litres of unsterilised loamy sand collected from the surface inside a recently cropped cut-flower glasshouse without a history of Fusarium wilt infestation. Stems of stocks (*Matthiola incana*) infested with *Fusarium oxysporum* f. sp. *mathioli* were collected from another glasshouse in June 2014 for use as inoculum in both years. Additional soil was collected from the same glasshouse from below cultivation depth ten days before commencing each experiment and a "starter" product, Herbie 67P, incorporated and the soil sealed in a lidded bucket indoors to incubate the bacteria naturally present in the soil.

In 2014, a four replicate experiment was set up on 25 July. The soil was treated with one of three products (Herbie 14.1, 14.2 and 14.3) with or without the addition of the Herbie starter product. It was left to run for eight weeks but the inoculum was sampled after two weeks and so two net bags containing six infested stem sections were buried at 100 mm depth per container.

In both years, treated and untreated pots containing inoculum were set up in a polytunnel at ADAS Boxworth and given 5 mm of water over the soil surface before being made air-tight and left to allow microbial activity to take place. Infested stems were retrieved after the stated intervals and the presence of viable *Fusarium* determined by isolation onto agar.

In summer 2014, the pots with Herbie 14.3 plus starter Herbie 67P were the only ones to have a significantly lower proportion of stems with viable *Fusarium* (33%) after two weeks compared with 81% in the untreated. However, more stems showed *Fusarium* in all treatments after eight weeks.

In 2015, a six replicate experiment was set up on 12 February. The soil was treated by the most effective treatment combination in 2014 of starter Herbie 67P plus Herbie 14.3 at the original 33 ml/L of soil rate and also at 53 ml/L. When the net bag of stem pieces was retrieved after two weeks from each pot, there was no difference relative to the untreated, with over 90% of the stems in the treated pots having viable *Fusarium*, and no benefit shown from having added a higher rate of the Herbie 14.3.

In 2014, analysis of the soil after eight weeks showed that the percentage organic matter was higher in all six Herbie treatments compared with no treatment. Sulphates were higher in all six Herbie treatments and greatest in treatment seven (14.3 + starter) which appeared to be the most effective treatment. This treatment (14.3 + starter) also showed the greatest amount of available nitrogen, phosphorus and potassium. In 2015, the two Herbie treatments had higher phosphorous and potassium levels.

Action points for growers

- Be aware *Fusarium* remains viable on infested stems for at least nine months when not buried and even when buried the pathogen survives for at least eight weeks, so crop removal prior to soil treatment will assist in reducing the level of inoculum present.
- The addition of fast-metabolised processed products such as Herbie 14.3 to the soil followed by covering with plastic has the possibility of reducing fungal pathogens in soil through the enhanced activity of anaerobic bacteria causing the production of chemical by-products. However, the effectiveness of this anaerobic disinfestation procedure is likely to be greater at summer soil temperatures than those experienced in the winter and growers should test the procedure on a limited area first.

SCIENCE SECTION

Introduction

Soil-borne pathogens invariably build up in the soil with intensive mono-cropping as is generally practiced for high-value specialty crops such as cut flowers. After the loss of methyl bromide and the restriction of Basamid (dazomet) use to once in every three years, soil disinfestation for cut flowers grown under protection now largely relies on steam sterilisation. This method, whilst effective, is not without risk and is costly, labour-intensive and not environmentally sustainable in the long-term. Alternative methods of soil disinfestation that are effective, sustainable and practical to apply are urgently required. The major soil-borne pathogen of ornamental crops in the UK is *Fusarium* (e.g. affecting lisianthus, column stocks) and to a lesser extent species of *Verticillium* (e.g. chrysanthemum), *Pythium* (e.g. stocks, chrysanthemum) and *Sclerotinia* (several crops). *Fusarium* wilt of column stocks (*Fusarium oxysporum* f. sp. *mathioli*) is an ongoing major problem in the UK and will be investigated here.

New chemical fumigants for soil disinfestation are unlikely to become available in the medium term. In Southern Europe, soil solarisation is feasible as a broad-spectrum treatment option and increasingly used; but in Northern Europe soil solarisation is not very practical due to unpredictability of periods with high temperatures and the desire to fully utilize glasshouses to grow crops in during summer. Anaerobic soil disinfestation (ASD) is a potential non-chemical alternative for glasshouse and field crops. It involves incorporation of specified organic matter (e.g. with a known C/N ratio and protein content) into soil at a high moisture content and covering with oxygen-impermeable film for four to six weeks. Efficacy is believed to arise from production of low molecular weight fungitoxic acids and other chemicals. There is strategic work on the technique by Wageningen University, seeking to understand the mechanisms of activity with a view to optimising effect and reducing treatment time to two weeks (Runia *et al.,* 2012), and applied research by a commercial company in the Netherlands (Thatchtec BV). The technique has been used in commercial organic tomato production in the UK and initial results look promising (Brian Moralee, Wight Salads Group, pers. comm.).

The technique was trialled in the UK on *Verticillium dahliae* prior to planting trees, using an early method of the technique when ryegrass was used as the source of organic matter. Although not as effective as chloropicrin, ASD treatment significantly reduced levels of *V. dahliae* in the soil and *Verticillium* wilt in Tilia trees (O'Neill *et al*., 2010).

The development of the technique in the Netherlands at Wageningen University and by the company Thatchtec B.V. (www.thatchtec.com) has increase interest in this approach. Results have shown disinfestation of soil against nematodes and *Verticillium dahliae* is possible using ASD with Herbie H7022 (consisting of organic by-products from the food processing industry) within fewer

weeks than earlier research (Ludeking *et al*., 2011). Organic materials (wheat, potato, soy or maize based granules provided as specifically coded “Herbie” formulations) are incorporated into soil, irrigated and covered with a virtually impermeable film for two weeks in summer. The anaerobic conditions control the target organisms. ASD with the organic product “Herbie” from Thatchtec has been found to reduce *Verticillium dahliae* (Runia *et al*., 2012) and the bacteria *Ralstonia solanacearum*. Thatchtec have worked on soil contaminated by Fusarium from asparagus crop debris in which it was suggested that the bacteria in the soil that may be involved in the disinfestation can be primed or boosted in some way by a preliminary incorporation of the “Herbie” product prior to carrying out the full procedure including covering (Henk Meints, pers. comm.).

Scientific assessment of the level of control of *Fusarium* which could be achieved by the use of organic fermentation products such as ‘Herbie’ is required, as this could readily be utilised by stocks growers as an alternative soil sterilant.

The specific objective of this work is:

- To determine the efficacy of Herbie organic material products against *Fusarium oxysporum* in soil

Aims

2015:

1. To test the best treatment from summer 2014 (Herbie 14.3 + Starter 67P) using the Herbie product at a higher rate in order to determine whether this might allow a shorter (2 week) period of covering than the 8 weeks tested previously
2. To record any change in effectiveness of the best treatment of summer 2014 when used at the same rate as before, but during colder temperatures in winter

Materials and methods

The experiment in 2015 was designed to mimic conditions experienced in a commercial cut-flower nursery soil substrate glasshouse at the end of a cropping year in when the soil would be rotavated and sheeted for steam sterilisation during winter. The set-up procedures used were the same as carried out at the end of the summer crop in 2014. The experiment was carried out in sealed 9.5 L pots in a polytunnel at ADAS Boxworth in February 2015,

Soil collection

Un-cultivated sandy loam soil was collected on 3 February 2015 from a commercial stocks grower, (J. A. Collison & Sons, Terrington St John) from a recently harvested glasshouse crop of lilies, the previous crop finishing in June 2014 having been stocks (*Matthiola incana*) not showing symptoms of Fusarium wilt. Soil was taken from the surface and to around a spade’s depth of approximately 250 mm. The “clean” soil (approximately 250 L) was collected and stored in a “bulk bag” at

ambient conditions in a barn so that the microbial flora and moisture content changed little after its collection. The top of the soil bag was covered, but not sealed tight. Prior to use the soil was thoroughly mixed by hand to mix the depths and avoid any pockets of different micro-flora.

Preparation of the starter

The soil for use in preparing the Herbie 67P starter was also collected on 3 February 2015 from the same glasshouse, but from the pathway. Soil was required to be moist, with an active bacterial population, and so needed to be collected just before its use to make the starter mix (Henk Meins, pers. comm.). The top 200 mm of soil was removed and wet soil below this depth dug out. Within two hours of collection this soil was used to make up the "starter". This was achieved by layering soil and Herbie 67P starter meal within a container to achieve an even a mix as possible (guidance from Thatchtec BV recommends evenly mixing the starter through the soil however this was not possible as the soil was cohesive). The layers were compressed to exclude air and 800 ml of tap water was poured over the top of the compressed soil. The container was sealed and knotted in a black plastic dustbin bag and left in the laboratory to incubate for 10 days at 20 °C.

After 10 days, on 12 February 2015, just before it was used in the treatment pots, the Herbie 67P starter soil was further prepared by tipping out the stock mix and thoroughly combining the layers.

Preparation of inoculum

Every treatment was inoculated with Fusarium infested column stock stems. Growing flowering stocks (*M. incana*) plants dying with wilt were pulled up and collected from within an area of a commercial glasshouse on 4 June 2014. Samples collected included both dead plants showing external mycelium and yellowing plants with leaf mottling and vascular staining. All the infected plants were spread out and left to air dry indoors for a month to encourage the production of resting spores. The spores were examined under a microscope and isolations made onto agar to confirm the presence of *F. oxysporum*. A couple of isolates from this were sent to Andrew Taylor at Warwick Crop Centre who confirmed by molecular diagnosis that although they were different colours (cherry purple and pinky-red) they all had identical elongation factor sequences despite some different morphology on PDA and all were confirmed as *F. oxysporum* (and said he had found it was not uncommon for even the same isolate of this fungus to produce different colours at different times on the same agar).

On 22 January 2015 two pieces were cut from each of the Fusarium infested stocks stems intended to be used in 2015 and incubated on agar in order to confirm that the pathogen was still viable.

After confirming Fusarium growth from all the tested stems they were cut into 20 mm lengths. From this inoculum six stem pieces were placed into plastic net “Tea” bags with a mesh size around 3 mm x 3 mm. Each bag was produced from a folded strip of net and stapled shut to leave a pocket about 40 mm x 30 mm containing the stem pieces. The stem pieces placed in parallel and in a

single layer per bag. Polypropylene string was fixed to one edge of each net bag to assist its recovery from burial. Eighteen bags were prepared, for six replicates of three treatments.

Treatment application, inoculation and trial set up

For each treatment the collected soil was passed through a coarse sieve to break up any large lumps and collect out any leaf pieces and spread out onto a clean plastic sheet. Three piles of soil were created. The two piles of soil (for T2 and T3) to be treated were then evenly sprinkled with a watering can with a fine rose with the required volumes of the liquid Herbie 14.3 product per litre of soil (Table 3). For treatments two and three the Herbie 67P starter soil was added at this stage by scattering small lumps over the piles. Both the starter and the Herbie product were then mixed thoroughly in the soil mimicking the product application followed by spading-in that is done in commercial crops (Henk Meints, pers. comm.).

The experiment was set up in a polytunnel at ADAS Boxworth. The high density polyethylene (HDPE) black pots were part-filled to a depth of 50 mm. One net bag containing the inoculum was placed centrally in each pot. A temperature logger probe (Delta T) was put nearby to be buried with the bag. Pots were then filled with the remaining treated soil allowing soil to fill in around the bags (avoiding air pockets) and to bury them to a depth of 100mm. Soil was compressed to further exclude air. 600 ml of cold tap water was applied to each pot (this was measured as being equivalent to an irrigation depth of 5 mm, the amount advised by Thatchtec).

Straight after watering each replicate, each pot was sealed with a transparent sheet of polythene taped tight around the pot rim (Appendix F). Each pot was then sealed in a double layer of black polythene (two dustbin bags) and tied shut. The container sides were protected from direct sunlight (in order to prevent the outer layers of the soil from temperature fluctuation and so mimic a soil bed) by placing a guard ring of filled pots all around the six replicate blocks of pots. The top was not shielded from the heat of the sun as the soil surface would normally be exposed to sunlight in a commercial crop. A heavy black plastic sheet was used to drape over the top of the whole trial including the “guard” pots and held down by bricks to further seal the pot tops to aid achieving anaerobic conditions.

Site and crop details

Table 1. Test site and plot design information

Test location:	
County	Cambridgeshire
Postcode	CB23 4NN
Soil type/growing medium	Sandy loam ex. glasshouse J A Collison, Terrington St John (following rotavation after a lily crop)
Nutrition	None added
Crop	None planted
Glasshouse* or Field	Polytunnel
Date of planting/potting	12.02.2015
Pot size	9.5 L (material HDPE 02)
Trial design (layout in Appendix C)	Randomised block design
Number of replicates	Six
Plot size w (m), l (m), total area (m²)	1 pot
Method of statistical analysis	ANOVA

*Temperature and relative humidity settings are given in Appendix B

Treatment details

Table 2. Detail of products tested (or not known, nk)

MOPS code number	Active ingredient(s)	Manufacturer	Batch number	% a.i	Formulation type
1. Untreated	-				
2. Herbie 14.3	Not disclosed	Thatchtec bv.	nk	nk	liquid
3. Herbie 67P (starter)	Not disclosed	Thatchtec bv.	nk	nk	meal

Table 3. Treatments

Product name or MOPS code number	Application timing	Dosage rate per litre soil	Spray volume (L/ha)
1. Untreated			
2. Herbie 14.3 + Herbie 67P	A1	33ml + 67 g	N/A
3. Herbie 14.3 + Herbie 67P	A1	53ml + 67 g	N/A
Application timing			
A1	12.02.2015		

Table 4. Application details

Application No.	**A1**
Application date	12.02.2015
Time of day	All day
Application method	By hand
Temperature of air – max/min (°C)	Not recorded
Relative humidity (%)	Not recorded
Soil temperature (Delta probe)	*(To be put into a graph)*

*Includes soil temperature and moisture details where relevant

Target pest(s)

Table 5. Target pest(s)

Common name	Scientific Name	Infection level pre-application
Fusarium	*Fusarium oxysporum* f. sp. *mathioli*	Infested stems introduced to pots

Assessments

After two weeks, on 27 February 2015, the inoculum bag was retrieved from each container. Any adhering soil was brushed off the stems. Each of the six stem pieces per bag was then cut in half longitudinally to expose the centre. As they were cut then either side was put into a separate beaker to give two replicates of each of the six pieces. No surface sterilisation of the pieces was used (this was done to half the pieces in the summer experiment). Stem pieces were transferred aseptically onto potato dextrose agar amended with streptomycin (PDA+S) (with either six stem surface sterilised, or unsterilized pieces per plate), placing the cut face down. Plates were incubated at 20°C. The number of half-pieces with Fusarium outgrowth was recorded after three and seven days. The later recording allowed the Fusarium colonies to be confirmed by the pink colouration that develops. The earlier assessment was made in case Fusarium growth occurred, but became overgrown by colonies of other fast growing species, such as Mucor.

A soil sample from the untreated sieved soil on 12 February and then two replicates of each treatment at the end of the experiment on 27 February 2015 was assessed for soil moisture content by drying in an oven set at 80 °C for 48 hours. A sample was also set to NRM Ltd. for nutrient analysis including % organic matter, pH, potassium, phosphorus and magnesium from the untreated soil at the start of the experiment, and from two plots per treatment from central plots 2, 5, 8, 11, 14, and 18 down the experiment length from various replicates at the end (Appendix D).

Table 6. Assessments

Assessment No.	Date	Timing of assessment relative to last application	Assessment type(s) (e.g. no./% LAI/crop safety)
1	02.03.2015	17 days	Agar plate counts after 3 days incubation
2	06.03.2015	21 days	Agar plate counts after 7 days incubation

Results

Control of Fusarium

On removing the seal from the pots on 27 February 2015, those with the Herbie treatments had fungal growth with abundant aerial mycelium on the surface and this was confirmed by microscope examination to be *Mucor* sp. *Mucor* sp. growth also developed in agar plates of the stem pieces.

Isolations onto agar after two weeks treatment (Table 7) started to produce Fusarium growth after three days, but more stem pieces were seen to still be infested after giving further incubation with assessment after seven days. At least a mean 90 % of the twelve pieces per bag had viable Fusarium in both the original and higher dose pots of Herbie 14.3, which was not significantly fewer than the 97 % of pieces with viable Fusarium from the untreated pots. Similar lack of difference between the treatments was seen when the six halves from the different stems per bag were analysed (data not show) a separate replicates.

Table 7. Effect of treatments, after two weeks' burial, on Fusarium sp. colony growth after 3 and 7 days incubation. Results show % of stems out of six from which Fusarium sp. was re-isolated.

Product name or MOPS code	**Assessment 1 after 3 days**	**Assessment 2 after 7 days**
1. Untreated	15.3	97.2
2. Herbie 14.3 (33ml) + Herbie 67P	11.1	90.3
3. Herbie 14.3 (53ml) + Herbie 67P	15.3	94.4
F value (10 d.f.)	0.939	0.477
l.s.d.	30.0	12.33

Table 8. Soil nutrient analysis at the start of the trial on 12 February 2015, showing organic matter, pH, available phosphate, potassium and manganese

% soil moisture	**organic matter WB % w/w**	**pH**	**P mg/l (index)**	**K mg/l (index)**	**Mg mg/l (index)**
13.8	7.6	7.5	89.4 (5)	1013 (6)	210 (4)

After two weeks sealed the soil analysis showed (as in 2014) that the moisture content was a little higher in the treated plots probably as a result of the addition of the saturated soil containing the starter Herbie 67 P in addition to the 5 mm of irrigation given to each pot. The organic matter was slightly raised, even in the untreated. The soil had become more alkaline in both the treatments, but

remained around pH7.5 in the untreated. There was good replication between plots of the same treatment. The phosphorus index had increased in all the pots, although less so in the untreated. The potassium had slightly fallen in the untreated and risen in both treatments and manganese had risen slightly in all pots.

Table 9. Soil nutrient analysis after two weeks sealed in pots showing, available phosphate, potassium and manganese with samples taken to 27 February 2015 for two replicates sampled

Treatment	**% soil moisture**	**% organic matter content**	**pH**	**P mg/l (index)**	**K mg/l (index)**	**Mg mg/l (index)**
1. Untreated Plot 2	19.65	11.3	7.5	103 (6)	946 (6)	229 (4)
Untreated Plot 11	19.47	11.7	7.3	102 (6)	970 (6)	238 (4)
Mean Untreated	**19.56**	**11.5**	**7.4**	**102 (6)**	**958 (6)**	**233 (4)**
2. Lower rate Herbie 14.3 + Herbie 67 P Plot 5	23.41	12.2	8.3	110 (6)	1026 (6)	217 (4)
Lower rate Herbie 14.3 + Herbie 67 P Plot 14	21.58	11.8	8.5	124 (6)	1117 (6)	233 (4)
Mean lower Herbie 14.3 + Herbie 67P	**22.50**	**12.0**	**8.4**	**117 (6)**	**1071 (6)**	**225 (4)**
3. Higher rate Herbie 14.3 + Herbie 67P Plot 8	24.19	11.3	8.4	117 (6)	1129 (6)	224 (4)
Higher rate Herbie 14.3 + Herbie 67P Plot 18	23.96	12.2	8.4	114 (6)	1140 (6)	230 (4)
Mean higher Herbie 14.3 + Herbie 67P	**24.08**	**8.4**	**8.4**	**116 (6)**	**1134 (6)**	**226 (4)**

Table 10. Mean soil temperatures in the pots through the course of the trial

	Temperatures inside Herbie pots °C		
Treatment	**Min**	**Max**	**Mean**
1. Untreated	4.5	11.92	7.46
2. Herbie 14.3 (33ml) + Herbie 67P	4.68	12.41	8.08
3. Herbie 14.3 (53ml) + Herbie 67P	4.79	11.1	8.03
External probe	-1.75	14.05	4.90

Crop vigour

Not Applicable

Crop damage

Not Applicable

Formulations

Treatments were not spray-applied.

Making up the Herbie starter for incubation proved difficult as the soil was required to be from an irrigated crop and so the soil was not friable thus making even distribution of the starter organic meal through the soil difficult. This was mitigated by layering the soil with the starter mix in the incubation container.

Effect on non-target

No effects observed

Discussion

After two weeks of treatment in winter temperatures Herbie 14.3 with the addition of the starter product, had not given any reduction in the *Fusarium oxysporum* on the plant material extracted (number of stems with Fusarium) when compared with the untreated control. It should be noted that the use of infested stems covered with the resting spores and mycelium of Fusarium as inoculum (a standard procedure for testing chemical soil fumigants and steam) was an unnaturally hard challenge for the biological control method under test involving the production of metabolites by bacteria. In a commercial crop the plant material is gathered up and anything left is smashed by rotavation and so the Fusarium would not be protected by enclosure inside the hollow or stems and within intact dead tissue. Extraction of loose chlamydospores from the soil would be difficult as they are considerably smaller than the micro-sclerotia of Verticillium sampled by the Harris test. Molecular techniques might be able to detect a reduction in Fusarium DNA, but would not distinguish between live or dead Fusarium.

It is likely that completely anaerobic conditions were not achieved during the period of the trial as the *Mucor* sp. growth seen in the treated pots would be expected to have been suppressed. It is likely that the *Mucor* sp. was feeding saprophytically on the Herbie material that had been added to the pots to “feed” the anaerobic bacteria. The bacteria thus had more food still to use and produce metabolites that might have been able to act against the Mucor and Fusarium in the pots.

As in 2014 in summer, soil treated with Herbie 14.3+ starter in winter 2015 had higher phosphorus and potassium levels after two weeks of being sealed. The products added were not analysed and

so it is not known if they contributed directly to this or this was a byproduct of chemical reactions in the soil.

It is believed that the more times the Herbie products are used between crops in the same soil, the greater the shift in the bacterial population to favour those that create the conditions in which pathogenic fungi are controlled (Henk Meints, pers. comm.). In 2016, use of the product in a commercial crop could be investigated, perhaps including comparison of single and repeated treatment in a year.

Conclusions

There is potential for the use of Herbie 14.3 + Herbie 67P starter in soil in summer, to reduce Fusarium levels, but the experiment in 2015 indicated that treatment in the cooler soil temperatures of winter is less likely to give any reduction in Fusarium within a fortnight.

References

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Appendix A – Study conduct

ADAS are officially recognised by United Kingdom Chemical Regulations Directorate as competent to carry out efficacy testing in the categories of agriculture, horticulture and biologicals. Internal QMS guidelines were followed for the study.

GLP compliance will not be claimed in respect of this study.

Relevant EPPO/CEB guideline(s)	**Variation from EPPO**
N/A	

Appendix B – Meteorological data

Location of the weather station		On site (ADAS Boxworth)		
Distance to the trial site		0 m		
Origin of the weather data		Weather station		
Long-term averages from Boxworth 30 year mean				
Month/period	**Av temp (ºC)**	**Min temp (ºC)**	**Max temp (ºC)**	**Rainfall (mm)**
N/A				
Average conditions during the trial				
Month/period	**Av temp (ºC)**	**Min temp (ºC)**	**Max temp (ºC)**	**Rainfall (mm)**
N/A				

Weather at treatment application:

Month/period	**Min temp (ºC)**	**Max temp (ºC)**	**Rainfall (mm)**
N/A			

Logger data from inside the pots is shown in Table 10.

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Appendix C – Agronomic details

Growing system

Soils and stocks plant material were collected from a glasshouse grown commercial crop without *Fusarium* infection. No plants were grown in the experiment.

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Appendix D – *Trial layout*

1 **1** **3**	**2** **1** **1**	**3** **1** **2**
4 **2** **1**	**5** **2** **2**	**6** **2** **3**
7 **3** **2**	**8** **3** **3**	**9** **3** **1**
10 **4** **2**	**11** **4** **1**	**12** **4** **3**
13 **5** **3**	**14** **5** **2**	**15** **5** **1**
16 **6** **2**	**17** **6** **1**	**18** **6** **3**

PLOT
BLOCK
TREATMENT

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Appendix F – *Photographs*

Figure 1. Grower's glasshouse soil in a pot, with string tag from buried net bag containing stocks stems with Fusarium

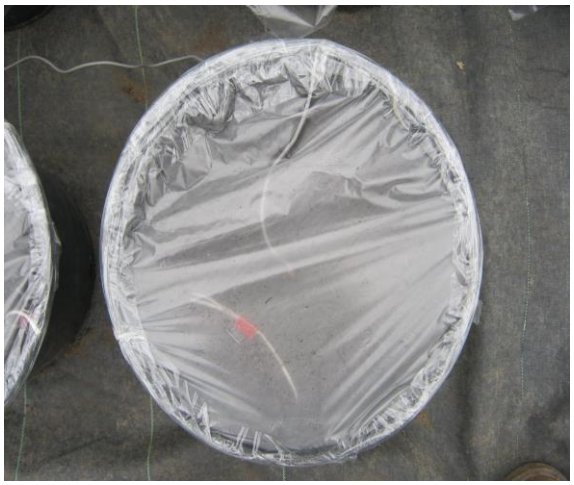

Figure 2. Buried Fusarium stem bag in a pot sealed from the air by a plastic sheet

Figure 3. Pots in randomised block with first plastic cover and some already sealed in black plastic bags to further ensure a seal

Figure 4. Sealed pots under a thick black plastic sheet as might be used to cover soil for treatment in a commercial glasshouse

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Figure 5. Mucor mycelium on soil surface when the plastic cover was removed after 2 weeks of Herbie treatment	**Figure 6.** Mucor sporangium head under high power microscope magnification from sample taken from soil

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