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:	Annual report, December 2014
Previous report:	None
Key staff:	Erika Wedgwood Robert Drummond Jonny Kerley Harriet Roberts
Location of work:	ADAS Boxworth Battlegate Road Cambridge CB23 4NN
Date work commenced:	01 March 2014
Date work completed (or expected completion date):	31 October 2014

DISCLAIMER

While the Agriculture and Horticulture Development Board seeks to ensure that the information contained within this document is accurate at the time of printing, no warranty is given in respect thereof and, to the maximum extent permitted by law the Agriculture and Horticulture Development Board accepts no liability for loss, damage or injury howsoever caused (including that caused by negligence) or suffered directly or indirectly in relation to information and opinions contained in or omitted from this document.

© Agriculture and Horticulture Development Board 2015. No part of this publication may be reproduced in any material form (including by photocopy or storage in any medium by electronic mean) or any copy or adaptation stored, published or distributed (by physical, electronic or other means) without prior permission in writing of the Agriculture and Horticulture Development Board, other than by reproduction in an unmodified form for the sole purpose of use as an information resource when the Agriculture and Horticulture Development Board or AHDB Horticulture is clearly acknowledged as the source, or in accordance with the provisions of the Copyright, Designs and Patents Act 1988. All rights reserved.

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
Pathologist
ADAS UK Ltd

Signature Date 16 December 2014

Report authorised by:

John Atwood
Project Leader
ADAS

Signature Date 16 December 2014

© Agriculture and Horticulture Development Board 2014. All rights reserved.

CONTENTS

Grower Summary……………………………………………………………………………4

Headline.. 5

Background and expected deliverables 5

Summary of the work and main conclusions... 6

Action Points.. 8

Science Section 9

Introduction 9

Materials and methods... 10

Treatment details 13

Target pest(s).. 14

Assessments.. 15

Results.. 16

Control of *Fusarium*.. 16

Crop vigour... 20

Crop damage 20

Formulations 20

Effect on non-target.. 20

Discussion 20

Conclusions 21

References 22

Appendix A – Study conduct 23

Appendix B – Meteorological data 24

Appendix C – Agronomic details... 24

Growing system 24

Appendix D – Trial layout.. 25

Appendix F – Photographs 26

© Agriculture and Horticulture Development Board 2014. All rights reserved.

GROWER SUMMARY

Headline

Anaerobic soil disinfestation of soil using three high-protein plant based products from Dutch manufacturer Thatchtec showed one treatment (Herbie 14.3 + Herbie 67P starter) to significantly reduce the proportion of infested plant stems with *Fusarium* in debris buried for two weeks.

Background and expected deliverables

Soil-borne pathogens invariably build up in the soil with intensive mono-cropping as 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 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 (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 4-6 weeks. Efficacy is believed to arise from production of low molecular weight fungitoxic acids and other chemicals. There is strategic work on the technique, known as soil setting, by Wageningen University. Applied research by a commercial company in the Netherlands (Thatchtec BV) is seeking to understand the mechanisms of activity with a view to optimising effect and reducing treatment time to 2 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 Herbie treatment

© Agriculture and Horticulture Development Board 2014. All rights reserved.

Summary of the work and main conclusions

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).

9.5 L polypropylene pots were filled with 8 L of un-sterilised loamy sand collected from inside a recently cropped glasshouse at a column stocks nursery. This soil was treated with one of three Herbie products with or without the pre-addition of a Herbie starter product prior to filling. Treatments were incorporated by hand mixing the product with soil. Two net bags (on strings to allow retrieval) containing plant material from *Fusarium oxysporum* infested stocks plants were buried at a cultivation depth of about 100 mm per container. These pots were watered with 600 ml tap water and each container was covered tightly with a black polythene cover and left in a polytunnel aiming to achieve temperatures up to the optimum for Herbie anaerobic soil disinfestation of 25°C for 8 weeks. The six Herbie treatments were compared with two untreated controls which comprised of inoculated buckets without any Herbie products, replicated four times.

Temperature inside each container was recorded throughout the experiment. Inoculum bags were retrieved after two and eight weeks of treatment, and plant material was isolated onto agar and the percent *F. oxysporum* re-isolation was assessed after three and seven day's incubation. Soil was sent for nutrient analysis at the end of the trial from each treatment and the control to assess for changes to soil mineral content and percent organic matter. Data was analysed by analysis of variance.

After two weeks of ASD treatment, Herbie 14.3 with the addition of the starter product showed significantly less *Fusarium oxysporum* on the plant material extracted when compared with the untreated control (Figure 1). Other Herbie treatments did not show an effect.

© Agriculture and Horticulture Development Board 2014. All rights reserved.

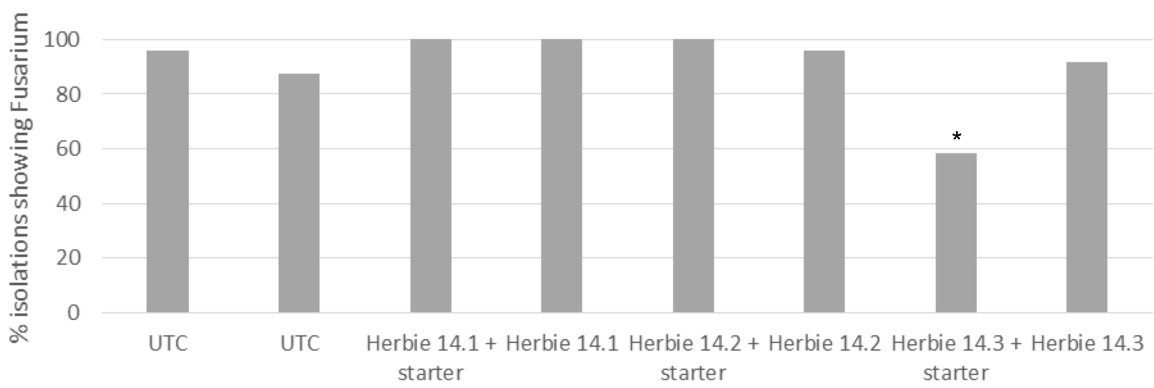


Figure 1. Assessment of plant material isolations retrieved after two weeks ASD. Percentage of infested stocks stems showing *Fusarium oxysporum* after 7 days incubation on agar**.** * denotes statistical significance at the 95% confidence level.

After eight weeks of ASD treatment, Herbie 14.3 with the addition of the starter product continued to show lower levels of Fusarium however this result was no longer statistically significant.

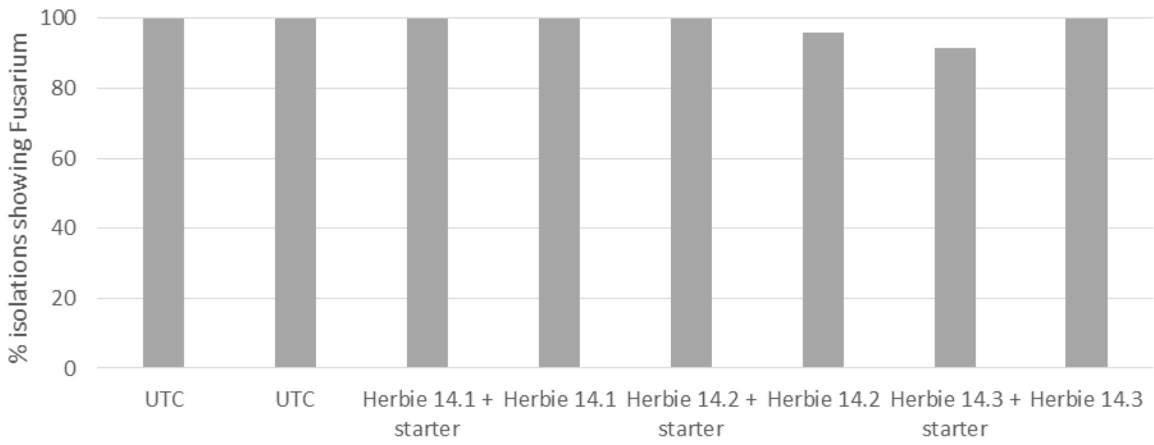


Figure 2. Results of the seven day assessment of plant material isolations made after eight weeks ASD, graph shows % plant material showing *Fusarium oxysporum*

Soil temperatures on average ranged between 15 and 30 °C through the eight weeks of the trial. During the first week of the trial, temperatures in the untreated control were on average 1 °C cooler than the six Herbie treatments suggesting the microbial action in these treatments may be raising the soil temperature slightly. This trend did not continue through August and September however. There was some concern that completely anaerobic conditions were not achieved throughout the trial because of the loss of tightness of the seal around the logger cables where they exited the pots.

© Agriculture and Horticulture Development Board 2014. All rights reserved.

Analysis of the soil after eight weeks showed that the three treatments which used the Herbie starter had on average 2 % greater soil moisture, and this was probably because the starter mix was produced by incubation of the Herbie 67P starter product in saturated soil. Interesting trends in other components were noted. The percentage of organic matter was higher in all six Herbie treatments compared with the control. 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.

Action Points

The most effective Herbie treatment (14.3 + starter) will be used in a further pot trial in 2015 to investigate soil treatment under cooler temperatures i.e. winter when sterilisation is normally carried out post-Christmas in the between-crop gap.

© Agriculture and Horticulture Development Board 2014. All rights reserved.

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 grow crops in glasshouses 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 4-6 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 2 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 increased interest in this approach. Results

© Agriculture and Horticulture Development Board 2014. All rights reserved.

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. 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

Materials and methods

The experiment was designed to mimic glasshouse conditions after the first crop of the year of stocks is harvested from the soil (end May – early June). The experiment was carried out in sealed 9.5 L pots in a polytunnel at ADAS Boxworth between July and September 2014.

Soil collection

Uncultivated sandy loam soil was collected on 4th June 2014 from a commercial stocks grower, (J. A. Collison & Sons, Terrington St John) from a recently harvested glasshouse crop of stocks 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 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 collected on 14 July 2014 from the same glasshouse from the pathway within a new crop of lilies. The 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

© Agriculture and Horticulture Development Board 2014. All rights reserved.

(Henk Meins, pers. comm.). 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 nine times 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 (Appendix F figure 5 and 6). The container was sealed and left in lab to incubate for 10 days at 20 °C in the dark.

After 10 days the Herbie 67P starter soil was further prepared by tipping out the stock mix and thoroughly combining the layers before sprinkling evenly at the rates shown in Table two onto the bulk soil for treatments three, five and seven at the same time as the Herbie 14.1, 14.2 or 14.3 treatments were applied before potting

Preparation of inoculum

Every treatment was inoculated with *Fusarium*-infested column stock stems. Growing flowering stocks (*Matthiola incana*) plants dying with wilt were pulled up and collected from within an area of a commercial glasshouse (Appendix F figure 1 and 2). 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*. Once dry, the stems were chopped up into 10 mm length pieces. 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. Each bag contained 6 replicate stem pieces of 10 mm length placed in parallel and in a single layer. A handle of polypropylene string was fixed to one edge of the net bag to assist the bag's recovery (Appendix F Figure 4).

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 (Appendix F Figure 3). This soil was then evenly scattered with the product granules or liquid of the particular Herbie product for that treatment. Quantities per litre of soil are given in Table 3. For treatments three, five and seven the Herbie 67P starter soil was added at this stage. Both the starter (where this was used) and the selected 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.).

High density polyethylene (HDPE) black pots without open drainage holes were part-filled to a depth of 50 mm. Two net bags containing the inoculum were placed in each pot separate from each

© Agriculture and Horticulture Development Board 2014. All rights reserved.

other and not touching the sides (Appendix F Figure 4). 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 100 mm. Soil was compressed to further exclude air and 600 ml of tap water was applied (measured as being equivalent to an irrigation depth of 5 mm as advised by Thatchtec).

Straight after watering each replicate, each pot was sealed with a double layer of black polythene (a dustbin bag) held in place by tying with twine under the pot rim. Temperature probes (Delta T) were placed at the centre-point of each pot, at the height of the inoculum bags (100 mm from the top of the soil) and were used to monitor the temperatures achieved (Appendix F Figure 7). The cables were put through each plastic lid and sealed in place with electrical tape.

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 the pots "pot tight" and surrounding the four replicates with a ring of filled guard pots (Appendix F Figure 8). 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 to further seal the pot tops to aid achieving anaerobic conditions.

Table 1. Test site and plot design information

Test location:	

© Agriculture and Horticulture Development Board 2014. All rights reserved.

County	Cambridgeshire
Postcode	CB23 4NN
Soil type	Loam ex. glasshouse J A Collison, Terrington St John (from recently grown column stocks crop)
Nutrition	None added
Crop	None planted
Glasshouse* or Field	Polytunnel
Date of potting	24.07.2014
Pot size	9.5 L (material HDPE 02)
Trial design (layout in Appendix C)	Randomised block design
Number of replicates	Four fold replication
Plot size w (m), l (m), total area (m^2)	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. Untreated	-				
3. Herbie 14.1+ Herbie 67P	Not disclosed	Thatchtec bv.	nk	nk	meal
4. Herbie 14.1	Not disclosed	Thatchtec bv.	nk	nk	meal
5. Herbie 14.2 + Herbie 67P	Not disclosed	Thatchtec bv.	nk	nk	meal
6. Herbie 14.2	Not disclosed	Thatchtec bv.	nk	nk	meal
7. Herbie 14.3 + Herbie 67P	Not disclosed	Thatchtec bv.	nk	nk	liquid

© Agriculture and Horticulture Development Board 2014. All rights reserved.

8. Herbie 14.3	Not disclosed	Thatchtec bv.	nk	nk	liquid

Herbie products were received on 3 June 2014 and stored in the dark at ambient room temperature

Table 3. Treatments

Product name or MOPS code number	Application timing	Dosage rate per Litre of soil	Spray volume (L/ha)
1. Untreated		-	N/A
2. Untreated		-	N/A
3. Herbie 14.1+ Herbie 67P	A1	9.0 g + 67 ml	N/A
4. Herbie 14.1	A1	9.0 g	N/A
5. Herbie 14.2 + Herbie 67P	A1	11.0 g + 67 ml	N/A
6. Herbie 14.2	A1	11.0 g	N/A
7. Herbie 14.3 + Herbie 67P	A1	33 ml + 67 ml	N/A
8. Herbie 14.3	A1	33 ml	N/A
Application timing			
A1	24.07.2014		

Table 4. Application details

Application No.	A1
Application date	24.07.2014
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)	28 °C after sealing pots

Target pest(s)

Table 5. Target pest(s)

Common name	Scientific Name	Infection level

© Agriculture and Horticulture Development Board 2014. All rights reserved.

		pre-application
Fusarium	*Fusarium oxysporum* f. sp. *mathioli*	Infested stems introduced to pots

Assessments

After two weeks, one of the inoculation bags was retrieved from each container using the string. The remaining bag was removed from each container after eight weeks burial (Appendix F Figure 9). After recovery from soil, any adhering soil was washed off the stems by rinsing in sterile distilled water. Each stem piece was then cut in half transversely. One section was dried on filter paper, with no further treatment. The second half was surface sterilised in 90% ethanol for 10 seconds. Surface sterilisation was done because after contact with the soil it was possible that a the stem pieces would have become colonised externally by bacteria that could out-compete *Fusarium* when incubated on the agar plate, swamping the *Fusarium* mycelium and so giving difficulty in recording *Fusarium* presence. The *Fusarium* inside the stems would be expected to grow out through the sterilised surface. Stem pieces were transferred aseptically onto potato dextrose agar amended with streptomycin (PDA+S) (with either six surface sterilised or unsterilised stem pieces per plate). Plates were incubated at 20°C. The number of pieces with *Fusarium* outgrowth was recorded after three and seven days (Appendix F Figure 10). The later recording allowed the *Fusarium* colonies to be confirmed by their pink colouration. The earlier assessment was made in case *Fusarium* growth occurred, but became overgrown by colonies of other species.

Soil moisture content and standard analysis was made of the soil just before it was used to fill the pots. A soil sample from each treatment was also assessed for soil moisture by drying in an oven set at 80 °C for 48 hours. A subsample was also set to NRM Ltd. for nutrient analysis including % organic matter, available nitrogen, potassium, phosphorus and magnesium cation exchange capacity and extractable sulphate.

Table 6. Assessments

Assessment No.	Date	Timing of assessment relative to last application	Assessment type(s)

© Agriculture and Horticulture Development Board 2014. All rights reserved.

1	11/08/14	2 weeks after application	Agar plate counts after 3 days incubation
2	15/08/14	2 weeks after application	Agar plate counts after 7 days incubation
3	22/09/2014	8 weeks after application	Agar plate counts after 3 days incubation
4	26/09/2014	8 weeks after application	Agar plate counts after 7 days incubation

Results

Control of *Fusarium*

After two weeks a bag was retrieved from each pot by lifting the plastic and pulling the bag out by the attached twine. At assessment 1, *Mucor* sp. and another white fungus had developed in many of the agar plates containing surface sterilised stems. The white growth was difficult to distinguish from any initially small, white, colonies produced by the *F. oxysporum,* and so zero records for *Fusarium* were registered (Table 7) with the anticipation that the presence or absence of *Fusarium* would become clear by assessment 2 as the colonies developed pink colouration. *Mucor* sp. had been found in an isolation made from the soil used in the pots. In the unsterilised stem dishes the *Fusarium* was able to grow out and develop colour quicker than from sterilised stems.

Isolations after two weeks of ASD and 3 days incubation of non-surface sterilised stems (Table 7) showed levels of *Fusarium* re-isolation between 40 and 95 %. No clear differences were observed between treatments. No *Fusarium* was re-isolated yet on pieces which had received surface sterilisation. After 7 days incubation, levels of *Fusarium* were between 58 and 100 % of non-surface sterilised stems with treatment seven, Herbie 14.3 + Herbie 67P, showing significantly less *Fusarium* compared with the untreated controls and all other treatments. No other significant results were observed. *Fusarium* was detected in all surface sterilised samples after seven days incubation. Treatment seven, Herbie 14.3 + Herbie 67P, showed lower levels of 33 % re-isolation compared with around 80 % in the untreated and other treatments, although this was not statistically significant.

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. 'SS' were surface sterilised to remove soil microbes to aid detection of *Fusarium* sp. infestation.

© Agriculture and Horticulture Development Board 2014. All rights reserved.

Product name	Mean % of stem pieces showing Fusarium			
	Assessment 1 after 3 days	**Assessment 1 after 3 days (with SS)***	**Assessment 2 after 7 days**	**Assessment 2 after 7 days (with SS)**
1. Untreated	54.17	0.00	95.83	79.17
2. Untreated	79.17	0.00	87.50	83.33
3. Herbie 14.1+ Herbie 67P	58.33	0.00	100.00	75.00
4. Herbie 14.1	79.17	0.00	100.00	87.50
5. Herbie 14.2 + Herbie 67P	95.83	0.00	100.00	70.83
6. Herbie 14.2	83.33	0.00	95.83	87.50
7. Herbie 14.3 + Herbie 67P	41.67	0.00	58.33	33.33
8. Herbie 14.3	75.00	0.00	91.67	62.50
F value (21df)	**NS**	**-**	**0.025**	**NS**
LSD	**49.67**	**-**	**23.86**	**36.53**

* No Fusarium was recorded as although there was colony growth it was smaller than without surface sterilisation (SS) and thus had not produced the pink coloration used to confirm identity

After eight weeks, the bags were dug out and a sulphurous smell was noted. At assessments 3 and 4, there was little difference in the proportion of stems with *Fusarium* sp.; only colony size increased between the 3 and 7 day incubation. Isolations after eight weeks of ASD and 3 days incubation (Table 8) showed levels of *Fusarium* sp. between 90 and 100 %. On pieces which received surface sterilisation, *Fusarium* sp. re-isolation was between 58 and 100 %. No significant differences were shown between treatments, however Herbie 14.3 alone and in combination with Herbie 67P starter tended to show lower levels of *Fusarium* sp.. After seven days incubation, levels of *Fusarium* sp. were the same. *Fusarium* sp. in the stems may have produced growth from the resting spores, or increased in the net bags, and this may have allowed colonies to develop quicker on the agar than after the two week burial.

Table 8. Effect of treatments, after eight 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. 'SS' were surface sterilised to remove soil microbes to aid detection of *Fusarium* sp. infestation.

© Agriculture and Horticulture Development Board 2014. All rights reserved.

Product name	**Mean % of stem pieces showing Fusarium**			
	Assessment 3 after 3 days	**Assessment 3 after 3 days (with SS)**	**Assessment 4 after 7 days**	**Assessment 4 after 7 days (with SS)**
1. Untreated	100.00	79.17	100.00	79.17
2. Untreated	100.00	100.00	100.00	100.00
3. Herbie 14.1+ Herbie 67P	100.00	75.00	100.00	75.00
4. Herbie 14.1	100.00	87.50	100.00	87.50
5. Herbie 14.2 + Herbie 67P	100.00	100.00	100.00	100.00
6. Herbie 14.2	95.83	70.83	95.83	70.83
7. Herbie 14.3 + Herbie 67P	91.67	62.50	91.67	62.50
8. Herbie 14.3	100.00	58.33	100.00	58.33
F value (21df)	**NS**	**NS**	**NS**	**NS**
LSD	**9.87**	**34.14**	**9.87**	**34.14**

Soil analysis before adding the Herbie products is given in Table 9. Soil analysis after eight weeks ASD (Table 10) showed that soil moisture was greater in the three treatments which received the Herbie treatment plus starter. Soil pH ranged from 7.3 to 7.7. Although Herbie 14.3 was a liquid this had not affected the moisture level. The organic matter percentage was greater in all six Herbie treatments compared with the untreated control. Extractable sulphate was also greater in all six Herbie treatments compared with the untreated control, and was highest in treatment seven, Herbie 14.3 + Herbie 67P. Herbie 14.3 and to a slightly lesser extent 14.1 in general also showed greater levels of available nitrogen, phosphorus and potassium. Estimated cation exchange capacity was in the medium range of 12-25 meq/100g across the treatments.

Soil temperature (Table 11) was fairly similar between treatments over the majority of trial ranging between 15 and 30 °C through the eight weeks of the trial. However in the first week after the trial was established pots receiving the Herbie treatments were on average 1 °C warmer than the untreated controls. August was on average cooler outside than the long term average at the Boxworth site, and with a maximum air temperature of only 19.8°C (compared with 22.0°C). However, September 2014 was warmer (Appendix B).

© Agriculture and Horticulture Development Board 2014. All rights reserved.

Table 9. Soil nutrient analysis at the start of the trial, showing pH, available phosphate, potassium, manganese and nitrogen

% soil moisture	pH	P (mg/l)	K (mg/l)	Mg (mg/l)	N (mg/l)
12.54	7.7	78.0	925	193	331.0

Table 10. Soil nutrient analysis after eight weeks sealed in pots showing, available phosphate, potassium, manganese, nitrogen, sulphate and cation exchange capacity

Treatment	% soil moisture	% organic matter content	P (mg/l)	K (mg/l)	Mg (mg/l)	N (mg/l)	Sulphate (mg/l)	CEC (meq/ 100g)
1. Untreated	25.06	6.5	82.0	990	225	331.0	490.4	21.7
2. Untreated	24.11	6.3	82.6	963	231	357.6	481.1	21.7
3. Herbie 14.1+ Herbie 67P	26.59	7.5	88.4	955	224	630.6	508.1	22.1
4. Herbie 14.1	24.55	7.1	85.2	1074	256	901.8	525.2	23.1
5. Herbie 14.2 + Herbie 67P	27.70	7.2	94.2	972	216	491.3	529.0	20.9
6. Herbie 14.2	25.25	7.2	95.4	979	226	470.8	515.2	21.3
7. Herbie 14.3 + Herbie 67P	26.04	7.5	111.0	1044	243	1028.5	617.7	22.2
8. Herbie 14.3	24.34	7.2	112.6	1164	263	1149.8	573.0	24.3

Table 11. Mean soil temperatures in the pots through the course of the trial

	Mean soil temperatures °C		
Treatment	**July**	**August**	**September**

© Agriculture and Horticulture Development Board 2014. All rights reserved.

1. Untreated	24.84	21.62	19.78
2. Untreated	24.93	22.01	20.08
3. Herbie 14.1+ Herbie 67P	26.82	22.28	20.98
4. Herbie 14.1	25.81	22.00	20.16
5. Herbie 14.2 + Herbie 67P	25.59	22.15	20.30
6. Herbie 14.2	25.81	22.36	20.77
7. Herbie 14.3 + Herbie 67P	26.66	22.29	20.34
8. Herbie 14.2	26.08	21.42	20.04
F value (15 df)	**0.010**	**NS**	**NS**
% cv	**2.37**	**1.91**	**2.80**

<u>Crop vigour</u>

Not Applicable

<u>Crop damage</u>

Not Applicable

<u>Formulations</u>

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.

<u>Effect on non-target</u>

No effects observed

Discussion

After two weeks of ASD, treatment seven, Herbie 14.3 with the addition of the starter product, showed significantly less *Fusarium oxysporum* on the plant material extracted (number of stems with *Fusarium*) when compared with the untreated control. Other Herbie treatments did not show

© Agriculture and Horticulture Development Board 2014. All rights reserved.

an effect. *Fusarium* was not eradicated by any treatment. It is believed that the more times the Herbie products are used between crops 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.).

After eight weeks of ASD treatment seven, Herbie 14.3 with the addition of the starter product, continued to show lower levels of *Fusarium* sp. compared with the untreated control and other treatments however this result was no longer statistically significant. There may have been initial suppression of growth of *Fusarium* sp., but after longer burial mycelial growth may have developed between stems within the net bags. However, these would probably be further apart when found as crop debris.

Soil temperatures on average ranged between 15 and 30 °C during the eight weeks of the trial, with a mean 21 °C, below the 25 °C optimum for the Herbie products recommended by Thatchtec. During the first week of the trial, however average temperature was 25 °C, with temperatures in the untreated control on average 1 °C cooler than the six Herbie treatments suggesting the anaerobic breakdown of the organic materials in the Herbie product by microbial action in these treatments might have been raising the soil temperature slightly. This trend did not continue through August and September however. There was some concern that completely anaerobic conditions were not achieved throughout the trial due to the possible loss of seal around the data logger cables in the plastic covers, and possible disturbance with the removal of the first inoculum bag after two weeks.

Soil nutrient analysis after eight weeks showed treatment differences. The three treatments which used the Herbie starter product had on average 2 % greater soil moisture, however although it might be expected that greater moisture would improve ASD this was only shown in one of the three treatments. Although 14.3 was a liquid this did not significantly increase the moisture content of the soil. Percentage organic matter was higher in all six Herbie treatments compared with no treatment. Sulphates were also higher in all six Herbie treatments. This suggests anaerobic conditions were experienced in the pots at points during the trial, and the sulphurous smell in the pots may support this. Levels of sulphates were greatest in treatment seven (14.3 + starter) which appeared to be the most effective treatment. This treatment (14.3 + starter) also showed the highest available nitrogen, phosphorus and potassium figures suggesting ASD amendments such as Herbie may show increased efficacy with altered C:N ratios.

Conclusions

Results have shown one of the ASD treatments tested (Herbie 14.3 + Herbie 67P starter) can significantly reduce levels of Fusarium, with efficacy shown after the shorter burial period of two weeks. Treatment caused substantial changes in some soil parameters. This approach, with

© Agriculture and Horticulture Development Board 2014. All rights reserved.

development could hold potential for use in the short turn-around period between summer crops when soils are warm and there is not usually time for soil sterilisation. Further investigation is required to develop this technique.

The most effective Herbie treatment (14.3 + Herbie 67P starter) will be used in a further pot trial in 2015 to investigate soil treatment under cooler temperatures i.e. winter, when sterilisation is normally carried out post-Christmas in the between-crop gap. This will seek to determine the efficacy of this treatment as an alternative to the declining range of available chemical soil sterilants.

References

O'Neill, T.M., Locke, T. and Dyer, C.J. (2010). A comparison of four pre-plant soil treatments for control of Verticillium wilt in field grown trees. Acta Horticulturae 883:235-242.

Runia, W.T., Molendijk, L.P.G, Ludeking, D.J.W. and Schomaker, C.H. (2012). Improvement of anaerobic soil disinfestation. Comm. Appl. Biol. Sci. Ghent University, 77/4, 753-762.

© Agriculture and Horticulture Development Board 2014. All rights reserved.

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	

© Agriculture and Horticulture Development Board 2014. All rights reserved.

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 (°C)**	**Min temp (°C)**	**Max temp (°C)**	**Rainfall (mm)**
July	17.3	12.7	22.9	45.4
August	17.5	13.6	22.0	54.6
September	14.5	10.1	19.0	51.6
Average conditions during the trial				
Month/period	**Av temp (°C)**	**Min temp (°C)**	**Max temp (°C)**	**Rainfall (mm)**
July	18.1	13.6	23.1	42.0
August	15.5	11.5	19.8	173.8
September	15.15	6.9	24.15	19.0

Weather at treatment application:

Month/period	**Av temp (°C)**	**Min temp (°C)**	**Max temp (°C)**	**Rainfall (mm)**
24/07/2014	23.4	14.5	27.3	0

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.

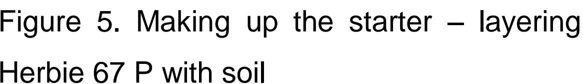
© Agriculture and Horticulture Development Board 2015. All rights reserved

Appendix D – Trial layout

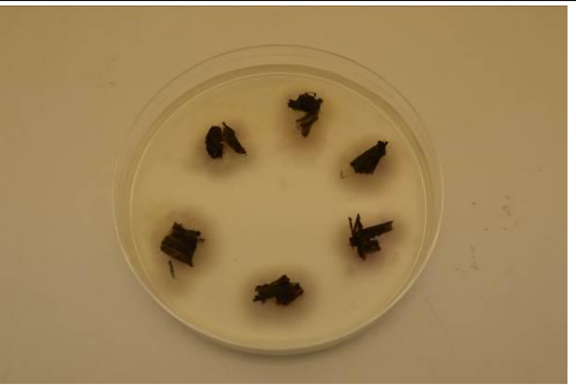
Plot	1	9	17	25
Block	1	2	3	4
Treat.	4	8	1	5
Plot	2	10	18	26
Block	1	2	3	4
Treat.	5	2	3	4
Plot	3	11	19	27
Block	1	2	3	4
Treat.	8	3	6	8
Plot	4	12	20	28
Block	1	2	3	4
Treat.	7	4	4	7
Plot	5	13	21	29
Block	1	2	3	4
Treat.	2	1	5	2
Plot	6	14	22	30
Block	1	2	3	4
Treat.	6	7	8	3
Plot	7	15	23	31
Block	1	2	3	4
Treat.	1	5	7	6
Plot	8	16	24	32
Block	1	2	3	4
Treat.	3	6	2	1

© Agriculture and Horticulture Development Board 2015. All rights reserved

Appendix F – Photographs

Figure 1. *Fusarium* wilt in stocks	Figure 2. *Fusarium* wilt in stocks
Figure 3. Soil sieved into pots	Figure 4. Pot set up, with mesh bags of stocks plant material, soil placed on top +/- starter
 Figure 5. Making up the starter – layering Herbie 67 P with soil	Figure 6. Making up the starter – wetting up before incubation for ten days

© Agriculture and Horticulture Development Board 2015. All rights reserved

Figure 7. Pot sealed with polythene with soil temperature probe inserted	Figure 8. Trial after potting on 24.07.14, before covering with a heavy plastic sheet
	
Figure 9. Extracting the second mesh bag after eight weeks sealed under the soil	Figure 10. Agar plate tests, *Fusarium* re-isolation from six infested plant stem pieces

© Agriculture and Horticulture Development Board 2015. All rights reserved