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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

The use of Trichoderma-based biopesticides for management of soil-borne diseases was evaluated on Choisya and Dianthus. Within the constraints of the experiment, the biopesticide programme was found to perform as well as a standard fungicide programme. In research to evaluate the effect of different application configurations on spray deposition to poinsettia, it was found that lower water volumes were the most efficient at depositing spray liquid on the plant.

Background

Pests and diseases (P&D) are a major constraint on the production of protected edible, and protected and outdoor ornamental crops. Chemical pesticides can no longer be relied upon as the sole method of P&D control, as significant losses of pesticide actives are occurring as a result of government legislation and the evolution of pesticide resistance in target P&D populations. Many growers already use Integrated Pest and Disease Management (IPDM), in which different crop protection tools are combined, including chemical, biological and cultural methods. IPM is now a required practice under the EU Sustainable Use Directive on pesticides. In order to make IPM successful, it is vital that growers have access to a full range of control agents that can be used as part of an integrated approach.

Biopesticides are plant protection products based on living microorganisms, plant or microbial extracts, or semiochemicals (behavior–modifying substances). A small number of biopesticides have been available to UK growers for some time, and an increasing number will be entering the market in the next few years. Within 10 – 20 years, the number of biopesticide products available is likely to exceed the number of conventional chemical pesticides. Biopesticides have a range of attractive properties, in particular they are low risk products for human and environmental safety and many are residue-exempt, meaning they are not required to be routinely monitored by regulatory authorities or retailers. While some biopesticides work well in IPM, UK growers have found others to give inconsistent or poor results, and the reasons for this are often not immediately obvious. Clearly, growers need to get the best out of biopesticide products in order to support their IPM programmes.

AMBER (Application and Management of Biopesticides for Efficacy and Reliability) is a 5-year project with the aim of identifying management practices that growers can use to improve the performance of biopesticide products within IPM. The project has three main parts: (i) to understand the reasons why some biopesticides are giving sub-optimal results in current

commercial practice; (ii) to develop and demonstrate new management practices that can improve biopesticide performance; (iii) to exchange information and ideas between growers, biopesticide companies and others in order to provide improved best-practice guidelines for biopesticides.

Summary

This year of the project saw the last of a series of “benchmarking’ trials completed. The aim of this work was to observe how a number of different biopesticides perform when used in commercial practice. Previous trials had included work on cucumber, pepper, chrysanthemum and cyclamen crops. The benchmarking trial in this year was the final part on a long-term experiment to evaluate the performance of Trichoderma-based biopesticides for managing soil borne diseases on Choisya and Dianthus. The biopesticide T34 Biocontrol (based on the fungal antagonist *Trichoderma asperellum*) was drenched onto Choisya and Dianthus growing in pots on either two or three occasions over the autumn and spring. This was compared to a chemical fungicide programme of one drench application each of Previcur Energy followed by Horti-Phyte. All the pots had been treated with a granular mix of Trianum G (*Trichoderma harzianum*) at the time the plants were potted up in September. Within the constraints of the trial (which included using natural disease infestation rather than artificially inoculating with soil-borne diseases), we found that the biopesticide programme used by the grower performed as well as a standard chemical fungicide system. All of the drench treatments were based on applying large volumes of water to the crop as per label guidance (application volumes equivalent to 10% of pot volume were used); this takes a lot of time to apply and can also increase waste, and there is a need to determine whether smaller volumes can be used that would still deliver the product to the root zone, but which would give savings in terms of the reduced time needed to treat the crop.

Research was also done to investigate biopesticide spray application to crop foliage. Because most biopesticides currently on the market work by contact action, achieving optimum activity depends on delivering an effective dose directly to the target pest or disease. However, there is little information available to growers on how different spray configurations affect biopesticide spray deposition on a crop. In this part of the project, research was done at the track sprayer facility at Silsoe Spray Applications Unit to quantify how different spray configuration affected the deposition of spray liquid onto poinsettia foliage, using a spray tracker dye. The work started with an observational trial done at a commercial nursery to observe application with a boom sprayer of a fungal biopesticide to a poinsettia crop, and which is used as part of a whitefly management programme. Following this, six different spray configurations were tested back in the laboratory at Silsoe that are typically available to

growers using boom sprayers with ornamental crops. The variables tested were nozzle type, forward speed, pressure, nozzle flow rate, applied volume, boom height, nozzle angle and nozzle configuration. The spray volumes applied covered the typical range of water volumes recommended for a biopesticide product, from 500 to > 1000 litres per ha. It was found that although (i) the lowest applied volume resulted in lower doses on the plant than the higher volumes (ii) the lowest volume was the most efficient at depositing spray liquid on the plant, as it resulted in a greater proportion of the spray volume adhering to the plant foliage. The data suggest that the most efficient application strategy is to apply a higher concentration of biopesticide product in a lower volume of water. As part of the same study, the application of the fungal biopesticide to the commercial poinsettia crop was studied to help develop a method for measuring the numbers of fungal spores per unit leaf adhering to the crop foliage. The plan is to be able to use this method to directly compare the deposition of fungal spores with the deposition of spray liquid on the crop.

Work was also started to improve our understanding of the relationship between arthropod pest population dynamics and microbial biopesticide efficacy. Microbial biopesticides are slower acting than conventional synthetic chemical pesticides, and, in some cases, it takes 5 – 7 days for the biopesticide to cause mortality. If the target pest has a short life cycle, and pests reach the adult life stage before they are killed by the biopesticide, then the slow mortality rate of a biopesticide may not be sufficient to stop the pest population from growing. The effect of the biopesticide will also be influenced by the susceptibility of different pest life stages, by environmental conditions, and by crop type. However, the details of how these factors affect the performance of biopesticides are not well understood. This is partly because it is difficult to study the effects of multiple combinations of factors using conventional experiments. An alternative is to put existing data sets from individual factors into a mathematical model of pest population dynamics. Modelling allows predictions to be made about the effects of particular combinations of treatments, a subset of which can then be investigated experimentally. For the Amber project, Dr Dave Skirvin at ADAS has started to develop a so-called “boxcar train” model to describe how pest populations increase over time, and which can be used to investigate mathematically the influence of pest life stage factors, environmental conditions and plant variety on the efficacy of biopesticides applied at different times during the development of a pest infestation. The model is based on a mathematical simulation of a pest transitioning from one life stage to the next until it reaches adulthood and reproduces. The rate of population growth depends on the number of life stages, the development time of each life stage, the natural mortality occurring in each life stage, and the number of offspring produced per adult. The model has been developed initially for glasshouse whitefly, although the basic ideas behind the model allow it to be used for any

pest for which there is adequate data about its life history and susceptibility of different life stages to biopesticides. At the time of writing, the model ran successfully with dummy data, and in the next stage of the project it will be tested using real world data for glasshouse whitefly taken from the scientific literature and from laboratory tests of fungal biopesticides against whitefly nymphs and adults.

Financial Benefits

It is difficult to comment on the financial benefits given the early nature of results. However any improvements to the performance of biopesticides - including issues such as improved efficiency of spray applications, and improved efficacy and reliability - would allow growers to use biopesticides more cost effectively and to reduce over reliance on synthetic chemical pesticides at a time when their availability is declining, and when growers generally are under increasing pressure to produce crops with zero detectable pesticide residues.

Action Points

Growers should ensure that spray applications are done according to best practice guidelines in order to get the best out of biopesticides. No other specific actions are being recommended at this stage until more research has been done.

SCIENCE SECTION

1. Project background, aims and objectives

Growers face a serious challenge to protect their crops from pests and diseases without over-relying on synthetic chemical pesticides. Synthetic chemical pesticides are important tools for crop protection, but overuse can lead to unwanted effects on non-target organisms and control failures through the evolution of resistance in pest and disease populations. Legislation (The Sustainable Use Directive) is now in place throughout Europe which requires farmers and growers to use Integrated Pest and Disease Management (IPDM) wherever practical and effective in order to manage pesticide applications more sustainably. IPM uses combinations of crop protection tools (chemical, biological, physical and cultural controls, plant breeding) together with careful monitoring of pests, diseases and natural enemies.

Biopesticides are plant protection products based on micro-organisms, substances derived from plants and semiochemicals. Biopesticides can make a valuable contribution to pest and disease control when used as part of IPM. Most biopesticide products are recognized as posing minimal risk to people and the environment and they often have a low harvest, re-entry and handling intervals. Biopesticides are usually applied with existing spray equipment, and some microbial biopesticides may reproduce on or in close proximity to the target pest / plant pathogen, which could give an element of self-perpetuating control. Most biopesticides are residue-exempt and they are not required to be routinely monitored for by regulatory authorities or retailers. As alternatives to conventional chemical pesticides, they offer new and multiple modes of action so can help reduce the selection pressure for the evolution of pesticide resistance in pest populations and there is also evidence that some biopesticides stop the expression of pesticide resistance once it has evolved. However, there are disadvantages of biopesticides compared to conventional chemical pesticides and a balanced approach to evaluating them is required. These may include a slower rate of control and often a lower efficacy, shorter persistence, and greater susceptibility to changing environmental conditions. In particular, because biopesticides are not as “robust” as conventional chemical pesticides, and they have multiple modes of action they require a greater level of knowledge on behalf of the grower to use them effectively.

A small number of biopesticides have been available to UK growers for some time, and an increasing number will be entering the market in the next few years. Within 10 – 20 years, the number of biopesticide products available is likely to exceed the number of conventional chemical pesticides. While some biopesticides seem to be working well in IPM, UK growers have found others to give inconsistent or poor results, and the reasons for this are often not

immediately obvious. Clearly, growers need to get the best out of biopesticide products in order to support their IPM programmes.

AMBER (Application and Management of Biopesticides for Efficacy and Reliability) is a 5 year project funded by the Agriculture and Horticulture Development Board (AHDB project code CP158). The research team is made up of crop protection scientists at Warwick Crop Centre, ADAS, Silsoe Spray Applications Unit, as well as two consultants in IPM and biopesticides, Dr Rob Jacobson and Dr Roma Gwynn. The research team receives advice from an Industry Steering Group which is comprised of some of the UK's leading growers, backed up with expertise from AHDB management staff.

The aim of AMBER is to have UK growers adopting new practices that have been demonstrated to improve the performance of individual biopesticide products within commercial integrated pest and disease management (IPDM) programmes. The systems will be developed and demonstrated using approved biopesticide products. Once in place, the systems can be applied to other biopesticide products that become approved in the future.

The project is focused on biopesticides for use in three broad crop sectors: protected edible crops (primarily salad crops such as pepper, cucumber and tomato, as well as protected herbs, and we are also doing targeted work on mushroom crops; however the project does not include any work on protected soft fruit crops at this stage); protected ornamental crops; and outdoor ornamental crops such as nursery stock. These industries are economically important and rely heavily on having effective systems of pest and disease management.

The project has three component objectives:

1. Identify gaps in knowledge that might be causing biopesticides to be used sub-optimally.

2. Develop and demonstrate management practices that can improve biopesticide performance.

3. Exchange knowledge and share experience with growers, biopesticide companies and other industry members in order to provide improved best-practice guidelines for optimum use of biopesticides within more robust IPM.

There are too many biopesticide products, crop types, and pest and disease problems to work on everything. Instead, we are focusing on a targeted number of commercially available biopesticides and on a selected number of pests and diseases on crops with different crop architectures. The general principles developed will then be extrapolated and tested on other

crops later in the project. Once in place, these systems can then be applied to other biopesticide products that become approved in the future.

Objective 1. Identify gaps in knowledge that might be causing biopesticides to be used sub-optimally: Benchmarking the performance of Trichoderma-based biopesticides for management of root rots on Choisya and Dianthus

1.1 General Introduction

In year 1 the project team obtained baseline information on the use and performance of some representative biopesticide products on protected crops. Most of this work focused on benchmarking the performance of six different biopesticide products against six different plant pests and diseases (P&D). A meeting of the Industry Steering Group identified eight priority P&D. These infest a wide range of PE, PO and HNS crops, can be difficult to manage with conventional chemical pesticides due to pesticide resistance and other problems, and cause significant financial losses if not controlled. The selected priority P&D are: (1) western flower thrips; (2) aphids; (3) glasshouse whitefly; (4) two-spotted spider mite; (5) Botrytis; (6) powdery mildew; (7) root rots (Pythium / Phytophthora); (8) downy mildew. Note that a separate work package is being done on mushroom disease management and does not form part of this report. Six different P&D were selected for study in biopesticide benchmarking experiments using crops that represent different types of plant architecture and growing conditions (Table 1).

Table 1: Pests, Diseases and Biopesticides identified for benchmarking experiments.

Pest / Disease	**Crop**	**Biopesticides tested (Mapp Number)**
Powdery mildew	cucumber	AQ10 (17102)
Botrytis	cyclamen	Prestop (15103)
Root rots	Choisya & Dianthus	T34 Biocontrol (17290); Trianum G (16740); Prestop (15103)
Aphids	sweet pepper	Botanigard WP (17054); Majestik (17240)
Western flower thrips	pot chrysanthemum	Botanigard WP (17054) tank mixed with Majestik (17240)

Glasshouse whitefly	mint	Naturalis L (17526) (tbc before start of trial)

The benchmarking had two objectives:

- To assess the use of biopesticides at recommended rates against invertebrate pests and diseases in commercial crops.
- To observe and record data on how the grower uses the biopesticide product(s) as part of Integrated Pest and Disease Management (IPDM), including product storage, application, and pre- and post-application monitoring. The component relating to application was, in this early project stage, designed more as an observation exercise, rather than to quantify application performance. The reasons for this were:
    - The knowledge relating to current practice in application for protected crops is limited since research in this area is negligible and equipment tends to be bespoke, rather than standardised
    - Experience suggests that obtaining information about application from a survey is usually of limited value: a conversation with the spray operator is always the most successful approach, so this was the main objective for each of the 'observation' exercises
    - Quantifying application performance can be costly and there are particular challenges with high-value crops
    - The resources available for application research in AMBER are sufficient for a small number of focused experimental studies rather than covering a wide range of trials
    - It was necessary to find out what equipment and expertise is available at the potential trial sites before any application study could be devised

Because of positive engagement by growers, there was some intervention by the AMBER project team in some of the trials, however for the most part, the biopesticide use was undertaken according to the spray operators' own procedures and the team merely observed. Some visual assessments were made, and non-intrusive measurements were made to allow the approximate application volumes to be determined.

For this part of the project, benchmarking trials of biopesticide performance were done in year 1 on the following pests / disease and crop combinations: (1) aphids on an organic pepper crop; (2) western flower thrips on chrysanthemum; (3) powdery mildew on cucumber; (4) Botrytis on cyclamen. A fifth trial was started in year 1 to investigate control of root rots on choisya and dianthus, This trial was done from autumn of year 1 to spring of year 2 and the

results are given below. Trial number 6 was planned in year 1 to investigate biopesticide performance against glasshouse whitefly on mint, however it could not be done because of operational issues experienced by the host grower. By the end of year 1, it was evident that issues around spray application were key to improving biopesticide performance. Therefore, we decided to substitute the whitefly trial from year 1 with a case study in year 2 to investigate the performance of a horizontal boom sprayer used to apply biopesticides against whitefly on a commercial poinsettia crop. This is reported on as part of Objective 2 in this report.

1.1.2 Benchmarking the performance of Trichoderma-based biopesticides for management of root rots on Choisya and Dianthus

Pythium / *Phytophthora* root infecting pathogens have a wide host range and potentially cause severe damage in the PE, PO and HNS sectors. The activity of the biofungicides currently available is not confined to these oomycetes, but includes root infecting fungi such as species of Fusarium and Rhizoctonia. Dianthus finals grown overwinter (in unheated glass) are susceptible to Fusarium wilt and Pythium root rot. In a study of plant clinic reports (HNS 169) Choisya were shown to be susceptible to *Thielaviopsis basicola* (black root rot), *Fusarium* spp., *Pythium* spp., and *Phytophthora* species including *P. citricola*, *P. cryptogea* and *P. cinnamomi* with wilting symptoms resulting from root rot often following when plant stress has been caused such as at potting or when temperatures rise over 30°C. The *Pythium ultimum* group are, however, favoured by temperatures between 10°C to 15°C and wet growing media. Work on a *Pythium* / *Phytophthora* root infecting pathogens rather than a downy mildew was adopted, because there are currently no UK biofungicide products with recommendations against downy mildews and would provide a contrast to the foliar pathogens with regard to optimisation of product application.

In this benchmarking trial, the performance of the fungal biopesticides Trianum G (*Trichoderma harzianum* strain T22) and T34 Biocontrol (*Trichoderma asperellum* strain 34) were evaluated against root rots on Choisya and Dianthus grown as commercial glasshouse crops. Trianum G is approved for use on protected ornamentals and is used routinely in the growing media delivered to the host nursery for potting-on liners and so was present in all treatments. T34 Biocontrol was selected as it was already in use on the nursery on species susceptible to root rots and has label recommendation for use against Fusarium wilt on Dianthus, but is known to have wider activity including oomycetes and can be used under EAMU 1118 of 2012 for protected ornamentals.

1.1.3 Methods

The Choisya cv. Sundance to be potted as finals were kept from receiving any curative chemical fungicides in addition to their preventative biofungicide programme following plug potting as liners. This was to ensure no adverse effect on the biopesticides by chemicals and to aid the build-up of plant resistance to disease from the biofungicides. The plug plants imported in April 2016 and destined for the experiment received T34 Biocontrol on 2nd May 2016 and Prestop on 16th May and 11th July 2016. However, by early August 2016 7% of the liners (16 out of 240 trays of 15 plants) which had received these drenches were dying following a period of drought stress. No losses were observed in plants from the same delivery which had instead been treated with Previcur Energy (Fosetyl-aluminium + propamocarb hydrochloride), Horti-Phyte (Potassium phosphite) and then Promess (propamocarb hydrochloride) on the aforementioned dates. It was shown that some of the Choisya plugs had pathogens present on their roots on arrival, although they looked healthy. Of the three symptomless Choisya plugs kept back from potting and laboratory tested in May the roots of one had *Thielaviopsis basicola* and another both *Phytophthora* sp. and *Pythium* sp.

Choisya, showing no visible symptoms, were selected in September 2016 from the biofungicide treated liners. The Dianthus modules arrived on site in September 2016 without information on their treatment history. Dianthus cultivars Shirley Temple and Cosmopolitan were potted on 15 September 2016 into 1.5 L pots and Choisya cv. Sundance into 3 L pots using a peat/bark mix with Trianum G incorporated. Plants were grown in a Venlo glasshouse complex maintained frost-free, without artificial lighting, and stood on sand beds with overhead watering by hand. The nursery deploys pest biocontrol organisms as required, rather than use chemical pesticides.

All three cultivars underwent three treatment programmes (Table 2). Use of T34 Biocontrol was under EAMU 1118 of 2012 for protected ornamentals, otherwise all uses were Authorised/on-label. One programme of chemical products (P1) and two of the biological product T34 Biocontrol (P2 and P3), with all initially receiving Trianum G from incorporation into the nursery's standard growing media for finals by the suppliers ICL. All drench treatments were given at 10% of pot volume (a standard industry practice and recommended on the product technical notes). The first drench date, in October 2016, was followed by another after two months to keep in step with the label for T34 Biocontrol. The third drench was in spring when the plants were starting to grow again.

Table 2: Treatment programmes (P1 to P3) used on Dianthus and Choisya against root rots.

P	Date applied	Treatment at potting [MAPP code]	Active ingredient	Rate of use	Description of treatment	Comments
1	15.09.16	Trianum G [16740]	*Trichoderma harzianum*		Biofungicide granules incorporated in the growing media in the bulk bale	Supplied ready-mixed by ICL
2	15.09.16	Trianum G	*Trichoderma harzianum*		As for P1	
3	15.09.16	Trianum G	*Trichoderma harzianum*		As for P1	
P		**Second Treatment**	**Active ingredient**	**Rate of use**	**Description of treatment**	**Comments**
1	04.10.16	Previcur Energy [15367]	Fosetyl-aluminium + propamocarb hydrochloride	3 ml / m² with water at 10% pot volume	10 % of pot volume drench	
2	04.10.16	T34 Biocontrol [17290*]	*Trichoderma asperellum* Wettable Powder	10 g per 1000 L growing media. Irrigation rate	10% of pot volume drench	Pre-soaked T34 for 1.5 hours. Repeat every 2-3 months
3	04.10.16	T34 Biocontrol	*Trichoderma asperellum*	10 g per 1000 L growing media. Irrigation rate	10% of pot volume drench	As for P2
P		**Third Treatment**	**Active ingredient**	**Rate of use**	**Description of treatment**	**Comments**
1	08.12.16	Horti-Phyte	Potassium phosphite	200 ml / 100 L water	10 % of pot volume drench	Not a registered fungicide
2	08.12.16	T34 Biocontrol	*Trichoderma asperellum*	10 g/ 1000 L growing media. Irrigation rate	10 % of pot volume drench	Pre-soak T34 30 mins. Repeat after 3 months
3	08.12.16	None	n/a	n/a	n/a	n/a
P	**Date**	**Fourth Treatment**	**Active ingredient**	**Rate of use**	**Description of treatment**	**Comments**
1	23.03.17	None	n.a.	n.a.		Grower standard = no treatment at this timing if no visible problems
2	23.03.17	T34 Biocontrol	*Trichoderma asperellum*	10 g / 1000 L growing media. Irrigation rate	10 % of pot volume drench	Pre-soak 30 mins. Experimental high rate per timing
3	23.03.17	T34 Biocontrol	*Trichoderma asperellum*	5 g / 1000 L growing media. Irrigation rate	10 % of pot volume drench	Pre-soak 30 mins. Lower repeat rate recommended

Trial design

Plants were set out in plots with 200 Choisya (arranged 10 pots wide and 20 pots long). There were also two plots of 100 Dianthus per treatment (each 10 pots wide and 10 pots long), with all plants for each treatment adjacent on the bed (Figure 1). Plots were separated by approximately 0.5 m wide pathways.

Figure 1: Plants on the sandbed after potting in September. Choisya plants (front left of picture) in blocks of 200 plants per treatment and Dianthus with two cultivars each of 100 plants per treatment (right front and across the rear).

Treatment application assessments

Observations were made of nozzle type and likely pressure and flow rate and applied volume was calculated subsequently.

Sample pots of Choisya and Dianthus from across the width of plots were weighed before and after treatment to see how much liquid was added to the pot by the drench and any variation across the bed. In addition containers were placed on the sand bed between some of the pots before they were sprayed in order to catch the spray falling and give an indication of the evenness of the spray coverage. In October containers were used with a 56 mm diameter rim, whereas the greater number used in December had a 65 mm diameter rim. A digital thermometer was used to record the air temperature and the temperature of the water in the spray tank at the time of the spray applications.

The T34 product was pre-soaked in order to obtain a faster colonisation of the pots by the beneficial fungus (as directed by the product Technical Leaflet). On 4th October the T34 Biocontrol was added to a 10 L capacity bucket 90 minutes before use. The product dispersed fully to create a blue-tinged liquid which left no deposits in the bucket. The opened foiled packet was then stored in a sandwich box in the fridge in the pesticide store. On 8th December, the T34 Biocontrol treatment for the three plots of P2 were each made up in 2 L plastic jugs and stood in the pesticide store for half an hour while the chemical for P1 was applied. The grower used water to give a 10 % of pot volume: in October, this meant that the 600 L of Choisya (200 pots of 3 L) for each of P2 and P3 were treated with a total of 6g

of T34 Biocontrol prepared in 60 L of water. The two varieties of Dianthus per treatment totalled 300 L in volume (200 pots of 1.5 L), therefore requiring 3g T34 Biocontrol prepared in 30 L of water. In December P2, but not P3, was re-treated.

Application was via a lance with two FF110 – 20 nozzles which produced spray fans in parallel with the lance (i.e. the spray was directed forwards and backwards from the lance end). The lance was on a hose reel to a 300 L tank with the pump. The sprayer was operated at 3 bar on the pressure gauge on the tank (there was no gauge on the lance) but probably < 1.0 bar at the nozzle. The plastic lance was about 1 m long so that, by lifting it up almost horizontal, the spraying was able to reach the far side of a plot. A separate sprayer tank and lance was used for the T34 Biocontrol and the chemical product (either Previcur Energy in October or Horti-Phyte in December) to ensure there was no cross-contamination. No chemical was used in P1 in March 2017, in line with grower practice.

For applications in October and December, samples were taken of the dilute T34 Biocontrol product in the spray tank and from the lance and the powder from the product packet into sterile 25 ml universal tubes. Each tube was agitated and 1.5 ml taken off and spread thinly over the whole surface of a 90 mm plate of potato dextrose agar (PDA). In October, another plate was also prepared using a sample of the T34 Biocontrol product taken directly from the packet, placing six specks on the agar and then tapping it so that the powder covered the agar surface. All plates were incubated inverted at 20°C in the dark for seven days and then examined for colony growth of *Trichoderma*.

Crop Assessments

Prior to each drench, the plants were examined for disease symptoms and the development of any signs of wilting. The number of plants with any symptoms were counted and the severity of their symptoms noted. Plant vigour (excluding those wilting) was recorded for each plot overall using a 0 (dead) to 9 (strong and healthy) index. Foliar phytotoxicity was also recorded and an index of 0 (no phytotoxicity) to 9 (dead) used. Any plants which start to die were returned to the laboratory for assessment.

At the final assessment in spring 2017 (close to the point of sale) the plants were assessed for plant vigour and phytotoxicity as described above. One Choisya plant, in the same position in each of the five assessment blocks was un-potted and the root coverage of the root ball and the proportion of roots that were a grey colour (an indication of Pythium root rot) was recorded. The foliar vigour index (0-9) based on plant size and the amount of new growth was also recorded for each of these pots.

Data loggers to record temperature in the compost and temperature and humidity in the crop canopy recorded throughout the benchmark.

1.1.4 Results

Treatment application

Different interpretations of the label led to a lack of clarity about the required application volume for the particular application conditions. The decision was made to use volumes appropriate to incorporation in growth media, i.e. 10% of pot volume. This extraordinarily high volumes is unlikely to be practical for large areas.

On 4 October the lance output suggested that poor pressure control observed was likely as a result of high flow rates and constriction at the nozzle. This however did not affect application apart from slowing it down. The spray operator was observed treating the pots by walking up and down alongside the pots and moving the 1.5 m long lance back and forth over the trial area and taking for example 10 minutes to drench 400 pots of Choisya.

The samples from the spray tank and the lance and the packet when plated onto Potato Dextrose Agar (PDA) grew to produce the green sporulation typical of *Trichoderma spp.* by seven days. Each 90 mm agar plate received 1.5 ml of suspension and produced similar colony counts of around 25 colonies per 10 mm x 10 mm. It was noted that the colonies from the lance were single and evenly spread over the plate, whereas those from the tank tended to be clumped in twos and threes.

Crop Assessments

Foliar vigour, wilting, and any phytotoxicity as a result of the 4 October 2016 treatment applications were assessed prior to further treatments being made on 8 December 2016. A few of the 200 Choisya in each of three treatments had some leaf bleaching unlikely to be associated with a root rot (seven pots in P1, four pots in P2 and 10 pots in P3) but plants were otherwise showing excellent uniform vigour (Index 9). In the Dianthus, a few of the 100 plants of cv. Shirley Temple were wilting in each of the treatments (two pots in P1, three pots in P2, and in P3 two pots wilted plus another three having poor vigour (Index 3). The dead plants had not made any growth in the three months since potting suggesting that the plants were already infested at potting. The remaining cv. Shirley Temple had an excellent vigour index of 9, as did all the 100 cv. Cosmopolitan in each of the treatments. Pots with dead plants were removed according to grower hygiene procedures, but plants with poor vigour were left to see if they recovered or failed. Laboratory isolation from a pot of cv. Shirley

Temple from each of the three treatments confirmed the presence of Fusarium, which from the pale pink colony colour and the symptoms was probably the wilt pathogen *Fusarium oxysporum*.

On 6 April, the Choisya plants all appeared healthy and were assessed for foliar vigour (0 dead to 9 excellent) together with the grower who stated that all plants of Choisya were marketable, with a few starting to flower. The variation across the 200 Choisya plants in all three treatments was recorded by division of plants into blocks of 40 plants. Plants given the lower total dose of T34 Biocontrol (P3) had on average less-vigorous plants, with no block of plants given a very good vigour score of 8 (Table 3) in contrast to plants given either three full doses (P2) or the chemical fungicide treatment.

Table 3: Foliar vigour Indices (0 all dead to 9 all excellent) for Choisya on 6 April 2017 (i.e. at the end of the trial) for the grower standard chemical programme (P1), the full T34 Biocontrol programme (P2) and the reduced dose and less frequent application programme (P3).

Position on bed (scoring block)	Foliar vigour index (0 to 9)		
	Choisya in P1	Choisya in P2	Choisya in P3
A	6	7	6
B	7	7	6
C	6	8	7
D	7	8	5
E	8	6	6
Mean vigour index	6.8	7.2	6.0

For Choisya on 6 April 2017, the roots of a plant in each of the five assessment blocks were examined for the root coverage of the root ball and the proportion of roots that were a grey colour (an indication of Pythium root rot) rather than white and healthy (Table 4). The moistness of the compost was noted and was found to be uniform across the samples. Only a small amount of Pythium root rot (5% or zero on most plants) developed in the Choisya. One plant in the full programme, P2, had 20% of roots grey, but otherwise the incidence and severity of root rot was low and similar for plants given different treatments.

More roots (a mean 34% root ball cover) were produced by Choisya in P3 (Table 4) given reduced application of T34 Biocontrol, than plants of P2 (with 22% root ball cover) given the greatest accumulated total of T34 Biocontrol of 30 g / 1000 L of growing media over three applications. However, the best root production (47% root ball cover, more than twice that of P2) followed the use of Previcur Energy (fosetyl-aluminium + propamocarb hydrochloride) followed by Horti-Phyte (potassium phosphite), but as there were no untreated pots it was not

known whether or not this was a benefit of these treatments or conversely whether T34 Biocontrol application had reduced root production.

The foliar vigour index (0-9) based on plant size and the presence of new growth for each of the five sampled Choisya pots per treatment (Table 4) showed no difference (a mean of 7, denoting good vigour). Foliar vigour was unaffected by the differences in root coverage up to this date.

Table 4: For five Choisya plants per treatment on 6 April 2017, the proportion of growing media surface around the pot face covered by roots (% root ball cover) and the proportion of these roots that were grey with Pythium. Foliar vigour Indices (0 = dead to 9 = excellent). Grower standard chemical programme (P1), the full T34 Biocontrol programme (P2) and the reduced dose and less frequent application programme (P3)

Position on bed (scoring block)	Choisya in P1			Choisya in P2			Choisya in P3		
	% root-ball cover	% of roots grey	Foliar vigour index	% root-ball cover	% of roots grey	Foliar vigour index	% root-ball cover	% of roots grey	Foliar vigour index
A	40	5	6	20	0	7	40	0.1	8
B	45	5	7	20	0	7	40	5	8
C	50	5	7	12	0	8	33	5	7
D	50	0	7	45	5	8	33	0	8
E	50	0	8	15	20	6	25	5	6
Mean	47.0	3.0	7.0	22.4	5.0	7.2	34.2	3.0	7.4

The Dianthus were also assessed on 6 April 2017, but for foliar vigour only (not roots) as wilt symptoms from *F. oxysporum* arise as a result of vascular plugging in the stem after entering roots, not from root rotting. The number of plants that had wilted (including those removed previously for diagnosis) were counted per treatment for each of the varieties. The plants in both varieties which received either P1 or P2 and had not wilted were all very uniform across the treatment block and were given a vigour index of 9. However, plants of cv. Shirley Temple in P3 (at the back of the bed) were more variable and received a vigour index of 7 and in addition had six plants that were dead or dying, three more than previously, indicating (assuming equal distribution of infected plants) that this treatment was less able to stop wilt disease progress. For cv. Shirley Temple, P2 had four dying plants, two more than previously, whereas the grower standard P1 still had two. No plants of cv. Cosmopolitan had any signs of wilting. A sample of sand from under the Dianthus was taken to the laboratory to check for infestation by putting it onto agar, and no Fusarium isolated.

Air temperature rose over 25°C in September not long after potting (Figure 2). The glasshouse at the nursery is not heated and so air temperatures fell after September, as expected. In December and January, frost protection air circulation in the glasshouse kept the temperature falling below zero (Figure 3).

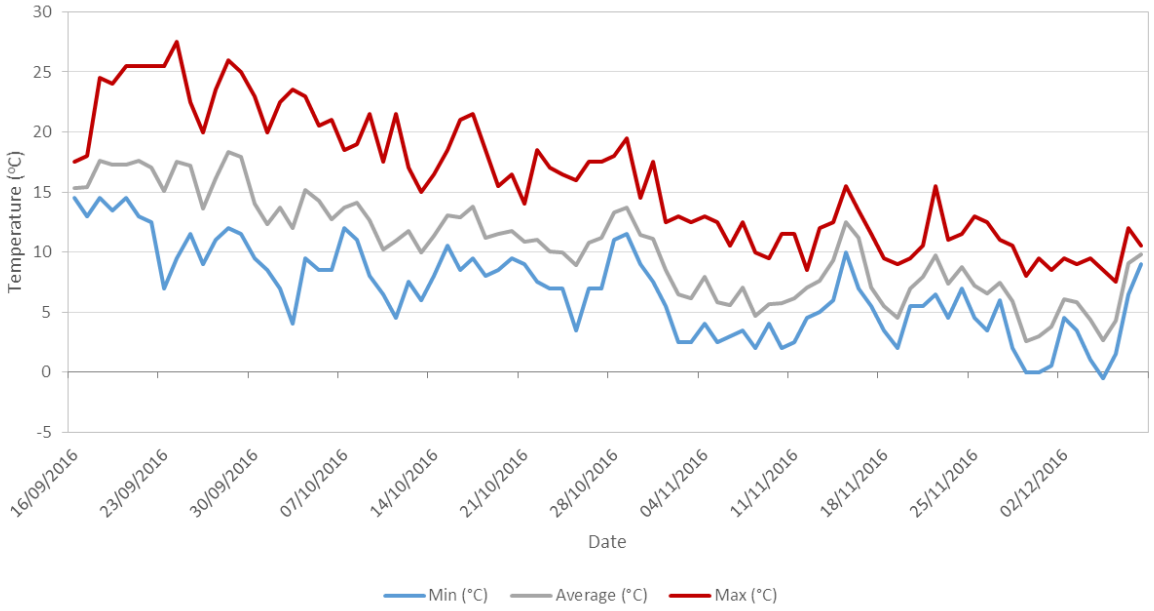

Figure 2: Air temperatures (minimum, maximum and mean daily) at Choisya plant canopy height from 16 September 2016, just after potting. Readings are continued on Fig.3.4.

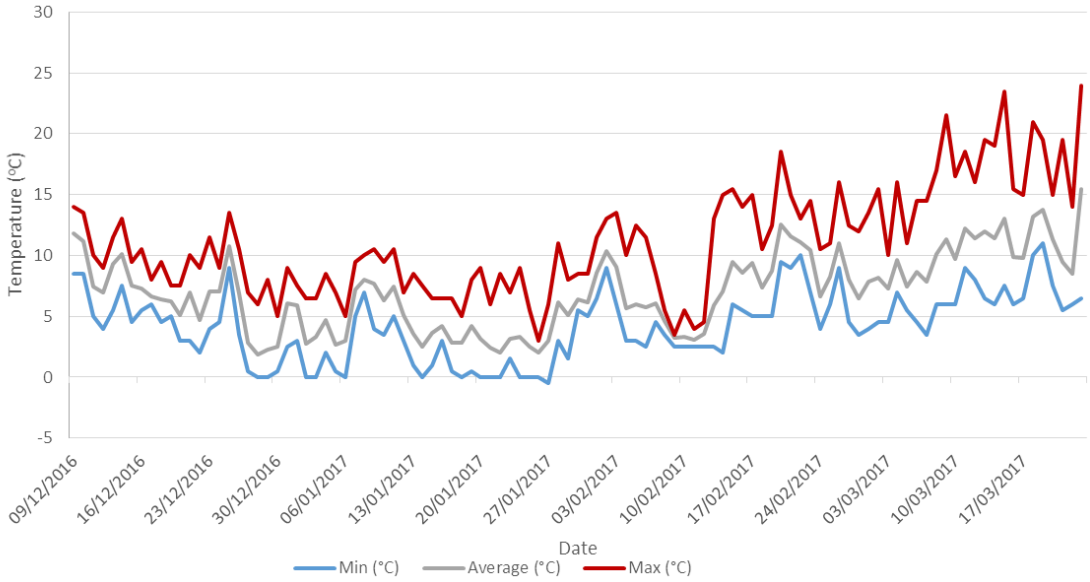

Figure 3: Air temperatures (minimum, maximum and mean daily) at Choisya plant canopy height continued, up to the date of the final biofungicide application on 23 March 2017.

The relative humidity at plant canopy height was most variable between September and November 2016, falling at times to below 60% r.h. (Figure 4). Humidity was thereafter rarely below 80% until February 2017 (Figure 5).

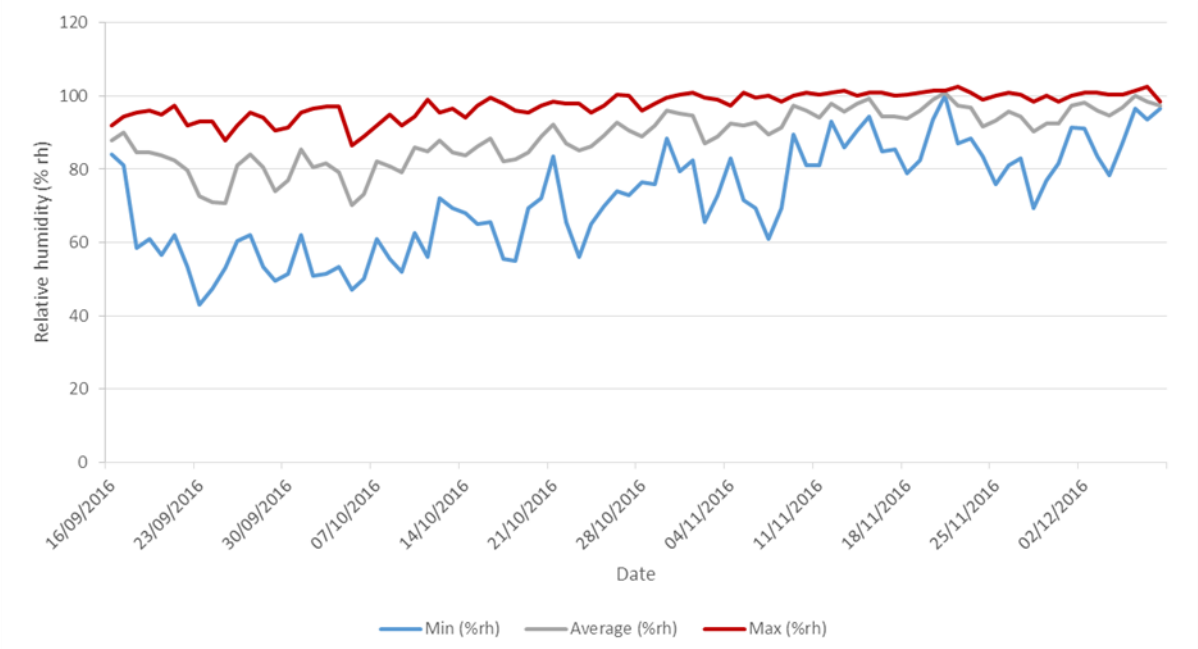

Figure 4: Relative air humidity (minimum, maximum and mean daily) at Choisya plant canopy height from 16 September 2016, just after potting. Readings are contd. on Fig. 3.6.

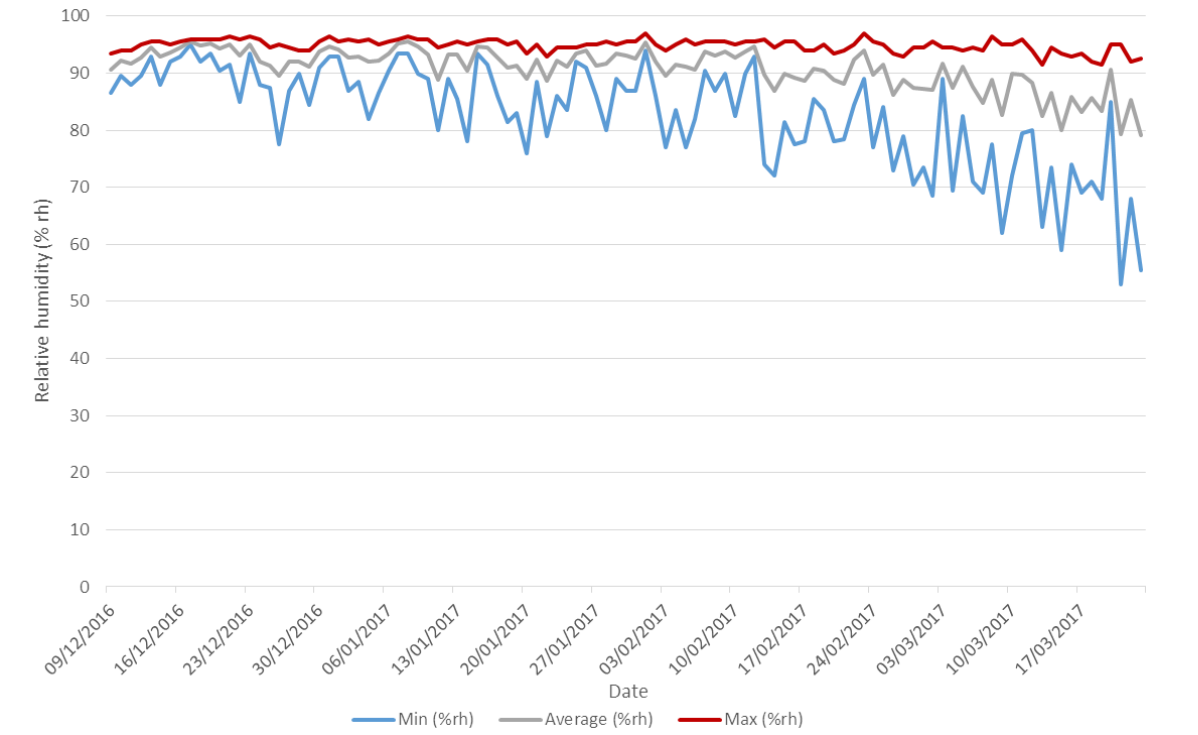

Figure 5: Relative air humidity (minimum, maximum and mean daily) at Choisya plant canopy height continued, up to the final biofungicide application date on 23 March 2017.

Temperature in the growing-media between September 2016 and March 2017 was a few degrees cooler than the air temperature during sunny periods and a few degrees warmer when air temperatures dipped towards freezing in December and January (Figure 6). The moisture probe, calibrated for the growing media used at the nursery (Figure 7), showed that in the P2 Dianthus pot the water content was 16% at the first drench day on 4 October and 7% on the 8 December. Apart from in February and March 2017 the sandbed irrigation provided the pot with at least 10% water by volume (i.e. field capacity). The drier period in November was unintentional, and would have advanced the expression of any Fusarium wilt in the Dianthus.

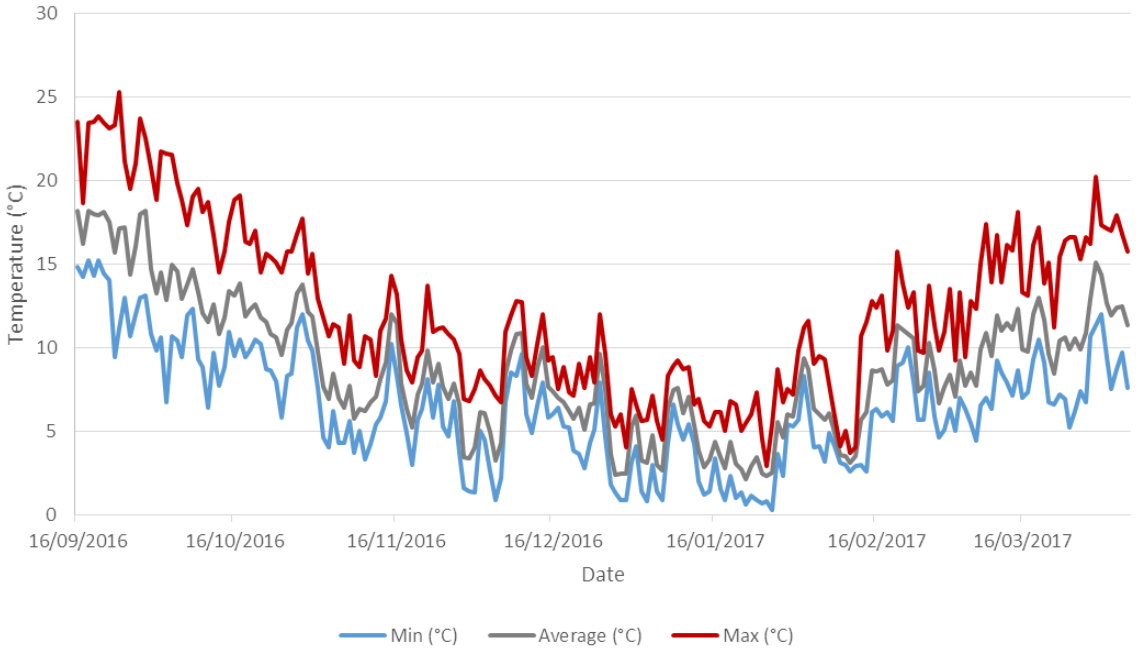

Figure 6: Temperature (minimum, maximum and mean daily) recorded in the peat-based growing media of Dianthus plant from the day after potting in September 2016 until the date of the final biofungicide application on 23 March 2017.

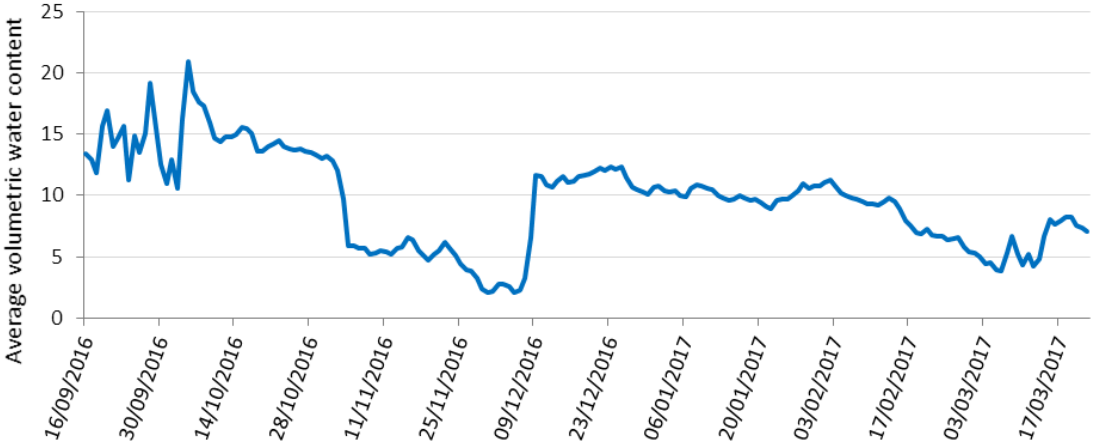

Figure 7:. Mean daily Average Volumetric Water content recorded in the peat-based

growing media of a Dianthus plant in P2 from the day after potting in September 2016 until the day before the final biofungicide application on 23 March 2017.

1.1.5 Discussion

The Choisya and Dianthus used in the experiment were purchased by the nursery and it was found that a proportion of them were non-symptomatically infected by various root rot pathogens. Biofungicides work as protectants (not curatives) and so further losses were expected and this project allowed comparison with the chemical fungicide treatments applied prophylactically to the same batches of plants. The T34 Biocontrol label and EAMU are confusing to read with apparently conflicting information on the dose for drenching and whether this is covered by the word “irrigation”. Some clarification was obtained from Fargro and the issue appears to arise because of the way text is abstracted by the UK pesticide regulators.

Further work is required to determine whether bio-fungicides applied to growing-media survive and can potentially multiply given conditions suitable to them, and whether these conditions can be achieved during plant production at commercial nurseries. Conditions probably required, such as maintained humidity, nutrients (from root exudates) and fewer temperature extremes, are more likely to be achieved in containers or the soil than on leaves and so survival is more likely. No particular conditions are stipulated on the product information sheet as being required for the activity of T34 Biocontrol after application to plants. Beneficial organisms working by competition for nutrients with pathogens will however need to be kept at high populations to be effective and it is likely more information will be able to be sourced on microbial interactions in the soil now that techniques are becoming available for the molecular quantification of organisms in soil and water.

1.1.6 Overall Conclusions

In the work with Choisya and Dianthus the T34 Biocontrol (*Trichoderma asperellum*) powder dispersed readily in the water when made up for application (in contrast to the clumping seen for AQ10 and Prestop used in other experiments). Only a small amount of Pythium root rot developed in the Choisya finals. In the absence of a true control to provide data on absolute efficacy, we can conclude that the T34 performed as well as the standard chemical treatment under the conditions of the experiment. More roots were produced by Choisya when the second application was delayed and this repeat dose halved, compared with when they were given three full rate applications. However, the best root production followed the use of Previcur Energy (fosetyl-aluminum + propamocarb hydrochloride) followed by Horti-Phyte

(potassium phosphite), but as there were no untreated pots it was not known whether this was a benefit of the standard treatments or conversely whether the higher (30 g total rather than 15g) T34 Biocontrol application had reduced root production. There is little evidence from the scientific literature to indicate that T34 inhibits root growth, however internet searches shows that some agronomists are stating on line that Previcur Energy promotes root growth. Further observations are needed, but there was an indication that Choisya given three full rate applications of T34 Biocontrol had more-vigorously growing foliage at the time of marketing although the root growth was poorer. A few Dianthus of one cultivar were affected by Fusarium wilt, but too few to draw conclusions on product efficacy. It was believed that Dianthus infection occurred before application of protectant T34 Biocontrol. Neither plant species suffered any foliar phytotoxicity from drenching T34 Biocontrol over the foliage.

Objective 2. Develop and demonstrate management practices that can improve biopesticide performance.

2.1 Evaluating the performance of a horizontal boom sprayer for biopesticide application to poinsettia

As described above, a case study was done in year 2 to investigate the performance of a horizontal boom sprayer used to apply biopesticides against whitefly on a commercial poinsettia crop.

2.1.1 Site visit and sprayer set up.

A visit to the grower's site (Borden Hill, Stratford) was made by the Amber team, with the new sprayer being the focus of attention. The grower had recently invested in a manually operated horizontal boom sprayer, but had not yet used it. The boom sprayer was to replace applications using a Ripa gun. The aim of the application was to target the biopesticide (Naturalis) at the underside of leaves, where the whitefly are present.

We identified some possible improvements to the sprayer set up at this point, which involved reducing the boom height, increasing the pressure and angling the spray by twisting the entire boom. This would result in a higher droplet velocity and a more horizontal trajectory that would have a better chance of passing underneath leaves. This was tested with a water spray and water-sensitive paper, which was unable to show any improvements, but did demonstrate that some spray was reaching the underside of the leaves.

A second visit was made to the site to observe the application of the biopesticide.

- General sprayer setup; Hypro FF110-03VP nozzles angled 50° forward facing.
- Pressures: Pump 7.0 bar, sprayer 2.5 bar, nozzle 2.0 bar. Speed of sprayer was 1.39 km/h
- Volume delivered was estimated to be 850 l/ha.
- It was observed that the tank system did not allow agitation during spraying.
- Assuming that the glasshouse was full, the Grower calculated that application using a Ripa gun would cost £380 per spray and with the boom £70 per spray. A total saving of £310 per spray.
- Some plants were then brought back to Silsoe to enable different spray configurations to be tested for deposition on each side of the leaves using a tracer dye.

2.2 Evaluation of the effect of different application configurations on spray deposition to poinsettia using a track sprayer

2.2.1 Methods

Six different treatments were tested, with 6 plants per treatment. All plants were sprayed using the Silsoe track sprayer. Photographs of the plants were taken following the application. All the leaves were sampled from each plant once dry, and the upper and lower sides of each leaf were washed separately. Then the total leaf area was determined by image analysis. The ratio of the quantity of spray on the upper to lower leaf surface can be calculated, as well as the total quantity per unit area per plant on upper and lower leaf surfaces.

The treatments tested are given in Table 5. The aim was to explore the kind of configurations which could be achievable at Borden Hill. Treatment 6 could be considered a 'control', although it was different from the actual settings used for the first Borden Hill application – the pressure was higher, and the boom height was possibly greater. However, this would be considered more typical.

Table 5: Spray configurations evaluated for effect on spray deposition on poinsettia leaf surfaces

Trt	Nozzle	Forward speed, km/h	Pressure, bar	Nozzle flow rate l/min	Applied vol, l/ha	Boom height, cm	Nozzle angle	Config
1	FF110-015	1.4	3.0	0.6	514	30	30	Alt. forw /back

2	FF110-03VP	1.4	3.0	1.2	1028	30	30	Alt. forw /back
3	FF110-015	1.4	3.0	0.6 * 2	1028	30	30	Twin
4	FF110-02	1.4	4.0	0.92	792	30	30	Alt. forw /back
5	3D-02	1.4	3.0	0.8	685	35	40	Alt. forw /back
6	FF110-03VP	1.4	3.0	1.2	1028	35	0	Vertical

2.2.2 Results and discussion

Figure 8 shows the total quantity of spray liquid per unit leaf area for the different treatments. The variability is relatively high, partly because there were two different growth stages amongst the plants that were used and therefore the likelihood of statistically significant differences is low. However, it can be seen clearly that the lowest applied volume (treatment 1) does result in lower doses on the plant than the higher volumes.

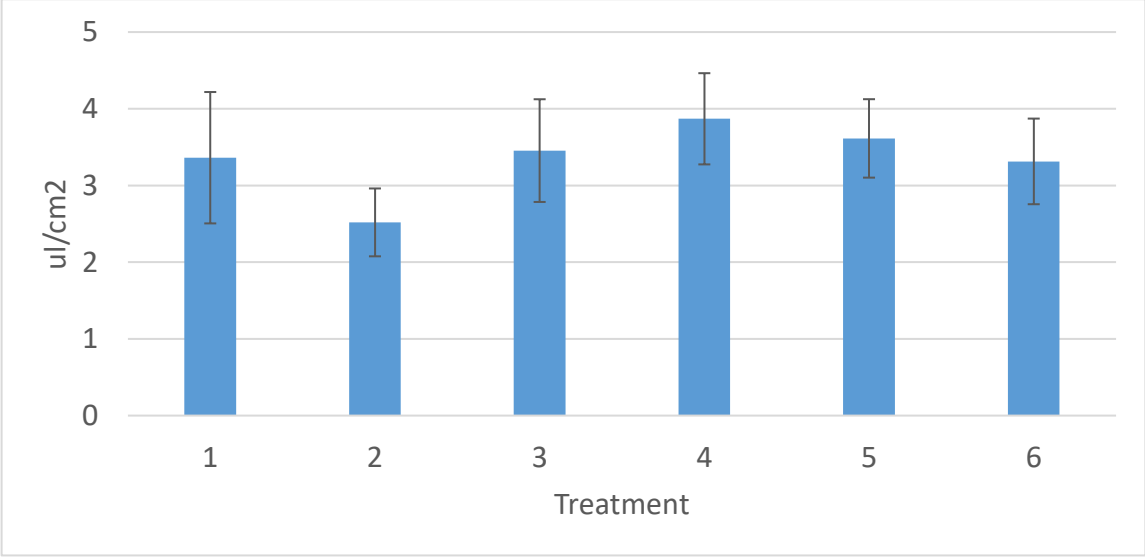

Figure 8: Total spray deposit per unit leaf area per plant.

The distribution between upper and lower leaf surfaces is given in Figure 9. A very small percentage of the spray is deposited on the underside of the leaves.

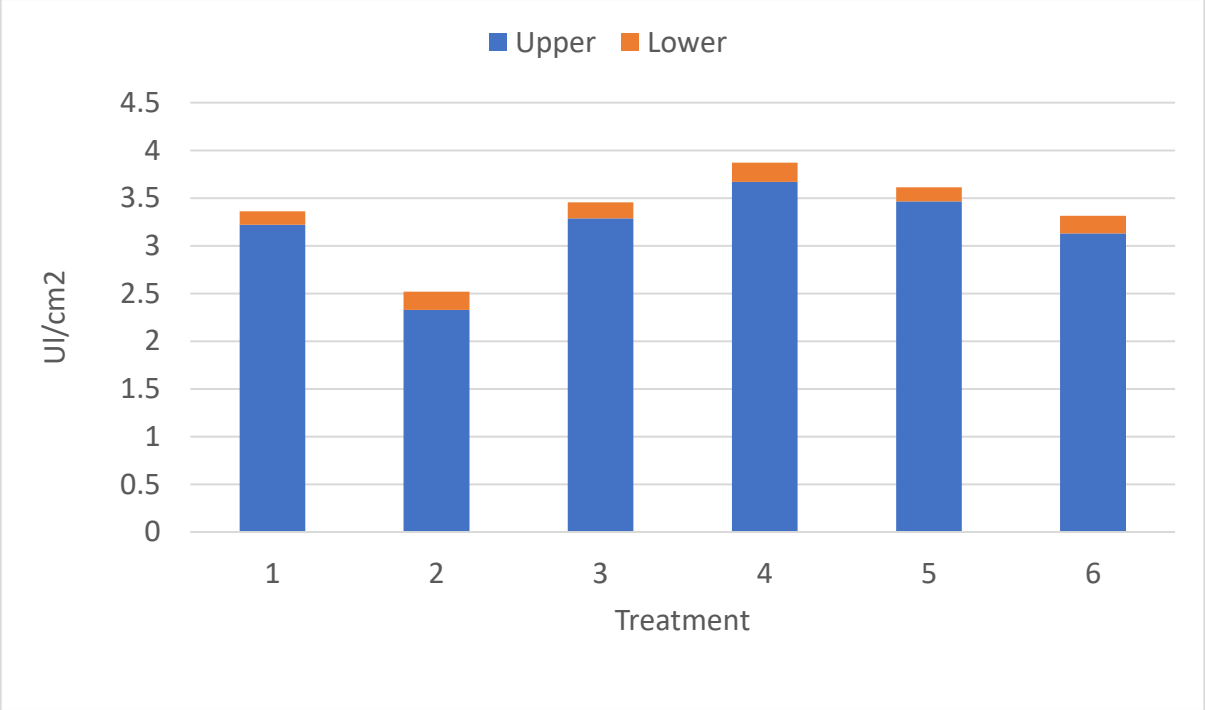

Figure 9: The proportion of spray deposited on upper and lower leaf surfaces

Figure 10 shows the same data as in figure 8 but normalised to 500 L/ha. This relates to the proportion of spray that is deposited on the plant, and demonstrates that the lowest volume is the most efficient and depositing spray liquid on the plant. Figure 11 shows the normalised deposit underneath the leaves, again demonstrating that the lower volumes are most efficient at delivering spray to the underside of leaves. There is a slight suggestion that a higher pressure, which would result in higher droplet velocities and more air movement, might increase the quantity on the undersides of leaves.

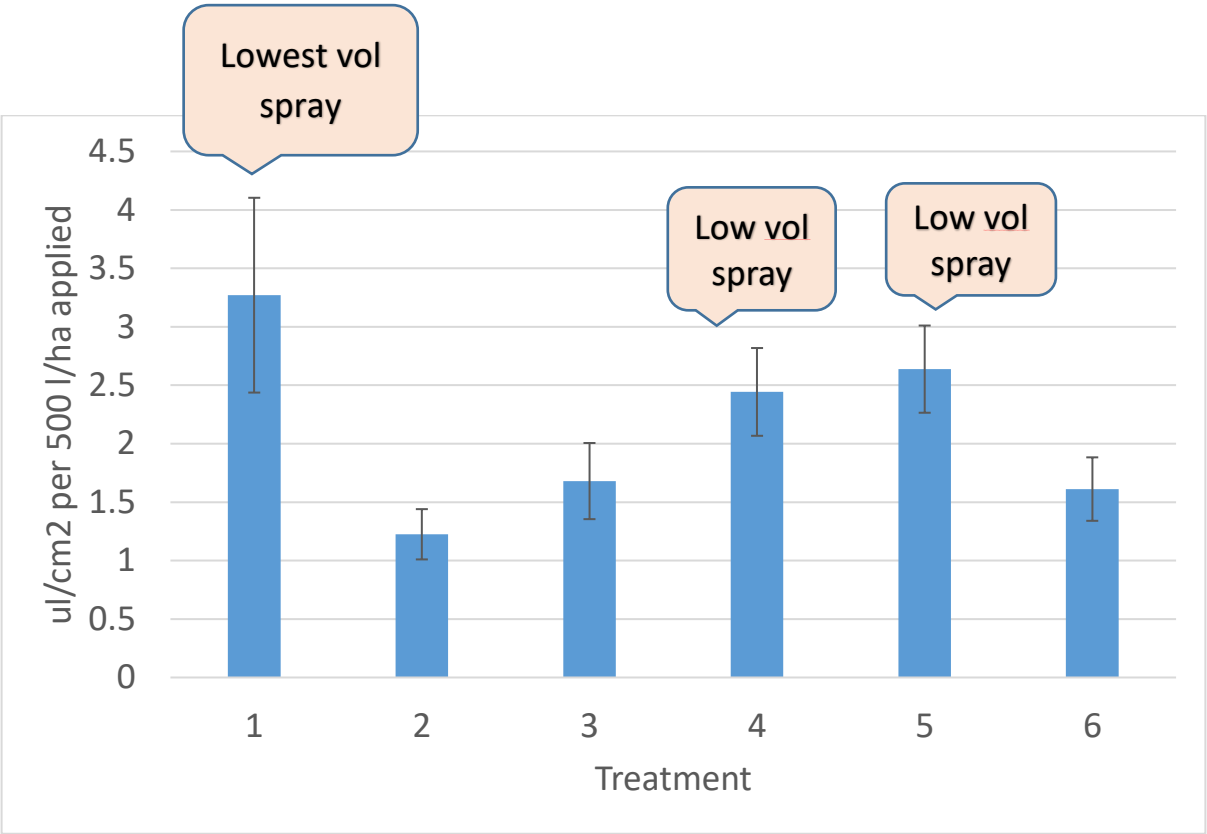

Figure 10: Normalised total spray deposits.

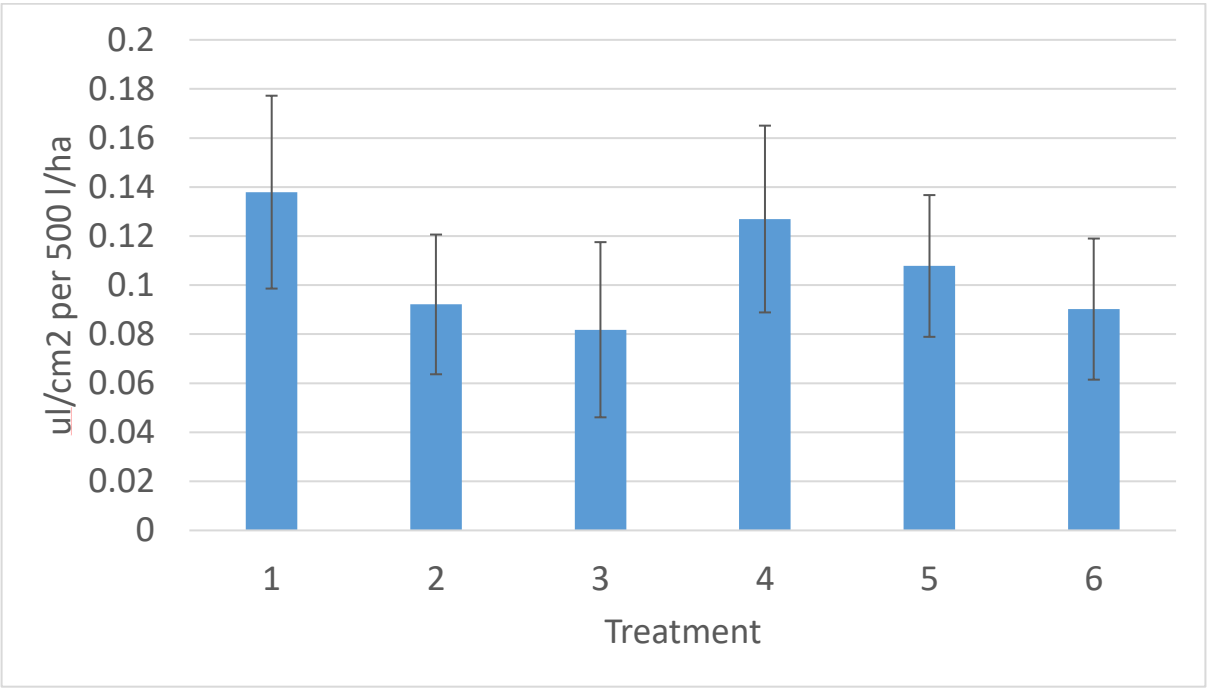

Figure 11: Normalised deposits on the lower leaf surface.

Figure 12 shows that a small number of plants had curled leaves, which would increase the measured quantity on the underside of the leaf. However, this is unlikely to result in improved control, since the whitefly would not be present on this part of the leaf. Figure 13 shows that the highest applied volume results in very high levels of run-off.

Figure 12: Poinsettia plant with one curled leaf.

Figure 13: Application at 1028 L/ha showing a very high level of run-off

The product manual for Naturalis requires spraying to 'just before run-off' with a fine spray. It also suggests around 1000 L/ha. For a poinsettia crop, this would be well beyond run-off. Figure 14, which shows the relationship between deposit and volume, suggests that just

before run-off (defined as the volume at which maximum deposit is achieved) could be around 750 L/ha or less. However, as Figure 10 shows, this is not the most efficient volume for applying a spray to this crop, and the same dose applied in a volume of around 500 L/ha (i.e. a concentration of 0.6%) would result in significantly more product on the plant than 1000 L/ha at 0.3%

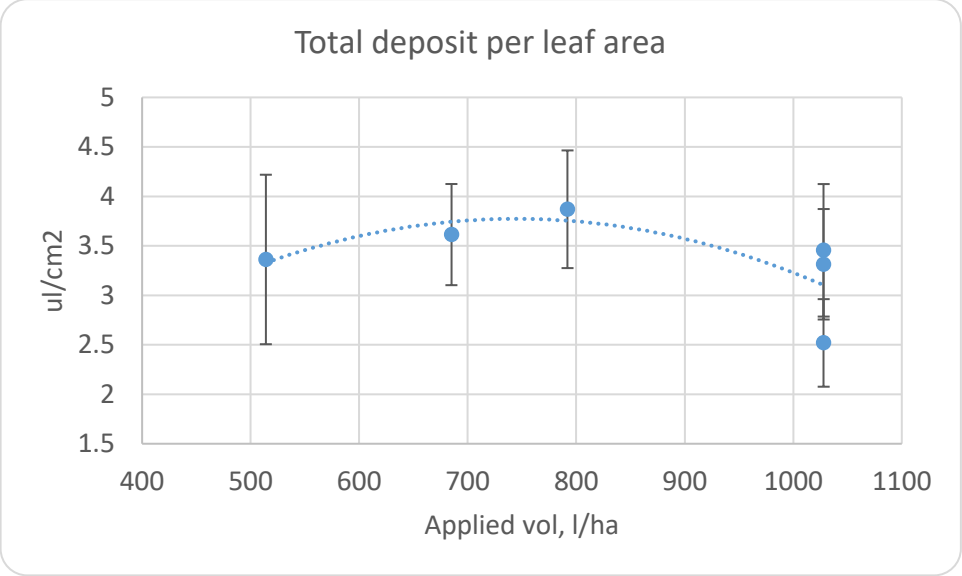

Figure 14: Relationship between volume and quantity deposited on the plant

2.2.3 Conclusions

The results of the track sprayer experiment indicates that the proportion of spray reaching the underside of leaves is very small. Reducing the applied volume (but not necessarily the applied dose) is the best way to maximise the total quantity of active substance on the leaves, as well as the quantity of active substance on the underside of leaves. However this potentially means that the concentration of the product needs to be increased. Angling nozzles did not improve the total quantity on the leaves or the quantity on the underside of leaves. There is also a suggestion from the data that increasing pressure might increase the quantity on the underside of leaves

2.3. Evaluation of the number of spores of *Beauveria bassiana* (Naturalis) deposited on leaf surfaces of a poinsettia crop sprayed using a manual, horizontal boom sprayer

As part of the poinsettia study, an evaluation of the number of spores per unit area on and under leaves was evaluated following the application of Naturalis by the grower to the crop using the horizontal boom sprayer. One hundred and eighty leaf samples were taken, from

the sprayed plants, 24h after application and imprinted (upper and under side) onto selective agar media. The agar plates were incubated for 5-7 days at 23ºC, in darkness and the number of *Beauveria* colonies counted. Overall there was a mean of 6.89 *Beauveria* spores per cm^2 recovered from the leaves; 5.3 and 8.3 spores per cm^2 were recovered from the upperside and the underside of the leaves respectively. The volume of liquid applied to the crop was then extrapolated using data on the concentration of the spore suspension. This was compared with the quantity of spray liquid determined in track sprayer experiments at Silsoe shown in Figure 15.

The two measurements were made at different times and using different application techniques and therefore we would not expect them to be the same. However, they are the same order of magnitude. The major difference is that the spore measurement suggests that the same volumes were applied to the underside of the leaves as to the top surface, whereas the track sprayer results indicate that most of the application was to the top surface.

Further work is needed to develop these techniques and to identify the reason for the apparent differences. This work should involve evaluation of the recovery efficiency from different leaf surfaces and determining if the quantity of spores retained on a leaf is correlated with the quantity of spray liquid deposited.

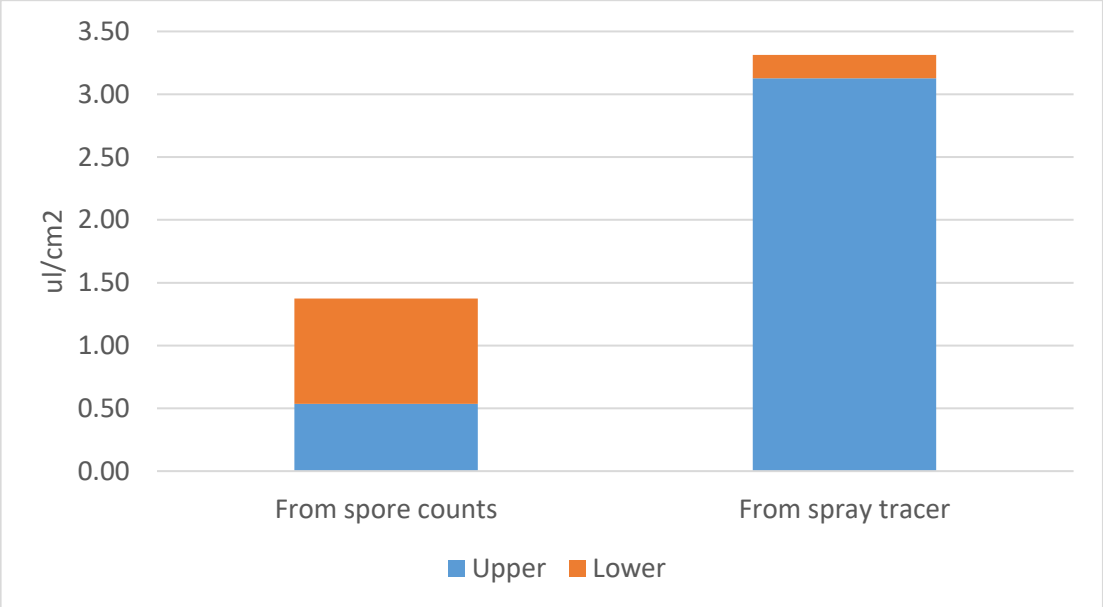

Figure 15: Comparison between two different techniques for evaluating the quantity of product deposited on leaves. The left hand bar shows the volume of liquid deposited on leaf surfaces estimated from data on Naturalis spore deposition taken from the crop at Bordon Hill. The right hand bar shows the volume recorded from tracer dye spray applications using a track sprayer at Silsoe.

2.4 Reviewing biopesticide product labels

A spreadsheet with a summary of application requirements for all biopesticides relevant to the Amber project has been produced. Some analysis of the information in the spreadsheet is required, which will be undertaken next year. Following this, discussions with manufacturers will be held where possible to explore the underlying data and knowledge that has resulted in the particular recommendations for each product so that we can identify situations where there is potential for further optimisation.

An initial discussion was held with Certis, where they provided some useful information relating to application conditions in their regulatory trials, and other research data.

3. Understanding the relationship between pest population dynamics and microbial biopesticide efficacy

On protected crops, pest populations can increase exponentially if left untreated. The aim of Integrated Pest Management is not to eradicate pest populations entirely, but rather to reduce them below an economic action threshold. This requires knowledge of the population dynamics of the target pest. Microbial bio-insecticides based on insect pathogenic fungi, bacteria and viruses work by infecting and then killing individuals of the target pest species. Unlike conventional chemical pesticides, death of individual pests is not instantaneous, and in some cases it takes 5 – 7 days to cause mortality. If the target pest has a short life cycle, then the slow mortality rate of a biopesticide may not be sufficient to stop the pest population from growing (i.e. individual pests may become infected, but if it takes a long time to kill them, they may well reproduce before they die). In addition, different pest life stages can vary in their susceptibility to a biopesticide. For example, larval instars can rid themselves of a fungal biopesticide by moulting, which reduces their susceptibility.

In order to make best use of a biopesticide, it is important to understand how it impacts on pest population dynamics. However, few studies have been done in this area. In particular, there is a need to determine the most suitable timing for application based on an understanding of the following key biological factors: (i) the time to development of different pest life stages and the reproductive rate of adults; (ii) the distribution of the life stages of the pest; (iii) the susceptibility of the life stages to the biopesticide; (iv) the time taken for the biopesticide to kill the life stage.

In this part of the project, Dr Dave Skirvin at ADAS has started to develop a boxcar train model (where each boxcar represents a pest life stage) to describe how pest populations increase over time, and which can be used to investigate the influence of pest life stage factors on the efficacy of biopesticides applied at different times during the development of a pest infestation. The model is based on a pest transitioning from one instar to the next until it reaches adulthood and reproduces. The rate of population growth depends on the number of life stages, the development time of each life stage, the natural mortality occurring in each life stage, and the number of offspring produced per adult. The model runs successfully with dummy data. Model runs have been done with different initial starting numbers of individuals in one or more life stages. It needs to be tested using real world data on (i) development times of different life stages, natural mortality and reproductive rate of selected pest species; (ii) the effect of biopesticides on the survival of different life stages and adult reproduction; (iii) effect of host plant on development times and reproductive rate; (iv) a combination of biopesticides and different crop types. This data will be obtained initially from the scientific literature and unpublished data from colleagues, and then it will be followed by using data from our own experiments where there are data gaps or where we need to do validation.

4 Overall Conclusions

This phase of the Amber project has seen the last of the benchmarking trails completed. The broad aim of this work is to find out how biopesticides perform in commercial practice when applied by growers in their own IPM systems. The study of the performance of Trichoderma-based biopesticides for managing soil borne diseases on Choisya and Dianthus was a relatively long experiment running for close to a year. The results indicate that the standard biopesticide programme performed as well as the standard chemical fungicide system within the constraints of the trial: these included the lack of a no-treatment control and the fact that the trial relied on natural disease infestation rather than artificially inoculating plants with disease (as would be the case with a standard efficacy trial). Because Trichoderma works as a preventative measure, then crop biosecurity will have a critical role to play in reducing the opportunities for plants to become infected in the first place, and thus reducing the overall level of pathogen pressure. Two issues stand out from the trial that are consistent with the other benchmarking trials done earlier in the project. The first was the observation that the biopesticide product label needed to be clearer, particularly with respect to guidance on water volumes, product dose and timing and frequency of application. In general we have found that growers find the labels difficult to interpret for their specific production systems. This is a

result of the formatting of the label required by the government pesticide regulator, which results in a label that provides generalised information. In this particular case, the label could be interpreted in different ways with respect to the dose of the product. The biopesticide companies provide additional guidance, for example through providing technical notes, online video guides, and direct conversations. These are all critically important. It is clear that the official labelling system is not working as well as it could. It also emphasises the need for growers to take advice from agronomists / crop protection consultants with experience of working with biopesticide products. The second issue concerns the large amount of water used to drench the biopesticide onto the crop. An application volume equivalent to 10% of pot volume was used, which is an accepted practice in the industry. However, using such large volumes has significant costs in terms of the time needed to treat the crop and can also increase waste. There is a need to understand whether smaller volumes can be used, which can be applied faster (this would include understanding whether smaller volumes enable the product to reach the root zone effectively), and whether systems such as applying the product in the irrigation lines are effective.

Because biopesticides work largely by contact action (i.e. they have no systemic or translaminar activity), it is important that they are applied to the right location, at the most effective concentration, and at the right time. It is critical that growers use appropriate application technology, and that all sprayers are calibrated, serviced and used correctly. In our case study at Bordon Hill, we observed that the grower could make significant savings as a result of investing in a horizontal boom sprayer for application of a biopesticide used in a whitefly management programme, which replaced application with a Ripa gun. These savings were in terms of the reduced volume of application and, associated with this, the shorter time needed to apply the product. The spray applications team at Silsoe helped to set up the new sprayer, emphasising the need for growers to be able to access specialist help when adopting new sprayers.

The subsequent experiments done using the track sprayer at Silsoe, in which different possible spray parameter configurations for the Bordon Hill poinsettia crop were tested under controlled conditions, provided hard evidence that the spray volumes being recommended for biopesticides on protected crops are generally too high and will mean that products are applied at well past the point of run off. The typical range of water volumes recommended for a biopesticide product is 500 – 1500 L/ha. In the poinsettia example, the proportion of spray that was deposited on the crop was highest at 500 L/ ha, whereas the manufacturer's recommended volume for this crop was 1000 l/ha. The data suggest that the best application strategy is to apply a higher concentration of biopesticide product in a lower volume of water.

This could create problems for the grower if the optimum water volume and biopesticide concentration for a particular crop are outside the limits defined on the product label. One of the long term aims of Amber is to get product labels changed so that they allow growers to apply the most efficient water volumes and product concentrations.

Our experience so far in Amber is that there is a false perception among growers that increasing the water volume gives better spray application to the crop. There is a significant opportunity to improve all areas of biopesticide application, including product storage, product preparation and mixing, spray equipment set up and maintenance, optimising application volume, and tank cleaning. These issues were explored in detail in a successful spray applications workshop at Silsoe in October 2017 and are summarised in the Knowledge and Technology Transfer section of this report (see below).

Knowledge and Technology Transfer

Presentations

Chandler, D. Amber – Improving biopesticide performance. Presentation at the BPOA Spring Conference 2017 'Using technology- serving the grower's needs', 18th January 2017, Whittlebury, Northants.

Chandler, D. Introduction to the AMBER biopesticide project (AHDB CP158). Presentation to the British Herbs Technical Meeting "Developing Best Practice for Herb production, 1st February 2017, Kenilworth, Warwickshire.

Chandler, D. Introduction to the AMBER biopesticide project (AHDB CP158). Presentation at the 2nd BCPC Pests and Beneficials Annual Review "Achieving sustainable pest control – Hard lessons from the pyrethroid story and implications for an IPM future", 26th January 2017, Harpenden, Herts.

Chandler, D. Introduction to the AMBER biopesticide project (AHDB CP158). Presentation at the AHDB Herbaceous perennials technical discussion group meeting, 8h February 2017, RHS Garden Wisley, Woking, Surrey.

Chandler, D. Introduction to the AMBER biopesticide project (AHDB CP158). Presentation at the Northern meeting of the Royal entomological Society "Pre and post-harvest insect pest management", 22nd February 2017, Cawood, Yorkshire.

Chandler, D. Introduction to AMBER. Presentation to the leafy Salads technical group, March 2017, Wellesbourne.

Chandler, D. The future of crop protection: the role of biopesticides in IPM. Presentation to the NFU, June 2017, West Sussex.

Chandler, D. Helping growers to get the best out of biopesticides: the UK AMBER project. Presentation at SIP, August 2017, San Diego.

Chandler, D. Update on the AMBER project: developing tools to improve the performance of biopesticides. Presentation to the Tomato growers association, September 2017, Kenilworth.

Bennison, J. Potential for biopesticides in IPM programmes for control of western flower thrips. Presentation at AAB conference 'Advances in IPM 2017', September 2017, Newport.

Lane, A., Prince, G. & Chandler, D. An introduction to biopesticides and improving spray application. British Herbs Technical day, September 2017, Surrey.

Chandler, D. Update on the AMBER project: developing tools to improve the performance of biopesticides. Presentation to the Hardy Nursery Stock panel, October 2017, Leamington.

Chandler, D. Biopesticides and their practical uses in IPM. Update to Dutch growers, October 2017, Wellesbourne.

Ramsden, M. Update on the AMBER project: developing tools to improve the performance of biopesticides. Presentation at the Cucumber and Pepper conference, October 2017, Essex.

Chandler, D. The use of biopesticides in IPM: the UK AMBER project. Audax Conference, October 2017, Reading.

Chandler, D. The use of biopesticides in IPM: the UK AMBER project. AHDB Agronomist conference, December 2017, Peterborough.

Articles

AHDB News: Spray application for biopesticides in protected crops, March 2017

AHDB Crop Protection Review: AMBER (CP158), July 2017

AHDB News: Introduction to Biopesticides, July 2017

AHDB News: Getting the most out of Biopesticides, October 2017

Website

The website went live in June 2017.

Table 6: Website summary statistics

	2017							
Page	June	July	August	September	October	November	December	Total
AMBER project	1006	268	352	216	226	187	131	2386
What are biopesticides?	152	70	45	131	138	123	52	711
Biopesticides - pros and cons	101	43	35	110	146	135	89	659
Project details	114	76	37	55	74	73	34	463
Research plan	68	26	25	39	50	56	28	292
Project team	407	33	27	53	62	71	28	681
Links	68	33	18	20	27	58	33	257

Workshop

During the two years that the AMBER project has been running, there have been a number of key areas explored by the project team aiming to improve the efficacy and understanding of biopesticides for horticultural businesses. From the benchmarking trials carried out on commercial sites in year one, it became apparent that the topic of application practice was one of the areas of importance where there were information gaps, and that further work and knowledge exchange within industry was required.

A workshop at Silsoe Spray Application unit was held, in October 2017, and attended by growers, agronomists, spray operators and biopesticide manufacturers. Through a series of practical demonstrations, presentations and discussions the workshop highlighted the importance of the following areas to maximise the efficacy of biopicesticdes through application:

In order to maximise the efficacy of biopesticides through application, the workshop highlighted the importance of the following areas:

Cleaning

Thorough tank pre-cleaning is essential to ensure the integrity of the product is maintained and optimal control achieved.

Although it is normally assumed that washing your tank and lines three times will remove residues of conventional products before using biopesticides, it was demonstrated by using soluble dyes that even washing six times still left identifiable residues. This could affect the viability of the biopesticide and ultimately reduce its efficacy.

If possible, consider investing in a separate tank for biopesticides and conventionals.

Nozzles and filters should be checked and cleaned prior to application, and on a regular basis.

Mixing

To ensure a full dose of biopesticide is applied at the nozzle, ensure you thoroughly mix/pre-mix the formulation before application to release active ingredient. Sustained agitation of the formulation during application is also essential with most products to maintain equal dispersal of the active ingredient.

Storage

Some biopesticides are based on living micro-organisms and therefore require the appropriate storage conditions to maintain the optimum viability of the product; it shouldn't be assumed that they can be stored in the same way as conventionals. Make sure that you read all product information in advance to establish the required conditions.

Calibration

Accurate calibration of spray equipment on a regular basis is crucial to optimise the dose applied to the crop and ensuring that legal requirements for use of the products are met It will also enable the spray operator to identify when equipment is worn or damaged, and needs replacing to maintain performance.

Label guidance

Many growers report difficulties with label guidance and the limited technical information that is present on the product label. It is important to speak directly with the relevant manufacturer to gain additional technical information that they may hold to assist in optimising application techniques.

Application

It is critical that growers and spray operators get the basics right when applying biopesticides

- Investing in appropriate equipment and ensuring that it is maintained well. If possible, dedicated equipment for biopesticides should be used.

- Understanding the pest life cycle. Control may vary depending on the susceptibility of the development stage of the target. The AMBER project is specifically looking at this in more detail during the next stage of the work.
- Taking account of crop structure. Vine crops and bedding plants are going to require different approaches to application.
- Understanding where the spray needs to reach and using equipment that can best achieve this
- Optimising the applied volume (which in many cases will mean a reduction) to maximise the total quantity of active substance in the right place on the crop and improve its distribution
- Being aware of your forward speed – this has to be part of your calibration. Increasing your forward speed is a more effective way to reduce volume than reducing pressure, but might not be possible without some automation. If you compare 0.4m/sec (a typical walking speed for manually operated spray booms) with 1m/sec – the lower volume applied at the higher speed resulted in less active ingredient on the floor.
- One size doesn't fit all. You need to adapt the application to fit your growing system

The workshop was a great opportunity for attendees to learn more about application best practice, there was plenty of opportunity for discussion between growers and manufacturers. Biopesticide manufacturers are aware of the importance of application accuracy, and are becoming increasingly aware of the need to liaise with industry through work such as the AMBER project, to ensure the maximum potential of biopesticides is achieved on commercial sites.

Appendices

AHDB CP158: Application and Management of Biopesticides for Efficacy and Reliability (AMBER)

Revision to Programme of Work (11.07.17)

Objective 2: Develop and demonstrate management practices that can improve biopesticide performance

WP2.2 Identify and investigate management practices that have potential to optimize biopesticide performance (Years 2 & 3, Warwick, ADAS, SSAU; Warwick to lead).

The results from benchmarking experiments (WP 2.1) done in 2016 indicated that work to develop biopesticide Potential Optimization Practices (POPs) in 2017 should concentrate on three areas: (i) providing better technical guidance for growers on how to optimize spray applications; (ii) provide information for growers on the persistence on foliage of plant disease control agents, to enable them to adjust the timing and frequency of applications; (iii) to provide better understanding of the relationship between microbial biopesticide efficacy and pest population size. The work programme has been adjusted to enable us to focus on these priority areas. The new work will be done through a combination of (i) sharing information with biopesticide manufacturers, other researchers and regulators (ii) analyzing the scientific literature (iii) conducting laboratory and controlled environment experiments. It is likely that the work programme will have to be refined further as it proceeds, e.g. the design of experiments can only be finalized once information has been obtained from manufacturers. For this reason, the new work programme will be reviewed regularly by the AMBER team in consultation with AHDB managers and the Chair of the Industry Steering Group. Meetings with AHDB will be held monthly to review progress and discuss changes to the programme. Notes will be taken at these meetings so that a record is kept of how the programme has been modified as the work progresses.

<u>WP2.2.1. Better delivery of biopesticide to the target (SSAU, RG, RJ, ADAS, Warwick)</u>

At present, the label provided to growers by biopesticide manufacturers / distributors usually contains only general recommendations about spray application. The label specifies the dose of biopesticide to be applied (i.e. the amount of biopesticide per ha that can be applied), gives a range of water volumes that are permitted on the crop (where water acts as a carrier for the biopesticide), the frequency of applications and the maximum number of applications per

crop. The benchmarking work done in AMBER indicated that growers need more specific guidance on the quantity of water to be sprayed for different crops. The specific objective is to determine the lowest volume of water required to provide efficacy, as this reduces waste and is more efficient, provided that it does not contravene the minimum water volume stated on the label, as this is a legal requirement. This will be done as follows:

Analysing the current technical guidance on water volumes for biopesticides

- Information already available within the consortium will be reviewed and collated. A database will be prepared that lists the currently approved biopesticides and their respective label guidance (which includes guidance on application) – this will be used to identify gaps in knowledge about the application requirements for different biopesticides and crop types.
- Where additional information is needed, Amber staff will meet with the manufacturers of the microbial biopesticides on the UK market to discuss how the current label recommendations and technical guidance information were developed. Ideally we would like to understand what experiments were done to determine the dose of biopesticide and volume rates so that we are in a better position to consider the costs / benefits of changing these. However we realize that this may be confidential information that the companies are not willing to share.
- Information provided by the biopesticide companies will be interrogated. It will be compared against other information from the literature, plus in-house information held at SSAU, to determine whether the current recommendations on dose and water volumes are optimum for different crop types. We will divide crops according to the type of crop architecture: low/dense canopy crops (e.g. bedding/pot plants, lettuce, herbs); tall row crops (tomatoes, peppers, cucumbers); large container crops such as poinsettia, HNS; and isolated plant crops such as field grown trees.
- If the recommendations are considered to be optimum, then work will be done to determine how growers can achieve them, i.e. to make applications accurate and efficient. This may involve making recommendations on the most appropriate type of spray equipment to use for different crops.
- However, if there is scope for improving the recommendations, then work will be done to address this first. Research on water volume rates and nozzle designs is likely to consist of experiments at SSAU's track sprayer facility. The preferred option is to set up a case study, in which we 'walk through' the application of a particular biopesticide on a particular crop type. Information will be obtained on the biology of the chosen pest (pest life-cycle,

timing of arrival in the crop, preferred feeding location in the crop, dose of biopesticide required for control). In addition, trial data will be obtained on where the product has worked best. The application team will then use this information to determine the best way (in terms of water volume, spray pressure, nozzle selection) to deliver the required dose of biopesticide to the target crop using tracer dyes.

- There is a specific issue concerning the application of some biopesticide products to tall row crops. The technical guidance information provided by the manufacturers gives the quantity of biopesticide to be applied per unit floor area. For tall row crops, this needs to be translated into the quantity of biopesticide per unit 'wall' area. Discussions will be held with the UK regulator, the Chemicals Regulation Division of the Health and Safety Executive, to look for ways to solve this particular problem.
- Finally, research will be done to quantify the application of a biopesticide to a crop when applied by a commercial grower under best practice guidelines (i.e. a 'real world' situation), and compare this to an 'ideal world' situation in which the biopesticide is applied under replicated, controlled conditions using the Silsoe track sprayer. This work will be done using the fungal biopesticide Naturalis, applied to a commercial poinsettia crop. Naturalis is used as part of the IPM programme for this particular crop. Immediately following application by the technical manager of the nursery, Amber staff will take samples from the crop and quantify the amount of fungal spores deposited per unit leaf area. Data will also be recorded on water volume, pressure, walking speed, nozzle type, sprayer calibration, environmental conditions within the nursery etc. The application will then be replicated at Silsoe under controlled conditions using the track sprayer. This will enable us to determine any variance between application by a technically skilled grower under real world conditions and application under 'ideal' conditions.

<u>WP 2.2.2 Better understanding of biopesticide persistence (Warwick, ADAS, Silsoe).</u>

This work will be done in order to inform the timing and frequency of biopesticide spray applications to crops. The interval before replenishment is required affects the cost of use, whilst long intervals in situations of poor persistence risk crop damage. The focus will be on biopesticides used in the UK against foliar diseases: the microbial pest control products (MPCPs) AQ10 (Manufacturer CBC, specific against powdery mildews); Prestop (Lallemand Plant Care); and Serenade ASO (Bayer CropScience) which will be used against Botrytis.

- Discussions will be held with the manufacturers of the products to understand how their recommendations on timing and frequency of application were developed for different crop types. This will include discussing experiments that may have been done by the manufacturers to quantify persistence of the MPCP as well as persistence of activity.

- The scientific literature will be reviewed to describe the current state of knowledge about the persistence of AQ10, Prestop and Serenade ASO on crop foliage including the factors that may determine the length of persistence of effective doses. Where possible, comparisons will be made with other microbial biopesticides that are not currently available in the UK in order to identify any general patterns. We are particularly interested in being able to quantify the rate of decline of microbial populations on foliar surfaces in relation to the effective dose.
- As part of this, we will work with Dr Xiangming Xu at NIAB-EMR who is currently investigating the use of molecular biology methods to quantify MPCP persistence on foliage through an AHDB studentship.
- We will write a summary document for growers explaining what is known about persistence of an effective dose of the biopesticides on foliage and make recommendations about how this information can be used to inform the timing and frequency of application. If possible, we would like to produce graphs showing how the MPCP changes over time in the absence of a host and which growers can use to inform their spray application programmes. At the time of writing, we would hope to be able to include data from the manufacturers but they may be unwilling to put this into the public domain.
- Experimental work will be done if we identify gaps in knowledge. The plan for this is as follows, but will be reviewed closer to the time and adapted as necessary:
    - One or two representative plant types will be chosen to study the persistence of AQ10, Prestop and Serenade – probably a herb crop and cyclamen or begonia, but the final decision will be made in consultation with the rest of the science team. Plants will be grown within controlled environment chambers under temperature / humidity / light regimes that match those used in crop production. Plants will be sprayed with the test MPCPs at manufacturers' recommended doses (water volume to be determined in consultation with Silsoe) and samples will be collected on at least seven occasions (up to 21 days depending on pilot studies). For sampling, leaves will be removed from plants, washed in a surfactant and the MCPC enumerated by counting CFUs from aliquots plated onto agar based media. Additional work will be done in which the top or lower surface of leaves will be imprinted onto agar to investigate differences between persistence on different leaf surface. Pilot work will be done to study the usefulness of this method: an alternative would be to shield upper and lower leaf surfaces during spraying and then wash MPCP from leaves. The spray application method will probably need to be modified to ensure that sufficient MPCP is deposited onto the lower surface

of leaves for reliable measurements to be taken. Untreated controls will be used to measure background levels of the MPCP and adjust treatment values as appropriate. This method is not as 'in depth' as using strain specific molecular probes to determine MPCP quantities by qPCR but the data sets should be good enough for our purposes. Samples will be taken at standardized plant heights on tomato, using plants of the same age. Data will be recorded for temperature, day length, humidity, light intensity.

- Following this, work will be done to quantify persistence of activity. Plants will be sprayed with MPCPs, left for increasing periods of time under controlled conditions, before addition of pathogen spore inoculum (some pilot work may well be required to determine the dose of pathogen inoculum required). This protocol may be modified where label recommendations strongly advise two applications in quick succession at the start of a programme. The effect of the MPCPs on plant health (visible symptom incidence and severity) will be recorded over time.

WP2.2.6. Understanding the relationship between pest population dynamics and microbial biopesticide efficacy (ADAS, Warwick, RJ, RG).

This WP will investigate how the efficacy of microbial bio-insecticides is influenced by the biology and demographics of their target pest species. We will use a discrete time, stage-structure population dynamics model to investigate the influence of different factors on the efficacy of biopesticides applied at different times during the development of a pest infestation. The model will consist of two populations, a susceptible population (uninfected) and a population infected with the biopesticide. The number of individuals in each life stage, their age, and, for the infested population, the length time that they have been infected with the biopesticide will be explicitly represented within the model. This will enable us to accurately represent the distribution of the different instars in the total population, and to incorporate potential escape effects due to moulting of life stages.

- The growth rates of the life stages, the reproductive rate of the adult life stage, the susceptibility and time taken for the biopesticide to kill each life stage will be input values to the model. These inputs will be able to be varied to represent different insect pest species.
- The model will then be run with different initial starting numbers of individuals (in one or more life stages) and different application timings for the biopesticide to identify whether there are application strategies (timing and number of applications) that can lead to an effective reduction in the pest population.

- We will aim to work with 4 pest types that have different life histories – aphids, whitefly, spider mites, and western flower thrips. However, the exact number studied will depend on the existing availability of input data and whether we need to run experiments to obtain data where none exist. EPF will be used as exemplar biopesticides.
- The population dynamics model will be written by the ADAS modelling team.
- Warwick and ADAS entomologists will mine the scientific literature to provide input values for the model. We will also consult with biopesticide industry researchers to obtain data on the biopesticide susceptibility and mortality rate of pest life stages if this data is not already within the literature. As a last resort, we can fill any data gaps by running our own laboratory bioassays.
- It may not be possible in this year to validate the model, but it may be possible to compare model predictions to the results from efficacy trials, and we will design validation experiments to be done in the next season.

