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Key staff:	Jude Bennison,Clare Butler Ellis John Clarkson, Roma Gwynn Rob Jacobson, Andy Lane, Gill Prince, Mark Ramsden, David Talbot, Erika Wedgewood
Location of project:	University of Warwick Warwick Crop Centre, School of Life Sciences, University of Warwick, Wellesbourne, Warwick CV35 9EF UK
Industry Representative:	Rob James, Thanet Earth Marketing Ltd, Barrow Man Road, Birchington, Kent, UK, CT7 0AX
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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.

Dr D Chandler

Principal Research Leader

University of Warwick, Warwick Crop Centre, School of Life Sciences, University of Warwick, Wellesbourne, Warwick CV35 9EF UK

Signature ..... Date .....

Report authorised by:

[Name]

[Position]

[Organisation]

Signature ..... Date .....

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Headline

Different biopesticides were evaluated on commercial nurseries against a range of pests and diseases of protected edible and ornamental crops. Opportunities to improve biopesticide performance by altering local management practice have been identified. These include modification to spray applications, and improved understanding of how biopesticide efficacy is affected by P&D population size.

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 identify gaps in our knowledge about biopesticides that are causing them to be used sub-

optimally 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

In the first year of the project, the research 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 five different biopesticide products against five different plant 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, and cause significant financial losses if not controlled. The 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). Experiments on glasshouse whitefly had to be postponed until year 2 to fit in with the host grower, but benchmarking at the five other nurseries was done successfully.

Table 1. Combinations of pest / disease, crop and biopesticides selected for benchmarking in year 1

P/D	Crop	Biopesticides tested	MAPP number
Powdery mildew	cucumber	AQ10 (CBC / Fargro)	17102
Botrytis	cyclamen	Prestop (Lallemand Plant Care)	15103
Root rots	Choisya & Dianthus	T34 Biocontrol (Biocontrol Technologies / Fargro); Trianum G (Koppert); Prestop (Lallemand Plant care)	17290 16740 15103
Aphids	sweet pepper	Botanigard WP (Certis); Majestik (Certis)	17054 17240
Western flower thrips	pot chrysanthemum	Botanigard WP tank mixed with Majestik (Certis)	17054; 17240
Glasshouse whitefly	mint	Naturalis L (CBC / Fargro) (tbc before start of trial)	17526

For all benchmarking experiments, the biopesticides were applied by the host grower to naturally occurring populations of P/D and done according to the best practice guidance supplied with the product. The products were incorporated into the existing IPM systems used by the grower. The intention of this work was to observe the performance of the selected biopesticides under 'real world' commercial production conditions. As expected when working on commercial crops, in the majority of cases it was not possible to include untreated controls. The research team observed how the grower used the biopesticide product(s), and data were obtained on the following: product storage conditions; application (spraying equipment, pressure, water volume, product concentration); deposition on the crop; persistence; amount of P/D control; environmental conditions in the crop.

Benchmark 1: Powdery Mildew on Cucumbers (17 August - 7 September 2016)

The objective of this experiment was to compare preventative application of AQ 10 (based on the mycoparasitic fungus *Ampelomyces quisqualis* strain AQ10) for management of cucumber powdery mildew (*Podosphaera xanthii*) compared to grower current practice (curative spray applications of the chemical fungicide isopyrazam once mildew was observed). AQ10 was applied to a three week old cucumber crop (two varieties were used, var Bonbon, which has intermediate levels of mildew resistance, and Bonifacio, which has mildew susceptibility) on two occasions using a hand-pushed trolley with a vertical boom with five pairs of nozzles (FF80 02) angled 45 degrees upwards, (3620 L/ha), with a hose attached to a 1000 L static water tank with agitation. The trolley was pulled backwards over a pair of rails along the space between the crop rows, with the operator setting his pace based on his own experience. Assessments (% powdery mildew and phytotoxicity) were undertaken before spraying and four days after the second spray (21 days from first spray). Samples from the spray tank, nozzles and leaf canopy were taken back to the laboratory for further assessment.

The water volume selected by the grower was done on the basis of his general knowledge and experience, and it is likely that the grower underestimated the volume that was applied. The AQ10 label guidance on water volumes was not found to be informative. The mixing of the product for this particular experiment was problematic: Granules (possibly the carrier) were visible as brown clumps in the water in the spray tank after leaving the AQ10 the recommended 30 minutes to hydrate. The same problem did not occur in follow up laboratory tests done with a different batch of the product, and further investigation is needed to find out the reason for poor mixing in this particular case. Some other biopesticide manufacturers are now posting YouTube videos for growers on how to mix and prepare their products and it would be worth having something similar for AQ10. The spray operator aimed to deliver

visually wet plants and adjusted his walking speed accordingly between rows. Speed was therefore slow. Viable spores of *A. quisqualis* were not recovered from spray or leaf samples taken on the first spray application. Despite this, AQ10 sprayed rows had lower powdery mildew infection than untreated plants. There was evidence that AQ10 worked more effectively when used as part of an IPM approach with the mildew resistant variety BonBon, with only trace levels of mildew seen on AQ10 treated plants compared to average levels of 4.5% on untreated plants.

Benchmark 2: Botrytis on cyclamen (12th July – 8th September 2016)

The objective of this experiment was to evaluate the effect of Prestop (based on the antagonistic fungus *Gliocladium catenulatum* strain J1446) on a natural infection of botrytis on cyclamen under commercial production. Two treatments were compared, consisting of (i) Prestop and (ii) an alternating fungicide programme of Rovral WG (Iprodione) and Amistar (azoxystrobin). Both treatments were applied to a six week old cyclamen (var. Picasso Verandi – Mixed) crop on two occasions at three week intervals. Application was made using a RIPA nozzle on the end of a hose from a Brinkman 200 L tank sprayer. Assessments (% *Botrytis sp.* sporulation, phytotoxicity) were undertaken before each Prestop application and samples from the spray tank, nozzles and leaf canopy were taken back to the laboratory for further assessment. Application of Prestop reduced the incidence and severity of botrytis on the leaves compared with an alternating spray programme of Amistar and Rovral WG at the same application interval. Neither treatment programme provided total control of the disease, with botrytis being recorded on over half of the plants in both treatments: at the final assessment, 56% of Prestop treated plants and 84% of the chemical fungicide programmes had botrytis sporulation, with a mean of 1.4 and 2.44 leaves per plant affected, respectively. Only 16% of the affected plants treated with Prestop had botrytis rot progressing back from the leaves into the petioles, whereas 52% of the affected chemically-treated plants had softened petioles. Leaf imprints showed that most of the Prestop product was applied to the upper surface of leaves. The finding that Prestop appeared to give superior control compared to the conventional chemical fungicide programme is worth noting. However, adequate mixing of the product required supplementary diagrams provided by the product supplier and could be improved by more detailed label guidance. The manufacturer, Lalllemand, has since put up videos on YouTube instructing growers how to prepare the product, and has also developed a tool that enables growers to detect the presence of *Gliocladium* on plants after spraying. The experiment highlighted a number of areas where application of both the biopesticide and the conventional chemical fungicides needs to be improved. In particular, there is a requirement to deliver spray to the older leaves at the base of the plant and deep within the crown, which could be addressed with improved application technique, while the

very high water volumes used for the product combined with wide plant spacing used for this particular crop meant significant spray waste.

Benchmark 3: Root rots on Choisya and Dianthus (15 September 2016 to May 2017)

The objective of this ongoing work is to evaluate the effect of biopesticides in IPM programmes for root rot pathogens on both Dianthus and Choisya. The grower already uses three different biopesticides as preventative treatments have been applied. When older, the plants are potted into media with an incorporated biofungicide. The benchmarking experiment compares two different types of disease treatment that are incorporated into the growing medium when the plants are first grown and then potted on. Choisya are treated with T34 Biocontrol (*Trichoderma asperellum* strain T34), Prestop (*Gliocladium catenulatum* strain J1446) and Trianum G (*Trichoderma harzianum* strain T22), while Dianthus receive a preventative treatment of Trianum-G. The grower then supplements these biopesticide treatments with drenches of conventional chemical fungicides (Previcur Energy and Horti-Phyte) later in the year. For the benchmarking trial, the standard IPM programme is being compared against one in which the conventional fungicide drenches are replaced with drenches of T34 Biocontrol applied either two or three times from autumn to spring. The idea is that the *Trichoderma* asperellum strain T34 fungus in T34 works by growing and colonizing the root zone, and hence only one or two drench applications are needed to achieve this, in contrast to chemical fungicides that need to be applied more frequently. The drench treatments were applied at 10% of pot volume using a lance (2 x FF110 – 20 fan nozzles; 75-100 thousand L/ha) on a hose reel to a 300 L tank with pump. Assessments (foliage health, phytotoxicity) is being done over the winter of 2016 / 17 with assessment of roots for rots in March 2017. Initial points to note on the drench applications included an observation of significant waste of spray product running from the pot surface on to the bed. Viable T34 Biocontrol colonies were quantified from growth medium and observed at similar levels both in the spray tank and from the lance. The experiment has already highlighted a number of areas where application could be improved, including the need for clearer, more informative guidance in the product label, elimination of run-off to beds, and reducing the time required for drench applications through improved pressure control.

Benchmark 4: Western flower thrips in pot chrysanthemum (7 July – 28 July 2016)

The objective of this experiment was to assess the use of Botanigard WP (based on the insect pathogenic fungus *Beauveria bassiana* strain GHA) and Majestik (a product based on maltodextrin) at recommended rates against invertebrate pests in pot chrysanthemum, particularly western flower thrips (WFT), *and Frankliniella occidentalis*. Two treatments (Nemasys® *Steinernema feltiae*, BASF UK (current practice used by the grower) and Botanigard WP + Majestik tank mix) were applied, three times at weekly intervals from bud break to the week before open flower and dispatch, along two parallel rows of benches. The treatments were applied using an automated 16 nozzle spray boom, with 03 flat fan nozzles spraying vertically downwards, 1089 litres of water per hectare. Assessments (the number of WFT, the presence/absence of aphids, aphid mummies, leaf miners, presence/absence of WFT damage on the leaves and petals and phytotoxicity) were taken from bud break to the week before open flower and dispatch on two cultivars that varied in their susceptibility to thrips damage. Samples from the spray tank, nozzles and leaf canopy were taken back to the laboratory for further assessment.

WFT and damage were recorded during the experiment, but numbers were very low in both treatments, despite the experiment being done at a time of year when WFT normally increased to levels that could cause crop damage if left unchecked. During the trial the WFT population levels were lower than normal, also indicated by sticky traps placed within the glasshouse. Numbers of WFT in the Botanigard WP treatment were not different from those receiving the standard nematode treatment. Viable *Beauveria* sp. colonies were found in similar numbers both in the spray tank (foam and suspension) before and after spraying and from the nozzles. Viable *Beauveria* sp. colonies were observed on both upper and lower leaf surfaces, with the majority of spores being located on the upper surface of the leaves. The results suggest that at low WFT pest pressure, the Botanigard WP and Majestik treatment applied was as effective as the application of entomopathogenic nematodes. The spray equipment operated well and complied with the label requirement. Exploratory experiments at Silsoe investigating different spray application scenarios suggested that the label recommendations are not likely to result in the highest doses of Botanigard on the plant buds and flowers.

Benchmark 5: Aphids in organic sweet pepper (23 June – 11 July 2016)

The objective of this benchmark experiment was to assess the use of Botanigard WP and Majestik at recommended rates against invertebrate pests in organic pepper, particularly the peach-potato aphid, *Myzus persicae*, which had recently reached high numbers on the crop. Four treatments (Untreated control, Botanigard WP, Majestik, Botanigard WP + Majestik tank mix) were applied twice, six days apart along both sides of a 130m long x 2.5m high sweet pepper row. The treatments were compared along four parallel rows, with untreated buffer rows between each treatment. Applications were made using a trolley with a vertical boom consisting of four pairs of 80° hollow cone 03 size nozzles, angled at 45° upwards; average volume 1377 L/ha, 500 – 1500 L/ha target volume. Assessments (the number of aphids, aphid mummies, hyper-parasitised mummies, and aphid predators) were taken on 15 selected leaves at each of three heights within the crop canopy; this was done immediately before the first spray, and then at day 6 and day 12. Samples of Botanigard WP were collected from the spray tank and nozzles during spraying, while leaf samples were taken from the canopy after spraying. These were taken back to the laboratory to estimate the concentration of viable fungal spores in the spray and on leaves.

Numbers of aphids per leaf were highly variable in all four treatments, but mean numbers were very high; around 175 aphids per leaf on untreated plants. With this aphid population density, none of the treatments reduced aphid abundance compared with abundance on untreated leaves. Viable *Beauveria* sp. colonies were found at similar numbers both in the spray tank before and after spraying and from the nozzles. Viable *Beauveria* sp. colonies were also observed on both upper and lower leaf surfaces but were variable between samples. Immediately after the benchmarking experiment, laboratory experiments were done to measure the susceptibility of individual *M. persicae* reared from the population infesting the crop. This showed that Botanigard WP killed *M. persicae* within six days of application.

For this particular experiment, we found that the spray equipment operated well. Excessive foaming was observed when the product was mixed in the spray tank but did not appear to impede application of Botanigard WP to the crop. Calculating the optimum application volume for the biopesticide was not straightforward, as no information was given for how to adjust for the height of vertical crops. This highlights the need for growers to be able to adjust spray tank water volumes to cope with different crop heights and structures. It was also noted that the spray volume applied and therefore the dose, is likely to fluctuate along the crop because of changing trolley speed and pressure during spray runs. The spray boom was also in close proximity to crop which may result in poor distribution of spray.

Botanigard WP is recommended for control of whitefly on various protected crops, however, it is known from the scientific literature that it is also effective against aphids, and this was confirmed in our own laboratory bioassays with *M. persicae*. The main question raised is, if Botanigard WP is able to infect and kill aphids under laboratory conditions, why was there no significant reduction in the aphid population on the crop? Temperature and humidity conditions recorded within the crop were within the limits recommended by the supplier. There is some background evidence that the fungal spores of Botanigard WP are susceptible to damage by UVA and UVB radiation (which is not filtered out from sunlight by glass). It is also possible that the speed of kill of the biopesticide was not fast enough to reduce the net reproductive rate of the aphid population sufficiently. Aphid nymphs may also have been able to 'escape' infection by fungal spores through moulting. Both of these effects may be more apparent at high pest population densities.

Summary of biopesticide application assessments

For all benchmarking experiments, observations and evaluations were made of how the partner growers in the project were applying the biopesticides to their crops. Some general conclusions can be drawn from this. Product, dose and timing are crucial parameters in the performance of biopesticides. Observations at this early stage of the project showed that high spray volumes were being used across all crops which are unlikely to be consistent with optimum deposition of product on the crop and maximum efficiency of the application process. More knowledge is needed about the optimum conditions required for good performance of each biopesticide in order to identify potential improvements in application. This includes quantity of product, quantity of water, location within the crop that should be targeted, and other environmental parameters that could influence performance. The sites chosen for year 1 benchmarking studies had a wide range of equipment for application but encountered common problems: (i) Mixing and dispersion of biopesticide products; (ii) Calibration of equipment and accurate dosing; (iii) Interpretation of labels to comply with legal requirements and best practice; (iv) Achieving uniform distribution over the crop. As part of this, there is a question about whether current label requirements can be modified to make the application more efficient, more efficacious and easier to deliver in practical situations. The label is a regulated document and text changes cannot be made without the approval of the regulator, however it is possible for manufacturers to add advisory information to the label or issue technical notes.

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

No specific actions are being recommended at this stage until more research has been done, however we would highlight to growers the need to ensure that spray applications are done according to best practice guidelines in order to get the best out of biopesticides.

SCIENCE SECTION

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 CP 158). 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.

Introduction

A selected number of growers were interviewed about their experience of biopesticides, how effective they found them, and what they consider to be the gaps in knowledge regarding successful biopesticide use in different types of crop and production systems. These interviews will form the basis for future grower surveys, and document how growers are using the biopesticides currently available on the market and confirm which pests and diseases are the most pressing for growers (in the context of biopesticides and IPDM). Questions include information on application equipment, product rates, water volumes, typical application conditions, application timing, and any special considerations being made such as pest life stage.

Methods

Interviews were carried out with the growers at the five sites used in the 2016 benchmarking trials. The interviews were carried out prior to the trials taking place to avoid influencing grower responses. Each interview took place at the host grower's nursery, though some additional information was subsequently provided where information was not available on the day. Additional brief surveys were handed out to additional growers at grower events organised by AHDB and completed *in situ.*

Results

The brief survey and the questions and grower responses of the detailed survey are reported in Appendix 1; Tables 1 and 2. While all growers were aware of biopesticides, there was limited detailed knowledge of what a biopesticide is, how they differ from conventional plant protection products, and the application specifications may change in order for them to be effective. In one case, the grower had been using *Bacillus thuringiensis* subspecies *kurstaki* strain ABTS-351 (Dipel DF) without recognising that it was a biopesticide. All respondents identified a lack of free, independent guidance on biopesticide applications, and all had either direct or indirect experience of biopesticides being unreliable. We also found that the growers had not adapted equipment and/or practice for biopesticide application, but more often used biopesticides as a direct replacement for an existing pesticide.

Conclusions

The key messages from these interviews were:

- There is a lack of free, independent advice on which biopesticides to use under which circumstances, and how they should be applied.
- Biopesticides were applied using current equipment and practices, which may restrict their impact on the target pests.
- Growers perceived biopesticides to be unreliable, potentially as a result of sub-optimal or incorrect applications.

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

General Introduction

In year 1 the project team has worked to obtain baseline information on the use and performance of some representative biopesticide products on protected crops. Most of this work has 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 to be studied in year 1.

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.

Benchmark 1: Powdery mildew on cucumber

Introduction

Powdery mildew (*Podosphaera xanthii*, formerly *Sphaerotheca fuliginea*) is common on glasshouse cucumbers, increasing in importance during the year because three crops follow in quick succession. It is important to stop it infecting fruit. Some varieties have intermediate resistance. Mycosphaerella and Botrytis are the other key diseases. The Cucumber Growers' Association provides its members with a list of fungicides so that alternation of mode of action groups is possible. The list includes the biofungicides AQ10 (*Ampelomyces quisqualis* strain AQ10) against powdery mildew and Serenade ASO (*Bacillus subtilis* strain QST 713) against botrytis. AQ10 was selected for this benchmarking trial at a host site where the product had only been tried once with a product sample and had not been taken up subsequently as the grower did not notice any obvious benefit. The grower did not routinely use any protectant plant protection products against any disease. Applications were made only when powdery mildew started to be seen. This was principally because fruit was picked daily and harvest intervals after the use of conventional chemical pesticides are between one and three days. Furthermore, there was concern that detection of any residues by the companies that are supplied could cause adverse comment. Crop coverage by sprays was believed to be an issue by the grower as the crop grows very rapidly (being stopped by taking off the top shoot around 2 m high), requiring water volume of at least 1000 L/ha, depending on crop height. The main aim was to keep the mildew from infecting the fruit. The variety comprising the main area, cv. Bonbon, was selected by the grower for its intermediate resistance to powdery mildew (the best level available), but four rows of cv. Bonifacio (also bred by Rijk Zwaan) of known mildew susceptibility were being grown to evaluate the variety. Insect biocontrol agents were used in the crop rather than conventional chemical pesticides.

Methods

The cucumber plants were bought-in on rockwool cubes (Plantop) and placed in the Venlo glasshouse on coir slabs (Botanicoir MIST) on 4 August 2016 as the third crop of the year. Each cube was fertigated by dripper. Plants were pruned to produce two stems held on a V-shape of vertical strings with the fruit produced up the strings and (unlike tomatoes) the lower leaves were left on the plant throughout cropping. Each row of plants was 50 m long with 1.5

m between cubes on opposing row faces. The path floor between the rows was covered in white plastic.

The treatments applied are shown in Table 2. Prior to the site visit, the product label and other technical information were evaluated to determine the application conditions that were required. At the site, a combination of observation and discussion was used to determine as much as possible about the equipment available and how it would be used.

At the first application on 17 August and the second on 3 September AQ10 was the only product applied. However, the grower became concerned that mildew was developing in the rest of the crop and so he applied Reflect (isopyrazam) to the rows out into the crop either side of those which had been treated by the AQ10 on 1 September.

AQ 10 (batch 3215815 11.11.2015) was given to the grower (after brief storage after delivery in the ADAS cold store) and was kept in the grower's fridge. The AQ10 was weighed out by the grower in the glasshouse and mixed according to the product label directly into the tank.

Table 2. Treatments and rates applied to the cucumber crop by the grower on the dates given. Treatment 1 and then 3 were applied to the whole crop of cv. Bonbon, Treatment 2 was applied to the experimental area of cvs Bonbon and Bonifacio only.

	Treatment	**Products [MAPP code]**	**Active ingredient & formulation**	**Rate of use**	**Application date / comments**
1	Untreated	n/a	n/a	n/a	The grower left the crop unsprayed until mildew was seen
2	Biofungicide	AQ10 [17102]	*Ampelomyces quisqualis* strain AQ10 (58% w/w minimum 5 x 10^9 spores / g of product) Wettable Gran**ule**	14 g / 200 L water (aiming for 70 g / ha for crops above 125 cm)	17 August 2016 Actual rate achieved estimated as 253 g/ha No harvest interval
				15.9 g / 300 L water (aiming for 53 g for crops 50 to 125 cm)	3 September 2016 No harvest interval
3	Chemical fungicide on Untreated if mildew seen	Reflect [17228]	Isopyrazam (125 g/L) Emulsifiable concentrate	0.1 L / 100 L	1 September 2016 when mildew seen Maximum of two applications per crop one day before harvest

Trial design

The grower was willing to apply the AQ10 down four pathways of the crop. The mildew susceptible variety Bonifacio was available in four rows and so the treatment was shared between these and the grower's main variety with intermediate resistance, Bonbon. This resulted in direct application of spray to three row faces of Bonifacio (and five untreated) and five row faces of Bonbon (and more than five untreated) (shaded black in Figure 1). When the spray was applied on 17 August, droplets were observed on leaves in the next row to the one being sprayed and so a further row each side of the sprayed rows was also counted as having some AQ10 on the leaves (shaded grey rather than white in Figure 1.1).

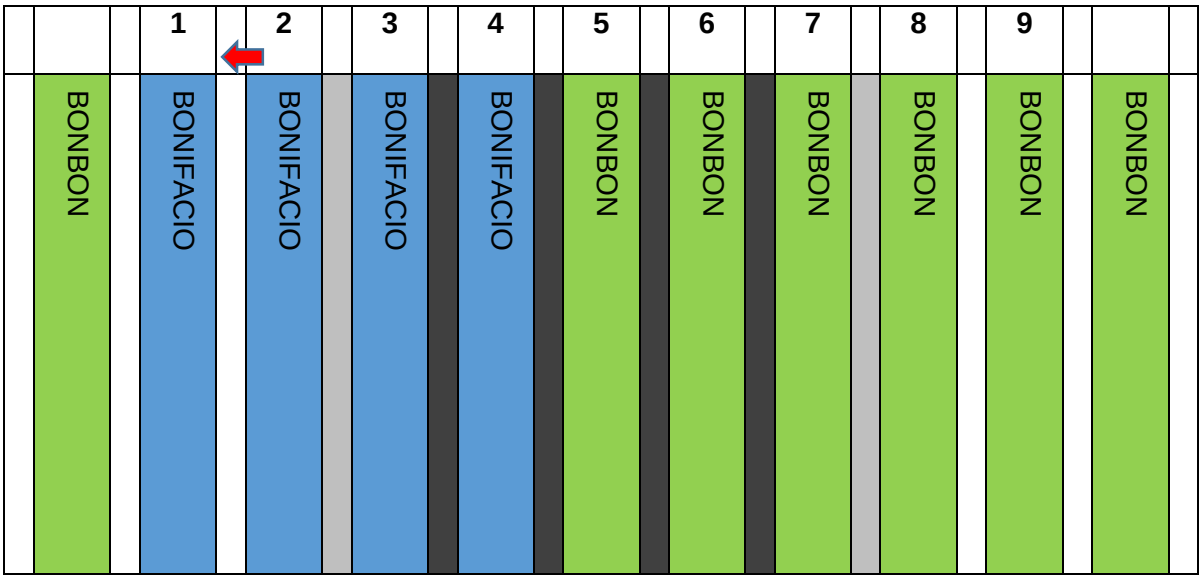


Figure 1. Rows of the cucumber varieties Bonifacio and Bonbon. The AQ10 sprayer travelled down the pathways shaded black on the diagram, with nozzles facing to spray the crop faces either side. Spray drift was observed into neighbouring pathways (grey shaded). There was no or minimal spray drift through or over the crop to the rows facing the white shaded paths. Arrow indicates which face rows were assessed for mildew on 7 September.

Figure 2. Vertical spray boom on rails in crop (left picture) and static sprayer tank (right).

Treatment application

A hand-pushed trolley with a vertical boom with five pairs of nozzles (FF80 02) angled upwards, 7 bar; 1.15 km/h was used with a hose attached to a 1000 L static water tank (Figure 2). The trolley was pulled backwards over a pair of rails along the space between the crop rows, with the operator setting his pace based on his own experience. Measurements were made of walking speeds; nozzle types, pressures and flow rates were noted, and applied volumes were calculated subsequently. On the 17 August the four lower pairs of nozzles were used. Heights of the nozzles above the ground were 0.45 m, 0.80 m, 1.15 m, and 1.5 m. All parts of the application process were observed as far as possible and there was no observation of tank and equipment cleaning following the application. On the 3 September the four upper pairs of nozzles were used to treat the crop where the fruit was unpicked and where the treatment was still preventative on the majority of younger leaves.

The pH and temperature in the spray tank was measured. A sample of the spray suspensions were collected in a 30 ml sterile tubes from the spray tank and one of the sprayer nozzles. These were streaked over PDA plates in the laboratory and incubated. The same agar and incubation was also used with a sample of AQ10 different to that used at the nursery in order to confirm the culture method and the colonies to be expected. Straight after spraying with AQ10 a number of discs were cut with a cork borer from a sample of leaves. While still in the glasshouse, each leaf disc was lightly briefly pressed sprayed side downwards onto a PDA plate. The plates were then returned to the laboratory for incubation and assessment.

Crop Assessments

Crop assessments were carried out prior to the two applications of AQ10 to record the incidence of powdery mildew on each of the crop rows. At the first assessment, all the row faces of the plants in rows 1 to 9 were examined for the presence of any mildew. At the second assessment, when mildew was found on the leaves throughout the crop, because of the long length of each row available to be assessed, examination was restricted to one to give a total of nine faces (four of cv. Bonifacio and five of cv. Bonbon). At this time each row was divided into four approximately 12 m sections in order to record whether there was any variation along the row. Within each length of row the crop was assessed separately above and below 1.5 m as this was the height of crop on 17 August and thus the leaves above 1.5 m would not have been present to have received the first AQ10 applications.

Any phytotoxicity, such as scorch or distortion, was noted at the second assessment. Assessments were based on EPPO guidance document PP 1/135(4e) “Phytotoxicity

assessment". A temperature and humidity logger was placed under a sun and rain shield in the crop at 1 m height for the duration of the assessments.

Environmental conditions (air temperature and relative humidity) were recorded electronically using a logger suspended in the crop.

Statistical analysis

No analysis was performed on the data as there was no replication of the two treatments.

Results

Treatment Application Assessments (Observations only made on 17th August)

The target volume was not defined on the label. After leaving the AQ10 more than the recommended 30 minutes to hydrate the product (possibly the carrier) was clearly visible as brown clumps in the water in the spray tank. Residue was still visible at the end of the application. Application commenced at 10:05 and finished by 10:30. Spray operator aimed to deliver visually very wet plants and adjusted walking speed accordingly between rows. Speed was therefore very slow. Much of the spray from the top nozzle went over the top of the crop and there was also penetration through the crop into the next row (Figure 1). Significant over-dosing because actual applied volume was much higher than the spray operator thought. The configurations of the nozzles were designed to give optimum under-leaf coverage, although not necessarily required for this application and the nozzles might be too close to the crop for optimum distribution. An uneven vertical distribution was noted. The leaves still held droplets after 1 hour, although it was sunny and over 20 °C in the house.

No *Ampelomyces* sp. growth developed on PDA agar from the AQ 10 taken from the tank before and after application and from a spray nozzle. No *Ampelomyces* sp. grew from the sample cores from sprayed leaves impressed on agar plates.

The second application was intended to be within 7 to 10 days of the first (as given in the Technical Notes for the product), but the grower was unable to fit this in with staff working in the crop and so it was done after 17 days on 3rd September. Prior to this the grower had seen mildew in the crop and in the AQ10 area, and the grower had applied a chemical fungicide on 1st September outside the five AQ10 sprayed rows. No observers were present at either of these applications.

Crop Assessments

At the first AQ10 application on the 17th August the crop was around 1.5 m tall, with on average 17 leaves up each stem of the cucumber plant, which had not reached the top wire.

Powdery mildew was only seen on Bonifacio in row 4 where two adjacent leaves (each four up from the ground) and one leaf further along the same row had a patch of mildew no wider

than 10 mm. It was however, noted that there was severe mildew infection on older plants by the door in the next glasshouse compartment 20 m away.

At the final assessment on the 7th September 2016, very rapid colonisation of powdery mildew on the Bonifacio was observed with lower leaves having a mean 18%, with a trend of increase moving away from the sprayed rows 3 and 4. For Bonbon the lower leaves of the sprayed rows had traces of mildew when the unsprayed had 4.5% (Figure 3). Where mildew had recently become visible on the new growth this was the lowest for the Bonifacio row 4 which had been AQ10 treated four days earlier. The treated Bonbon in rows 5 and 6 had zero mildew with traces visible in the unsprayed rows (Figure 3). Similar mildew levels on either lower or upper portions of the plant were seen along each row (data not presented). No phytotoxicity was seen at this assessment.

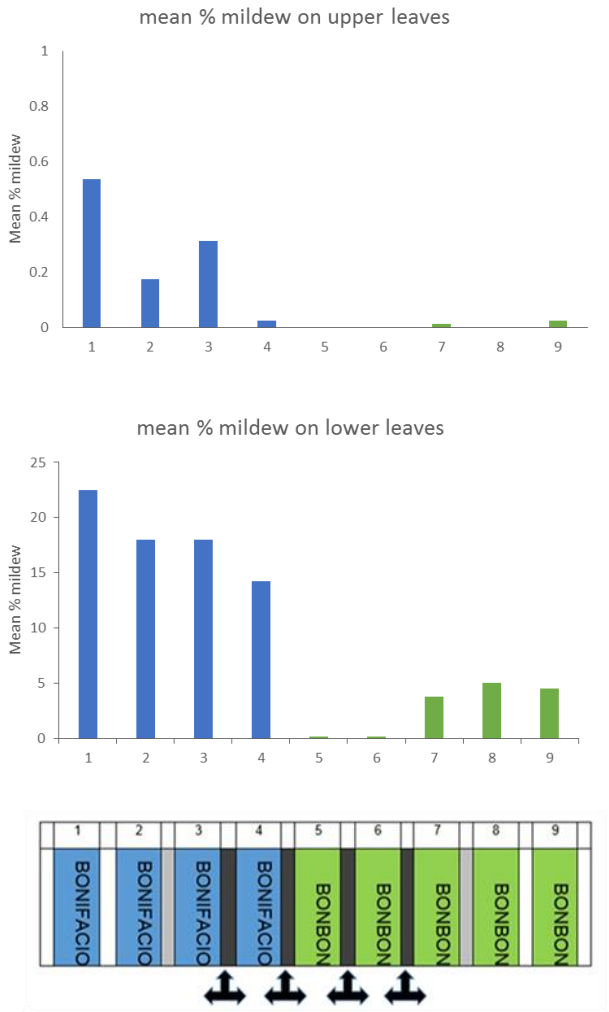
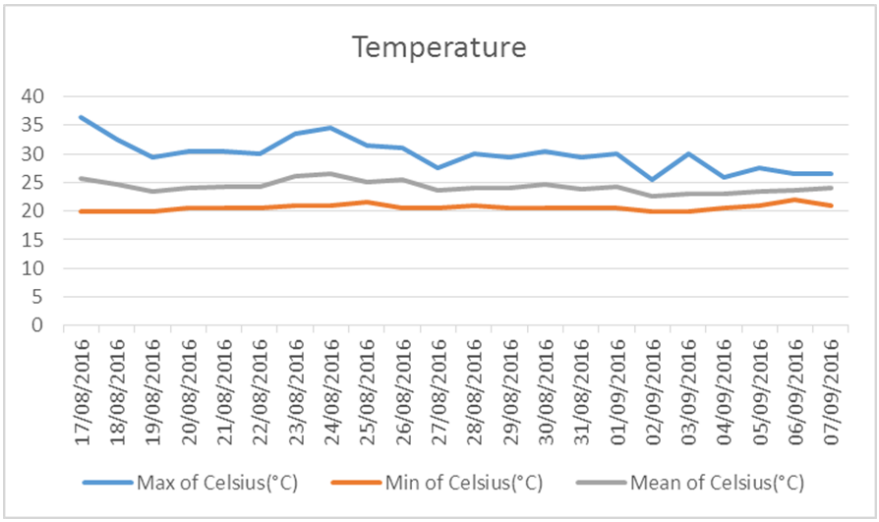


Figure 3. Mean % mildew on cucumber leaves on 7th September, cv. Bonifacio rows 1-4, cv. Bonbon rows 5-9. AQ10 application to the lower 1.5 m of rows 3 to 7 on 17 August 2016 and to the upper leaves on 3rd September.

Figure 4. Mildew on lower leaves of cucumber plants on 7 September 2016.

The glasshouse temperature was maintained between 20°C and 30°C. Minimum humidity was more erratic between days, although with a daily mean around 70% RH which is favourable to the powdery mildew (Figure 5). Low humidity was measured on 17th August when the first spray was made and the logger was installed in the crop. The temperature recording following application shows that the second, but not the first biofungicide applications were followed by good conditions for its growth (Figure 6). The rise in afternoon temperatures probably also resulted in the observed decrease in humidity on the 17th August.



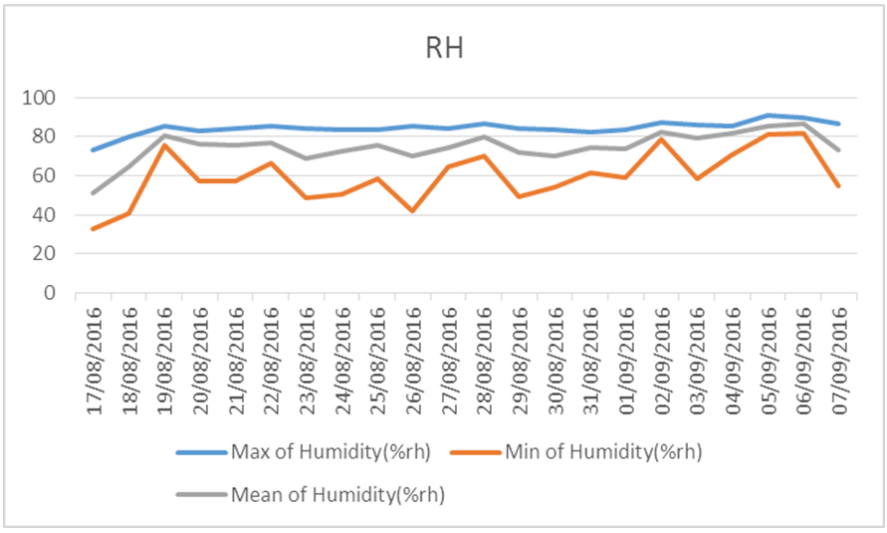


Figure 5. Temperature and relative Humidity within the crop canopy.

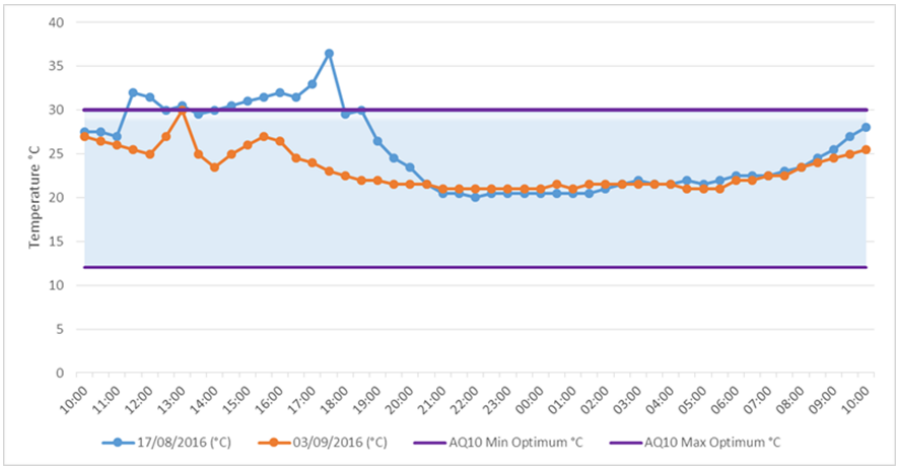


Figure 6. Temperature chart from the logger in the canopy in the glasshouse for 24 hours after each spray application on 17 August (upper line) and 3 September 2016. Blue shaded strip is the optimum temperature range for *Ampelomyces quisqualis* strain AQ10. according to the AQ10 manual.

Discussion

The water volume required was estimated by the grower based on his experience, according to crop height. However, more spray was applied in this experiment than had been estimated by the grower. The AQ10 product manual gives a sliding scale for product dose, with more product required for taller plants. The grower stated that he sometimes sprays every other row because plants in the "unsprayed" rows do receive product. This may however, be less suitable for products such as biologicals where contact is needed and there is no systemic activity (other than plant stimulation).

The "intermediate resistant" variety gave good disease suppression and so preventative fungicides are likely to have a better chance of reducing this fast spread pathogen than on the susceptible variety. The AQ10 application interval needed could be different (perhaps outside of the 7-10 days recommended) for different varieties. By using untreated plots to compare with the biofungicides then disease pressure was higher than if that neighbouring area had been treated by either a chemical or biological product.

There may have been an issue with the viability of the AQ10 used as it was not able to be cultured from the spray tank. The method used to quantify spore viability (plating spores on to PDA media) has been used on other batches of AQ10 and found to work well. It was possible that with sampling near the surface that the spores had not fully circulated, but this does not explain why the lance sample was negative as the outflow into the lance was from the tank base. However, at this stage, we think that the most likely explanation for low AQ10 viability would be the effect of pesticide residues in the spray tank. Although the grower had washed the spray tank after the previous chemical use, it is unlikely that a water rinse would remove all residues. The greatest concern would be for fungicide residues. We also cannot discount the combined effects of residues of different pesticide substances, particularly if these had synergistic inhibitory effects on AQ10. In principle, any residue would have been diluted by the 200 L of water added to the spray tank, although it is also possible that the formulants used in the AQ10 could have taken any pesticide residue back into solution in the spray tank. The AQ10 samples taken from the spray tank and the spray lance were held for a number of hours (ca 3 hours) while they were transported back to the laboratory for plating onto PDA. This prolonged period of exposure could have amplified any inhibitory effect of tank residue on the AQ10 in comparison to the shorter period of exposure that the fungus would have experienced between being placed in the spray tank and then sprayed onto the crop. This would then account for the fact that the AQ10 did demonstrate some efficacy on the crop. Unfortunately a sample to check on product viability was not collected from the packet of AQ10 before adding it to the tank, although it is reasonable to assume that the product would have been viable as the sample had been received direct from the suppliers

(Fargro) and the nursery fulfilled the requirement to store in a refrigerator. The issue of the potential for agrochemical residues to impact negatively on microbial biopesticides is a complex one to address, since it is likely to vary according to individual grower practice – but it is an area that warrants further consideration in AMBER. Observations suggested that there might be areas of poor coverage, but no quantitative data was obtained to support this. Further work can consider how this might be improved through application technique and potentially by the use of adjuvants.

Conclusion

- AQ10 sprayed rows had lower powdery mildew infection than the unsprayed rows, with very little mildew seen on the treated plants of the more resistant variety (cv. Bonbon) compared with cv. Bonifacio.
- Improvement in product dispersal in the spray tank is required. There was also a query about *Ampelomyces quisqualis* strain AQ10 spore viability at the trial site.
- Application technique was slow and inefficient, and the applied dose was too high at the first application.

Benchmark 2: Botrytis on cyclamen

Introduction

Botrytis (*Botrytis cinerea, grey mould*) affects cyclamen causing rotting of the lower leaves in contact with the growing-medium and also affecting the petioles and flower stem bases where high humidity favours infection. Left unchecked the whole crown of the plant can rot. Spores landing on and infecting petals cause spotting.

On nurseries preventative conventional chemical pesticide sprays are routinely applied to the crop. These are made every two to three weeks, depending on the speed of production of new growth. Prestop (*Gliocladium catenulatum* strain J1446) has full approval for use on all protected edible and non-edible crops. It is stated that it gives moderate control of Botrytis and some other named diseases.

Methods

Cyclamen of a mixed flower colour, cv. Picasso Verandi, was potted at the end of May 2016 (week 22) into 105 mm diameter pots using ICL 40% peat bedding plant mix without controlled release fertiliser and no fungicide incorporated. The crop was grown under polythene in wide-span, high tunnels each around 30 m x 18 m and able to be opened at the sides and doors and with computer controlled automatic vents and fans in the roof. Natural daylight was not supplemented. Pathways were concrete.

The plants were ready-spaced from potting, being held in position in Teku ST12B black plastic open base six-hole carry-trays 390 mm x 280 mm, with three plants arranged in alternate holes (so that pots were about 100 mm apart). The plants were stood on a white perforated plastic cover over capillary matting with a black plastic below (Figure 7). The crop received overhead watering as required until the roots were established and then irrigated via drip tubes onto the capillary matting. Mains water was used for irrigation.

Table 3. Treatments used on cyclamen.

Treatment	Products [MAPP code]	Active ingredient & formulation	Rate of use	Application dates / Comments
1. Biofungicide	Prestop [17223]	*Gliocladium catenulatum* strain J1446 (32% w/w nominal 2 x 10^{8} cfu/g). Wettable powder	500 g / 100 L water	12 July (Week 28), 2 August 24 August *minimum 3-week interval required
2. Chemical	Rovral WG [13811]	Iprodione (750 g/kg) Wettable granule	67 g / 100 L water	in alternation with Amistar 28 June (Week 26) 2 August
	Amistar [10443]	Azoxystrobin (250g/L) Suspension concentrate	1 ml / 1 L water	In alternation with Rovral WG 12 July (Week 28) 24 August

The biopesticide treatment investigated was Prestop and it was compared with the nursery's standard preventative control programme for Botrytis on cyclamen, of an alternation of Rovral WG (iprodione) and Amistar (azoxystrobin) at 21 day intervals (Table 3).

Trial design

There were two treated areas of plants, each 3 m across the width of the bed and 10 m down the bed length (Figure 7 and 8). There was no untreated area and there were no replicate blocks.

Figure 7. Arrangement of cyclamen plants in trays and area of experiment in the glasshouse on 12 July just before the first application of Prestop to the cyclamen in the bed to the right of the path. An alternation of Rovral WG and Amistar was applied to the rest of the crop and monitored in the bed left of the path.

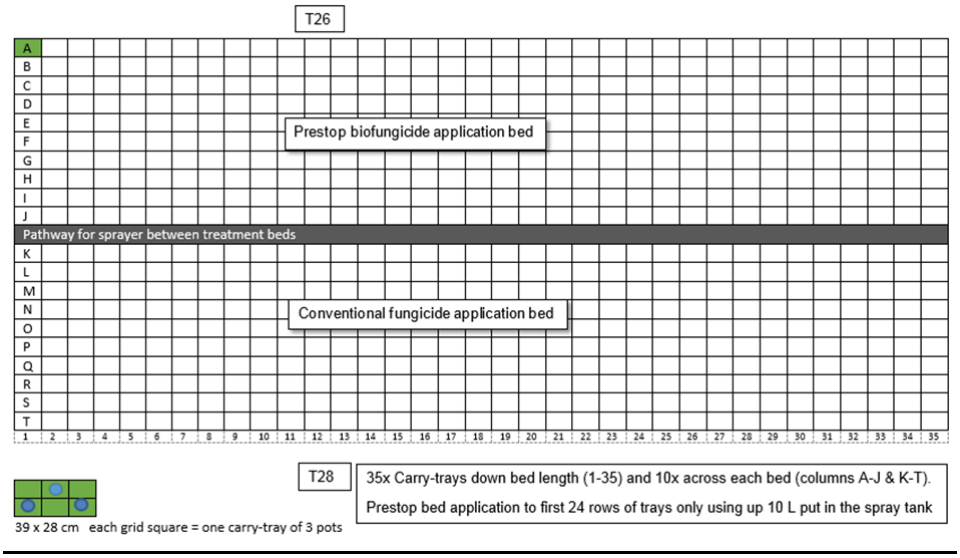


Figure 8. Layout of the trial area showing the columns of trays A to T and the rows 1 to 35, each tray with three plants.

Each treatment was applied to a bed of 10 carry-trays wide (columns) and 24 trays down the length (rows) with spraying carried out moving in the direction from row 1 to full coverage of row 24 and then a little further until the tank was empty (to give 20 x 72 lines of pots for each bed down either side of a pathway (Figures 7 and 8).

Treatment application assessments

Prior to the site visit or trial, the product label and other technical information were evaluated to determine the application conditions required. At the site, a combination of observation and discussion was used to determine as much as possible about the equipment available and how it would be used. At the start of the trial the nozzle used and lance output was recorded. The RIPA nozzle was already adjusted to deliver 7 L / minute at 15 bar pressure

from the Brinkman 250 sprayer. The nursery's spray operator was observed by a researcher during mixing and application of the products on 12 July and 2 August 2016. The grower had received instruction that sprayer should be rinsed out well of previous chemical fungicides.

A 100 g foil packet of Prestop Lot number W3406612 (dated 06-2017) was received for the trial and kept in cold storage at 4°C before transport in a cool box to the site. A further supply of two packets of 100 g of the same Lot number (dated 08-2017) was received for the third application sent direct to the grower and kept unopened stored in their refrigerator normally used for storing seeds.

The pH and temperature in the spray tank was measured. Samples of the diluted product were taken from the tank and lance for laboratory assessment of biocontrol agent viability and to see colony density. Contact prints onto Petri dishes were taken from both sides of the Prestop sprayed leaves and incubated in the laboratory to visualise viability and colony density.

Mixing

On 12th July, mains water was taken from the tap into a 10 L bucket and about 1 L of this taken in a plastic jug to add the product. Weighing out of the product was carried out with the nursery's scales in the tunnel where the trial was located. The product was in a foiled sachet that had to be cut open with sharp scissors and a researcher resealed it with electrical tape and a bulldog clip (otherwise it would have been left open). The product was left in the tunnel during the trial procedures, but then returned with a researcher for freezer storage (as there was not one on the nursery site).

Using mixing instruction pictures provided by Lallemand, 50 g of the Prestop was added to a litre of water in a jug half an hour before making up to 10 L for use. When the product was added to the water in the jug, lumps floated on the surface which took seven minutes to dissipate. After discussions with Lallemand, for the next application the product was first put in the jug to create a "cream" in a smaller amount of liquid and then more water added to the jug and stirred before adding into the tank water after half an hour for subsequent applications.

Application

Application was made using a RIPA nozzle (Figure 9) on the end of a hose from a Brinkman 250 L tank sprayer. The outlet to the delivery hose is at the base of the tank.


Figure 9. RIPA spray gun attached to delivery hose and close-up of nozzle at host nursery.

On 12th July four cups, and on 2nd August ten cups, 60 mm diameter across the rim were randomly placed out in the Prestop area to give preliminary observations of the volume of spray liquid applied. They were placed as far into the crop as could be reached from the sides of the bed.

Crop Assessments

Plants were examined for Botrytis sporulation before the first two Prestop applications on 12th July and 2nd August, assessing plants in the centre of each carry-tray 1 to 25 in the second row in from either side of the path (rows I and L). Plants assessed within the Prestop bed were labelled with a number 1 to 25 to allow re-assessment, and plants directly opposite were examined in the chemical treatment bed. No assessment visit was made just before the 24 August applications as the grower carried this out without a researcher present.

At the final examination, on 8th September, when plants were in full flower (Figure 10) individual plants were picked up and the lower leaves lifted to look for Botrytis under the canopy. One plant in each of the 25 trays within the second line of trays from the path were assessed from both treatment beds for the number of leaves with Botrytis sporulation (a mean total of 45 leaves present per plant) and the number of flowers with any spotting on the petals or softening of the petioles (a mean total of 25 flowers raised up/plant).

Phytotoxicity was assessed after each application date based on EPPO guidance PP 1/135(4e) “Phytotoxicity assessment”.

Environmental conditions (air temperature and relative humidity) were recorded electronically using a data logger suspended in the crop with the sensor at a level 30 mm above pot surfaces.

Figure 10. Cyclamen in full flower on the 8 September 2106 assessment day viewed looking from column A in the Prestop bed towards row 25 and the chemical fungicide treated bed.

Statistical analysis

No analysis was performed on the data as there was no replication of the two treatments.

Results

Treatment mixing and application

The Prestop label says for foliar applications to apply at high volume to just before run-off, ensuring thorough coverage of the crop, paying particular attention to wounds when applying for Botrytis. Label guidance is given for strawberries (similar rosette-type crop architecture to cyclamen) of 1200 L per hectare, whereas 500 plants of tomato, pepper or cucumber could be treated with 10 L. The estimated applied volume was 3000 L/ha. There was probably significant overdosing, although no dose is specified, because of the high volumes used. Evenness of application is the biggest problem with hand-held systems, but to compensate the grower applied in very high volumes. The big gaps between pots in this benchmark meant significant waste of product.

On the first application on the 12th July, it was observed that some plants had pools of liquid held in cupped leaves (which remained wet for at least an hour) (Figure 11) and the growing media was saturated. Measuring-cups of 60 mm diameter placed in the Prestop treatment bed in empty tray-holes at tray-positions 3, 15 and 23 during application contained variable amounts of spray liquid; 0.48 g, 1.14 g and 0.74 g of liquid, respectively.

Figure 11. Pooling of Prestop spray suspension on cyclamen leaves on 12 July 2016 and showing how upper leaves can prevent those underneath from being treated.

At the second application on the 2nd August, the product was creamed with a small amount of water, but had not dispersed after 30 minutes. More water was added before mixing it into the tank. The tank was washed out and sprayed over another crop before applying the conventional chemical fungicide to the other bed of cyclamen. Similar variation in spray volume throughout the area was observed with less collected between rows 9 and 17 down the bed length (Figure 12), whereas across the bed from the path there was a range of both higher and lower volumes captured (data not shown). The pattern down the bed length has some similarity to the rise in the number of leaves affected by Botrytis on 8 September between rows 14 to 17 of the Prestop area.

The third application was carried out on the 24th August by the nursery without researchers being present. The new batch of Prestop was used and applied following the same procedures as before.

A fourth application was due on the 14 September, but was not done because a brief rise in air temperature meant that the crop came into open flower sooner than had been predicted, with plants ready to be sold by the assessment visit made on 8 September.

Gliocladium sp. grew on PDA from the sample of liquid taken from the Prestop spray tank and the spray lance on both the sampled spray dates, on 12 July and 2 August 2016.

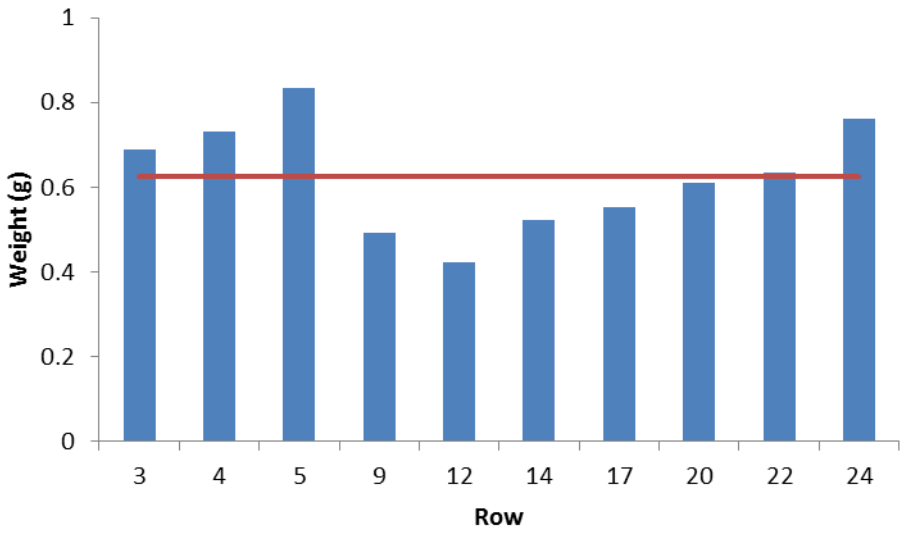


Figure 12. Weight of Prestop spray caught in measuring cups placed in the crop on 2 August 2016. Showing variation above and below the mean 0.62 g (Std. Error 0.041) as the spray operator moved down the path towards row 24, with less delivered half way down the bed to plants of equal canopy size.

Crop Assessments

Before the first Prestop application, no Botrytis was visible from above looking across the beds and so 25 plants were picked up and checked underneath the canopy all-round the pot for any signs of disease. No active sporulation or Botrytis was seen, but one dry shrivelled leaf in the Prestop area had the brown residue of sporangial growth.

Prior to the second spray applications, of the 25 plants assessed per treatment 15 of the conventional chemical fungicide treated plants (60%) and seven of the Prestop treated plants (28%) had Botrytis sporulation on mainly only one leaf (Figure 13). Plant vigour was good throughout the crop.

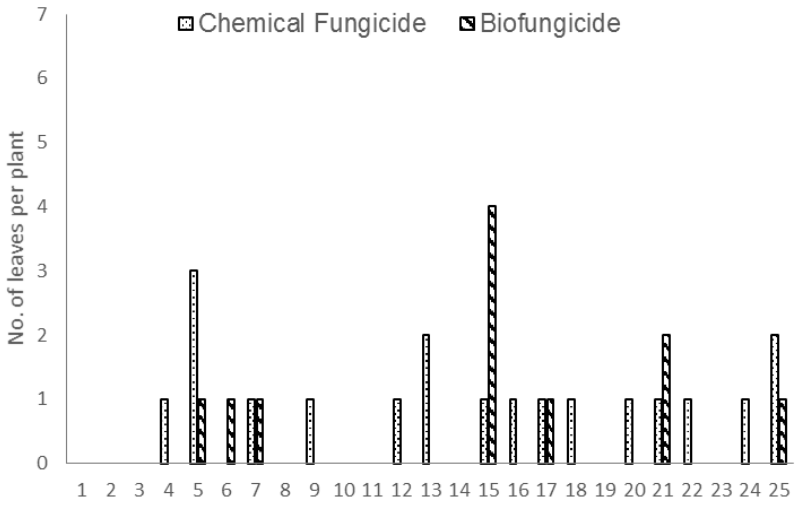


Figure 13. The number of leaves per plant on 2 August 2016 with Botrytis symptoms, for plants at positions 1 to 25 in the chemical (dotted bars) and Prestop (striped bars) beds.

Two weeks after the last spray applications to the crop there had been an increase in the incidence and severity of Botrytis in both treatments. Of the 25 plants assessed per treatment 14 from the biofungicide (56%) and 21 from the chemical fungicide (84%) programmes had Botrytis sporulation (Figure 14), with a mean 1.44 or 2.44 leaves affected, respectively (Figure 15). On the affected plants only four Prestop treated plants had Botrytis progressing back from the leaves into the petioles, whereas 13 chemically treated plants had softened petioles. Most of the sporulating leaves were in contact with the growing-media and under the canopy of the younger leaves in the rosette. Flower petal or stalk symptoms were uncommon, with three plants with an affected flower in the Prestop treatment and four in the chemically treated.

Figure 14. Botrytis sporulation on an old leaf under the rosette canopy in the Prestop sprayed bed on 8 September 2016. The Botrytis has not spread into the neighbouring petiole.

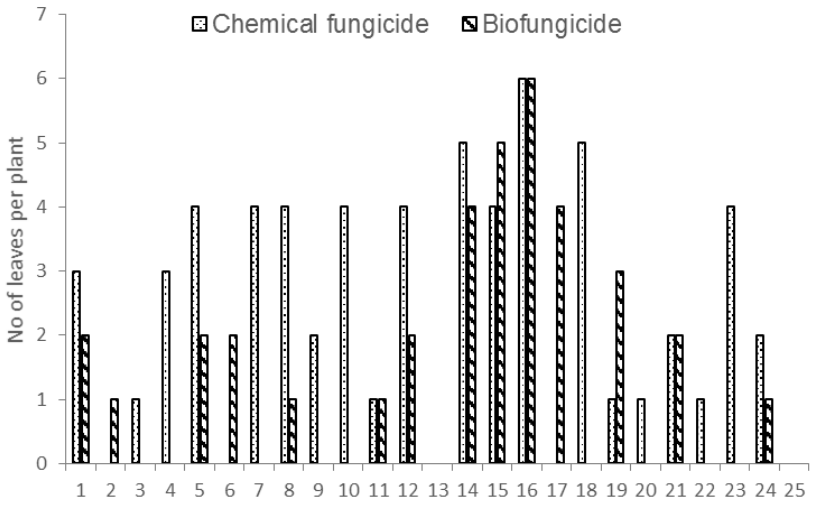


Figure 15. The number of leaves per plant at marketing stage on 8 September with Botrytis symptoms, for plants at positions 1 to 25 along either side of the pathway in the direction of spraying in the chemical (dotted bars) and Prestop (striped bars) beds.

No phytotoxicity was seen on either the leaves or the flowers, and vigour as measured by leaf and flower colour and leaf production was equally good between the two treatments. The grower considered all plants to be marketable. Plants are picked-over by the nursery just before sale to remove any damaged flowers or leaves. It is not possible to easily do this earlier before sale as the trays are packed tightly on the wide bed without a means of easy access.

The polytunnel housing the experiment had vents, doors and fans which enabled some adjustment of temperature and humidity for the crop, but on sunny days temperatures under the shade cup of the logger at crop height commonly reached over 30°C. Relative humidity around the logger varied between around 40% and 90% (Figure 16). For four to five hours after application it was above the minimum optimum humidity for growth of the *Gliocladium catenulatum* strain J1446 in the Prestop, but as temperatures rose the humidity fell below the optimum on the 12 July and 24 August although it had remained ideal for 24 hours following the spray on the 2 August (Figure 17).

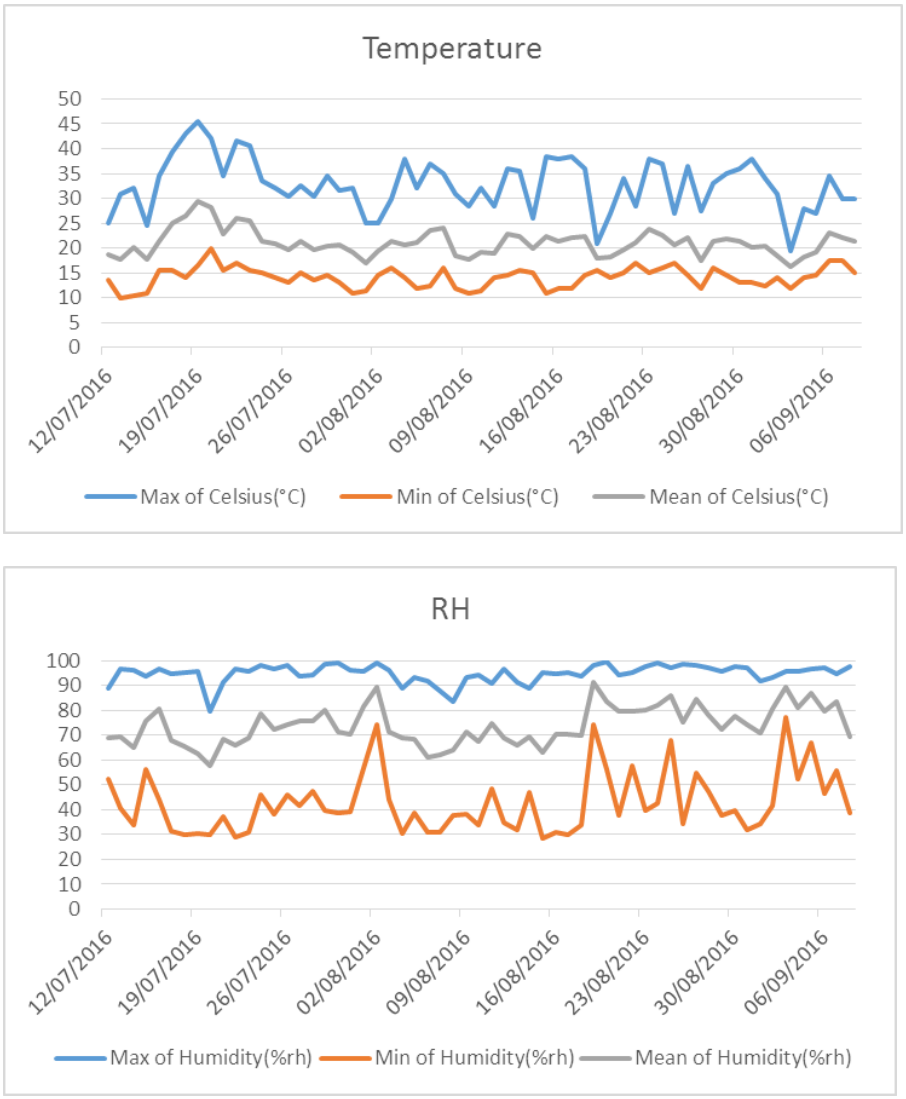


Figure 16. Temperature and humidity within the crop canopy.

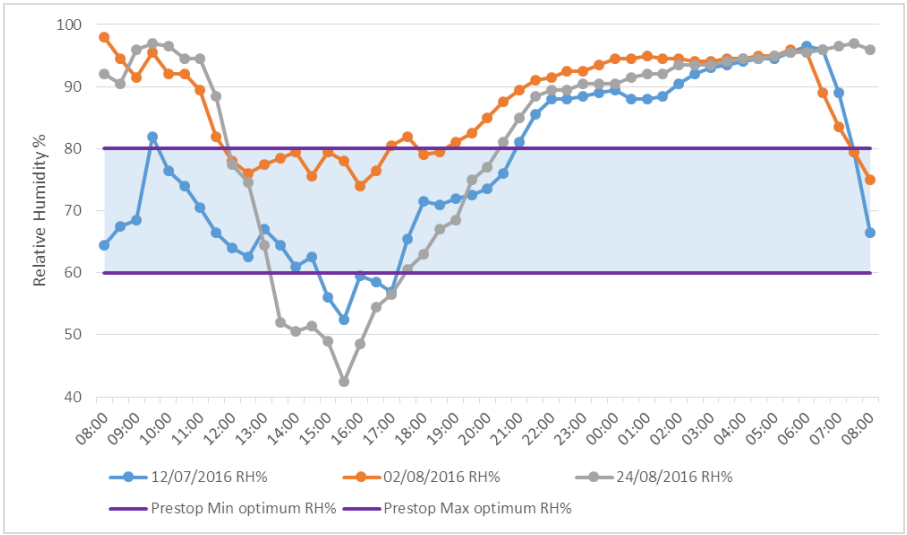


Figure 17. Relative humidity in the crop canopy over the 24 hours post spray applications. All spray applications took place between 08:00 and 09:00. The shaded area represents the optimal conditions for *Gliocladium catenulatum* strain J1446.

Discussion

The Prestop foil packaging meant that it was not easy to reseal the product to prevent it absorbing moisture and allowing germination to commence in storage. However, the pack size of 100 g might only cover 60 m² at the high water volumes used at the host nursery using 100 g / 20 L. The host nursery had not used biofungicides before and so had no refrigerator or freezer designated for pesticides and so the recommended way to store opened product was not possible on site. It was also noted that if the nursery practice is to make up the product where the sprayer is filled then the packet could become warm before the remainder was returned to the store for next time.

Two different ways of mixing the product were tried at the nursery. These were discussed with Lallemand who have since produced You-Tube videos of how to mix the product:

https://www.youtube.com/watch?v=TMcjL9Fu_3I Full video

https://www.youtube.com/watch?v=sQSQ68tc6tA No agitation system video

https://www.youtube.com/watch?v=3r5NLlfqvbc With agitation system video

In addition, Lallemand have recently developed a ready to use tool called Prestop Vision that will allow users of Prestop to reveal the presence of alive *Gliocladium catenulatum* strain J1446 on plants that have been sprayed (https://icl-sf.com/uk/news/fight-a-fungus-with-a-fungus-prestop/?_cldee=YWdvdWdoQGxhbGxlbWFuZC5jb20%3d&recipientid=contact-dd0b9254a208e61180f602175d08c221-296783839ce2422b91035fb1bd980592&esid=99d6ac22-acb0-e611-8101-02175d08c221)

Lallemand are able to supply some Prestop Vision kits to evaluate growers' use, and the one grower asked so far has expressed interest in utilising this kit to verify that the biofungicide has come through the spray equipment and is alive on the plant.

The rosette-shape of the plant means that spray penetration to the older leaves at the base of the plant and deep within the crown is difficult. It is possible that a treatment of the growing-media in order to contact where the leaves rest (by uptake from below, where this is possible) could be effective.

It was noted that on two of the application days the optimum humidity would have been held for longer if the applications had taken place after 18:00h, but on the other the conditions

were good for the beneficial fungus all day (and also good for Botrytis). Changes in grower working scheduling to evening spraying in summer would in general give greater assurance that the product would be given the best conditions to become effective against pathogens. Lallemand Plant Care's laboratory supplied the project with further information on conditions for *Gliocladium catenulatum* strain J1446 growth. Prestop's *Gliocladium catenulatum* strain J1446 in a mixture with water maintains its activity for 7 days at +4°C or 5 days if kept under +8 °C or 24 hours at ambient temperature. There is no evidence suggesting any advantage or disadvantage in leaving Prestop to soak longer than 30 minutes before use. *Gliocladium catenulatum* strain J1446 can grow on wet, moist or relatively dry leaves. Dry atmospheric conditions are what can be detrimental to *Gliocladium catenulatum* strain J1446 growth and survival but this factor is also detrimental to many pathogens such as Botrytis.

Conclusions

- Application of Prestop at three week intervals reduced the incidence and severity of Botrytis on the leaves of cyclamen more than an alternating spray programme of Amistar and Rovral WG at the same application interval.
- Neither treatment programme stopped Botrytis appearing and sporulating on over half of the plants.

Benchmark 3: Root rots on Choisya and Dianthus

Introduction

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.

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 will undergo each of three treatment programmes (Table 4). Use of T34 Biocontrol will be under EAMU 1118 of 2012 for protected ornamentals, otherwise all uses will be 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 will be 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 date might be delayed to three months when the plants are starting to grow again rather than treat in February 2017.

Table 4. Treatment programmes (P1 to P3) used on Dianthus and Choisya against root rots.

P	Treatment at potting [MAPP code]	Active ingredient	Rate of use	Description of treatment	Application date / Comments
1	Trianum G [16740]	*Trichoderma harzianum* strain T22		Biofungicide granules incorporated in the growing media in the bulk bale	15 September 2016 Supplied ready-mixed by ICL
2	Trianum G	*Trichoderma harzianum* strain T22		As for P1	As for P1
3	Trianum G	*Trichoderma harzianum* strain T22		As for P1	As for P1
P	**Second Treatment**	**Active ingredient**	**Rate of use**	**Description of treatment**	**Comments**
1	Previcur Energy [15367]	Fosetyl-aluminium + propamocarb hydrochloride	3 ml / m² with water at 10% pot volume	10 % of pot volume drench	4 October 2016
2	T34 Biocontrol [17290*]	*Trichoderma asperellum* strain 34	10 g per 1000 L growing media. Irrigation rate	10% of pot volume drench (Choisya – 6g in 60L) (Dianthus – 3g in 30L)	4 October 2016 Pre-soaked for 1.5 hours. Repeat every 2-3 months
3	T34 Biocontrol	*Trichoderma asperellum* strain 34	10 g per 1000 L growing media. Irrigation rate	10% of pot volume drench (Choisya – 6g in 60L) (Dianthus – 3g in 30L)	As for P2
P	**Third Treatment**	**Active ingredient**	**Rate of use**	**Description of treatment**	**Comments**
1	Horti-Phyte	Potassium phosphite	200 ml / 100 L water	10 % of pot volume drench	8th December 2016 Not a registered fungicide
2	T34 Biocontrol	*Trichoderma asperellum strain 34*	10 g/ 1000 L growing media. Irrigation rate	10 % of pot volume drench (Choisya – 6g in 60L) (Dianthus – 3g in 30L)	8th December 2016 Pre-soak 30 mins. Repeat every 2-3 months
3	None				
P	**Fourth Treatment**	**Active ingredient**	**Rate of use**	**Description of treatment**	**Comments**

1	To be determined	To be determined			9 February 2017
2	T34 Biocontrol	*Trichoderma asperellum strain 34*	10 g / 1000 L growing media. Irrigation rate	10 % of pot volume drench	Pre-soak 30 mins.
3	To be determined	To be determined	To be determined	10 % of pot volume drench	Treatment with T34 an option

* T34 Biocontrol was also sold under MAPP no. 15603 (expiry 30/09/2017)

Trial design

Plants were set out in plots with 200 Choisya (arranged 10 pots wide and 20 pots). 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 18). Plots were separated by approximately 0.5 m wide pathways.

Figure 18. 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 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 for the three plots of P2 were each made up in 2 L plastic jugs 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 and thus in October it was calculated that for the 600 L of Choisya (200 pots of 3 L) for each of P2 and P3 that 60 L of water was required and at 10 g per 1000 L of growing media that 6g of T34 Biocontrol was required in the tank. The two varieties of Dianthus per treatment totalled 300 L (200 pots of 1.5 L) and so 3g T34 Biocontrol and 30 L of water was required. In December P2, but not P3, was re-treated.

Application in October and December 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.

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 in October and December. 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

The plants were examined to ensure they had no disease symptoms at the time of the first drenches with re-examination at subsequent drenches required to determine whether any plants developed any signs of wilting. Final assessment will be carried out close to the time of plant sale in spring 2017. 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 1 (poor) to 9 (strong and healthy) index. In between these records the

grower was asked to report any phytotoxicity symptoms and if any plants started to look less than healthy and photographs were received. Assessments were based on EPPO guidance document PP 1/135(4e) “Phytotoxicity assessment”. Any plants which start to die will be returned to the laboratory for assessment and will be counted and removed from the experiment. Any plants which are starting to look as if they might have root rot will be left to see if they recover or not (which is the procedure of the host nursery). A temperature logger was buried in the growing media of a pot of Dianthus when it was potted on 15 September and the pot placed in P1. A temperature and humidity logger was suspended at canopy height in a Choisya pot in P1, shielded from irrigation and sunlight by a white cardboard canister. A moisture probe was left recording in another pot of Dianthus (in P2). The loggers were set to record at 30 minute intervals for the duration of the benchmarking.

Statistical analysis

For this benchmarking study there is not envisaged to be any statistical analysis to compare between treatments because of the lack of replication.

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 weather was sunny, but cool and the air temperature was 20°C when P1 was sprayed at 10:45, but had risen to 25°C by 11:50 and the completion of spraying P3. The liquid in the spray tank was 17°C.

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 on 4 October 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

Assessments of the crop from 4 October onwards will be provided in the next report after the final drench and assessment of the roots of the plants.

The glasshouse at the nursery is not heated and so air temperatures fell as expected following potting of the finals (Figure 19). The ongoing logger data recording the temperature in the pot and the moisture meter will be obtained and reported on at the end of the benchmarking.

Discussion

Work is ongoing and results will be presented in the next annual report. The Choisya 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. The biofungicides used in this project work as protectants and so further losses may be expected and this project will allow comparison with the chemical fungicide treatments applied 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.

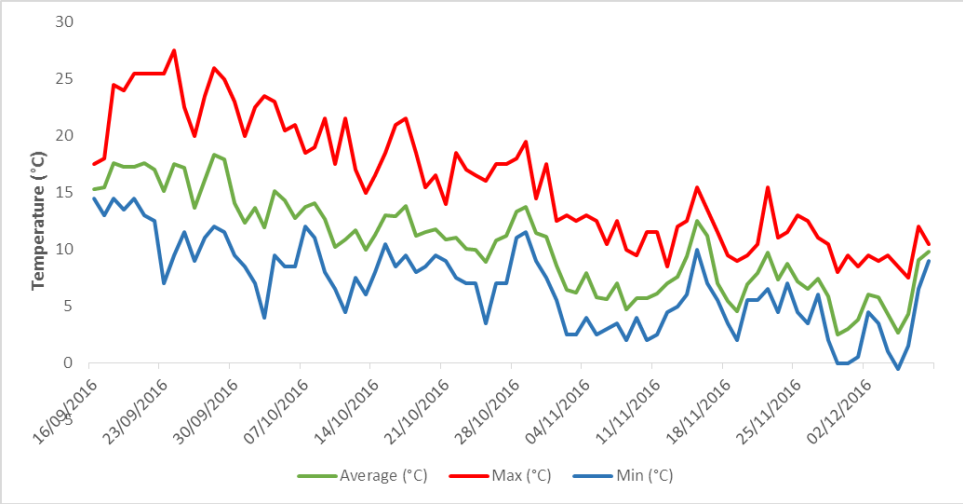


Figure 19. Air temperatures at canopy height from the date after potting in September to the first drench date on 4 October 2016.

Benchmark 4: Aphids in organic sweet pepper

Introduction

Aphids are a frequent and important pest of organic sweet peppers. The main aphid pest is the peach-potato aphid *Myzus persicae,* though the glasshouse and potato aphid *Aulacorthum solani* can also cause economic damage. Aphids can be present in pepper crops from very early in the year, feeding by sucking the plant sap, usually on the underside of leaves. Damage is caused both directly as a result of feeding, and by secondary damage as a result of honeydew deposited on leaves and fruit. Honeydew needs to be washed off the fruit prior to market, but can also lead to sooty mould outbreaks which also require the fruit to be washed before sale.

Aphid infestations in most sweet pepper crops, including organic crops, are managed using biological control, introducing aphid parasitoids and predators including the predatory midge *Aphidoletes aphidimyza* (Figure 20). While biological control can be effective in managing aphids, populations can increase rapidly and cause significant damage to the crop before their natural enemies are able to catch up. In addition, hyperparasitoids are commonly found in pepper crops and these can lead to breakdown in biological control by the primary parasitoids. Entomopathogenic fungi (EPF) are of interest to growers as they may provide an additional method of biological control which fits in to the Integrated Pest and Disease Management (IPDM) programme and meets organic production regulations.

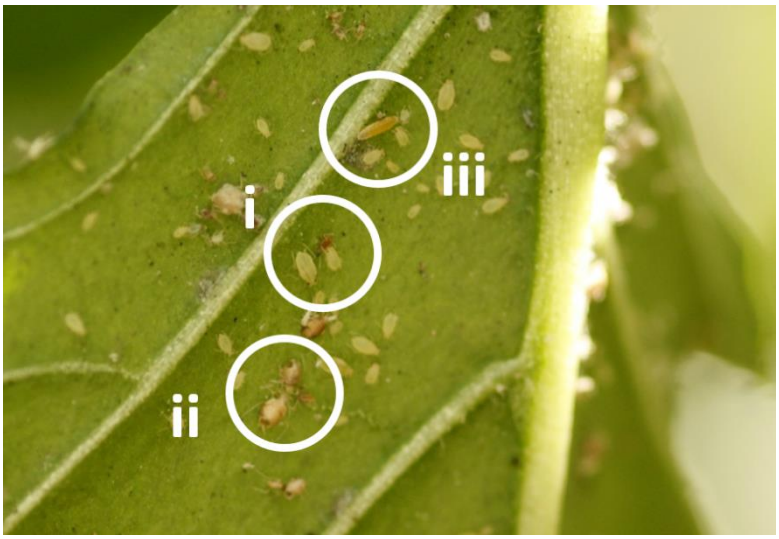


Figure 20. Peach-potato aphid *Myzus persicae* (i) on the underside of a sweet pepper leaf, *Aphidius*- parasitised aphids (‘mummies’ (ii), and an *Aphidoletes aphidimyza* larva (iii).

For this benchmarking trial, two products were selected; Botanigard WP and Majestik. Botanigard WP is a microbial biopesticide based on the entomopathogenic fungus *Beauveria bassiana* strain GHA*,* recommended for the control of whitefly; however there is evidence that it is also effective against aphids (Chandler & Prince, unpublished data). Majestik (maltodextrin) is recommended for spider mite control, but is recommended by the supplier to be used as a tank mix with Botanigard WP for improved whitefly control.

Methods

The sweet pepper crop was planted mid-January 2016 into 140m long irrigated soil beds. Aphid parasitoid wasps *Aphidius colemani* and *Aphelinus abdominalis* were released to control *M. persicae* and *A. solani* respectively. The generalist aphidophagous predators *Aphidoletes aphidimyza* and the hoverfly *Sphaerophoria rueppellii* were also released, and lacewing larvae *Chrysoperla carnea*, were introduced to hotspots of high aphid infestation. Natural enemies from the surrounding environment provided some additional biological control, e.g. the parasitoids *Aphidius ervi* and *Praon* spp., and the hoverfly *Episyrphus balteatus.* The crop was monitored by the grower fortnightly for pest infestations and natural enemy abundance in random locations throughout the crop. The site had no problems with either powdery mildew or botrytis, so no treatments were applied to manage these during this cropping season.

Spray applications were made on the 29 June and 5 July 2016. Pest assessments took place prior to both spray applications, and on the 11 July 2016, six days after the final application.

Table 5. Treatment and rates applied to the pepper crop. 'Biological control' refers to the grower's biological control programme used throughout the crop.

T	Product (MAPP code)	Active ingredient	Rate of use	Application dates / comments
1	Biological control	NA	Not recorded	Weekly, in response to pest pressure
2	Biological control + Botanigard WP (17054)	NA *Beauveria bassiana* strain GHA 4.4×10^{10} CFU/g	Not recorded 0.94 kg/L in 1377 L/Ha water	Weekly, in response to pest pressure 29 June & 5 July 2016
3	Biological control + Majestik (17240)	NA Maltodextrin (49% w/w)	Not recorded 25ml/L in 1377 L/ha water	Weekly, in response to pest pressure 29 June & 5 July 2016
4	Biological control + Botanigard WP (17054) + Majestik (17240)	NA *Beauveria bassiana* strain GHA 4.4×10^{10} CFU/g Maltodextrin (49% w/w)	Not recorded 0.94 kg/ha in 1377 L/ha water 25ml/litre in 1377 L/ha water	Weekly, in response to pest pressure 29 June & 5 July 2016

Trial design

The trial was designed in discussion with the grower to fit in with current practice, using the spray equipment available and following the grower's interpretation of the product labels and additional guidance where available.

The Botanigard WP and Majestik treatments were applied twice, six days apart along the whole length of beds of sweet pepper plants (130m long x 2.5m high). Each bed received the respective treatment from both sides, and two buffer beds with only the grower's biological control programme were used between each treatment (Table 5, Figure 21).

Two bays, each consisting of six beds, were selected, within the crop, in which aphid populations were abundant, under guidance from the grower. Within the selected bays, two beds were assigned two treatments at random. Within each treatment bed, the area was divided into three 30m lengths, with 20m buffers at either end. Within each 30m length, the crop was sub-set vertically into leaves in lower (0-80cm), middle (81-160cm) and upper (160cm and above) canopy sections (Figure 22).

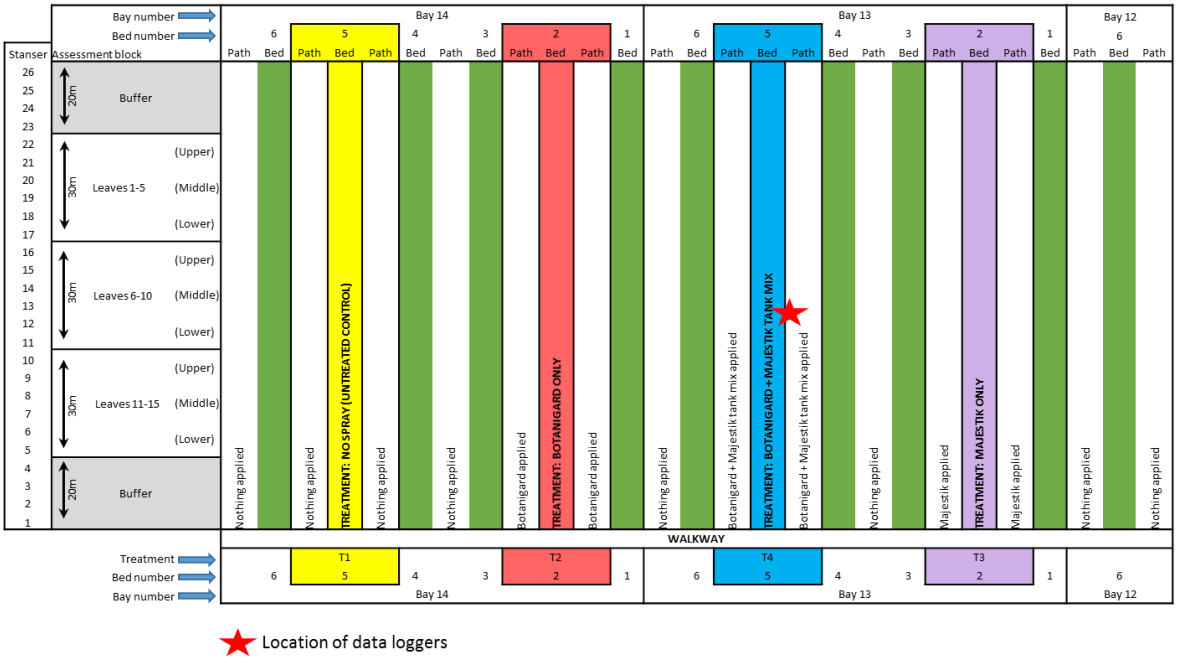


Figure 21. Trial layout.

Figure 22. Treatment path between two beds of organic sweet pepper plants. Aphid and natural enemy numbers were assessed at three sub-section heights; lower (0-80cm), middle (81-160cm) and upper (160cm and above).

Treatment application assessments

Prior to the site visit, the product label and other technical information were evaluated to determine the application conditions that were required. At the site, a combination of observation and discussion was used to determine as much as possible about the equipment available and how it would be used. The spray equipment was calibrated prior to the first applications using water on a row of plants outside the trial area. Nozzle type, sprayer speed and pressure were recorded and water volume applied per hectare was calculated. Samples of Botanigard WP were taken prior to and after spraying each of treatments 2 and 4. These were taken from the spray tank and the sprayer nozzles respectively. Each sample was

diluted and plated onto 90 mm plate of Sabouraud dextrose agar (SDA) and incubated at 23°C in the dark for five days and then examined for colony growth of *Beauveria* sp. Additional samples were taken from the foam created in the tank during the Botanigard WP and Majestik mixing prior to application. Samples were collected from five leaves, treated with T2 and T4 after spray application, at each of the three vertical sub-sets. Leaves were added to a single universal tube of wetting agent and the numbers of colony forming units (CFUs) of *Beauveria* sp. was measured by plating aliquots onto a selective medium. Additional leaves were imprinted onto selective SDA media.

Applications were made using a trolley with a vertical boom consisting of four pairs of HC 03 nozzles angled upwards; 5 bar, 3.6 km/h; 1377 L/ha, (500 – 1500 L/ha target volume).
The lowest pair of nozzles were 1.3 m above the base of the plants, and each subsequent pair was 360 mm apart, so that only the top 2/3rds of the plants were sprayed, as per usual grower practice to conserve natural enemies on lower leaves. Botanigard WP was applied at the recommended concentration (for whitefly control on sweet pepper) of 62.5g/100L. The water volume used for application was 1,337 L/ha. Therefore the product was applied at 0.86 kg/ha, slightly less than the maximum rate/ha on sweet pepper (0.94 kg/ha) due to slightly less water/ha being used than the maximum water volume recommended on sweet pepper (1,500 L/ha). Majestik was applied at the recommended concentration of 25ml/L in 1377 L/ha water. The treated area for T3 was 0.036ha, giving a total of 60 litres applied to the treated bed during each of the two spray applications. The tank mix of Botanigard WP and Majestik used the same rates as used in the treatments where each product was applied individually.

Crop assessments

Assessments took place prior to both treatment applications, and six days after the second application (three in total). In each treatment bed, 45 leaves infested with aphids were selected at the first assessment, labelled, and used for all subsequent aphid assessments. Fifteen leaves were assessed in each of the three sub-sections of each row, and in each sub-section five leaves were selected from each of the three vertical sub-sets (lower, middle and upper). Leaves with pre-existing damage (e.g. caterpillar damage) were avoided during the initial leaf selection, and any subsequent damage was recorded. During each assessment the number of live and *B. bassiana***-** infected aphids, aphid mummies, aphid predators and any other insect pests and/or predators were recorded. The aphid species was also recorded, along with parasitoid species (where possible) and predator species.
Emergence holes from aphid mummies were inspected, and the proportion of hyperparasitised mummies (those with ragged rather than smooth exit holes) was recorded.

The leaf area with sooty moulds growing on aphid honeydew was scored as 1-3; 1 = slight (less than 10% leaf area affected), 2 = moderate (10-50% leaf area affected), 3 = severe (50-100% leaf area affected). All assessments were carried out separately for the lower and upper surface of each leaf.

Any phytotoxicity and photographs of any phytotoxicity symptoms were taken at each application date. Assessments were based on EPPO guidance document PP 1/135(4e) "Phytotoxicity assessment". If possible the percentage area with damage was also recorded. Two data loggers were placed in the crop canopy at 80cm and 160 cm height in the middle of the row treated with treatment 4 (Figure 21), to record the ambient temperature and relative humidity throughout the trial period at 30 minute intervals.

Laboratory test to assess Botanigard WP efficacy against *Myzus persicae*

Samples of the peach-potato aphid *M. persicae*, were collected from outside the trial area and used to initiate a fixed age culture at Warwick Crop Centre. Second instar nymphs were sprayed directly with Botanigard WP under laboratory conditions and compared with a water control. Assessments of mortality were made daily for seven days and any dead aphids removed and incubated on damp filter paper to confirm mortality due to *Beauveria* sp. infection.

Statistical analysis

Data were subjected to analysis of variance in GenStat (16th Edition). For the purposes of this trial, all species of aphid, aphid parasitoid, hyper-parasitoid, and aphidophagous predators were grouped, respectively for analysis. Each group was log transformed prior to analysis, and outputs were back transformed to provide estimate averages per leaf. Parasitised and hyper-parasitised aphids were analysed with respect to aphid and parasitised aphid abundance respectively, using generalised linear models with Genstat regression.

Results

Treatment application

The spray equipment operated well. The spray boom was moved by a motor winding in the hose and since the diameter of the reel increases as the hose is wound in, the speed of movement will increase. Greater pressure reduction will result from a 'wound in' hose than from a straight hose. Applied volume is likely to fluctuate because of changing speed and pressure, due to the way the spray boom is moved. If so, then the dose will also change. There is a need to be able to adjust volume applied to cope with different crop heights and structures, which was done through selecting the number of nozzles in use on the vertical boom. There may be poor distribution of spray due to close proximity of crop to spray boom

– nozzles are designed to operate at a minimum distance from the target, dependent on the spray angle, to get an even distribution (although this is based on a horizontal boom).
Viable *B. bassiana* colonies were found at similar and expected levels both in the spray tank before and after spraying and from the nozzles. Viable *B. bassiana* colonies were observed on both upper and lower leaf surfaces but were variable between samples (Figure 23).

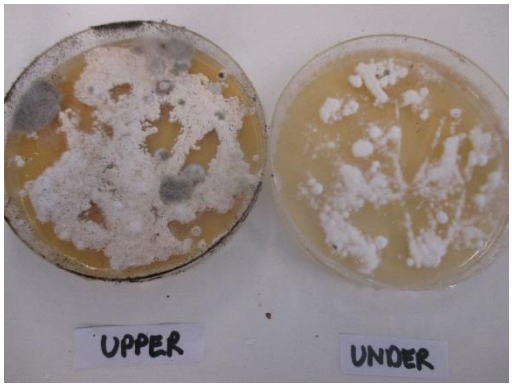


Figure 23. Leaf imprints from upper and lower leaves treated with Botanigard WP.

Crop assessments

The most common aphid species present was *M. persicae*, which represented more than 95% of all aphids recorded. The remaining 5% were *Aulacorthum solani.* Most aphids were found on the underside of leaves (>85%), and only numbers on leaf undersides were used in analyses.
Aphid numbers in the lower third of the crop canopy (on average 67.3, 99.3, and 221.7 aphids per leaf underside in the 1st, 2nd and 3rd assessments respectively) were around twice those in the upper canopy (29.5, 44.3, and 112.3, aphids per leaf underside respectively). The mean number of aphids on the underside of sweet pepper leaves increased over the course of the trial in all treatments (Figure 24). The number of aphids was greatest in the lower third of the crop canopy in all treatments in each of the assessments ($P<0.01$, Table 6). Given the variation between crop height sections, and the high background variation in aphid abundance, there was no significant difference in numbers of aphids between any of the treatments on any assessment date.

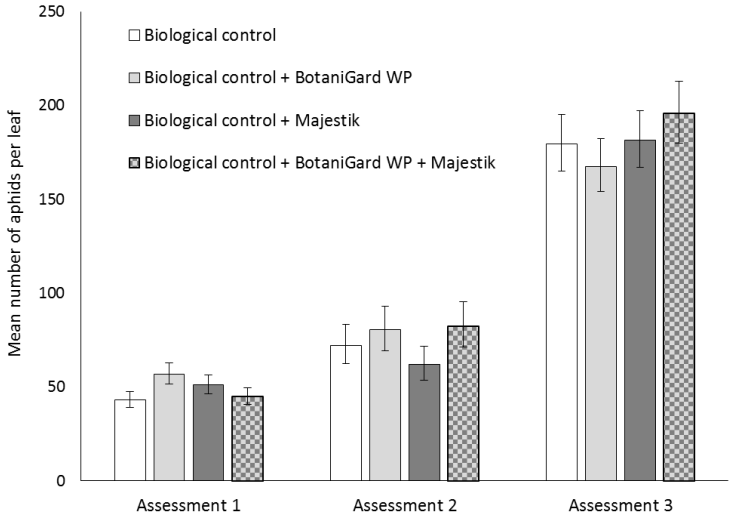


Figure 24. The mean number of aphids per leaf underside in each of the four treatments assessed prior to the first and second spray applications (assessments 1&2) and 6 days after the second spray application (assessment 3), on 29 June, 5 July, and 11 July 2016 respectively.

Table 6. Mean number of aphids per leaf underside in each of the four treatment rows at each assessment. Different letters after the treatments indicate where the mean number of aphids differs significantly within assessments between treatments and/or canopy positions.

	Assessment 1				Assessment 2				Assessment 3			
Treatment	Lower	Middle	Upper	*Mean*	Lower	Middle	Upper	*Mean*	Lower	Middle	Upper	*Mean*
1	68.3	31.6	30.0	*43.3*	125.8	62.6	28.1	*72.2*	220.5	178.9	139.5	*179.6*
2	86.8	46.7	37.2	*56.9*	90.2	83.7	67.4	*80.4*	154.0	183.6	165.2	*167.6*
3	64.1	63.8	26.0	*51.3*	80.7	71.0	34.3	*62.0*	233.5	261.4	49.8	*181.5*
4	49.9	60.0	24.9	*44.9*	96.4	104.1	47.2	*82.6*	278.9	213.2	94.9	*195.7*
Mean	*67.3a*	*50.5b*	*29.5c*	***49.1***	*98.3a*	*80.3a*	*44.3b*	***74.3***	*221.7a*	*209.3a*	*112.3b*	***181.1***

Three groups of aphid parasitoids were recorded; *Aphidius* spp. (85% of all parasitized aphids recorded), *Aphelinus abdominalis* (8%), and *Praon* sp. (7%). Mummies were found on both the upper and lower surface of leaves, but more commonly on the lower surface (82%). Variation in the proportion of parasitised aphids (hereafter 'aphid mummies') per leaf was high, and the relative distribution of aphid mummies was similar to that of live aphids in the crop canopy. A significantly greater proportion of aphids were parasitized lower down in the crop canopy than in upper and middle sections in all treatments and assessments ($P<0.01$, Table 7). Given this variation, there was no difference between treatments in the proportion of aphids parasitized in any of the three assessments.

Table 7. Mean proportion (%) aphids parasitized per leaf in each of the four treatments at each assessment. Numbers sharing the same letter are not significantly different.

	Assessment 1				Assessment 2				Assessment 3			
Treatment	Lower	Middle	Upper	*Mean*	Lower	Middle	Upper	*Mean*	Lower	Middle	Upper	*Mean*
1	10.6	15.1	2.6	*9.4*	4.2	4.8	2.4	*3.8*	4.2	2.6	1.9	*2.9*
2	11.5	10.5	0.9	*7.6*	6.6	4.4	1.2	*4.1*	4.6	3.2	1.7	*3.2*
3	8.5	5.6	1.8	*5.3*	4.9	4.0	2.8	*3.9*	2.5	2.0	1.6	*2.0*
4	14.7	6.3	2.5	*7.8*	6.0	3.9	0.7	*3.6*	3.7	2.9	1.1	*2.6*
Mean	*11.3a*	*9.4a*	*2.0b*	***7.5***	*5.4a*	*4.3ab*	*1.8b*	***3.8***	*3.8a*	*2.6ab*	*1.6b*	***2.7***

Hyperparasitism levels remained relatively low throughout the trial (Table 8). Initially hyperparasitism was significantly higher in the biological control only treatment bed prior to any treatments being applied ($P<0.05$). The proportion of hyperparasitised aphid mummies did not significantly differ between the upper, middle and lower third of the canopy. In the second assessment, there was no difference in hyper-parasitism within or between treatments. In the third assessment, hyper-parasitism was significantly higher in treatments 3 and 4 in the lower third of the canopy than in the upper and middle canopy sections ($P<0.05$), however, the hyper-parasitism rate during this assessment was very low.

Table 8. Mean proportion (%) of parasitized aphids showing evidence of hyper-parasitism per leaf in each of the four treatments during each assessment. Numbers sharing the same letters are not significantly different.

	Assessment 1				Assessment 2				Assessment 3			
Treatment	Lower	Middle	Upper	*Mean*	Lower	Middle	Upper	*Mean*	Lower	Middle	Upper	*Mean*
1	23.0	16.3	6.7	*15.3a*	4.3	9.8	0.0	*4.7*	2.0	1.1	0.0	*1.0a*
2	13.0	8.6	50.0	*23.9a*	7.8	3.8	6.7	*6.1*	2.5	3.8	0.0	*2.1a*
3	9.8	13.9	11.1	*11.6a*	11.2	8.8	4.0	*8.0*	12.3	0.0	4.4	*5.6b*
4	9.6	1.4	0.0	*3.6b*	7.8	17.1	18.0	*14.3*	13.9	9.1	0.0	*7.7b*
Mean	*13.8*	*10.0*	*17.0*	***13.6***	*7.8*	*9.9*	*7.2*	***8.3***	*7.7a*	*3.5ab*	*1.1b*	***4.1***

The most frequently recorded aphid predators during the trial were *Aphidoletes aphidimyza*, introduced as part of the grower's biological control programme. Aphid-predatory hoverfly larvae were also recorded, as well as lacewing larvae, *Chrysopidae spp.* and spiders, *Linyphiidae* species. At the first assessment there was no difference in predator numbers between the treatments, though significantly more predators were found lower in the crop canopy than in the upper and middle sections ($P<0.01$), Table 9). At the second assessment, significantly more predators were again found in the lower parts of the crop canopy than in

the upper and middle section (P<0.01), however in the treatment including Majestik (T3 & T4), predator abundance was significantly lower (around 50% lower) than in the other treatments (P<0.05). Predator numbers remained low throughout the trial, and in the final assessment there was no significant difference within or between treatments.

Table 9. Mean number of aphid predators per leaf in each of the four treatments during each assessment. Numbers sharing the same letters are not significantly different.

	Assessment 1				Assessment 2				Assessment 3			
Treatment	Lower	Middle	Upper	*Mean*	Lower	Middle	Upper	*Mean*	Lower	Middle	Upper	*Mean*
1	0.29	0.22	0.05	*0.19*	0.95	0.17	0.05	*0.39ab*	0.08	0.18	0.17	*0.15*
2	0.33	0.10	0.13	*0.18*	1.38	0.42	0.00	*0.60a*	0.67	0.15	0.10	*0.31*
3	0.54	0.29	0.05	*0.29*	0.20	0.13	0.05	*0.13b*	0.40	0.51	0.00	*0.30*
4	0.33	0.27	0.05	*0.22*	0.10	0.24	0.00	*0.11b*	0.00	0.10	0.00	*0.03*
Mean	*0.37a*	*0.22a*	*0.07b*	***0.22***	*0.66a*	*0.24ab*	*0.02b*	***0.31***	*0.29*	*0.24*	*0.07*	***0.20***

The temperatures in the crop canopy, at the first spray application (between 19:30 and 20:00 on 29 June) fluctuated between 23°C and 25°C and 83%RH and 93.5%RH (Figure 25 and 26). In the following 24 hours the temperature dropped to 16°C at 23:30, and then increased to 27°C at 12:00 on 30 June before dropping down to 24.5°C by 19:30 and the %RH remained above 90% until 12:00 the following day, when it dropped to 66%RH, and it remained below 75%RH until 16:30. The second spray took place between 15:30 and 16:30 on 5 July 2016. The temperatures in the crop canopy at this time fluctuated between 25°C and 27°C and the %RH fluctuated between 46% and 50%. In the following 24 hours the temperature dropped to 17.5°C at 00:00, and then increased to 31°C at 15:30 the following day and the %RH remained below 75% until 20:00. Between 20:00 and 08:30 the following day the %RH was above 75%, but then dropped to a low of 40% at 15:30 (Figure 26).

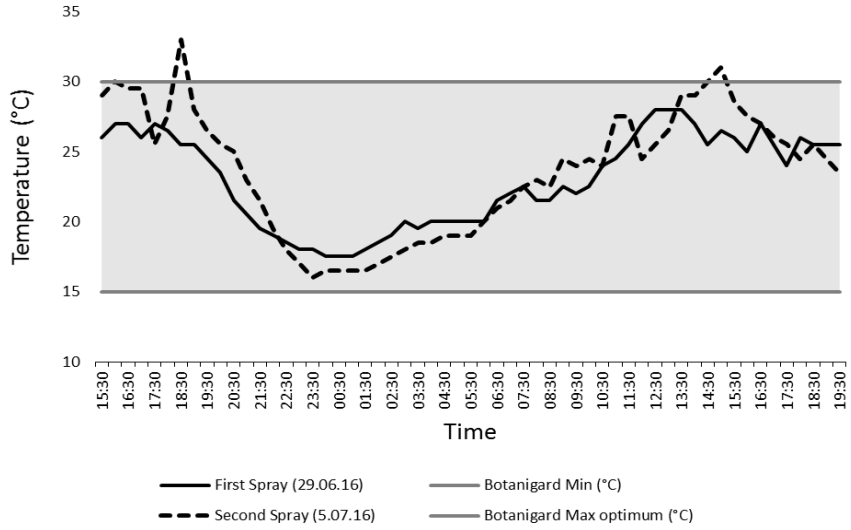


Figure 25. Temperature records in the crop canopy over the 24 hour periods at 30 minute intervals during and after treatment applications. The first sprays were applied between 19:30 and 20:30, the second sprays were applied between 15:30 and 16:30.

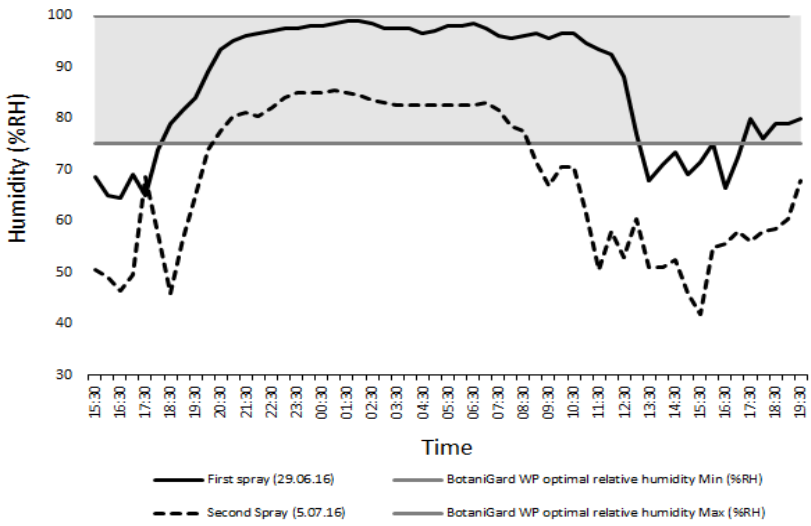


Figure 26. Relative humidity in the crop canopy over the 24 hours at 30 minute intervals post spray applications. The first sprays were applied between 19:30 and 20:30, the second sprays were applied between 15:30 and 16:30.

Myzus persicae, collected from Benchmark 4 site, were susceptible to Botanigard WP in the laboratory. No mortality was observed until four days after application but by day 7 post application 57% of those aphids treated with Botanigard WP were dead compared to 11% of those in the water treated control (Figure 27). Cause of death in the Botanigard WP treated aphids was confirmed as *B. bassiana* infection. Aphids in the bioassay were observed to reproduce up until the point of death.

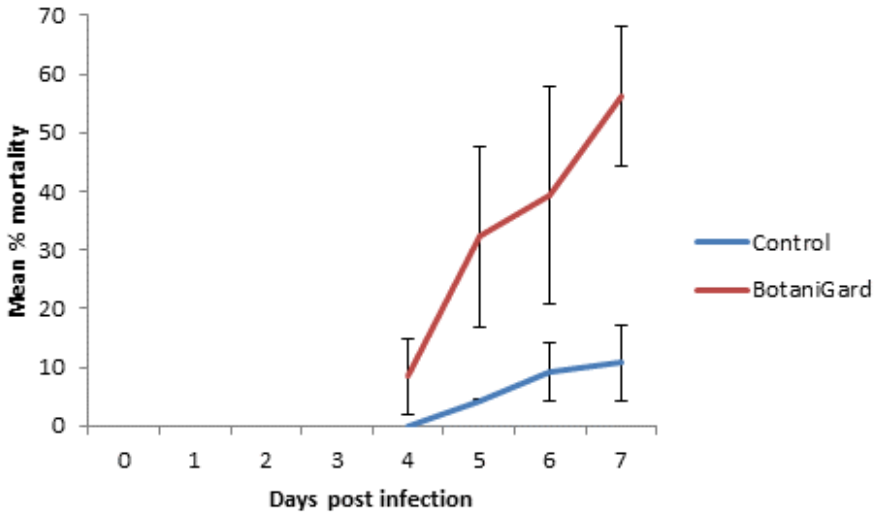


Figure 27. Mean percentage mortality of peach-potato aphids, *Myzus persicae*, over seven days post infection on pepper leaves treated with Botanigard WP or with a water control.

Discussion

Over the course of the trial aphid populations increased significantly in all treatments. Aphid numbers were highest in the lower third of the crop than in the upper and middle thirds, which is to be expected as these are the oldest leaves in the crop, and were not directly sprayed with any treatments to protect natural enemy populations, although some spray did run off from upper and middle leaves. Aphid natural enemies were also present in higher numbers in the lower third of the crop. There was some evidence that *Aphidoletes aphidimyza* larvae were adversely affected by Majestik, however numbers were too low to confirm this. Overall the numbers of predators were low in all treatments throughout the trial despite the increased availability of aphid prey.

Neither Botanigard WP, Majestik, nor the tank mix of the two were effective in reducing aphid numbers compared with those in the biological control programme only treatment, which acted as a negative control. There are three potential factors which may have contributed to aphid control failure:

- As both products require direct contact at the time of application, the application method may have failed to reach the target aphids.
- Contact may have been achieved, but the environmental conditions in the glasshouse may have been unsuitable for the product(s) to be effective. However, whereas Botanigard WP is recommended to be used at above 70% RH and in the late evening to avoid sunlight, Majestik is most effective in quick-drying conditions (i.e. hot and sunny) due to its physical suffocating action.

- The aphids may not have been sufficiently affected by the products or affected quickly enough to reduce population growth.

During calculation of the applied volumes and concentrations of the products, the treated area was taken to be the horizontal area under the sweet pepper crop. This does not account for the height or width of the crop. If the total height of the crop had been sprayed, rather than leaving the lower third of the crop unsprayed to preserve natural enemies, the grower considered that it would have been necessary to exceed the maximum water volume of 1,500 L/ha in order to achieve good coverage.

Viable *B. bassiana* colonies were found at similar levels both in the spray tank before and after spraying and from the nozzles, and on both the upper and lower leaf surfaces in both the Botanigard WP treatment on its own and in combination with Majestik. This demonstrates that Botanigard WP was being effectively applied onto the crop using the spray equipment available. However, there was variation in the density of *B. bassiana* colonies within and between treatments, and between the two treatment dates.

There was some concern at the time of application that Majestik caused foaming in the tank, and that when mixed with Botanigard WP this may have prevented consistent distribution of the *B. bassiana* spores. Assessment of the foam suggested that it contained the same numbers of spores as the liquid portion, so no impact on treatment efficacy was likely. The supplier is further investigating the issue of foaming.

While the equipment used was effective in delivering the treatments onto the leaves, the application volume is likely to have fluctuated due to the changing speed and pressure, because of the way the spray boom moved. This would impact on the dose rate along the length of the rows. It was also noted that the spray boom was in close proximity to the crop, which may have resulted in poor distribution of the spray when hitting leaves close to nozzles.

Temperatures in the crop canopy were within the recommended range (15-30°C) for Botanigard WP during the first spray application on 29 June, and over the following 24 hours. Shortly following the second spray application on 5 July, temperatures exceeded 30°C, reaching 33°C at 18:30 and 31°C at 14:30 on 6 July. The optimum temperature range for Botanigard WP is 20-30°C and temperatures higher than 35°C may kill the fungus (Certis Best Practice Guide for Botanigard WP). Relative humidity remained above the recommended level of 75 %RH during, and for the majority of 24 hrs after the first treatment applications. The second spray treatment was applied when %RH was below the recommended level of 75 %RH (50% RH at 15:30), and remained below 75 %RH for at least two hours following the spray application. Botanigard WP is recommended to be applied at

%RH greater than 70% for maximum efficacy, however the product label and best-practice guide does not state for how long these relative humidities need to be maintained. Spray applications on both dates took place prior to sunset and most growers are unlikely to be prepared to spray late evening. More information is needed on Botanigard WP susceptibility to UV in glasshouses where some of the UV is blocked out by the glass.

Botanigard WP is recommended for control of whitefly on various protected crops. While we have demonstrated that it will infect and kill the peach-potato aphid in a laboratory test, we also found a time lag between treatment and mortality of at least four days. Infected aphids continued to reproduce up until mortality. If the applications were as effective in delivering the fungal spores onto aphids in the pepper crop in the trial on a commercial nursery as in the laboratory test, around 40% of the aphids recorded during the first assessment should have been killed by the time of the second application. However, as those aphids could continue to have reproduced over the six days between assessments this would not give a 40% reduction in numbers. If reproductive rates still exceed mortality rates the population would continue to grow. The larger initial population size at the time of the second application would provide even greater resilience against treatment. While a significant difference may have been detectable between treatments over a longer period than used in the trial, control is unlikely to have been commercially acceptable. Botanigard WP is recommended to be applied as soon as pests are detected, thus efficacy may have been demonstrated if it had been used much earlier in the season. An additional factor may have been the relative impact Botanigard WP had on different life stages of the target aphid. For example, if nymphs are able to recover from fungal infection during moulting by shedding the old skin with fungal spores, this would result in treatment being ineffective.

Majestik was also not found to have a significant impact on aphid populations. The Majestik label states that some reduction of aphids may be given when applied for spider mite control, and the environmental conditions at the time of application should have been suitable for control to occur. Unlike Botanigard WP, Majestik causes mortality soon after application, as is blocks the insect spiracles. However, if the proportion of the aphid population killed was not sufficient for mortality to exceed subsequent reproduction over the following six days, the population would continue to grow. Furthermore, if the product varied in efficacy between different life stages e.g., by killing only the small nymphs, or had a detrimental impact on natural enemies (Majestik is safe to natural enemies once the spray deposit has dried but could adversely affect some of them during application), this would again reduce the effect on aphid numbers, thus masking any differences.

Conclusions

- Botanigard WP and Majestik as individual products or in a tank mix, used as a supplement to the grower's biological control programme did not give a reduction of an already large aphid population compared with the biological control programme alone
- The time delay between exposure of the peach-potato aphid to Botanigard WP and mortality may restrict this product's impact on large aphid infestations. In addition, the aphids' ability to continue to reproduce up until death even when infected may limit Botanigard WP efficacy.
- Temperature and relative humidity were both within recommended ranges for Botanigard WP during and following the first spray application. However, this application took place at 19:30-20:30, which is much later than most nurseries will operate. The second spray application, which took place between 15:30-16:30 is a better reflection of normal commercial practice, but was applied at a relative humidity much below recommended levels for Botanigard WP and when UV levels may have been detrimental to the product.
- Confusion in calculating correct dose on tall vertical crops, and product labels or guidance on use needs to be improved for growers to apply products effectively in three dimensional cropping systems. 'EPPO guidelines 'Dose expression for plant protection products' will be referred to when discussing application to tall row crops with growers in the rest of the project. Practical guidelines for growers will be given.
- The spray equipment operated well, but the equipment design is likely to have led to non-uniform spray application down the length of the sweet pepper beds. To gain full crop coverage (required by contact-based products), spray equipment needs to be able to adjust volume applied to cope with different crop heights and structures. The spray boom was in close proximity to the crop, which may have resulted in poor distribution of the products in the crop canopy.
- Excessive foaming was observed when product was mixed, however this was unlikely to have had an impact on application of the recommended dose.

Benchmark 5: Western flower thrips in pot chrysanthemum

Introduction

Western flower thrips (WFT) *Frankliniella occidentalis* is a serious pest for most growers of protected ornamentals. Feeding by WFT results in white flecking on leaves and petals and can also cause young leaf and flower distortion. WFT can also transmit tospoviruses e,g, Tomato spotted wilt virus (TSWV) and Impatiens necrotic spot virus (INSV) which like WFT have a wide host range including many ornamental and edible plants and weeds. WFT causes cosmetic damage to pot chrysanthemum when feeding on developing flower buds

and on petals (Figure 28). Damage is more noticeable in some varieties than others, depending on the flower colour and petal formation. If damage occurs on a small number of flowers, these can be removed without impacting the value of the plant, however if damage is more severe plants become unmarketable, leading to significant economic losses. Two aphid species, *Myzus persicae,* and *Aphis gossypii* can also cause commercial damage to pot chrysanthemum, as well as the leaf miner *Chromatomyia syngenesiae* and the two-spotted spider mite *Tetranychus urticae.* Powdery mildew and botrytis infestations can also occur.

For this benchmarking trial, two products were selected; Botanigard WP, and Majestik. Botanigard WP is a microbiological insecticide based on the entomopathogenic fungus *Beauveria bassiana* strain GHA*,* recommended for the control of whitefly, although there is also that it will give control of thrips. Majestik (maltodextrin) is recommended for spider mite control, but is recommended by the supplier to be used as a tank mix with Botanigard WP for improved whitefly control.

Figure 28. Damage caused by western flower thrips, leaf miners and aphids to pot chrysanthemums.

Methods

The study took place from 7 to 28 July 2016. Pot chrysanthemum were grown in 14cm pots in peat-reduced Bulrush substrate with 30% Forest Gold, on 12.7m^2 rolling benches on 24 parallel rows each containing up to 49 benches. Benches were moved between and along rows over time, so that plants moved from one end of the glasshouse to the other in line with their development during the production cycle. The crop is grown for 9-10 weeks (growth rate dependent on time of year and conditions). Ebb and flood irrigation was used throughout the glasshouse, and pesticides were delivered via an automated horizontal spray boom above the crop. In total approximately 30,000 pot mums were produced per week. The site had no problems with either powdery mildew or botrytis, so no treatments were applied to manage these during this cropping season.

The entomopathogenic nematodes *Steinernema feltiae* (Nemasys) were sprayed weekly for WFT in the winter, and twice weekly at half-rate in the summer, up to two weeks prior to flower opening. This system delivered approximately 115,000 nematodes per m^2 through the overhead spray boom. Spinosad (Conserve) could have been applied to later growth stages if necessary if WFT numbers increased during the summer, but this was not required during the trial period. Biological control agents were also introduced; a mix if aphid parasitoid species (*Aphelinus abdominalis, Aphidius colemani, A. ervi, A. matricariae, Ephedrus cerasicola, Praon volucre*) for aphid control, *Diglphus isaea* for leaf miner control. *Phytoseiulus persimilis* for spider mite control and *Amblyseius swirksii* for control of WFT if necessary. Yellow sticky traps between rows were inspected weekly in the winter and twice weekly in the summer, along with a random inspection of 50 plants. Additional biological control releases were made to spot treat pest infestations. The site had no problems with either powdery mildew or botrytis, so no treatments were in place to manage these during this cropping season.

Table 10. Treatments applied to the pot chrysanthemum crop.

T	Products (MAPP code)	Active ingredient	Rate of use	Application date / comments
1	Biological control + Entomopathogenic nematodes	NA *Steinernema feltiae* (Nemasys)	NA 115,000 nematodes/m^2	Weekly, in response to pest pressure Twice a week
2	Biological control + Botanigard WP (17054) + Majestik (17240)	NA *Beauveria bassiana* strain GHA 4.4 x 10^{10} CFU/g 0.625g/ litre Maltodextrin (49% w/w) 25ml/litre	NA 0.68 kg/ha in 1090 L/ha water 25ml/litre in 1090 L/ha water	Weekly, in response to pest pressure Weekly on 7, 14 and 21 July 2016

Trial design

Each treatment was applied to parallel rows separated by three current practice rows, over the final five weeks of the pot mum production cycle in the glasshouse (Figure 29). The treatments were repeated on four blocks of successive growth stages each planted one week apart. The youngest growth stage assessed was early flower bud, followed by bud break, flower opening and open flower stages (Figure 30). Each block consisted of between one and five benches, each of which contained 210 pots arranged as six rows of 35 pots each. Rows contained a mix of susceptible varieties, including Deejay Time (most susceptible to damage), Mount Aubisque Pink (moderately susceptible to damage), and Crystal Pink (least susceptible to damage). Pairs of the same variety were assessed over the course of the trial. Entomopathogenic nematode treatments and the combined Botanigard WP and Majestik treatments were applied three times, seven days apart along the whole length (622m^2) of benches of pot mums.

			Oldest plants west end								
					Biological control + Entomopathogenic nematodes				Biological control + BotaniGard WP + Majestik		
Potting date (week starting)	Block	Assessment date	16	17	18	19	20	21	22	23	24
9 May	Block 4	7 July			Deejay time				Deejay time		
					Mount Aubisque (Pink)				Mount Aubisque (Pink)		
									Mount Aubisque (Purple)		
					Chrystal Pink				Crystal Pink		
16 May	Block 3	7 & 14 July			Deejay time				Deejay time		
		14 July			Mount Aubisque (Pink)				Mount Aubisque (Pink)		
					Splash Sweet				Mount Aubisque (Purple)		
									Artistic Pink		
23 May	Block 2				Deejay time				Mount Aubisque (Pink)		
		7, 14 & 21 July			Crystal Pink				Crystal Pink		
					Rainbow Rosy						
					Inuit						
31 May	Block 1	7, 14, 21 & 28 July			Deejay time				Deejay time		
		21 & 28 July			Mount Aubisque (Pink)				Mount Aubisque (Pink)		
		28 July			Crystal Pink				Crystal Pink		
					Mount Aubisque (Pink)				Mount Aubisque (Purple)		
			Youngest plants east end								

Figure 29. Treatment allocations during benchmark trial.

Early flower bud

Bud break

Flowers opening

Flowers open

Figure 30. The four growth stages of the penultimate four weeks of pot chrysanthemum production.

Treatment application

A site visit before the applications was undertaken to evaluate the application equipment available. Prior to the site visit, the product label and other technical information was evaluated to determine the application conditions that were required. The speed of the automated spray boom, the nozzle sizes and types, and operating pressure that are routinely used in the glasshouse was recorded and applied volumes were calculated subsequently.

Applications were made using an automated horizontal boom consisting of 16 nozzles approximately 40cm above the crop. Fourteen FF110 03 nozzles sprayed vertically downwards across the rows, with 01 nozzles at either end of the boom spraying inward at an angle of 45 degrees. Botanigard WP was applied at the recommended concentration (for whitefly control on ornamentals excluding roses) of 62.5g/100L. The water volume used for application was 1090L/ha, slightly less than the maximum water volume recommended on ornamentals excluding roses (1,200 L/ha). Therefore the product was applied at 0.681 kg/ha, just under the label maximum water volume of 0.75 kg/ha

Samples of Botanigard WP were taken prior to and after spraying. These were taken from the spray tank and the sprayer nozzles respectively. Each sample was diluted and plated

onto 90mm plate of Sabouraud dextrose agar (SDA) and incubated at 23°C in the dark for five days and then examined for colony growth of *Beauveria* sp. Additional samples were taken from the foam created in the tank prior to application. Leaf samples were collected from Botanigard WP treated plants after spray application and imprinted onto SDA selective media.

Crop assessments

Assessments took place prior to each application, and seven days following the final treatment applications. During the first assessment, all blocks were assessed across all growth stages. At each subsequent assessment only the oldest two blocks were assessed. In each block 21 randomly selected pots from both sides of the bench were examined during the first assessment, and 42 randomly selected pots from both sides were examined on all subsequent assessments. The crop height, width, and total number of buds/flower per plant, were recorded on four randomly selected plants from each block in each treatment.

During each assessment the plants were assessed for the presence of thrips damage on the buds, flowers and leaves, and the presence/absence of aphids and/or parasitised aphids, along with evidence of any phytotoxicity. Any phytotoxicity and photographs of any phytotoxicity symptoms were taken at each application date as described previously, following EPPO guidance. The presence/absence of leaf miners was recorded for all plants during the third and fourth assessments. Each plant was then gently taped three time onto a white plastic tray held under the plant, turning the plant after each tap. The contents of the tray were inspected for WFT adults and larvae, and any WFT were then returned to the plant. The numbers of live WFT and any showing symptoms of infection with *Beauveria* sp. were recorded. Blue sticky traps were placed in each of the four blocks of Mount Aubisque (E/W orientation) in both of the treatment rows. The traps were collected and replaced each week, and the number of WFT were recorded on each trap.

One data logger, to record ambient temperature and relative humidity throughout the trial at 30 minute intervals, was placed in the crop canopy in each of the two treatment rows in the youngest block of Deejay Time plants.

Statistical analysis

It was not possible to carry out statistical analyses of the data due to the low numbers of insects recorded during the trial period in both treatments.

Results

Treatment application

The spray equipment used on this nursery was the best seen throughout the project; it operated well and allowed compliance with the label requirements. There were some minor problems with nozzles blocking (rust flakes from the delivery pipes), but this was considered to be due to the filters having been removed to avoid the nematodes blocking then and which good maintenance should solve. Foaming in the tank was observed with the Botanigard WP and Majestik combination but there was no evidence of any negative impact on the efficacy of the biopesticide. The supplier is further investigating the issue of foaming.

Viable *Beauveria sp.* colonies were found at similar levels both in the spray tank before and after spraying and from the nozzles. Viable *Beauveria* sp. colonies were observed on both upper leaf and lower leaf surfaces but less were observed on the lower surfaces.

Crop assessments

Western flower thrips, aphids, and leaf miners were recorded in both treatment rows during the trial, but numbers of all pests were very low.

Western flower thrips were observed in the rows treated with entomopathogenic nematodes and with the Botanigard WP + Majestik tank mix during the plant assessments and on the sticky traps. During the trial period WFT were recorded on 19 of the 896 pots inspected; a total of six in the Botanigard WP + Majestik treated row and 11 in the entomopathogenic nematode treated row, mainly in plants beyond bud break (Figure 31). Small amounts of thrips damage was present in both treatments, mainly found in open flowers (Figure 32). There was no indication that thrips damage differed between the treatments, and damage remained well below the grower's thresholds for any additional treatment to be carried out. More WFT were caught on blue sticky traps above the crop in earlier growth stages (Figure 33), but too few on which to carry out statistical analysis.

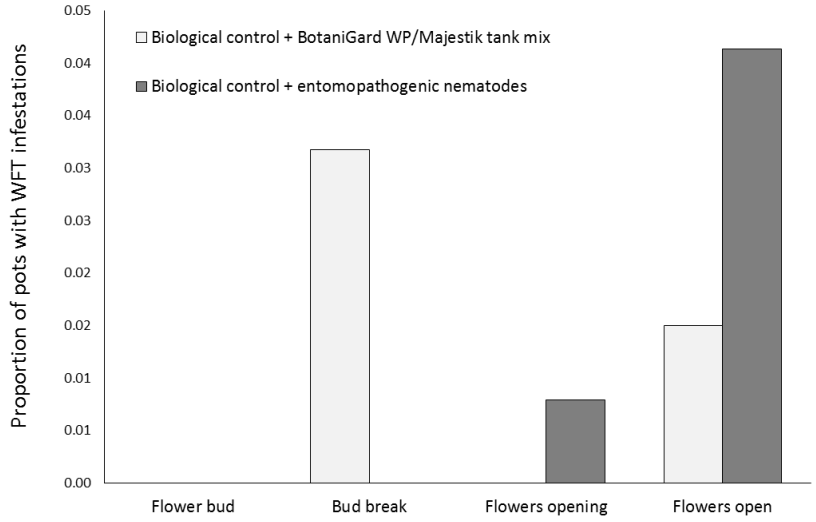


Figure 31. The proportion of chrysanthemum pots with WFT infestations in the four growth stages.

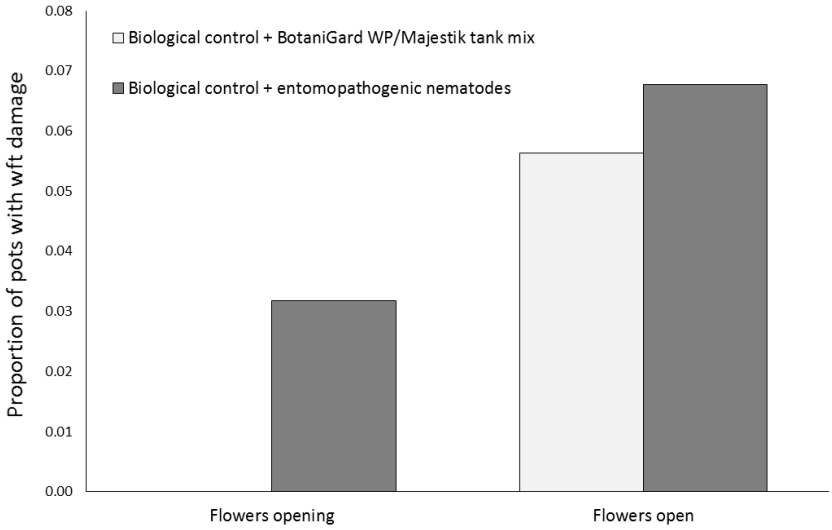


Figure 32. The proportion of chrysanthemum pots with WFT petal damage in the four growth stages.

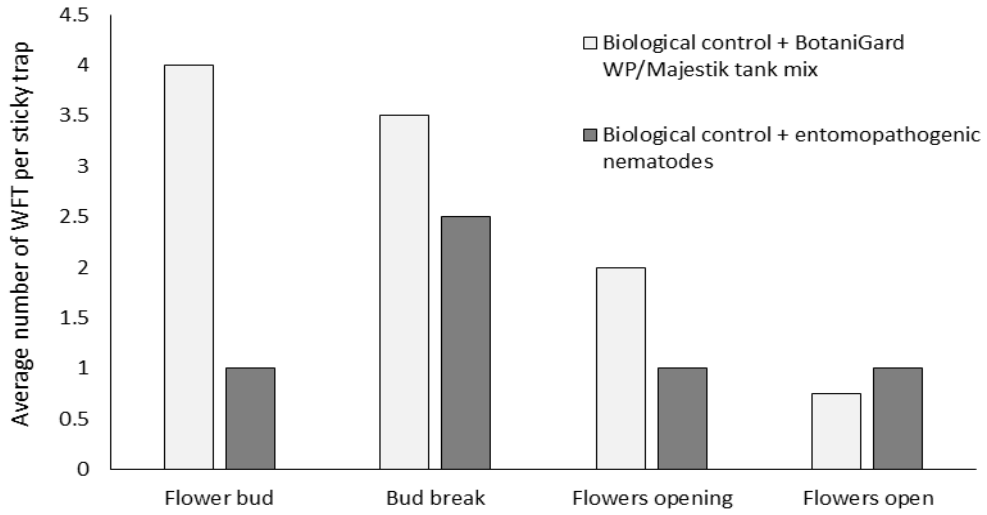


Figure 33. The mean number of western flower thrips per blue stick trap above the canopy in Mount Aubisque Pink benches in the four growth stages.

Aphid infestations were observed in both treatments, and the proportion of pots infested tended to increase in later growth stages (Figure 34). Very few leaf mines caused by leaf miner were recorded in both (Figure 35).

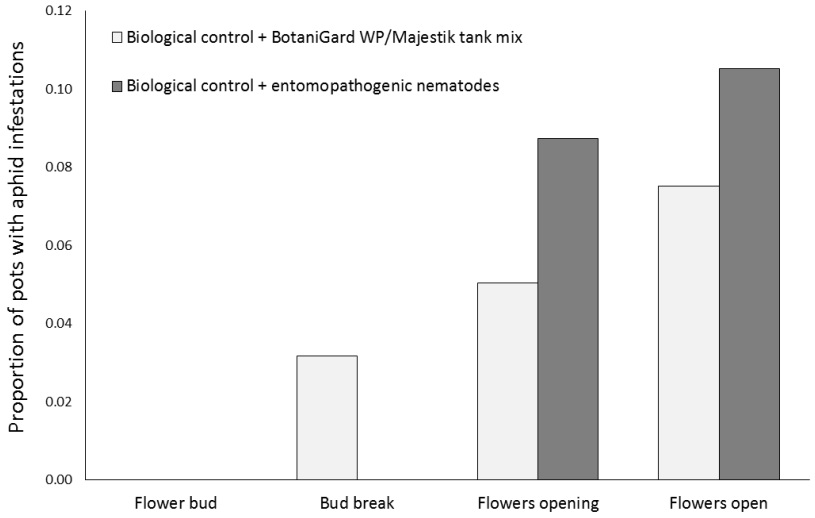


Figure 34. The mean proportion of chrysanthemum pots with aphid infestations in the four growth stages assessed.

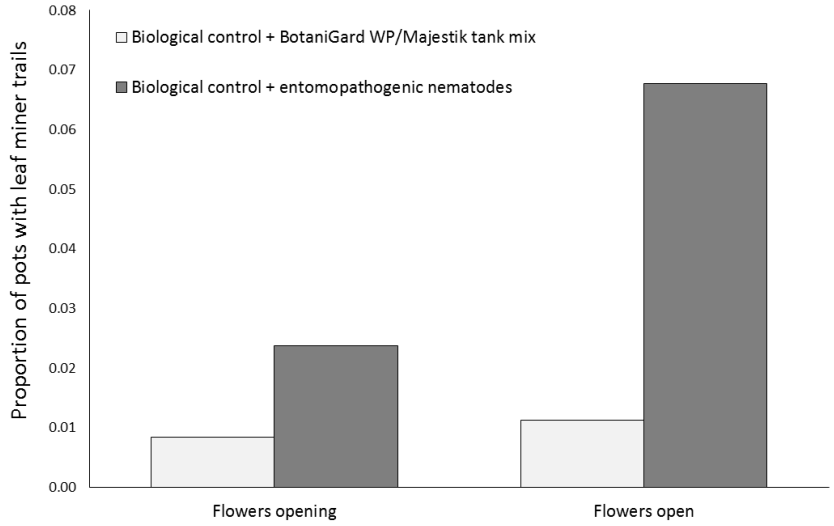


Figure 35. The mean proportion of chrysanthemum pots with leaf mines in the two growth stages assessed.

Spray applications took place between 15:30 and 16:30, on the 7, 14 and 21 July 2016. The temperature in the crop canopy fluctuated between 17°C and 32°C (Figure 36) and the percentage relative humidity (%RH) was below 75%, but increased rapidly to 84% within one hour. This reflects a slight delay between the data logger being placed in the crop and it recording %RH accurately (Figure 37). On each spray application day, %RH remained between 80-95% from 16:00 until 07:00 the following day, when %RH dropped to 55-80% and the temperature dropped from around 25°C to around 20°C at 01:00, and then increased from 08:00 the following day. Towards the end of the 24 hour period after the first and third spray, the temperature in the crop exceeded 30°C, in both instances after 12:00 the following day.

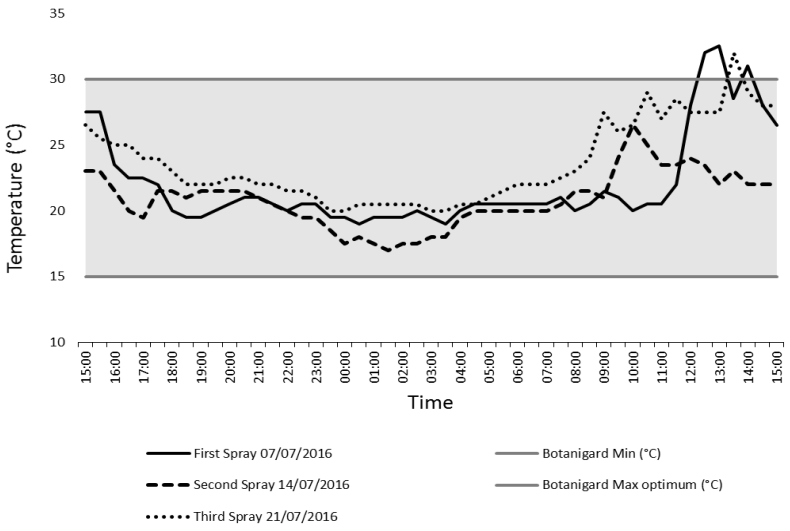


Figure 36. Temperature records in the crop canopy over the 24 hour periods during and after treatment applications. All spray applications took place between 15:30 and 16:30. The shaded area represents the optimal conditions for Botanigard WP application.

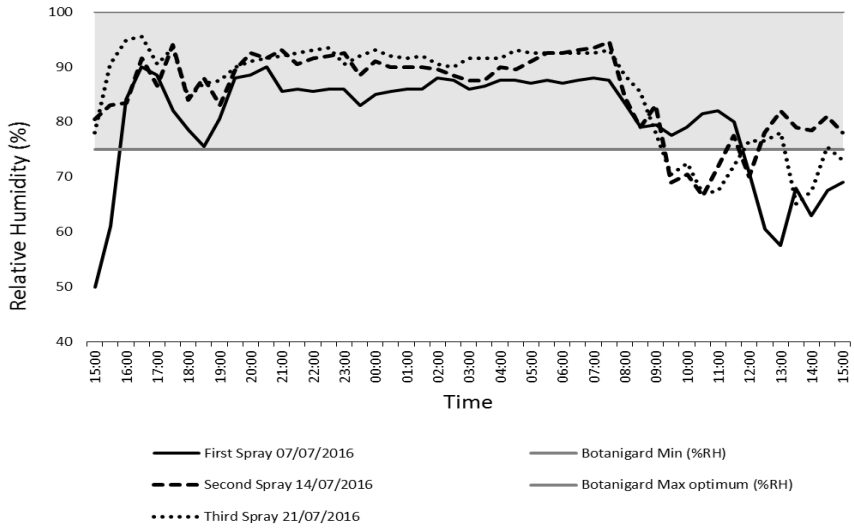


Figure 37. Relative humidity in the crop canopy over the 24 hours post spray applications. All spray applications took place between 15:30 and 16:30. The shaded area represents the optimal conditions for Botanigard WP application.

Discussion

Very low numbers of WFT, aphids, and leaf miners were present during the trial period on plants in both the benches treated with entomopathogenic nematodes, and with the Botanigard WP and Majestik tank mix. The low numbers of the pests, and the previous history of infestations requiring additional treatment at the site at this time of year, suggest that both treatments provided adequate control in this trial period, under these conditions.

Viable *Beauveria* sp. colonies were found at similar levels both in the spray tank before and after spraying and from the nozzles, and on both the upper and lower leaf surfaces. This demonstrates that Botanigard WP was being effectively applied onto the crop using the spray equipment available although fewer colonies were observed on the underside of the leaves However, there was variation in the density of *Beauveria* sp. colonies within and between treatments, and between the two treatment dates. As WFT are present mainly in flower buds and flowers when present rather than on leaves, spray application to flower buds is more critical than that to the leaves and has been studied in the laboratory (see section?)

Temperature and relative humidities in the crop canopy was within the recommended range (15-30°C; >70% RH) for Botanigard WP during the spray applications and for the majority of the following 12 hours. Botanigard WP is recommended to be applied at %RH greater than

70% for maximum efficacy, however the product label and best-practice guide does not state for how long these relative humidities need to be maintained.

Conclusions

- In the presence of low levels of western flower thrips, aphids and leaf miners, the Botanigard WP + Majestik treatment was as effective in managing pest infestations as the grower's currently used entomopathogenic nematodes within the biological control programme.
- The spray equipment operated well.
- Excessive foaming was observed when the two products were mixed, however this was unlikely to have had an impact on application of the recommended dose.

Benchmarking: spray application

As well as the observations and measurements taken at each benchmarking site which are reported within each benchmark some additional work was undertaken in the laboratory at Silsoe to test out some ideas for future measurements and to consider alternative application equipment for that used in benchmark 5.

Methods

A 3-nozzle boom with different nozzles and application volumes was mounted on a transporter and operated at the same pressure and speed as the sprayer at the site of the benchmark 5.

Artificial targets were added to five chrysanthemum pot plants so that each plant (representing a different growth stage) could be re-used for all treatments. The artificial targets represented flower buds, the central part of the flower and the soil surface. A tracer dye (Green S) and 0.1% non-ionic surfactant (Activator 90) was added to water and sprayed according to the treatments in Table 11. Artificial targets were then removed from the pot and washed in a known volume of deionised water. The rinsate was analysed with spectrophotometry to determine the quantity of deposited spray.

Table 11. Treatments used in laboratory experiments of deposits on chrysanthemums. Treatments A and D relate to the application conditions available at benchmark 5 site.

	Nozzle	Pressure	Orientation	Volume (l/ha)
A	FF02 XR	4.5 bar	vertical	725
B	FF 015	2 bar	alt F/B	362
C	FF 015	2 bar	twin cap	725
D	FF 03	4.5 bar	vertical	1089
E	Defy 3d 03	4.5 bar	alt F/B	1089

Results

Laboratory studies

The quantity of spray liquid recovered from the artificial flowers and buds on the plants at different growth stages (denoted 1-5) are given in Figures 38 and 39. This shows that if the product is applied at constant concentration, more spray liquid (and therefore more product) is retained on the different target sites with increasing volume. Small increases in quantity retained can be achieved with angled nozzle configurations.

The repeated use of the plants caused gaps to open up in their structure exposing the soil so that later treatments had much higher deposits on the soil than the earlier ones. Data relating to soil deposits were therefore misleading and not reported.

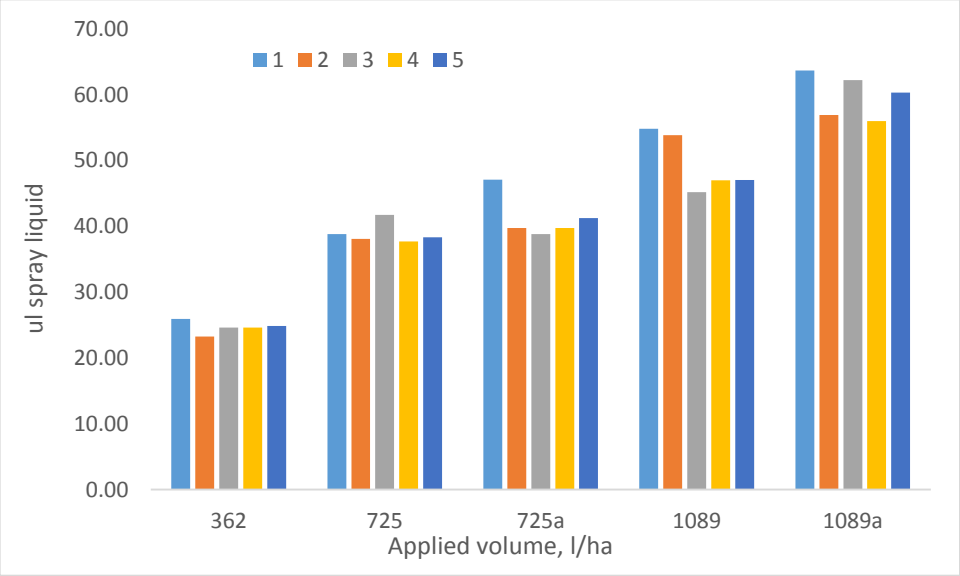


Figure 38. Deposit of spray liquid on artificial flower buds for different, growth stages applied volumes and some angled nozzles (denoted ‘a’).

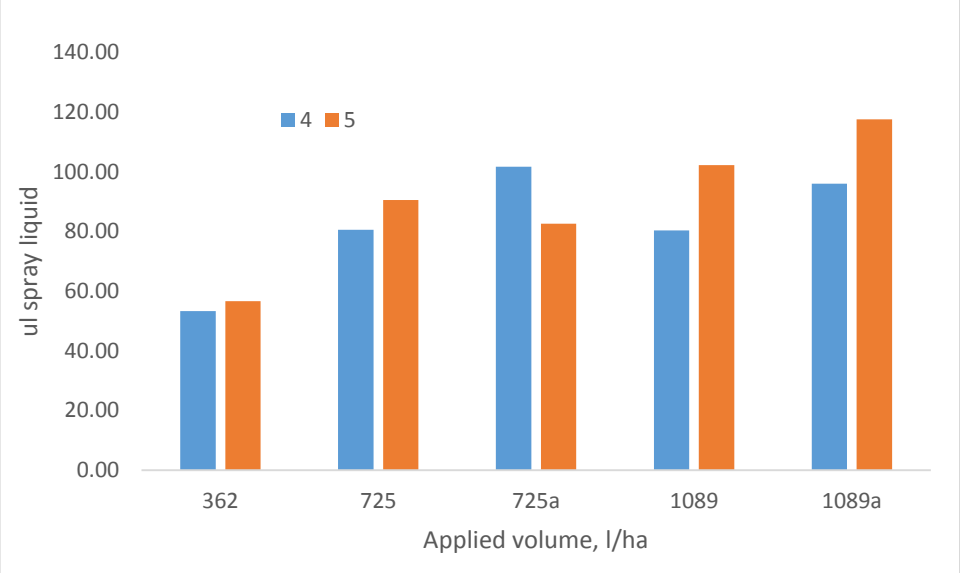


Figure 39. Deposit of spray liquid on a disc at the centre of the flower for different growth stages, applied volumes and some angled nozzles (denoted 'a').

Figures 40 and 41 show the same data normalised for applied volume. This therefore represents the proportion of spray retained on the different targets, or the quantity of product if applied at constant dose (i.e. the concentration is reduced as the volume increases). This shows that the greatest quantity of product is retained at the lowest volume.

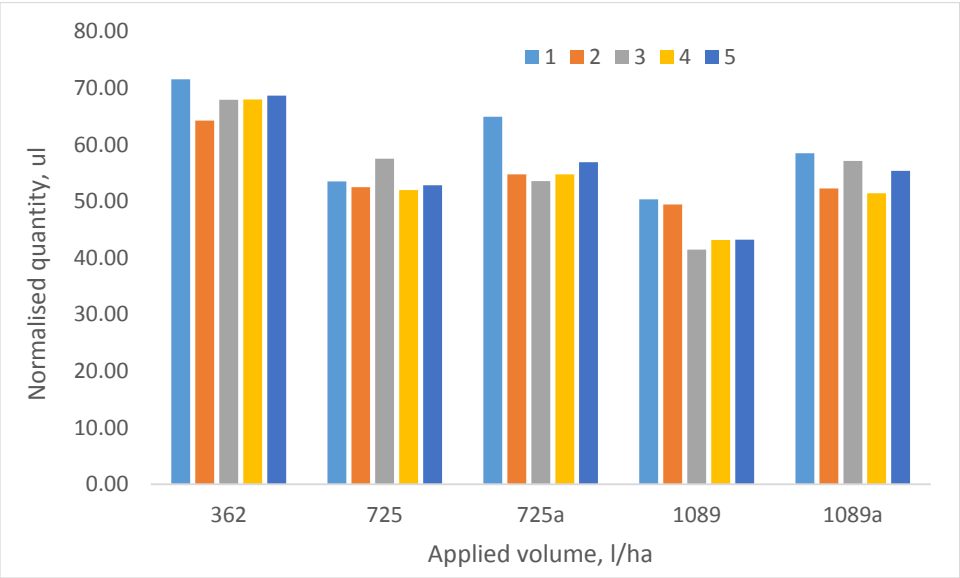


Figure 40. Normalised deposit of spray liquid on artificial flower buds for different, growth stages applied volumes and some angled nozzles (denoted 'a').

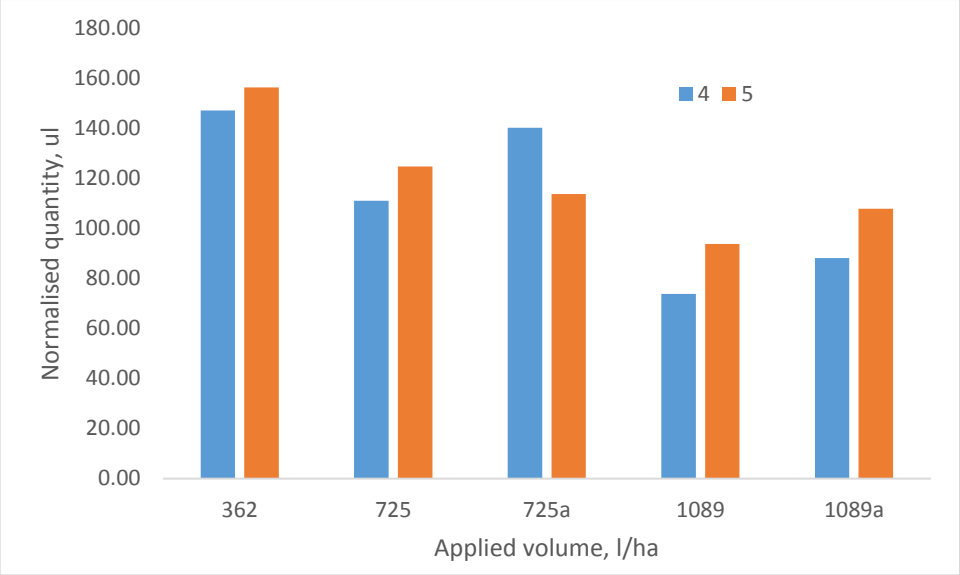


Figure 41. Normalised deposit of spray liquid on a disc at the centre of the flower for different growth stages, applied volumes and some angled nozzles (denoted 'a').

Generic observations from site visits and trials

All the spray operators that we encountered were engaged and interested, and all were trying to do as good a job as they could. Limitations on their ability to do this were (a) the quality of the guidance they are given relating to the application of the specific biopesticide and (b) the equipment available.

The label recommendations have subjective elements which are, at best, open to interpretation, and in some cases are very confusing. Instructions relating to run off, wetting and coverage are generally vague. Technical information generally does not expand on application, merely repeats label instructions. If there are restrictions in label wording imposed by the regulators, it would be useful to go into greater depth in the technical manual for the product. For example, where the label specifies an applied volume or dose in terms of litres per ha of floor area, simple calculation rules could be provided to convert this into an application to a vertical crop such as cucumbers and peppers.

Applying spray at a defined concentration is easier for the operator to comply with than at a defined dose – but then we have a poor idea of what the actual dose was unless a good calibration is done (especially with a tall crop).

In order to provide improved guidance, we need more detailed recommendations on application dose, water volume and required concentration, as relevant, and what the risks/benefits are in refining them. We need to engage with all the biopesticide manufacturers to explore this.

The lack of investment in spray equipment (in many cases) seemed inconsistent with the value of the crop and the financial risk relating to poor disease and pest control.

Mixing, dispersion and/or settling out is potentially a problem for some products. Agitation is variable across the different systems.

Discussion

Product, dose and timing are likely to be crucial parameters in the performance of biopesticides. The dose that is *received* by the target can be strongly influenced by application. However, we have to start with the dose that is *delivered* by the application equipment, which has been the biggest focus of this first year. Timing can be optimised with good logistics.

Observations show that very high volumes are being used within protected crops which are unlikely to be consistent with optimum deposit of product on the crop and maximum efficiency of the application process. However, application volume also influences surface wetting and drying, which are likely to affect the performance of a biopesticide. More knowledge is needed about the optimum conditions required for good performance of each biopesticide in order to identify potential improvements in application. This includes quantity of product, quantity of water, location within the crop that should be targeted, and other environmental parameters that could influence performance.

Manual application systems rely on a subjective assessment by the operator to deliver uniformity of spray across the treated area, which is extremely difficult to achieve.

The ability of growers to control either the applied dose or the water volume to their crops was poor in a number of situations, particularly manually-applied and vertical booms. The two situations where this was noticeable were

- Benchmark 1, where the applied volume was more than three times the operator's expectation, meaning that the dose was more than three times the label maximum;
- Benchmark 3, where an application to the soil was undertaken. Discussions amongst the project team following the application showed very different interpretations were possible of the label instructions. The applied volume that was used in the trial would not be practical on a larger scale.

The equipment available at the sites was largely fit for purpose, but in some cases, nozzles were old or damaged and pressure control was too far upstream. For future trials, we will need to ensure that all equipment is calibrated (including walking speeds) so that the applied doses and volumes are known to a reasonable level of accuracy. There may be advantages to bringing in our own equipment (such as knapsacks, lances, nozzles) so that we can have

consistent application conditions across the sites. We can also ensure no residues from previous chemical treatments in spray tanks.

Conclusions

- The sites chosen for year 1 benchmarking studies had a wide range of equipment for application but encountered common problems:
    - Mixing and dispersion of biopesticide products
    - Calibration of equipment and accurate dosing
    - Interpretation of label to comply with legal requirements and best practice
    - Achieving uniform distribution over the crop
- Could technical support information be modified to support making the application more efficient, therefore hopefully more efficacious and easier to deliver in practical situations? We believe this is the starting point for developing the experimental programme for application in year 2.

Overall Conclusions

The benchmarking studies done in Year 1 of the project covered a lot of ground, and the experiments included different biopesticides, sprayers, crops, pests and diseases. It is important to remember that these are not standard efficacy trials, but rather an investigation of how biopesticides performed when used by growers in IPDM systems against natural P&D infestations, to enable us to identify those areas of local management that can be modified in order to improve biopesticide performance. As such, benchmarking experiments in which biopesticides do not give high levels of P&D control are actually more informative than those in which biopesticides show high efficacy. Each experiment raised issues about biopesticide performance that were specific to that particular situation, and these have been flagged up during this report. There are also a number of generic issues arising from the research which we summarise as follows:

Grower "buy in" to the project. All our grower participants cooperated enthusiastically and put in considerable amounts of their own time and resources into the project. All growers followed the biopesticide application guidance, and demonstrated good application practice within the confines of the experiments.

More informative technical guidance notes are required from manufacturers, in particular with respect to mixing, dispersion and water volumes required for different types of crops. Water volumes are generally calculated on a basis of per hectare ground coverage and are not appropriate for tall row (i.e. vertical) crops. More use could be made of EPPO guidance on this and it may be useful to provide growers with and interpretation that can be used as guidance. Additionally, with more products being approved as Mutual Recognitions the label texts tend to reflect the growing conditions of the primary approval and may not fit UK conditions. Therefore additional advisory information is highly desirable.

In many cases, high spray volumes were being used, which are unlikely to result in optimum deposit of microbial biopesticides on the crop. It is likely that lower water volumes could be used to improve deposit on the crop and avoid wastage. This needs to be coupled with knowledge about the effective dose required for each product, expressed in terms of the number of microbial cells needed per unit area of the crop surface that needs to be targeted.

The test biopesticide used against aphids on pepper did not give any control, but the pest density in this case was particularly high. Biopesticide product guidance often states that the product should be used at the first sign of pest infestation rather than at high pest population levels, but no explanation is given as to why this should be the case. For conventional

chemical insecticides, which have a very fast speed of kill, the percentage kill obtained is, in principle, independent of pest population density. In contrast, some biopesticides work by reproducing within the target pest population and being transmitted to untreatednaïve pest hosts, in which case efficacy is dependent upon pest population density. However, this is not the case for Botanigard, as the fungus is used as an inundative biopesticide, whereby pest control occurs through the action of fungal spores sprayed onto the crop and not by transmission of spores between diseased individuals. Nevertheless, an apparent effect of pest population density will occur if two conditions are met; (i) the biopesticide does not cause a large reduction in the per capita reproductive rate of the pest; and (ii) the starting pest population size is high, which will make population effects more obvious to observers compared to small starting populations. The effect of a biopesticide on pest per capitata reproductive rate will depend on biopesticide speed of kill and lethal dose for each pest life stage, the rate of development of the pest from birth to adulthood, and whether the biopesticide has any pre-mortality effects on pest reproduction (e.g. castration). As a general rule, if speed of kill is constant, then pests with a long period of development from juvenile to adult will have a higher chance of death before reaching reproductive age compared to pests with a fast development time. ThereforeA priori, this is likely to explain why some pest species are more amenable to control with biopesticides than others. However, surprisingly, it is not something that has received much attention in the past. The way forward would be to use pest life history tables, coupled with information about speed of kill and effects on reproduction, and combined into a simple model of pest population dynamics

Environmental conditions for the biopesticides were within the recommended limits for activity supplied by the manufacturers in all of the benchmarking trials, but in cases where biopesticides are working under difficult biotic conditions (e.g. high pest density) or dose has been reduced by suboptimal application, then adverse environmental conditions could have major impacts on performance. One area where knowledge is missing is the effect of exposure to UVA and B, which in principle could reach harmful levels on some crops in summer.

Knowledge and Technology Transfer

Chandler, D. Future directions for R&D: IPM, biopesticides and the AMBER project. Presentation at "The Vine Weevil Summit", 12th July 2016, Banbury, UK.

Chandler, D. Overview of the AMBER biopesticide project (AHDB CP158). Presentation at "The Great British Tomato Conference, 29th September 2016, Kenilworth, UK.

Chandler, D. New biopesticides and their practical uses in IPM. Presentation at the Audax Seminar "Pesticide Risk management and Pesticide Updates", 11th October 2016, Reading, UK.

Chandler, D. Overview of the AMBER biopesticide project (AHDB CP158). Presentation at "The Cucumber and Pepper Growers Day", 12th October 2016, Essex, UK.

Chandler, D. Overview of the AMBER biopesticide project (AHDB CP158). Presentation at Gro South, 9th November 2016, Sussex, UK.

Butler Ellis, C. Application technology for biopesticides: What we know and what we need to find out. Presentation at "Advances in IPM 2016", 16-17Th November, Marston, UK.

Chandler, D. Overview of the AMBER biopesticide project (AHDB CP158). Presentation at "Advances in IPM 2016", 16-17Th November, Marston, UK.

Wedgwood, E. Practical issues around the use of biofungicides in commercial horticulture and their potential impact on disease control. . Presentation at "Advances in IPM 2016", 16-17Th November, Marston, UK.

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Appendices

Table 12. Grower survey handed out at AHDB grower events.

AHDB
AGRICULTURE & HORTICULTURE DEVELOPMENT BOARD

AMBER

Application and Management of Biopesticides for Reliability and Efficacy

1) NAME AND ADDRESS OF NURSERY

2) MAIN CROPS GROWN

3) ARE YOU USING ANY BIOPESTICIDE PRODUCTS FOR CROP PROTECTION? HAVE YOU USED THEM IN THE PAST? PLEASE NAME THE PRODUCTS USED.

4) WHAT SCALE IS THIS – FULL CROP OR A TRIAL ON A SMALL AREA?

5) WERE THE PRODUCTS USEFUL TO YOU?

6) IF YOU DID NOT GET A SATISFACTORY RESULT WITH THE PRODUCT, DO YOU KNOW THE REASONS WHY IT UNDERPERFORMED?

7) IF YOU WOULD BE HAPPY TO BE CONTACTED AND SHARE YOU EXPERIENCES AS PART OF THE AMBER PROJECT IN THE FUTURE, PLEASE PROVIDE YOUR EMAIL ADDRESS:

WARWICK
THE UNIVERSITY OF WARWICK

ADAS

RATIONALE BIOPESTICIDE STRATEGISTS

Silsoe Spray Applications Unit

Rob Jacobson Consultancy

Table 13. Grower responses to survey interviews carried out at five protected/ornamental crop production sites in the UK in May/June 2016. Responses from each of the five benchmarking sites are listed in boxes below each question.

Crop details
Bedding and pot plants (including hanging baskets). Tunnels and glasshouse with environmental control. Capillary matting is used on top of a plastic sheet. Plants are grown using trickle hose after initial overhead hand-watering until the roots have grown out. Baskets are hung over the crops and get drippers. Cyclamen are grown in peat reduced compost (40%).
Cucumbers in glasshouses, growing on Botanicoir slabs, with 4 plants in blocks. Rows 1.5m apart. Small plants bought-in from Holland on blocks and are 3ft within a week, 5 ft. in two weeks and about 6ft in three weeks. The leaves are a foot or more wide. 3 crops a year. Don't pressure wash or disinfect between crops and leaves are left in the house as the insect biocontrols are developing on the leaves (acknowledge there may also be diseases present). Second crop in mid-May will be finished by end July (3 months). Flowers formed within two weeks of planting. Each crop bay is 70 m long and 8 m wide. The crop is on strings, with each successive plants going in alternate directions so that there is a "V" shape over the slabs. New growth is fast to appear so requiring re-application at short interval.
Woody and herbaceous nursery stock including fruit trees. Outdoor container site and protected liner GH and tunnel site. Propagation material bought-in potted into 11 cm, overwintered and into 3L in the spring. 1 million saleable container plants/year all for garden centres (1L to 15L). Under protection growing on sand, but moving to woven ground cover.
Two hectare glasshouse (including walkways). Each bench is 12.7 m^2, 49 benches per row, 24 rows per block. Total growing capacity per block is 1.5 ha. In total around 30,000 pot mums produced per week. Environmental control, with temperature recorded on sensors in the nursery, and controlled using automated fans and vents. Keeps at around 23 degrees Celsius during the day, dropping down to around 12 at night. Blackouts used prior to, during, and following applications in the afternoon. Irrigation using ebb and flow benches. Plants are grown in Bulrush with 30% forest gold, with fertiliser applied via the irrigation system. Plants are initially grown for 4-5 weeks then spaced and finished along the rows in the main glasshouse area for the final 4-5 weeks. Control products are applied via a robotic horizontal boom down each row.
Organic and conventional pepper in glasshouses. Parallel rows of around 130-150m beds. All glasshouses have environmental control and irrigation.
1. What are the most common pests and diseases in your crops?
Aphids, moth caterpillars, powdery mildew, black root rot (not Pythium/Phytophthora)
Powdery mildew some years (but use resistant Bonbon). Mycosphaerella. Do not disinfect between crops. Spider mites (use biocontrol). Whitefly (use Encarsia) come in from outside. Both spider mites and whitefly require contact with the pesticides to be effective. Whitefly eggs need to be targeted (not adults).

Spider mite, leafhopper, white fly, aphids (use Amblyseilus, Swirski and Phytoseilus thus wary of Fenomenal use so use Prestop). Downy mildew (involved with trial). Black root rot in Cystus. Phytophthora found in Choisya from France. Rose P.mildew (tried forecasting programme, but can see if not PM weather). Root rots in choisya - Trianum in 3L as buy in bulk compost. Botrytis on stopped Dianthus use Prestop. Dianthus gets most losses from root rot in September to March batch than in spring, same for Choisya. Rhodanthemum can get root rot so gets Trianum G. Plants given a Fortify drench and propamocarb, if losses continue then get T34 Biocontrol and then Fenomenal and Previcur Energy.
Thrips, aphids, leaf miners, mildew (very rarely)
Aphids, caterpillars, sooty mould, powdery mildew
2. How frequently are pests monitored?
Not asked
Daily by the grower, focusing on known starting-points e.g. stanchion & heating pipes for spider mites (not in the middle). Mildew comes in where it is colder
Crop walking ongoing. Technical manager checks incoming material for pests and diseases and any problems a flagged up to the supplier.
Weekly monitoring by trained staff, plants assessed at random along edges of rows, and inspection of yellow stick traps alongside crop.
Weekly and whenever staff are picking, plus advisors come in once a fortnight and inspect random rows.
3. What level of damage triggers action?
Not asked
Mildewed leaves picked-off if only a bit, then if it spreads then treat unless can't get a three-day harvest interval. When plants are young the fruit are picked daily so the plant energy isn't used up. Fungicides need to be preventative. Insecticides only go on if biocontrols fail. Once fungicides are started can give every 5-7 days, usually only one or two applications (at very worst may have 10). Spray in the evening, or early morning around 6am if hazy, depending on when next harvest is due.
If an occasional plant affected will remove it from the bed
Damage flowers are removed, pots with heavy infestations are removed. If numerous (undefined) pots affected, additional control measures are put on (e.g. conserve used very occasionally where aphids or thrips get away from treatments).
No defined threshold.
4. How do you decide what form of control to use?
Advice from product reps e.g. Fargro, Karen Girard. Yearly store check from David Talbot
Chemical protection used only if mildew seen. Biocontrols first choice for pests. Spraying is costly so try not to spray.

Use Amblyseilus, Swirski and Phytoseilus rather than insecticides. Thus wary of Fenomenal use so use Prestop instead. Use Trianum in the compost, but can only do this for 3L pots because it is only technically feasible for the growing media supplier to mix it in to the large bales used for this, not for the liners. The product is dispensed from a hopper over a conveyor belt. It is not possible to have T34 Biocontrol even in 3L as the amount required is much too small to be able to be used with the hoppers. Prestop is used as a "sprench" straight after the Dianthus flower tip is cut out to get the plant to bush out as this protects to wound from infection.
Advice from product reps; biocontrols are first choice for pests, used to maintain low pest abundance.
Experience and advice from senior staff & product reps.
5. How do you monitor success of treatment?
Not asked
Daily inspection by the grower, focusing on known starting-points e.g. stanchion & heating pipes for spider mites (not in the middle). Mildew comes in where it is colder
Routine inspections
Routine inspections
Ongoing inspections
6. Have you previously used/currently use biopesticides?
Not initially recognised as a biopesticide, but Dipel DF is used against caterpillars on cyclamen. Macro-biologicals used include Thripex-V Phytoseiulus cucumeris and nematodes. Eradicoat is used against aphids and was good on fuchsia, but not on the curled leaves in apple trees so Gazelle had to be used.
AQ10 was tested 2 years ago when brought by the Fargro rep (Paul Tate).
Trianum on 3L pots e.g. Choisya, T34 Biocontrol used on Dianthus after Trianum G. T34 used via Dosatron. Prestop currently used. Have used AQ10 tank mixed with conventional products (ensuring AQ10 goes into fully diluted product). Met 52 was used in the growing media and would be interested in the liquid formulation. Dipel DF used as Totrix can be a problem.
Had previously tried using Botanigard as a soil drench on a few rows, but it was too expensive and didn't seem to be effective. Currently use nematodes, applied at half rate twice a week for thrips control
They have used DiPel DF for caterpillar control, Majestik, and Mycotal in the past, with variable results. They have also tried Serenade ASO and (AQ10 and Prestop) for botrytis and powdery mildew respectively.
7. How successful do you consider the biopesticides you have used compared to conventional plant protection products?

Dipel DF is effective, but no biofungicides have been used either in the compost or sprayer (and seemingly little knowledge of products available).
Control by AQ10 was not good
Biologicals continue to be used as preventative treatments (and so may not have got the problem). Both T34 and Trianum G will be followed by sprays of conventional products.
Variable success. Natural enemies are widely used, but pests can occasionally get away from them. Biopesticides have a similar issue that not 100% reliable, and more expensive. Biopesticides 'seem to help, but not remove' pests
Variable success. Natural enemies were the preferred control measure in organic peppers for pest control. Biofungicides seemed to be more effective, used in cucumbers not peppers
8. Do you think your use of biopesticides could be improved?
Not relevant
Application was done to give good coverage and using a bigger nozzle
There are queries about the performance of the biopesticides. Do they grow /survive in the growing media? Does the growing media affect the efficacy of Trianum G (they use peat+bark)? Like to use T34 Biocontrol as incorporation at the start, but the dose rate is so small that it is difficult to ensure even mixing through compost. What effect does storage of the big bales with Trianum G have on the viability (they are stored on a concrete pad in winter with dark film-wrap and plastic bag lid, and in summer in a shed). Would like to improve vine weevil control with biofungicides. Would like more information on the effects of biopesticides on beneficials.
The timing and location of application could be improved (not sure if they are best applied to soil or to leaves). Would like to be able to change the rates to get sufficient control, and to be able to better target it at the pests they encounter
Were interested in improving their application technology. Their current spray equipment was quite variable, and was constantly being repaired and tweaked. They weren't sure how this would affect the treatments, and as the biopesticides were already seen as variable this made the risk even greater
9. Have you ever used biopesticides and found control of the target to be unsatisfactory, do you know why?
Dipel DF is effective.
Yes have tried AQ10. Don't know why it wasn't effective.
Have ceased using Met 52 in the compost.
See above, tried as soil drench but didn't give 100% control.
Not asked
10. How easy do you find it to integrate biopesticides with your other crop protection practices?

Not asked
Pest control turns to chemicals instead if a problem develops.
Incorporation of biofungicides in the compost, used to use Fungarid against Phytophthora but that is not available now. Trianum G was selected as that was available from the growing media supplier via incorporation. Some other suppliers say they can mix in T34, but others say they can't. Followed with sprays of biofungicides and conventional. There is information on the internet of AQ10 and compatibility. Need to have more information on tank mixing and intervals between applications.
Used current shower boom, so fairly straight forward.
Just used current equipment and gave it a go.
11. Have you heard of other growers having good or bad experiences with biopesticides?
Not asked
No information.
No information.
They had heard that others had had good and bad experiences, and that Botanigard was less effective on aphids.
Not discussed
12. What do you think makes biopesticide application more or less successful?
Not asked
Need good coverage. Fungicides only work preventatively.
If tank mixing need to ensure it is added to the diluted conventional product or it could be killed. Application timing has to be in the day and have a re-entry protocol, ideally would do it early in the morning (4am) or 8-9pm, but this is not always possible. This would mean the products do not dry out as quickly.
Not discussed
Not discussed
13. What do you think the main challenges are to using biopesticides?
Information required on products and pests/disease targets
Technical information on good application and product efficacy needs to be clear. A consistent format for all pesticide applications would be good, in particular different products are given per ha or dose per water volume.
Need to have a knowledge of which crops are likely to be susceptible to root rot so can treat preventatively, including particular varieties. Susceptible include Aquilegia, Choisya (ternata, Sundance and White Dazzler) Clematis especially Avalanche, Cordyline, Cystis betandria,

Dianthus, Perennial wallflower, Euphorbia, Blackberry and hybrid berries, Perennial geranium/geum, Hebe, Lavender if in 7L pots, Potentilla, Rodanthemum.
They are not 100% reliable, and are expensive.
Conventional products are a known quantity, and can be relied on to take care of an infestation. In contrast, biopesticides can't guarantee a problem will be solved at the moment, making it difficult to justify their use
14. What do you find are the main advantages/ disadvantages to biopesticides over other methods?
Not applicable
The advantage would not having to worry about MRLs as exceeding these causes crop rejection. Also many products have a HI that is too long as pick every day when young to keep more fruit coming. The disadvantage for fungicides is that they are only used by the grower once mildew is seen and biofungicides are not curative. Once disease seen sprays go on repeatedly. Biocontrol organisms are used for pests, have not tried biopesticide control.
There are queries about the performance of the biopesticides. Do they grow /survive in the growing media? Does the growing media affect the efficacy of Trianum G (they use peat+bark)? Like to use T34 Biocontrol as an incorporation at the start, but the dose is too small to get it added to the compost by the suppliers. More information needed on compatibility - Syngenta and Fargro are good with technical notes, BASF less so.
They would prefer to move away from synthetic pesticides where they can.
Not asked
15. What training have you or your staff to enable optimisation of biopesticides?
Not asked
Fargro came to promote AQ10.
Have visits from suppliers of products. AHDB training courses such as the biologicals workshop. They also have independent advisors.
Mainly through product suppliers
Mainly through product suppliers
16. How do you store your biopesticides?
Dipel DF in the metal cabinet in the barn, but unsure if it needed to be kept cold. Two fridges are available in the barn and one currently stores seeds.
Have not stored biopesticides
Those requiring a fridge are kept in a domestic-type fridge inside the metal pesticide container. With Prestop 1kg usually the whole bag is used. T34 Biocontrol is hard to reseal to keep the moisture out. There is a quick turn-over on insect biologicals. Nematodes are stored in the fridge. When products are delivered the manager is notified to take them for correct storage.

In dry or wet locked chemical storage cabinets with other plant protection products, or in designated fridge when stored for longer periods.
17. What type of sprayers do you use?
One 200 L wheeled Brinkman sprayer with long hose and Ripa nozzle. A small knapsack also used on site (not seen).
Vertical boom sprayer running on the heating pipes. Nozzles are 45 degree angle and use Blue 03 F80 or Red 04 flat fan F80. Different nozzles and different angles used depending on the crop stage. Filters from tank, in boom and by nozzles. Six nozzles either side of the boom (to spray rows either side), the top ones are turned off for young crops. It takes 10 to 15 minutes to spray a row of 70m. When the crop is only a fortnight old there is spray-through and so every other row of crop will be missed out as this treats the 4 plants in each slab either side adequately.
250L sprayer (minimum fill 10L) for small areas lance with flat fans. Outdoor have a 15m boom wit nozzles pointing front and back with flat fans, but also have a hose and lance that can be used from the tractor tank.
700 L tank supplying automated 16 nozzle spray boom. 03 flat fan spraying vertically downwards, 01 nozzle at either end of the boom spraying inwards at 45 degrees. Forward speed = approx 1.62 km/hr
Semi-automatic vertical spray boom. 5 pairs of nozzles, bottom pair usually blocked to protect natural enemies when appropriate.
18. Do you use irrigation to apply biopesticides?
No
No information.
No
No
19. How old are your sprayers?
Not asked
Not very new. Boom made by Empass in NL.
Outdoor sprayer is old
Not asked, but not very old (within 5-10 years)
Old - not sure how old. Nozzles have been there as long as staff can remember
20. How often are nozzles replaced?
Not asked.
The ones installed were changed last year. Modern nozzles do not wear out as quickly as the old ones. It can be seen from the spray pattern when a new nozzle is needed.
Not asked

Not asked
Only if broken
21. What pressure are plant protection products applied at?
15 bar pressure at the pump. No other measured adjustment but can turn the nozzle to get stronger or weaker output i.e. the wider/more open the shorter the throw and more liquid on that area.
Pressure depends on what is to be sprayed 20 p.s.i to 40 p.s.i.
Not asked
4.5 bar
5 bar at boom
22. What water volume are they applied in?
Spraying of all pesticides is done to the point of run-off. The volume required is known by experience based on the bed width, pot density and crop height.
About 2000 L applied to a 2 acre block (2 tanks). More than 1000 L/ha (2500L?)
Not asked
approx 1000 L/ha
approx 1,400 L/ha
23. What is the water source and pH?
Mains water is used for irrigation. Not asked, but probably also used for the sprayer.
Bore hole water. pH not known
Rainwater off the roofs in winter, only if low is it topped up with borehole.
700 L tank from mains, warmed to approx 20 ^{O}C
Mains water to fill mobile tank.
24. How many times a year are sprayers calibrated?
Calibrate every season (year)
Not asked.
Not asked
Every season (once a year) and after repairs
Start of each season, and after repairs

25. What determines the timing and frequency of biopesticide applications?
On cyclamen use fungicides every 2-3 weeks depending on the speed of growth of the plants (faster growth requires earlier treatment to cover new growth)
Not applicable
Applications made according to crop requirements e.g. Prestop used after removing flower heads to protect wounded tissue or growing media with Trianum G incorporated to be present when plants are potted-on and roots establish.
Not asked
Not asked
26. What has the greatest influence on whether or not the business uses biopesticides?
A small range of pesticides are used which do not include any biofungicides, however keen to participate in the project to see whether biofungicides could be used instead of conventional products. Fungicides used are Amistar (botrytis), Fubol Gold, Rovral WG (botrytis), Plover, Systhane 20EW, Avatar (for damping off) and Cercobin WG (if hot conditions then drench pansy plugs after potting). Insecticides are Eradicoat, Dipel DF, Gazelle, and Chess. Growth regulators are B-Nine, Cycocel and Bonzi. Only Cycocel is used with a wetter. They have recently been sold Phorce from Plantsyence by Fargro to keep plants healthy - this is a foliar nutrient 5-38-15 containing phosphoric acid, potassium hydroxide and potassium citrate to be applied at 3-5 L/ha in 200 L water at 10-15 day intervals. Have heard that silica products are effective against disease.
The fungicides need to be able to control disease when it is seen as mildew sprays are not usually needed because a resistant variety is grown and it is only some years that mildew is seen and then a chemical fungicide is used from the list and the application rates provided by the cucumber growers' association and products alternated. Currently use Systhane 20EW a lot, Nimrod if the mildew gets very bad (but not a lot as it is hard on the plant). Amistar is used against Mycosphaerella. Haven't used the new products Reflect, Signum, Serenade ASO. Have tried potassium bicarbonate. Takumi insecticide was used but it didn't work. The plants from Holland are sent with a list of the products already applied.
1). The increasingly restricted number of chemical pesticides due mainly to EU regulation changes. The range available for outdoor ornamentals is particularly limited and so this causes concern for pesticide resistance management. 2) Pests are controlled using insect biological control agents and these can be affected by chemical fungicides. Biofungicides thus form part of the ethos of Integrated Crop Management 3) Some companies supplied by the nursery are asking for IPM to be used.
The main influence is the cost effectiveness of the product. If it achieves acceptable control at a lower cost to alternatives, they'll use it.
Main influence is where or not it is reliable enough to give the control they need. They want to protect their natural enemies, so biopesticides also need to be compatible with these.
Additional information

There is one cyclamen crop Picasso, Verandi-Mixed potted in Week 22 (wc/30 May) into 10.5 cm pots in 40% peat standard bedding mix from ICL with about 6000 plants across 5 areas of around 15m bed length in tunnels 27 and 28. N.B. 3 pots are placed in a Teku ST12B 39x28cm 6-hole carry tray and so the spacing is wider than expected for the number of plants/ha (no paths within each approx. 3m wide bed). Fungicide application every 2-3 weeks against botrytis which develops on the old leaves touching the compost and can follow early-flower removal. 10 leaves present and rooting starting at WK25, first spray thus needed WK26.
Tank cleaner is used and the spray waste is put onto an old crop. Adjuvant Codacide oil has been used when spraying.
Refrigerated storage for biopesticides is present in the pesticide store.
Not asked
Not asked

