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The results and conclusions in this report are based on an investigation conducted over a one-year period. The conditions under which the experiments were carried out and the results have been reported in detail and with accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results, especially if they are used as the basis for commercial product recommendations.

AUTHENTICATION

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

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GROWER SUMMARY

Headlines

- Controlled environment experiments showed that the mycoparasitic fungus *Ampelomyces quisqualis* declined markedly by 7 days after application to tomato as a representative crop plant.
- A pest control model has been developed to identify optimal biopesticide control strategies, using glasshouse whitefly and entomopathogenic fungi as a model pest and biopesticide.
- For spray applications to small plants with a horizontal boom sprayer, and where biopesticide products are used at a constant dose, the maximum active substance will be applied using the lowest water volume, providing that the maximum label concentration is not exceeded. Where the biopesticide is applied at a constant concentration, the maximum volume that should be used is 1000 L/ha, but there are likely to be benefits for smaller plants of reducing this down to around 500 L/ha.
- For tall plants such as tomato that are sprayed with a vertical boom, the quantity of active substance deposited on the plant appears to be relatively insensitive to volume. Thus, for biopesticide products applied at a constant dose, water volume can be chosen to suit other needs (e.g., use a low water volume to reduce the time needed to spray the crop). Where products are to be applied to tall crops at a fixed concentration, our studies suggest that the maximum volume that should be used is 1000 - 1500 L/ha applied to the crop.

Background

Pests (including invertebrates, plant pathogens and weeds) have a major impact on crop production, reducing yield and quality (it is estimated that about a third of the potential global crop yield is destroyed by pests before it is harvested). The standard method for pest control has been to use synthetic chemical pesticides. However excessive use is associated with a range of problems including harm to the environment, and concerns have also been expressed about safety to pesticide spray operators. Overuse has also resulted in the evolution of resistance in many pests, which has rendered some pesticides ineffective. In recent years, environmental legislation has resulted in a lot of these pesticides being removed from the market. Alternative pest controls are needed therefore. 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. One group of alternatives are ‘biopesticides’. These are pest control products based on natural agents, and there are three types; living microbes, insect semiochemicals and botanical biopesticides. These types of pest control agent are based on living organisms and so it takes more knowledge and understanding to use them successfully compared to traditional pesticides.

AMBER (Application and Management of Biopesticides for Efficacy and Reliability) is a 5-year project with the aim of identifying management practices that growers can use to improve the performance of biopesticide products within IPM. The project has three main parts: (i) to understand the reasons why some biopesticides are giving sub-optimal results in current commercial practice; (ii) to develop and demonstrate new management practices that can improve biopesticide performance; (iii) to exchange information and ideas between growers, biopesticide companies and others in order to provide improved best-practice guidelines for biopesticides.

Summary

Understanding the biology of biofungicides on crop foliage.

A small number of biofungicides are being used more widely in plant disease management programmes, but there has been a lack of independent information for growers about the length of time for which these control agents remain viable after they have been sprayed onto crop plants, which in turn will affect the optimum timing and frequency of application. In this part of AMBER, experiments were set up to investigate whether the persistence of the mycoparasitic fungus *Ampelomyces quisqualis*, which is used against powdery mildew, would depend on its application timing in relation to arrival of powdery mildew inoculum. This was based on findings from previous work in the project suggesting that the efficacy of this biofungicides is responsive to the population density of its powdery mildew host. Experiments were done in a controlled environment chamber, in which *A. quisqualis* was applied to tomato plants at different timings prior to, and after, the plants were inoculated with powdery mildew.

In overall terms, there was a marked decline in the presence of *A. quisqualis* on the leaves over 7 days, which is in keeping with previous observations. There was evidence that more *A. quisqualis* was present on leaves in which the mycoparasite was applied 2 or 7 days after inoculation with powdery mildew, compared to application 1 day before powdery mildew application, but this may also be related to initial differences in the amount of biofungicide applied to leaves.

Studies were carried out on a nursery to investigate late season applications of *A. quisqualis* against powdery mildew on Hebe and Rosemary. Hebe plants were inoculated with powdery mildew in mid-October and AQ 10 applied with or without Silwet L-77 wetter a week later. The incidence and severity of powdery mildew was compared weekly until early December with that of water-sprayed and wetter-only treated plants. Treatment to Hebe plants was done using a knapsack sprayer with a flat fan nozzle at the recommended commercial rate on two occasions at a 7-day interval at the end of October 2019. Leaf discs were sampled from central plants in plots directly after and again a week after each of these applications. *A. quisqualis* spore viability was assessed by washing leaf discs and spreading aliquots onto agar plates containing antibiotics to stop the growth of bacterial contaminants from the leaves. Viable *A. quisqualis* was detected seven days after AQ 10 application. Although three leaves had the first signs of powdery mildew on the first biofungicides application date, the disease did not establish much further, so that only seven plots had developed mildew by the end of the experiment, each on only one Hebe branch. Consequently, it was not possible to compare treatment efficacy. A parallel experiment was also done in the same nursery with Rosemary in early November 2019. A group of 24 plants were divided equally into those with either high (mean of 32%), medium (mean of 10%) or low (mean of 1%) mildew coverage. A single application of AQ 10 was made using a knapsack sprayer with a flat fan nozzle at the recommended rate to half of the plants while the remainder were treated with water as controls. Coverage of plants by powdery mildew changed by 5% or less up to the final observations at the end of December. on 20 December, without any difference between the AQ 10 treated or untreated plants. No greying of the mildew attributable to *A. quisqualis* parasitism was seen on any of the plants. Application coincided with a slowing of powdery mildew colonisation, but new growing tips continued to develop powdery mildew whether or not treated with the single AQ 10 application.

A pest control model to help identify optimal biopesticide control strategies.

The optimal use of biopesticides can differ markedly to that for conventional pesticides. This is because biopesticides can often take longer to kill individual pests than a conventional pesticide, can have different effects on different pest life stages, or have specific requirements for environmental conditions in order to work properly. All these factors influence how often they need to be applied, as well as things like the best time of day to apply them, and how quickly they can bring a pest population under control. Because of the large number of variables involved, identifying optimal application programmes for biopesticides using crop scale experiments is very time consuming and expensive. A better alternative would be to use mathematical modelling to simulate how pest populations respond to biopesticides, and

to computationally test out different biopesticide application scenarios to identify the ones that are likely to produce greatest improvements in control.

In this part of AMBER, a 'boxcar train' computer model was developed that simulates how pest populations grow over time. The model considers each individual in a pest population and mathematically describes their transition from one development stage to the next until they reach adulthood and reproduce. From this, the development of the whole population can be evaluated. The effect of biopesticide application on the pest population can also be simulated using data on the susceptibility of individual insects. A model was constructed for the glasshouse whitefly (*Trialeurodes vaporariorum*) and the tobacco whitefly (*Bemisia tabaci*) and control with the entomopathogenic fungi (EPF) *Lecanicillium* spp. and *Beauveria bassiana*. A literature review was carried out to identify model parameter values for each pest (e.g. time for completion of each developmental stage) and biopesticide (e.g. infection efficacy). Knowledge gaps were identified and filled using data from bioassays carried out in the project. The model developed here is a valuable research tool that allows different control programmes to be tested.

Making biopesticide spray application more efficient.

It has become increasingly apparent through AMBER that spray application of biopesticides to horticultural crops could be made significantly more effective than at present. The aim of this part of the project is to identify the optimum volume range to be used for biopesticides on representative crops, as this needs to be in place before appropriate spray equipment and other techniques for improving application can be explored. Growers are using relatively high volumes for biopesticides as set out by the product label recommendations, possibly because such labels need to cover a wide range of crop structures. Unfortunately, data is not available from biopesticide companies to support the volumes being recommended.

A set of experiments were done using a track sprayer and tracer dyes to investigate the effect of altering spray water volume on the amount of product applied per unit leaf area. By using this approach, a range of volumes can be applied to a crop through changing nozzle and forward speed. Because changing nozzle also changes droplet size, which influences the quantity retained on the plant, we chose to use the speed of the track sprayer to manipulate volume. The quantities of spray liquid deposited on different parts of plants were determined by washing detached, leaves in a known volume of water and then evaluating the rinsate using spectrophotometry. The weight of the plant material in each sample was determined so that results can be presented as quantity of spray liquid per unit mass of plant material. It is then also normalised for the applied volume, and presented as quantity of spray liquid per mass of plant material per 100 L/ha applied volume. This allows the quantity of active

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substance to be estimated on the assumption that concentration increases as volume reduces.

Experiments done using basil, as a representative short plant, sprayed with a three-nozzle horizontal boom, indicate that, where the biopesticide is applied at a constant dose, the maximum active substance will be applied using the lowest water volume providing that the maximum label concentration is not exceeded. Where biopesticide products are used at a constant concentration, the maximum volume that should be used is 1000 L/ha, but there are likely to be benefits for smaller plants of reducing this down to around 500 L/ha. This is considerably less than the upper water volume allowed for most biopesticides on the label (which is typically 1500 L/ha).

An experiment was then done using a vertical boom track sprayer erected within an experimental tomato crop, as a representative tall plant. In this case, the quantity of active substance deposited on the plant appears to be relatively insensitive to volume. Thus, for biopesticide products applied at a constant dose, water volume can be chosen to suit other needs (e.g. use a low water volume to reduce the time needed to spray the crop). Where products are to be applied to tall crops at a fixed concentration, our data indicates that the maximum volume that should be used is 1000 - 1500 L/ha applied to the crop.

Finally, a system was developed to investigate how control of water volume translates into effects on biopesticide efficacy. This was done using a fungal biopesticide sprayed against spider mite on tomato. The system allowed us to have precise control of water volume, quantify the number of fungal spores deposited per unit leaf area, and monitor mite mortality under controlled conditions. The data will be analysed in 2020 although the indications from the raw data are in keeping with our tracer dye experiments, i.e. that the best strategy for optimising control is to manipulate the water volume to achieve the highest concentration of biopesticide on the leaf surface (i.e amount of active substance per unit leaf area). The experiment shows significant promise as a cost-effective technique that can begin to explore the relationship between efficacy and application method without the need for costly field trials.

Financial Benefits

- Biopesticides can be more expensive and less forgiving of environmental conditions that conventional pesticides so understanding the optimal way to use them is crucial to maximising efficacy and minimising cost.
- Computers models are useful for understanding systems that involve complex biological interactions where there are multiple interacting factors. They can be used for rapidly

testing a large number of hypotheses to identify those hypotheses that should be further investigated. The model developed here is a valuable research tool that allows different control programmes to be tested. Once optimal control programmes are identified a subset of these will be tested experimentally to assess the accuracy of the model. Attempting to investigate all components of a spray programme in laboratory or grower experiments would be prohibitively expensive and time-consuming.

- Similarly, the systems developed in AMBER on biopesticide spray application also enable different spray systems to be investigated faster and cheaper than using field trials.
- At present, most biopesticides are used according to a constant dose model. The upper water volume recommendations for biopesticides are typically 1500 L/ha, and growers might be tempted to use this on the assumption that higher water volumes give better coverage on the plant. However, on short plants, a better strategy would be to use the lowest water volume providing that the maximum label concentration is not exceeded. In principle this means that the maximum active substance will be applied, which will maximise efficacy. Lower water volumes also mean that the time to spray will be reduced, saving on labour costs. On tall plants (tomato, cucumber, pepper) the quantity of active substance deposited on the plant appears to be relatively insensitive to volume, in which case - for products applied at constant dose - spraying at a lower volume will save time and money without affecting product efficacy in a negative way.

Action Points

- Biopesticide efficacy depends on good management practice, with attention to detail being paid at all stages of their use (storage, handling, mixing, application, monitoring).
- When using biopesticides at a constant dose, a sensible strategy is to the lowest water volume within the label limits providing that the maximum label concentration is not exceeded.

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SCIENCE SECTION

1. Project background, aims and objectives

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

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

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

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

AMBER (Application and Management of Biopesticides for Efficacy and Reliability) is a 5 year project funded by the Agriculture and Horticulture Development Board (AHDB project code CP158). The research team 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 working 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.

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2. Research results from Objective 2: Develop and demonstrate management practises that can improve biopesticide performance.

The current phase of the project is looking at management practices that have potential to improve biopesticide performance (Objective 2 of the project). Here we report on 3 areas: (1) understanding the persistence of biofungicides on crop foliage; (2) making biopesticide spray application more efficient; (3) developing a pest control model to help identify optimal biopesticide control strategies.

2.1. Biofungicides

Objective 2, Work Package WP 2.2.2 Better understanding of biopesticide persistence

Introduction

Microbial biofungicides are being used more widely in plant disease management programmes, but there has been a lack of independent information for growers about the length of time for which these control agents remain viable after they have been sprayed onto crop plants. Microbial biofungicides are normally used as preventative treatments and so they must be applied before their target plant disease is present in significant numbers. If the control agent does not survive for long, and it is applied too far ahead of the disease, then it will not provide any plant protection. Therefore, understanding the persistence of the control agent is important for determining the best timing and frequency of applications. Some microbial biofungicides work by colonising the leaf surface and preventing plant diseases from establishing by outcompeting them for space. These include *Gliocladium catenulatum* (used in Prestop for management of botrytis) and *Bacillus subtilis* (used in Serenade for botrytis management). Experiments done previously in AMBER have shown that Prestop grows well on crop plants, and about twice as many propagules were retrieved 7, 10 and 14 days after Prestop application as on the application day. Similarly, when Serenade ASO was applied to plants, the numbers of *Bacillus subtilis* bacteria recovered 7 days after application were similar to those recovered immediately after they were sprayed onto the crop, which again shows good persistence. Therefore, in these cases, the timing window for application is relatively wide. In contrast, biofungicides that are based on mycoparasites - such as the fungus *Ampelomyces quisqualis* (the active agent in the biofungicide AQ10), which is used against powdery mildew - function by colonizing the target plant pathogen itself, rather than growing on the leaf. In the case of *A. quisqualis*, the fungus is applied as spores which will only germinate when in physical contact with powdery mildew mycelium. Research in AMBER has shown that spores of *A. quisqualis* survive for less than four days when sprayed onto

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leaves in the absence of powdery mildew. Therefore, the timing window for using AQ10 is relatively short. If it is applied before mildew has arrived in the crop, then this has to be done within the 4-day window. One option is to use in-crop environmental monitors to warn the grower when conditions are favourable to mildew development and to then apply the mycoparasite in the next day or two. An important consideration is to apply the control agent before mildew becomes too established on the plant and outstrips the ability of *A. quisqualis* to control it. Another option is to inspect the plants regularly and wait until powdery mildew is first seen before AQ 10 application - again this has to be timed correctly so that the mycoparasite is able to control the disease before it passes the economic damage threshold.

WP. 2.2.2.1 Quantifying the persistence of the mycoparasite *Ampelomyces quisqualis* on tomato plants at different application timings

In this part of AMBER, experiments were set up to investigate how the persistence of AQ10 would depend on its application timing in relation to arrival of powdery mildew inoculum. This is based on our findings from previous work in the project suggesting that AQ10 has greater efficacy where there is the "right level" of powdery mildew colonisation of the plant, so that there is mycelium present that the *A. quisqualis* can feed within, but not so much that the mildew "gets ahead" of the parasite.

Methods

Experiments were run spanning three weeks in each of July, August and September 2019. The methodology used was essentially similar and so the details are given below combined for the three experiments and any differences noted that resulted from changes made after gaining information from the earlier experiments.

Powdery mildew host plants

Plants of tomato cultivar Elegance, 28 days old, were provided by Delfland Nurseries. In the experiments run in July and August, the plants had two expanded leaves, while in September the plants were at the three-leaf expanded stage. Plants were grown in individual plastic pots (9cm x 9cm x 10cm) in peat growing-media to allow handling and survival in the controlled environment cabinet. Plants were placed into a growth chamber one week prior to the start of each experiment to allow acclimatisation to the experimental conditions. After three weeks in the cabinet the plants were given a tomato liquid feed (Levington's Tomorite NPK 4-3-8 at 20 ml per 4.5 L) via the capillary matting, instead of the regular watering.

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Inoculation with disease was done using tomato leaves with visible powdery mildew that were obtained the day before use from a local nursery and which were placed in a cold store until required. The leaves were separated into piles for each replicate that comprised a similar total area of sporulating mildew colonies. In July, leaves with powdery mildew were shaken over the plants. In the August experiment, donor leaves had visible mildew colonies, but none were densely sporulating and so these were dabbed lightly (aiming to avoid displacing the *Ampelomyces* sp. on sprayed plants) three times onto each of the two oldest leaves per plant, using a fresh donor leaf (or leaf piece) for each recipient leaf. This resulted in rapid growth across the whole leaf surface which is unlikely to have reflected the natural infection process in a normal crop, which would be slower and result in separate colonies developing on the leaf. For this reason, the September inoculation was done without donor leaves touching recipient plants. Once inoculated, the plants were placed straight away into a humid environment to encourage powdery mildew spore germination. In July, the plants were sealed in transparent plastic bags: this was based on a method used to study AQ10 persistence in 2018, but in this case powdery mildew disease only developed to any visible extent after the bags were removed at the end of the leaf sampling. Because of this, the protocol was altered in the August and September experiments, and the plants were placed in transparent heavy gauge plastic sleeves, which covered the whole plant height and which created a lower humidity environment. The plants were well watered and stood on a double layer of moist capillary matting, so the plants continued to transpire and raise humidity around the leaves.

Experimental Conditions

Plants were maintained at 25 °C ± 1°C under a 16 h : 8 h, day : night schedule, with the light period occurring within normal day time. Light intensity of 800-1000 µmol m^2 s^{-1} was achieved with fluorescent lamps (22 Philips 55 W tubes, supplemented with four Philips 60 W tubes). Plants were sleeved in transparent plastic, held off the plants with stakes (Figure 1). Humidity was maintained at 70 % inside the open plant bags by ensuring that troughs containing the plants were refilled daily with water to keep capillary matting moist. Temperature and humidity were recorded every 30 minutes with a logger held beside the foliage on a stake in a pot, with a plant, set up as a spare.

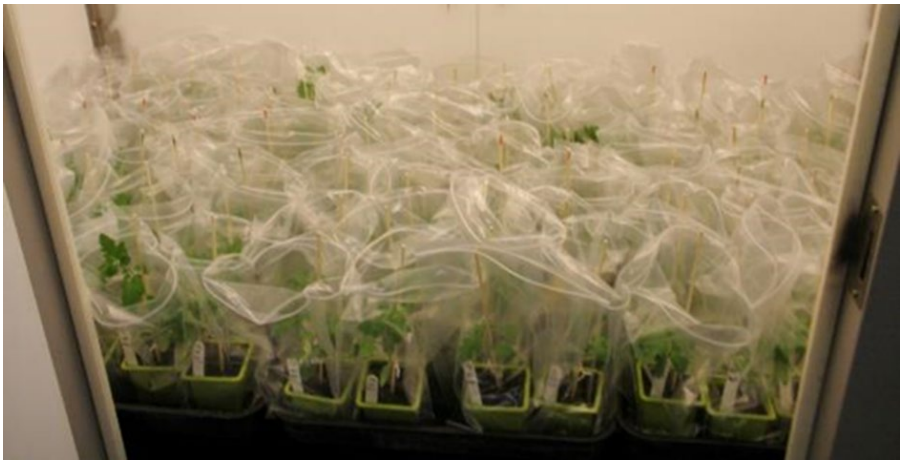

Figure 1. Tomato plants in the controlled environment cabinet

Experimental Design

The first experiment starting on 1 July 2019 used three replicates of four AQ 10 treatment timings, given either 24 hours before powdery mildew inoculation of tomato plants, or 24 hours, 4 days or 7 days after inoculation, plus an untreated and a water treated control (Table 1). Plants were sampled at 0, 2, 4 and 7 days after spraying (0 – 7 dpi) to assess *A. quisqualis* persistence. Powdery mildew colony growth and any greying of the mildew caused by parasitism was recorded in conjunction with the *A. quisqualis* colony sampling of those plants.

Table 1. Treatments in July were based on AQ 10 application timing in relation to artificial powdery mildew (PM) inoculation of tomato plants.

Code	Spray treatment in relation to mildew inoculation	Mildew inoculation	No. of plants (0 dpi)	No. of plants (2 dpi)	No. of plants (4 dpi)	No. of plants (7 dpi)
T1	AQ 10 24 hr pre-PM	Yes	3	3	3	3
T2	AQ 10 24 hr post-PM	Yes	3	3	3	3
T3	AQ 10 4 day post-PM	Yes	3	3	3	3
T4	AQ 10 7 day post-PM	Yes	3	3	3	3
T5	SDW as for T1-T4	Yes	1			
T6	No spray	Yes	1			

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The experiment was repeated in August and September but this time only three AQ 10 application timings were used: AQ 10 application 24 hours before powdery mildew spores, 48 hours after mildew spores, or 7 days after powdery mildew application. Water controls with and without powdery mildew were also included (Table 2). Plants were assessed at 0, 4 and 7 dpi. On all occasions, plants were arranged in a randomised block design, with each of the three replicates in a third of the cabinet width. The treatments were randomised in each replicate, but pots of the same treatment for sampling at each of the dpi were adjacent to each other.

Table 2. Treatments in August and September were based on AQ 10 application timing in relation to artificial powdery mildew (PM) inoculation of tomato plants (T1 to T4) and to application of water alone (T5 to T8) at the same timings.

Code	Spray treatment in relation to mildew inoculation	Mildew inoculation	No. of plants (0 dpi)	No. of plants (4 dpi)	No. of plants (7 dpi)	No. of plants for mildew records
T1	AQ 10 24 hr pre-PM	Yes	3	3	3	3
T2	AQ 10 48 hr post-PM	Yes	3	3	3	3
T3	AQ 10 7 day post-PM	Yes	3	3	3	3
T4	AQ 10 24 hr pre-PM	No	3	3	3	3
T5*	Water 24 hr pre-PM	No	1	1	1	3
T6	Water 24 hr pre-PM	Yes	1	1	1	3
T7	Water 48 hr post-PM	Yes	1	1	1	3
T8	Water 7 day post-PM	Yes	1	1	1	3

*No AQ 10 and not mildew inoculated to compare with T1. No plants were set up as water controls without powdery mildew at the T2 and T3 timings because of restricted space in the cabinet. Total 72 plants: 54 mildew inoculated, 18 plants received no mildew.

Preparation of AQ 10 suspension

AQ 10 was supplied By Fargro Ltd and stored at 4˚C until use. A new packet was used each experiment. The concentration applied in each of the three experiments was standardised at 1 x 10^6 cfu / ml (based on optimisation work carried out earlier within the AMBER project). An initial stock solution was made up with 0.6 g AQ 10 added to 50 ml sterile water, soaked for 45 minutes with agitation and a further 50 ml was added, continuing agitation of the suspension. In July and August, sterile distilled water (SDW) was used to make up the product, but in September this was replaced by sterile tap water because a research paper (Anglei *et al.,* 2016) determined that germination of *Ampelomyces* sp. was poor in SDW but

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improved if some elements were present. Following counting of the spores using a haemocytometer, this stock solution was diluted to make up a spray suspension with a final concentration of 1 x 10^6 cfu / ml. 20 µl aliquots of the spray suspension were pipetted and spread on PDA plates as a check of product viability by the growth of mycelium.

Treatment of plants with AQ 10

Plants for the water controls were sprayed before those due to receive AQ 10 to ensure there could be no AQ 10 spores present in the air when the controls were sprayed. In July and August SDW water was used, but this was replaced by sterile tap water in September. The freshly prepared solution was transferred into a hand sprayer with the nozzle was adjusted to produce a spray of fine-medium droplets. The plants were sprayed individually until the first droplets started to coalesce on the leaves. Approximately 30 ml was used per plant of three expanded leaves. After treatment, once the leaves had just dried, open-topped transparent bags were placed up around the foliage of the plants. These were sealed tight against the side of each pot to create a humid environment. Lower leaves remained sheathed and so at high humidity throughout, but new growth was outside the bags by the end of the experiments.

Leaf Sampling

Plants were sampled at 0, 4 and 7 dpi in the same, first half of the day in which they had been sprayed. Three AQ 10 treated plants and one water sprayed plant were sampled at each time point, with two leaves sampled from each plant (one mature and one younger leaf). Leaf discs were sampled to target any powdery mildew colonies when visible about a week after powdery mildew inoculation. A record was made of the presence of mildew on the sampled tissue and if any of the grey-brown colouring typical of Ampelomyces parasitism was present.

A leaf disc of 9.7 mm diameter was extracted by perforating each leaf using the top of a 1.5 ml Eppendorf tube (Figure 2A). Discs were suspended individually in each tube containing 1.5 ml SDW (Figure 2B). Tubes were vortexed for 20 minutes to dislodge spore and aliquots of 100 µl were spread onto 90 mm diameter agar plates containing potato dextrose agar (PDA) supplemented with ampicillin (200µg per L), streptomycin (200µg per L) and chlortetracycline (20µg per L). The spore suspension was distributed evenly over the plates using a fresh sterile plastic L-shaped rod. This resulted in six leaf disc washings per treatment, each spread over three PDA plates.

Figure 2. A) Leaf disc punched from a tomato leaflet using the lid of a sterile Eppendorf tube. B) Tube containing 1.5 ml of water with a leaf disc.

The agar plates were incubated at 21°C in 16h of light and 8hr of dark. *Ampelomyces* colonies were counted from eight days after plating, although samples of plates were re-checked after this to see if any further colonies had developed. High levels of contamination were observed by other fungal and yeast species, and for this reason counts were only done on those areas of the plate that were free of contamination using a template gauge placed against each plate, which allowed the number of *A. quisqualis* colonies to be extrapolated to the whole plate. Results are presented as the extrapolated colony counts on each agar plate.

Powdery mildew colony growth recording

Mildew colony growth was recorded when each plant was sampled, with the latest assessment being made 14 days after mildew inoculation. For the July experiment, no mildew had developed in the anticipated timespan of the leaf sampling and thus additional recording was carried out on plants that were unbagged. It was thought possible that the bags had inhibited mildew development, in particular the production of spores (which cause colonies to become white). Records were made for 22/23 and 28/29 days after inoculation. In August and September, measurements were carried out between 3 and 22 dpi, to be able to compare the speed of powdery mildew hyphal growth across the leaf for colonies that did and did not subsequently show *Ampelomyces* sp. parasitism.

The percentage mildew cover on each of the lower two leaves was recorded. If a leaf dropped off, the next leaf up was recorded instead. A 0-3 mildew index was recorded of the colony being measured. This was 0 = no mycelium visible, 1 = mycelium just visible, 2 = white, 3 = white and powdery because of abundant spores. A 0-3 *Ampelomyces* parasitism index for the measured colonies was established since parasitised mildew develops a grey / brown colouration because of the development of chlamydospores. The colour intensifies with the

period of hyper-parasitism and is not easy to distinguish initially. The index was 0 = no greying, 1 = slight grey/uncertain, 2 = grey, 3= mildew colony obviously grey.

Results

July: Viable *Ampelomyces* sp. colony counts on agar from leaf disc washings

Detailed results are not given of colony counts for the July experiment because they are not believed to be an accurate representation of the persistence of AQ 10. There were on average 44 colonies of *A. quisqualis* per agar plate (range 0 to 231 per plate) from leaf washings made directly after spraying the four AQ 10 treatments (0 dpi). However, areas of many plates were lost to leaf fungal contaminants, including salmon-pink and cream-coloured yeasts, and a relatively fast growing *Penicillium* spp. (Figure 3).

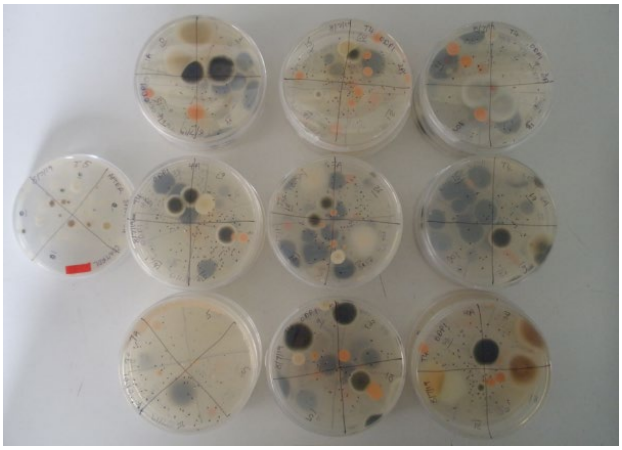

Figure 3. Underside of 18 agar plates of T4 (AQ 10 a week after mildew inoculation) from leaf washings on the day of spraying (0 dpi). Black dots mark *A. quisqualis* colonies

July: Development of powdery mildew and parasitism by *A. quisqualis*

Visible powdery mildew developed on only one of the plants (T4 for the 7 dpi counts on 15 July), when a colony of 5 and of 3.8 mm diameter was recorded on the third leaf up. After the final sampling of plants on 15 July, once the bags from replicate two were removed, more powdery mildew lesions started to be seen. By 19 July a further lesion had developed on T4 and a new lesion on T3. By 24 July, three plants of T1, one of T2, two of T3 and one of T4 had mildew lesions (ranging from 4 to 12.5 mm), but none were parasitised. By 28 / 29 days after powdery mildew inoculation (29 July) several grey areas of *A. quisqualis* parasitism were confirmed, principally on the lesions first seen on 24 July. Neither of the two powdery mildew inoculated control plants developed mildew.

All AQ 10 treatment timings in July had produced powdery mildew lesions on their second and third leaves (the first leaf having dropped off) with the preventative spray (T1) producing

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the most mildew lesions, with ten out of 17 lesions on one plant by 29 July. Visibly parasitised lesions were always the more mature (biggest) lesions on the leaves and no slowing of mildew growth was apparent, but new mildew spore production was reduced, with mildew colonies becoming velvety (and grey / brown) rather than powdery and white (Figures 4 - 5). Smaller mildew colonies were probably not started by the artificial inoculation, but from spores released by primary mildew colonies and so probably infected leaves when *A. quisqualis* persistence was likely to already be low.

Figure 4. Tomato leaf surface covered with powdery mildew mycelium, including erect conidiophores, the terminal cells of which had become brown because they contained pigmented *A. quisqualis* instead of terminating in chains of mildew spores. From a distance the colony appeared a buff-grey colour.

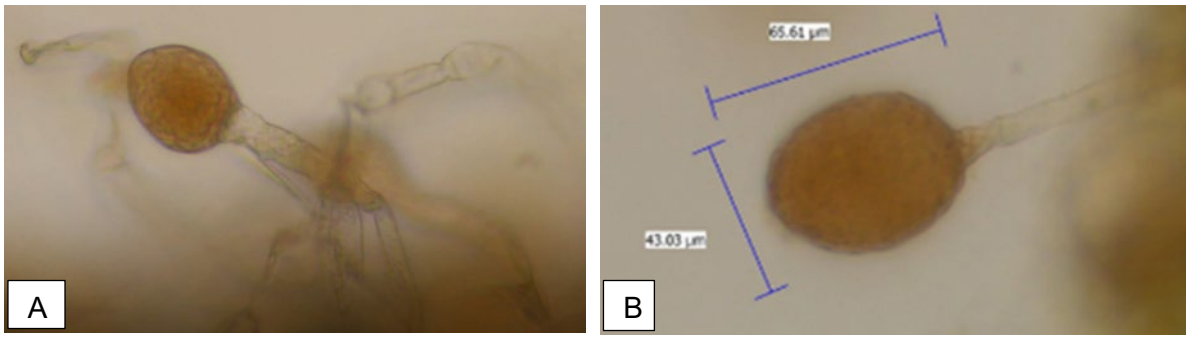

Figure 5. A) Terminal cell of powdery mildew conidiophore containing brown pigmented *A. quisqualis* which has started to form spherical bodies that will become spores. B) Powdery mildew terminal segment 65 x 43 µm of a conidiophore parasitised by pigmented *A. quisqualis*, to form the pycnidium of the parasite.

Greying attributed to *A. quisqualis* was only seen on colonies wider than 10 mm, before this diameter powdery mildew colonies were colourless (not white with mildew sporulation). The ratios of the number of grey lesions: white powdery mildew lesions for the three plants per treatment were 23%, 12%, 25% and 37% parasitised for T1-T4 respectively. One large lesion was half grey, half white (Figure 5B), all others were all grey and these could be only a few millimetres away from a white sporulating powdery mildew lesion of probable similar age. By six weeks the parasitised mildew colony growth was static (Figure 6B). When the tomato leaf

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upper surface was viewed under low power magnification, amongst the powdery mildew mycelium the erect terminal cells of the mildew conidiophores had become brown because they contain pigmented *A. quisqualis* instead of terminating in chains of mildew spores. The grey colouring of the colony was being produced by the mixture of healthy (white/colourless) and parasitised (grey-brown) mildew mycelium (Figure 4).

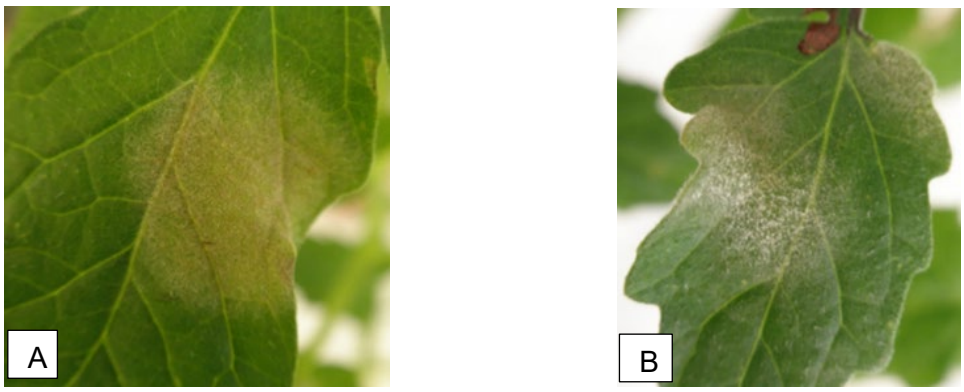

Figure 5. A) Powdery mildew mycelium on a tomato leaf caused to become grey and velvety through parasitism by *A. quisqualis* a month after AQ 10 applications and mildew inoculation. B) Abundant white sporulation of powdery mildew developing on part of a tomato leaf a month after AQ 10 application. The upper side of the colony has less sporulation and has become grey because of parasitism by *A. quisqualis*.

Figure 6. A) Powdery mildew parasitised by *A. quisqualis* following application of AQ 10 a month before. The mildew had covered half the leaflet and part of the midrib. Younger nearby powdery mildew colonies remained unaffected by the parasite. B) Six weeks after AQ 10 application a grey *A. quisqualis* colony remained over the powdery mildew colony it had halted. New powdery mildew colonies had subsequently established on the same tomato leaflet.

August: Viable *Ampelomyces* colony counts on agar from leaf disc washings

Overall, there was a decline in the presence of AQ10 on the leaves over 7 days, which is in keeping with previous observations. There was evidence that, at 4 days post application, there was more viable AQ10 on leaves for those treatments in which the AQ10 was applied

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after the powdery mildew (treatments T2 and T3) compared to the treatment where AQ10 was applied before mildew (T1) or where mildew was not applied at all (T4) (Table 3). However, this may have been caused partially by initial differences in the amount of AQ10 sprayed onto the leaves, as evidenced by differences on colony counts taken at day 0 (Table 3). By day 7, there was more viable AQ10 on leaves for treatment T3 (Q10 applied 7 days after powdery mildew) compared to the other treatments. There was no evidence that the AQ10 prevented the development of powdery mildew in this experimental system (see below).

Table 3. Mean colony counts from leaf washings in August from three replicate AQ 10 treated plants, two leaves per plant each producing three plates at 0, 4 and 7 days after *Ampelomyces* sp. inoculation.

		0 days after AQ 10 (0dpi)		**4 days after AQ 10 (4dpi)**		**7 days after AQ 10 (7dpi)**	
Treatment	**Mildew inoculation**	***A.quisqualis* colonies / plate**	**Mildew visible**	***A.quisqualis* colonies / plate**	**Mildew visible**	***A.quisqualis* colonies / plate**	**Mildew visible**
T1*	24h post T1	29.7ab	No	5.0a	No	6.2a	Yes
T2	48h pre T2	35.5bc	No	19.9b	Yes	4.2a	Yes
T3	7d pre T3	48.2c	Yes	25.6b	Yes	13.6b	Yes
T4*	No mildew	15.0a	No	6.7a	No	2.9a	No
s.e.d		8.44		6.06		3.61	
l.s.d.		16.83		12.09		7.20	
F. pr.		0.002		0.002		0.02	
d.f.		68		68		68	
Duncan's multiple range test		Within each assessment date treatments sharing the same letter do not differ significantly from each other					

* T1 and T4 leaf washings on the same date, washings were on later dates for T2 and T3

Fungal contaminants were present on the agar plates originating from the natural leaf flora of both the AQ 10 and the water sprayed plants for the leaf washings at all of the intervals after spraying. The colonies were principally of *Penicillium* spp., but also some salmon or white coloured *Fusarium* spp., and some leaves produced a salmon-coloured yeast. Across the experiment, the area of plate surface lost to contaminants was principally within the range of 3% to 25%. However, the plates for the last sprayed plants (T3 and T8) had worse contamination, with 26% to 80% of their surface areas lost. For most plates it was therefore necessary to extrapolate the number of *Ampelomyces* sp. colonies for the whole plate from the area that was able to be counted. No *Ampelomyces* sp. colonies were obtained from the leaf washings of T5, T6, T7 and T8 sprayed with water, confirming that this fungus was not

present on the tomato plants from either treatment at the nursery or by cross-contamination in the growth cabinet.

On the spraying days, counts ranged between 15 to 48 colonies on the agar plates across the treatments (Table 3; Figure 7). There was no significant difference between the mean numbers of colonies recovered from leaves of treatment T1 or T4. Treatment T2 (treated on 15 August) had a mean colony count similar to T1 and T3 (treated on 20 August). However, significantly fewer ($P<0.01$) colonies of *Ampelomyces* sp. (15 cfu) were washed off the T4 leaves than from either T2 or T3 (mean 42 cfu).

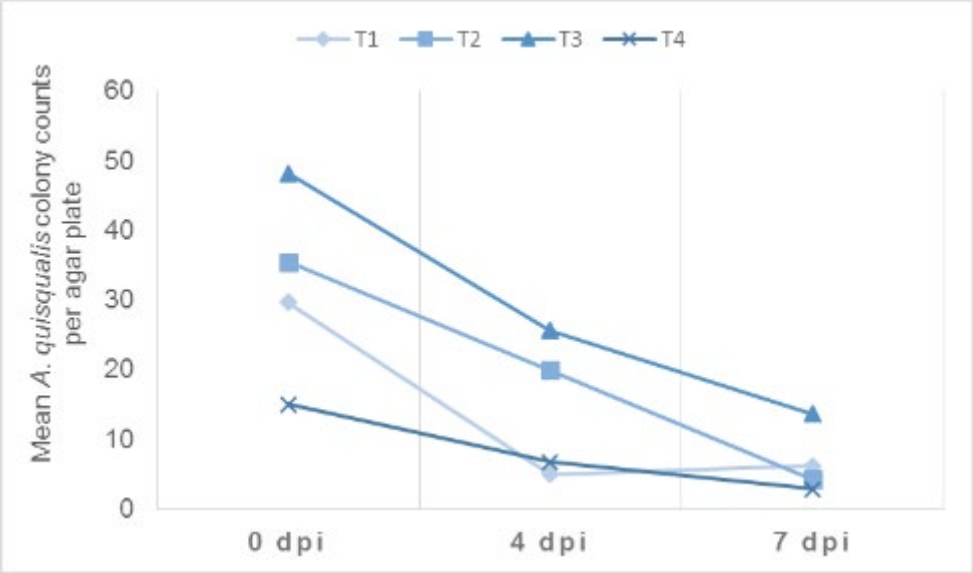

Figure 7. Mean *A. quisqualis* colonies per agar plate per treatment at three sample intervals 0, 4 or 7 days after spraying with AQ 10 in August 2019. Leaves were only taken once from each plant and so the lines do not show changes on the same leaf.

No mildew was visible at 0 dpi in treatments until it was seen on T3 and T8 on 20 August. Powdery mildew had been applied to T3 a week before they were sprayed with AQ 10 and powdery mildew hyphae had become visible (not yet powdery (index 1)) on two of the three plants of T3 due to be sprayed and sampled on 20 August. One plant had 15% mildew cover on each of the sample leaves, and the other had 30 and 20% on each of the leaves. No mildew was visible at 0 dpi in other treatments. The water control plant for T3, T8, had 15% mildew on its lowest leaf. None of these plants had any visible *A. quisqualis* parasitism.

By 4 dpi T1 counts had declined by the greatest amount to 16.8% of the original count. T4 had decreased to 44.7%, T2 and T3 showed a similar lower decline to 56.1% and 53.1% of their starting counts, respectively (Table 3; Figure 7). When T1 and T4 were sampled at 4 dpi on 16 August (three days after T1 had been mildew inoculated) their counts still remained

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statistically similar to each other, 5.0 and 6.7 cfu, respectively, but this was a noticeable decrease from the record at 0 dpi. On 16 August there was no mildew on either the AQ 10 sprayed plants or the water controls without and with mildew, T5 and T6. When plants of T2 were sampled at 4 dpi on 19 August (six days after mildew inoculation) just over half the number of colonies had grown than previously resulting in 19 cfu / plate. This was significantly more ($P<0.01$) than either T1 or T4 at the same interval. The T2 plants were heavily infested by powdery mildew (although not yet white), with lower leaves having 50%, 80% and 60% cover and no visible *A. quisqualis* parasitism. There was 40% mildew on each of the leaves of the inoculated water control plant (T7). The results for T3 on 23 August (10 days from mildew inoculation) at 4 dpi of 25 cfu / plate were similar to T2, but five times more ($P<0.01$) than either T1 or T4 at the same interval. Mildew had continued to develop on T3 plants.

By seven days after AQ 10 applications, the least decrease from the starting counts was in T3, with 28.2% of the original colonies viable. The greatest overall decreases (by an average 17.3%) occurred for T2 at 11.8%, T4 at 19.3% and T1 at 20.87% of their starting counts (Table 3; Figure 7). There was no significant difference between T1 and T4 at 7 dpi and on19 August there was still no significant difference between these treatments, with low colony counts again shown. The uninoculated T4 plants still had no mildew, but the lowest leaf on T1 replicate plants had 50%, 50% and 20% cover and two of the next leaves were also infested. The mildew was still not white, and no parasitism was seen. The control plant, T6, water sprayed before mildew inoculation, had only 10% mildew, not yet white, on each of its lowest two leaves and no parasitism.

By 7 dpi on 22 August, only 4 cfu / plate were retrieved from the mildewed T2 plants, similar to T1 and T4 after the same interval. Mildew, not yet white, on the three T1 plants covered 50%, 50% and 20% of their lowest leaves with some second leaves also mildew infested, but none on T4 at 7 dpi. No mildew colonies showed any greying due to *A. quisqualis*.

By 7 dpi for T3 on 27 August 13 cfu / plate were counted, significantly more ($P<0.05$) than in the other three treatments which either had no mildew inoculation (T4) or AQ 10 had been applied close to the time of mildew inoculation (T1 and T2). Powdery mildew continued to spread on the mildew inoculated plants for leaf sampling, so that by 27 August when the three T3 plants were sampled at 7 dpi the sampled lowest leaves of replicates 1 to 3 respectively had 75%, 50% and 98% cover and the leaf above had 1%, 30% and 30%. The mildew was white due to sporulation. The water sprayed mildew inoculated control plant for T3, T8, had 85% cover on its lowest leaf, and the mildew was also white.

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August: *A. quisqualis* parasitism development on repeat observation plants over one month

By 20 August, no indications of parasitism were present when mildew was first seen on all the inoculated treatments seven days after mildew inoculation. Fourteen days from mildew inoculation, one leaf on two separate T1 plants were given an *Ampelomyces* sp. index of 2 to record a grey colouration of a mildew colony. The mildew on these leaves covered 80% and 85% of the surface and had a mildew index of 3 (sporulating). By the next week, 22 days after mildew inoculation, one of the leaves with potential parasitism had become necrotic because of the mildew and had dropped off and on the other leaf the grey colouration could not again be detected. Twenty two days after mildew inoculation, a check was made for *Ampelomyces* sp. parasitism on any leaves on both the observation and the sampled plants. One or more lower leaves had dropped off by this time. Some apparent greying of the mildew on some remaining leaves was seen, but when this was checked under a dissecting microscope it was determined to be caused by underlying necrotic leaf tissue (resulting from the mildew). Thirteen leaf sections of mildew colonies from across the AQ 10 treatments were examined under the compound (high power) microscope for any parasitism not visible to the naked eye. On one observation plant (T3 in Replicate 2) there was browning within a mildew hypha on the leaf surface, and within mildew conidiophores (Figure 8). These structures resembled those of more advanced pigmentation and parasite spore formation seen in plants of the earlier experiment and confirmed to be *Ampelomyces* sp. No other parasitism was seen.

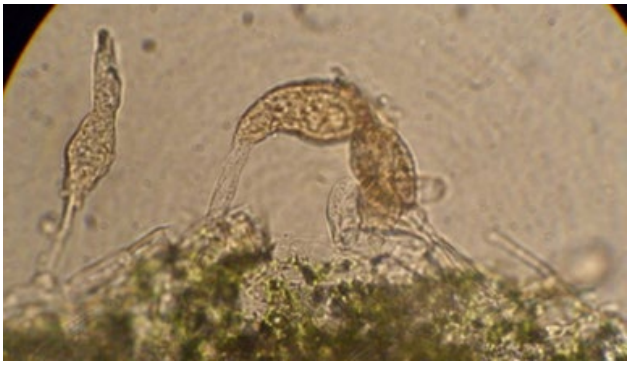

Figure 8. Tomato leaf cross-section (leaf tissue at base) with powdery mildew conidiophores on the leaf surface which have become pigmented by *A. quisqualis* parasitism.

August: Powdery mildew development on observation plants

Plants were temporarily removed from their plastic sheaths for observation of mildew development at three, seven, 14 and 22 days after mildew inoculation. Mildew cover was recorded on the leaves expanded at the time of inoculation (Figure 9; Table 4). After powdery mildew inoculation to all treatments except T4 and T5 no mildew was visible on the

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observation plants after three days (Figure 9). Mildew was visible on the assessed two lower leaves per plant in all inoculated treatments after seven days (Figure 9; Table 4).

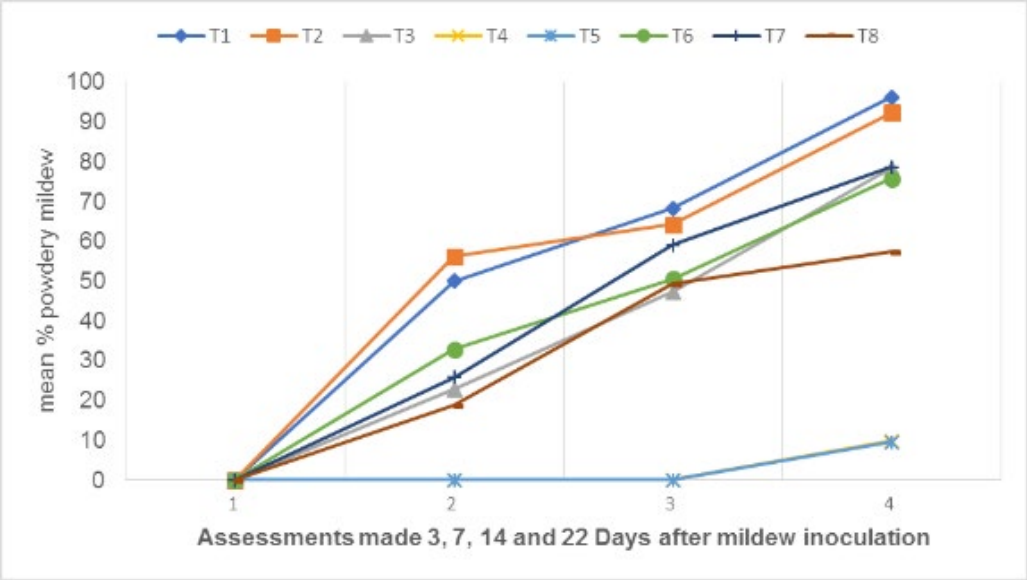

Figure 9. Mean % cover of powdery mildew on the lowest two leaves of three plants per treatment given repeated assessment at 3, 7, 14 and 22 days after powdery mildew inoculation of all but T4 and T5.

Table 4. Mean % powdery mildew visible across two leaves on each of three plants per treatment at 7, 14 and 22 days after plants were inoculated with powdery mildew on 13 August 2019 (except T4 and T5).

Treatment [to compare with water control]	**7 days after mildew inoculation** % mildew	**14 days after mildew inoculation** % mildew	**22 days after mildew inoculation** % mildew
T1 [T6]	50.0bc	68.3b	96.2c
T2 [T7]	56.2c	64.2b	92.3bc
T3 [T8]	† 22.8abc	47.2b	78.3bc
T4 no mildew	0.0a	0.0a	9.8a
T5 no mildew	0.0a	0.0a	9.5a
T6	32.9abc	50.5b	75.8bc
T7	25.8abc	59.0b	78.7bc
T8	† 18.7ab	49.2b	57.3b
s.e.d.	15.02	16.48	16.12

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l.s.d.	32.2	35.35	34.57
d.f.	14	14	14
F pr.	0.017	0.004	<0.001
Duncan's multiple range test	Within each assessment date treatments sharing the same letter do not differ significantly from each other		

† AQ 10 and water were sprayed directly after observations on this day.

After 7 days, T3 and T8 had a mean 20.7% powdery mildew and plants of T1 and T2 treated with AQ 10 around the time of mildew inoculation ranked above the other treatments, with a mean 53.1% mildew. The inoculated treatments T4 and T5 had no visible mildew and did not differ statistically from T8 untreated inoculated plants with a mean 18.7% mildew cover. T8 had less ($P<0.05$) than T2 with 56.2% cover. T2 plants had been sprayed with AQ 10 four days before. Observation plants of T3 had a mean 22.2% mildew cover on the lowest leaves on the day that T3 plants kept in the same growth cabinet received their AQ 10 spray. The plants due to be sprayed had a similar mean 13.3% cover on their two lowest leaves, resulting from leaf covers for Plant 1 of 0% and 0%, Plant 2 of 15% and 15% and Plant 3 of 30% and 20%. Mycelium at this time was just visible on the inoculated plants as a hyphal threads across the upper leaf surface (index 1), with no significant difference between treatments (Table 5).

Table 5. Mean mildew 0-3 index across two leaves on each of three plants per treatment at 7, 14 and 22 days after plants were inoculated with powdery mildew.

Treatment [to compare with water control]	**7 days after mildew inoculation**	**14 days after mildew inoculation**	**22 days after mildew inoculation**
	Mildew index	**Mildew index**	**Mildew index**
T1 [T6]	1.8b	3.0c	2.8c
T2 [T7]	1.8b	2.8bc	2.8c
T3 [T8]	1.0b	2.5b	2.2bc
T4 no mildew	0.0a	0.0a	1.2a
T5 no mildew	0.0a	0.0a	1.3ab
T6	1.5b	2.5b	2.7c
T7	1.3b	2.5b	2.2abc
T8	1.2b	2.5b	2.3bc
s.e.d.	0.37	0.21	0.44
l.s.d.	0.79	0.44	0.94
F pr.	<0.001	<0.001	0.010
Duncan's multiple range test	Within each assessment date treatments sharing the same letter do not differ significantly from each other		

A week later, 14 days after powdery mildew inoculation there was no significant difference in the mildew on inoculated plants (a mean 51.4%) (Figure 9; Table 4). There was still no mildew on the uninoculated plants. Mildew was clearly obvious on the inoculated plants, with leaves being white (index 2) and some with dusty abundant sporulation (index 3) (Table 5). The plants of T1 and T2 (the highest ranking treatments in mildew cover) had the most advanced colony development. All the leaves of T1 were sporulating abundantly, significantly (P>0.001) more than the plants of T6, T7 and T8 that had been sprayed with water instead of AQ 10.

At the final assessment of observation plants, 22 days after mildew inoculation, one or two lower leaves had been lost in most plants and records were switched to the next leaf or two up the stem (that would have been present at the experiment start). The only significant difference (P>0.001) between the inoculated treatments was between T1 (preventative AQ 10 spray) where the leaves were almost totally covered by mildew and T8 (water spray a week after mildew inoculation) where less than two thirds of the area was affected (Table 4). Powdery mildew, but not yet sporulating (Table 5) had spread from inoculated plants to the uninoculated plants, but with no difference between T4 (AQ 10 sprayed three weeks before) and T5 (water sprayed at the same time) resulting in a mean 9.6% leaf cover.

Viable *Ampelomyces* sp. colony counts on agar from September leaf disc washings

Overall, there was a decline in the number of viable *A. quisqualis* colonies over time on leaves, in keeping with the observations from the August experiment. The decline occurred over 7 days and did not appear to be related to the presence of powdery mildew. As before, there appeared to the differences in the amount of AQ10 applied initially to the leaves, with more AQ10 being applied in treatments T1 and T4 than T2 and T3 (Table 6). No *Ampelomyces* sp. colonies were obtained from the leaf washings of T5, T6, T7 and T8 that had only been sprayed with water on the same days as other plants received AQ 10. This confirmed from the 0 dpi sample that this fungus was not present on the tomato plants and from the 4 dpi and 7 dpi samples that there was not cross-contamination from AQ 10 sprayed plants within the growth cabinet. All colonies retrieved from the AQ 10 plants were thus from the product application. The results were thus analysed using only T1, T2, T3 and T4, with each having 18 replicate agar plates. The water sprayed and AQ 10 plants in the September experiment produced very few fungal colonies from the natural flora on their leaves in contrast to the August experiment.

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Table 6. Mean colony counts from leaf washings in September from three replicate AQ 10 treated plants, two leaves per plant each producing three plates at 0, 4 and 7 days after *Ampelomyces* sp. inoculation. T1 AQ 10 24 h pre-mildew, T2 AQ 10 48 h post-mildew, T3 AQ 10 7 days post-mildew. Discs from 18 replicate leaves washed / treatment

Treatment	P. mildew inoculated	*A.quisqualis* colonies / plate	*A.quisqualis* colonies / plate	*A.quisqualis* colonies / plate
T1*	24h post-T1	66.0b	14.0a	9.6ab
T2	48h pre-T2	19.2a	6.7a	5.7a
T3	7 d pre-T3	29.2a	13.9a	† 7.1a
T4*	No mildew	64.4b	33.0b	12.5b
s.e.d.		11.72	5.06	2.24
l.s.d.		23.39	10.09	4.47
F. pr.		<0.001	<0.001	0.019
d.f.		68	68	68
Duncan's multiple range test				

* T1 and T4 leaf washings on the same date, washings were on later dates for T2 and T3.
† T3 were the last leaves sampled (24 September) and the only leaves with powdery mildew.

On the spraying days, counts ranged between a mean 19 to 66 colonies on the agar plates across the treatments (Figure 10; Table 6). There was no significant difference between the mean number of colonies recovered from leaves of either treatment T1 or T4. There were significantly ($P<0.001$) fewer colonies washed from leaves for both T2 and T3, with similarly less than half the colonies recovered as from the other two treatments. Powdery mildew had been applied to T3 a week before they were sprayed with AQ 10, but unlike in August, no powdery mildew hyphae had become visible on the plants to be sprayed on 17 September.

When further plants of T1 and T4 were sampled at 4 dpi there had been a noticeable drop in *Ampelomyces* sp. colony numbers in T1, bringing the numbers down to meet those of T2 and T3 to give a mean 11.5 colonies. When leaf washing was done on another set of T1 and T4 plants at 7 dpi. T4 colony numbers had fallen to again be similar to those of mildew inoculated T1 (Figure 10; Table 6). Colony numbers were similar in T1, to those of T2 and T3 washed, a mean 7.5 colonies per agar plate.

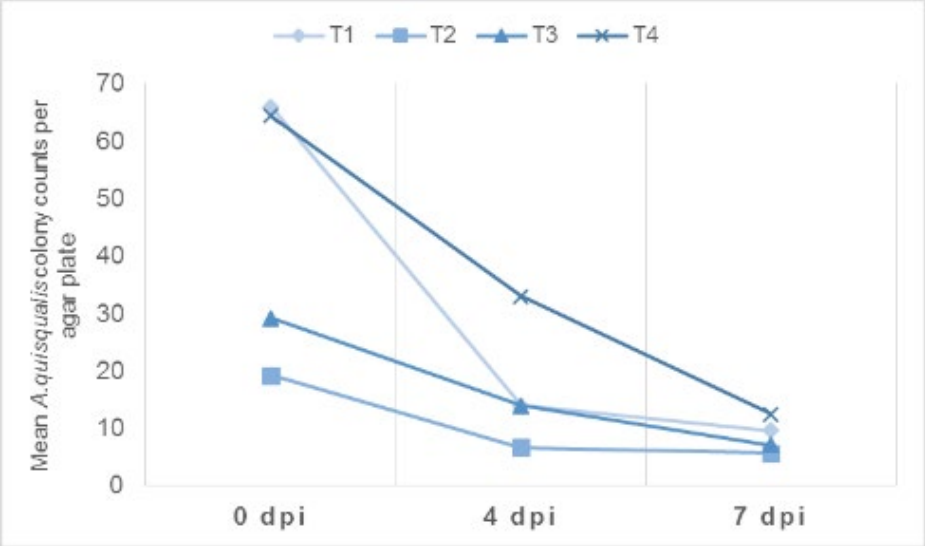

Figure 10. Mean *A. quisqualis* colonies per agar plate per treatment at three sample intervals 0, 4 or 7 days after spraying with AQ 10. Leaves were only taken once from each plant and so the lines do not show changes on the same leaf. Each agar plate held a 100 µl drop from 1.5 ml used to wash a 9.7 mm diameter leaf disc. September 2019.

Powdery mildew was first seen on any of the sampled plants by 24 September, it was on "leaf 3" of three plants of T3 and one of water control T8. By this time the lowest leaf on the plants had dropped off and so the one above "leaf 1" and the next up were sampled for the 7 dpi count. There was a faint mildew colony on each leaf which ranged from 3 mm to 9 mm diameter and these were taken for leaf washing. There was 30%, 2% and 50% mildew per leaf of T3 and 50% on the one T8 plant. There was no discolouration of the mildew colonies to indicate *Ampelomyces* sp. parasitism. Previously, in August, by this interval from inoculation, lower leaves on some sampled plants had over 50% powdery mildew cover.

Ampelomyces sp. parasitism was not visible on any of the leaves sampled for spore washing. On 26 September, 17 days after mildew inoculation, all the plants that had been sampled, and had been replaced in the growth cabinet, were re-examined carefully before disposal. Eight plants had potential grey areas and so suspect areas were either mounted directly for microscope examination or a sticky tape mount of the mycelium on the leaf surface was made on a microscope slide. They all had abundant powdery mildew conidia, but no brown mycelium or other structures produced by *A. quisqualis* were seen in either hyphae or conidia.

Some white powdery mildew was present on a few of the observation plants. The lowest leaf was usually yellow with the effect of powdery mildew infestation and had sometimes dropped off. On these leaves the mildew was not obvious (index = 1), but microscope checks showed that powdery mildew conidia were present. All the leaves on the plants were examined closely with a magnifying glass for signs of parasitism and no greying of colonies was seen. Mounts

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were made of the tissue and only on leaf 3 of T3 Replicate 3 plant was there any query, when a darker area of tissue, resembling the pycnidia of *A. quisqualis,* was seen in four of the mildew spores.

September: severity of powdery mildew on repeat observation plants

Observation plants for each treatment were examined for powdery mildew at three, six, nine, 13 and 17 days after inoculation. The percentage mildew cover on the lower leaves was assessed (Figure 11; Table 7) and an indication of the maturation of the colony was obtained by the mildew index recording the development of obvious sporulation (Table 8).

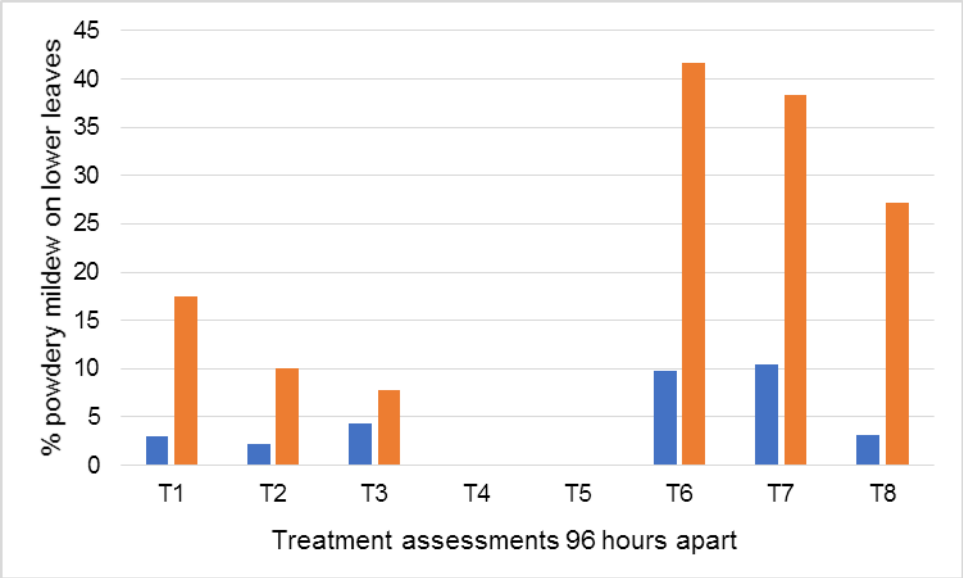

Figure 11. Mean % powdery mildew visible across two leaves on each of three plants per treatment at 13 days (blue bars) and 17 days (orange bars) after all except T4 and T5 plants were inoculated with powdery mildew on 10 September 2019. The water sprayed T6 and T7, and subsequently T8, differed significantly per date from the AQ 10 treatments T1, T2 & T3.

Table 7. Mean % powdery mildew visible across two leaves on each of three plants per treatment at 13, 17 days after plants were inoculated with powdery mildew on 10 September 2019 (except T4 and T5). No mildew was visible by nine days after inoculation.

Treatment [compare with water control]	**13 days after mildew inoculation** **% Mildew**	**17 days after mildew inoculation** **% Mildew**
T1 [T6]	3.0a	17.5abc
T2 [T7]	2.2a	10.0ab
T3 [T8]	4.3a	7.8ab
T4 no mildew	0.0a	0.0a

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T5 no mildew	0.0a	0.0a
T6	9.8b	41.7d
T7	10.4b	38.3cd
T8	3.1a	27.2bcd
s.e.d.	2.47	9.42
l.s.d.	5.29	20.21
F pr.	0.004	0.002
Duncan's multiple range test	Within each assessment date treatments sharing the same letter do not differ significantly from each other	

No mildew had developed by three or seven days after mildew inoculation. Thirteen days after mildew inoculation, plants which had received a water spray either a day before (T6), or two days after powdery mildew inoculation (T7) had significantly more (P=0.004) powdery mildew (a mean 10.1% of the lower leaves) compared with the other powdery mildew inoculated treatments T1, T2, T3 and T8 (Figure 11; Table 7). In T8, where the water spray was not given until seven days after powdery mildew inoculation, this higher level of mildew of the water controls was not seen, potentially indicating that mildew infection and spread was increased by wetting close to inoculation. This is supported by the mildew sporulation index on T8 being a little lower than on the other mildew inoculated plants, indicating that the colonies had started growth later (Table 8).

Table 8. Mean mildew 0-3 index across two leaves on each of three plants per treatment at 13 and 17 days after plants were inoculated with powdery mildew on 10 September 2019. Index 0 = no mycelium visible, 1 = mycelium just visible, 2 = white, 3 = white and powdery because of spores. At nine days after inoculation all treatments = Index 0.

	13 days after mildew inoculation		**17 days after mildew inoculation**	
Treatment [compare with water control]	**Mildew index**	**Duncan's test**	**Mildew index**	**Duncan's test**
T1 [T6]	0.39	b	1.56	c
T2 [T7]	0.33	b	1.00	b
T3 [T8]	0.44	b	1.33	bc
T4 no M	0.00	a	0.00	a
T5 no M	0.00	a	0.00	a
T6	0.33	b	1.67	c
T7	0.44	b	1.44	c

T8	0.28	ab	1.39	c
s.e.d.	0.137		0.164	
l.s.d.	0.295		0.352	
F pr.	0.023		<0.001	

By 17 days after powdery mildew inoculation, T6 and T7 had risen to cover a mean 40% of the surface of their lower leaves and there had been a jump in the mildew coverage on the other water control, T8, so that these were all not statistically different (Figure 11; Table 7). There was significantly (P=0.002) less mildew on all three AQ 10 treated plants and they were not statistically different from each other (mean 11.8% of lower leaf area). However, the preventative treatment, T1, was the worst affected and not statistically different from T8. Some of the mildew colonies by this time were becoming white, indicating the start of sporulation, but there was not the abundant sporulation (index 3) that had commenced in the August experiment using the same treatment regimes (Table 8).

Discussion

Overall, the results showed that *A. quisqualis* declined on leaves over a 7 day period, with a range of 11.8% to 28.2% of the original colonies present in the August experiment and 19.4% to 29.7% % in the September experiment. In addition, *A. quisqualis* was seen to persist on tomato leaves within parasitised powdery mildew until the end of the experiment after 30 days. Samples taken at 28 days after the July AQ 10 treatment, microscope examination showed abundant production of *A. quisqualis* conidia and the pigmented mycelium of the parasite in the mildew cells had not collapsed.

However, although the experiment was conducted at high humidity, the optimum condition required for *A. quisqualis* spore germination to occur within the first 10-20 hours after spraying (Kiss *et al*., 2010), control of powdery mildew was not good at any of the timings tested. In July, the powdery mildew was able to spread to colonies of 10 to 14 mm diameter before there was a change in the colour of the colony to indicate parasitism had taken place and most mildew colonies appeared unaffected. In August, powdery mildew had already turned white with mildew sporulation a mean 53% of the surface of the lower (sprayed) leaves by five days (T2) or eight days (T1) after AQ 10 application and only one leaf was recorded as having *A. quisqualis* in the mildew after a month. In September, powdery mildew mycelium was slower to grow than in August, but visible growing on the leaves 13 days after mildew inoculation. However, 4.3% of the leaf area was already covered in powdery mildew on plants sprayed seven days before (T3) (at the interval a re-spray is advised in crops), with earlier

sprayed treatments (T1 and T2) showing similar mildew at this time. No evidence of *A. quisqualis* parasitism of the mildew was found on any plants after a month from inoculation.

It is possible that the high humidity in the sealed bags in July delayed the sporulation of the powdery mildew, as tissue infection on tomato normally occurs within three to nine hours and, after haustoria develop to feed inside the leaf, conidia are produced in five days (White, 2012). White (2012) found there was no effect of humidity on *O. neolycopersici* sporulation on tomato, but quoted work by Jacob *et al*. (2008) in which sporulation was greater at 70% RH than at 85% RH at 22°C, with a significant reduction in sporulation at 99% RH.

The microscope examination of tomato leaves showed that discolouration of the powdery mildew resulted from the production of pigmentation in the parasitized cells. It was seen in the agar plates that the colonies took eight days to become pigmented at an incubation temperature of 21°C and it is possible that this could also be the case when growing in the host fungus. It is probable that the powdery mildew colony does not become visibly parasitised for over a week from infestation. Particularly if the powdery mildew mycelium and conidiophores have become dense then the increase in the strength of the pigmentation of the colony will be gradual and easily not seen initially (even with a hand lens). Once powdery mildew conidiophores are invaded then mildew spore production will cease, but previously formed mildew spore chains will still be adhering to the colony.

In curatively applied treatments T2 and T3, only on average around 50% the original number of spores were viable four days after application, however this was no better than for T4 which received no mildew. In contrast, the preventative treatment (T1) at 4 dpi had steeper drops in persistence of to 16% in August and 21% in September of the original count. This seemed to indicate that the arrival of powdery mildew spores within 24 hours of *A. quisqualis* arrival is detrimental to the parasite's survival but it may also reflect differences in the amount of AQ10 applied to the leaves. Alternatively, it is possible that this apparent lower viability after 4 days could have followed dislodgment in the leaf touching for powdery mildew inoculation in August, but this did not arise in September. Finally, if it is possible that the brief removal from the humid conditions in the growth cabinet caused the loss of viability of spores applied 24 hours before, then this would indicate that a continuous humid period for up to the first four days is crucial for the product.

In the September experiment, after a steeper initial decline overall there was less loss of viability than shown previously; T4 at 0 dpi had a mean 64 spores per plate (l.s.d. 23), falling to 51% of the starting count (33 spores, l.s.d. 10) and at 4 dpi they were 38% of the previous count (12 spores, l.s.d. 4) by 7 dpi. The final September count was 19.4% of the starting population, but this was an identical spore count (12 / plate) to the 2018 work.

In the August experiment the final count was lower (2.9), but only 15 colonies had been counted at 0 dpi. Interestingly, in August the overall reduction over seven days was to 19% of the starting count, the same as the September experiment. Re-application of the product is advised every 7 days and by this time from the 2019 work 19.4% of the original spores could be expected to have survived in the absence of powdery mildew (T4) and a similar mean 21.8% of the original number (range 11.8 – 29.7%) in the presence of mildew (T1, T2 & T3) under conditions of high humidity.

Whatever the starting colony count an assumption could be made that the proportion of spores losing viability over time will be similar. So, within 7 days of application at very most 30% of the original spores will still be viable (based on the 2019 experiments). Whether there would be control if powdery mildew spores arrive on leaves when *A. quisqualis* spores have been on leaves for a week was not specifically tested in these experiments. However delayed curative application control is unlikely to work as even when AQ 10 was applied close to powdery mildew inoculation the mildew then developed to cover half the lower leaf surfaces 7 days after mildew inoculation.

In the September experiment, plants sprayed with water either a day before or two days after powdery mildew inoculation (T6 and T7) had significantly greater mildew coverage than plants sprayed with AQ 10 at 13 days after inoculation. By 17 days, mildew had also increased significantly where water had been sprayed seven days after mildew inoculation (T8). This indicates that AQ 10 significantly reduced mildew development up to 13 days after application, (although without the recommended re-application of AQ 10 after a 7 day interval), then levels were then able to rise. However, the confidence in this statement is eroded because T8 (which received water 7 days after mildew inoculation, when T3 received AQ 10) also had as little mildew at 13 days after inoculation as the AQ 10 treated plants. Also, T3 had only been applied six days before M13 mildew observation so treatment effects might not expect to be seen so quickly. It is possible that water application increased the colonisation of powdery mildew in T5 and T6, perhaps by providing conditions for mildew germ tube development and plant host penetration, and AQ 10 had no effect on the mildew.

Evidence of parasitism was not seen in the August nor the September experiments (in all but one leaf). The conditions on the leaves inside the plastic sheaths provided a humid environment that is said to be required by the hyper-parasite, there was no chemical fungicide residue in the sprayers or on the plants that could have affected it, and by our calculations there should have been sufficient AQ10 present to give powdery mildew control.

In agar plates the *A. quisqualis* colonies each resulted from a cluster of hyphae which produced radiating hyphae resulting on a colony diameter of up to 2 mm after eight days of

incubation. It was not known if this speed of colonisation would have been the same within powdery mildew mycelium (or if hyphae grew without being inside a mycelium), but at a slow colonisation speed a collaborative attack would be required to penetrate the powdery mildew and enter at various positions. Powdery mildew mycelium grows rapidly on leaves, for example on tomato leaves over only four days a colony of 4.2 mm wide had grown to 10.1 mm wide, and another of 7.3 mm had spread to 9.9 mm, in records taken in the July experiment. It is clear that the *A. quisqualis* (as indicated by the manufacturers of AQ 10) needs to attack in the initial stages of powdery mildew colonisation.

Parasitism was observed in a few plants that had been inoculated in July with greying of the mycelium, the colouration being confirmed on microscope examination to be from the presence of *A. quisqualis* conidia in the mildew hyphae. This was seen 28 days after AQ 10 application. In some cases only half the mildew colony was grey, the remainder continuing to sporulate. The parasitism changed the colour of the powdery mildew colony and reduced mildew spore production, and so could be of use in a tomato crop, but for ornamental plants the marketability would still be lost whether the mildew was white or grey.

WP 2.2.2 Autumn / winter application of *Ampelomyces quisqualis* as a biofungicide against powdery mildew

The biofungicide AQ 10 is recommended for the preventative control of powdery mildew, or for application preventatively or when levels are below 3% coverage. AQ 10 contains the mycoparasite *Ampelomyces quisqualis* strain AQ10 that controls powdery mildew by developing inside the mildew forming its own spore-forming bodies within the mildew mycelium and the structures that would normally produce mildew spores. Good leaf coverage of the product is required, and it is unclear whether the addition of a wetter could aid this and increase efficacy.

Previous work in this project on AQ 10 was carried out in a controlled environment cabinet at 25°C on tomato plants in high humidity. Environmental conditions in UK unheated glasshouses in the last quarter of the calendar year will tend towards the lower temperature optimum for *A. quisqualis* infestation, but conversely the reduced sunshine and higher relative humidity compared with summer application could favour *A. quisqualis* persistence. Powdery mildew development is also likely to slow down in cooler conditions and so there is a possibility that any parasitism could keep pace with the pathogen.

Two efficacy experiments were therefore carried out in commercial crops of Hebe and Rosemary in different bays of the same unheated glasshouse between September and December 2019, to seek to record *A. quisqualis* spore persistence and the level of parasitism

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and powdery mildew control. In the Rosemary, plants with mildew below and above the 3% mildew cover guideline for AQ 10 application were compared. In-crop temperature and humidity loggers, as also being utilised by the nursery, were set up in the plots to record the conditions around the plants for the fungal pathogen and parasite.

Experiment 1: Hebe

AQ 10 is a bioprotectant containing the spores of the fungus *Ampelomyces quisqualis* strain AQ10. This fungus penetrates and invades fungal cells of powdery mildews including the spore producing structures, the dispersal spores (conidia) and the resting spores (chasmothecia) and kills by destroying the cytoplasm. It is approved for use against powdery mildew species on a range of named protected edible crops and has extension of use, EAMU 2646 of 2015, for use on a range of protected crops including ornamental plants and herbs. The technical notes provided by the UK distributors, Fargro (Anon, 2018) state application should be at 7 to 10-day intervals and that at least two applications are recommended (a maximum of 12 may be applied). The maximum individual application rate given on the EAMUs is 70 g / ha (the same as the label rate for protected strawberry, but approved rates on vertically growing protected edibles start at 35 g / ha for crops up to 0.5 m tall).

Application is recommended when humidity is increasing. Not in direct sunlight. At a temperature between 12°C to 30°C. Typically apply early morning or late evening. Apply at high water volume at high pressure with a fine spray. Thorough coverage of the leaf surface is required. No recommended water volume is given; sufficient should be used to ensure uniform coverage of the crop, without causing run-off (Anon, 2018).

The technical notes state that “the product should be applied when conditions are conducive to powdery mildew infection and development, but before mildew mycelium becomes established in the crop. It should be applied preventatively, or at low infection levels of less than 3% infected leaf area. Apply starting from the very first sign of mildew or when it is expected” (Anon, 2018).

Soaking the AQ 10 for 30 minutes to an hour before use is said to improve efficacy, greater than that produced by the use of an adjuvant. The product label recommends the use of AQ 10 with the adjuvant Nu Film P and the technical notes (Anon, 2018) explain that this is from mutual recognition of the German label and that adjuvants are not necessary. The notes give results from two trials on cucumber that show a small but statistically insignificant percentage increase in efficacy. The notes state that adjuvants are most valuable under conditions of low humidity and high temperature. Silwet L-77, Codacide Oil and Activator 90 are listed with Nu Film P as potential adjuvants. AQ 10 is stated to be compatible with Silwet L-77 and that for

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edible crops AQ 10 may have to be used at half the recommended rate based on the Silwet L-77 approval, but that efficacy at half rate is untested.

Following on from the information provided in the Fargro technical notes there were a number of areas of guidance to growers that merited following up and the efficacy of AQ 10 on measuring, particularly when considering its application on a crop permitted under EAMU. There were thus two considerations; a) if application is carried out before powdery mildew mycelium has become well established would the hyper-parasite survive until it is able to contact its host, and b) would there be any greater efficacy in using a spreader with the AQ 10? Two hosts, Hebe and Rosemary were selected that were usually prone to powdery mildew infection in autumn at the host nursery. These also provided different surface textures on which spray deposition and retention patterns could differ; Hebe plants have smooth, shiny flat ovate leaves, whereas Rosemary plants have needle-shaped leaves with a rough surface held fairly erect, and so were likely to differ.

Materials and methods

Two crops susceptible to powdery mildew were selected at the end of August 2019; *Hebe x Franciscana* "Variegata" (shrubby variegated form) and *Rosamarinus officinalis* "White Water Silver" (prostrate form). Both are infected by species of Erysiphe. Both sets of plants had just been potted-on into 3 L pots at the end of August 2019. The products applied and details of the treatments are given in Tables 9-11.

Table 9. Products applied, manufacturer, formulation type and ingredients

Product	Manufacturer	Type	Active ingredients
AQ 10	Biogard	Water dispersable granule	58% *Ampelomyces quisqualis* strain AQ 10. Minimum 5.0 x 10^9 spores g^{-1}
Silwet L-77	De Sangosse	Liquid	Minimum 80% w/w polyalkylene oxide modified heptamethyl trisiloxane. Maximum 20% w/w allyloxypolyethylene glycol methyl ether.

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Table 10. Products, registration numbers, treatment codes and application rates to Hebe in October and November 2019

Products	Numbers	Treatment codes	Application rates
AQ 10 (*Ampelomyces quisqualis* AQ 10) Fungicide	MAPP 17102 PCS 05801	T2 & T3	Maximum dose rate of 70 g / ha, applied at 700 L / ha (10 g AQ 10 / 100 L of rain / borehole water, as standard at the nursery)
Silwet L-77 Organosilicone non-ionic wetting agent	ADJ No: 0640 PCS No. 00400	T3 & T4	0.05% of final spray solution (0.5 ml / L). Added after 45 minutes pre-soaking of AQ 10 in half the final volume of water

Table 11. Treatment codes, and treatments applied to Hebe at each of two timings in 2019.

Treat-ment code	Treatment timings	
	23 October 2019, seven days after powdery mildew inoculation.	6 November 2019, applications repeated seven days after the first
T1	Rain / Borehole water (nursery supply)	Rain / Borehole water (nursery supply)
T2	AQ 10	AQ 10
T3	AQ 10 plus Silwet L-77 surfactant	AQ 10 plus Silwet L-77 surfactant
T4	Rain / Borehole water + Silwet L-77	Rain / Borehole water + Silwet L-77

Study design and layout

The area was centrally placed within the multi-ridge unheated Venlo glasshouse on a commercial nursery. The plots of the Hebe trial were on the opposite side of a main concrete pathway to a bed of Rosemary (Figure 12). Both crops were marked out in randomised block designs, but as no sprays were applied to the Rosemary (as it was not inoculated and did not show any natural infection) no further details of the Rosemary layout are provided. There was a floor-standing fan unit one bed up from the Rosemary, diagonally across from Hebe plot 401, and this was set by the nursery to come on automatically to move air to give frost protection.

Figure 12. Layout of Hebe plots within a nursery bed used for the AQ 10 efficacy trial in 2019. Square plots, of nine plants each, up either side pathway are indicated by corner marking Ringot pegs of the same colour. The pegs visible in the distance belonged to the Rosemary trial laid out in a similar way to the Hebe trial. At the front of the photograph with white Ringot pegs are the Rosemary plants included in the Hebe trial sprays, alongside a plot of Hebe plants, to compare *A. quisqualis* leaf washings from the two leaf morphology types. A circulating fan unit for frost protection (white rectangular unit) is shown between the two crops.

Treatment with AQ 10 was planned to follow label directions and be either once powdery mildew became visible in the crop, or when disease was expected to develop. By 16 October no mildew had been seen in either crop. No powdery mildew was available to inoculate the Rosemary and so treatments were not started on this crop but monitoring for mildew continued. No further details of the Rosemary trial are provided as no powdery mildew developed in it and so the crop was not given any product applications. Powdery mildew was found on Hebe plants in another bay of the glasshouse and these were used to inoculate the Hebe trial with treatment applications starting seven days later on 23 October. The experiment on the Hebe is therefore described fully.

The Hebe plants were arranged within a bed of 20 x 64 pots, marking out plots of three rows of three. A double pot guard was defined around each plot. All pots of (200 mm diameter) were kept at the normal commercial spacing of about 25 mm between rims so that each plot of nine assessed plants was 650 mm x 650 mm. Within each plot the plants were numbered 1 to 9 to be able to follow disease development on individual plants.

The four treatments were randomised within six replicate blocks, with replicates split equally between two sides of a bed so that access could be gained to the plots from the pathways. Two further square plots, of nine uninoculated Hebe plants and of nine Rosemary plants moved from the Rosemary trial area were also set up at the end of the Hebe trial area beyond the end plots 304 and 604. These were sprayed on the same two days as the main trial with only the AQ 10 (T2) spray and leaf material sampled just after spraying and seven days later in order to compare *A. quisqualis* colony counts from the two different leaf types.

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Timing of procedures

The experiments on Hebe and Rosemary were set up in late summer, this being the period when powdery mildew was normally observed on these crops. From spray applications in October and leaf disc sampling for *A. quisqualis*, weekly assessments of powdery mildew and *A. quisqualis* parasitism continued into December 2019 (Table 12).

Table 12. Timings of plant assessments and spray applications at the nursery site in 2019.

Date	Summary of procedures carried out
28/08/2019	Areas of Hebe and Rosemary selected for trial.
18/09/2019	Plots labelled. Assessed for powdery mildew.
02/10/2019	Assessed for powdery mildew. Two Zensie temperature & humidity loggers set up within canopies of Hebe and Rosemary beds.
16/10/2019	No powdery mildew present on Hebe and so plants inoculated.
23/10/2019	Powdery mildew assessed. Treatment sprays on Hebe. Hebe leaf discs sampled for 0 dpi colony counts.
30/10/2019	Powdery mildew assessed. Hebe leaf discs sampled for 7 dpi colony counts. Treatment sprays on Hebe. Hebe leaf discs sampled for 0 dpi colony counts.
06/11/2019	Powdery mildew assessed. Hebe leaf discs sampled for 7 dpi colony counts.
13/11/2019	Powdery mildew assessed on Hebe.
27/11/2019	Powdery mildew assessed on Hebe.
05/12/2019	Powdery mildew assessed on Hebe.

Inoculation with powdery mildew

No powdery mildew was visible on the Hebe trial by mid-October (by when some foliage had grown 30 mm beyond pot edges), but a small bed of Hebe plants in another bay of the glasshouse was found to be diseased. These Hebe plants with sporulating mildew (Figure 13*)* were used to infest the trial plants. Although there was also no powdery mildew on the Rosemary trial no potential spreader plants were available to inoculate them at this time.

Figure 13. Heavily sporulating powdery mildew on the older leaves of a variegated Hebe from another bay in the same glasshouse.

On 16 October, Hebe plants cv. Tricolour were selected out from the bed of infested plants in order to have 24 plants with a similar infection severity of around 65% powdery mildew cover. One spreader plant was allocated to each plot. A card disc covering was put over the bark topping and the plant held sideways and hit once from above with a Ringot peg starting at central plant 5 then at each of the four corner pots and then turning the plant with the other side down to finish diagonally so that all 9 pots received a tap**.** In some cases, spores were seen to puff off, and infected leaves frequently detached and scattered over the recipient plants and were not removed. Inoculation of the Hebe trial was started at plot 101 at 12:35h at 20°C with hazy sunshine and finishing all 24 plots by 13:15h when it had become cooler, at 16.7°C, when a light drizzle entered via the vents and the fan nearby cut in. The next day was cold (4°C outdoors) and misty until mid-morning and there was drizzle in the afternoon.

The percentage cover of the whole plant by visible powdery mildew was assessed for each of the nine plants per plot. If the colony became grey, and so indicated parasitism by *A. quisqualis,* the lesion area affected was still included within the estimate of mildew cover (as the grey area would still affect marketability).

To provide additional information on the appearance of the disease on the plant a record was also made of the number of leaves visibly affected by powdery mildew. This showed where new colonies developed on previously unaffected leaves. Increase in lesion size on already infested leaves was indicated by an increase in the overall percentage cover on the plant.

The progress of powdery mildew sporulation was also recorded using an index for each plant for the commonest colony appearance, the stronger the colony growth the whiter and more powdery the colony could become. Failure of a colony to become dense could also be an indication of the early stages of *A. quisqualis* parasitism developing. A 0-3 index was used:

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- 0 = no powdery mildew mycelium, 1 = mycelium just visible, 2 = white, 3 = intense white and powdery because of spore production.

Parasitism by *A. quisqualis* was also recorded per plant, for any incidence of greying seen on that plant and then the commonest stage of development seen. A 0-3 index was used:

- 0 = no greying, 1 = slight grey / uncertain, 2 = grey, 3 = mildew colony obviously grey.

Additional observations were also made of the proportion affected of any mildew colonies with parasitism and photographs taken.

Leaf disc samples for colony counting of *A. quisqualis* were targeted to include leaves with mildew (as survival of the hyper-parasite was anticipated to be better here than on mildew-free leaves). Leaf sampling was completed before any visible parasitism was expected to have developed, and so where leaves were observed to be parasitised these were available for subsequent records.

Treatment application

On 23 October, spray application was made using the equipment used on the nursery for treating small areas of crop. This was a 20 L Cooper Pegler back-pack nursery hand pump sprayer (H pressure set inside tank) with a single lance with a flat fan nozzle F110 – 04 (BCPC F110 1.6/3). The sprayer, pipes and lance had been thoroughly washed out using the detergent All-Clear and well rinsed with water prior to using with AQ 10.

The recommended dose of AQ 10 for ornamental plants was used (70 g/ha) and selecting a water volume of 700 L / ha suitable to give coverage of the size and canopy density of the plants and as used on earlier experiments on the site. 0.2 g of AQ 10 was used in 2 L of nursery reservoir tank water. AQ 10 granules from a new packet were used and resealed and returned to a refrigerator in the laboratory for further use 7 days later. On both days, the product was made up and left to stand for 45 minutes in a part filled spray tank before stirring well and topping-up. The suspension was then kept agitated by walking movement and the manual pump-action. Spray-guard walls were placed around each plot in turn, between the pair of guard pots, and the plants given a pass with the lance (from the back to the front of each plot) aiming for the calibrated 1.6 seconds per plot of nine pots to deliver the required volume (Figure 14).

Figure 14. A plot of Hebe plants being sprayed with a single-nozzle lance from a knapsack sprayer. A single pass was made across the area. The neighbouring plots were protected from the spray using spray boards held between the guard plants during spraying.

Leaf disc sampling to count viable *A. quisqualis* colonies at 0 and 7 days after AQ 10 sprays

The counting of *A. quisqualis* colony numbers on agar plates was supplementary to the recording of mildew levels and parasitism as seen on the leaves. It was anticipated that in the glasshouse there would be a high level of surface contaminants on the plants including fungal and bacterial spores (both saprophytic and pathogenic), and that these would reduce the accuracy of plate counts by covering some of the agar plate surface that could have supported *Ampelomyces* sp. colonies. The colony count results were thus obtained to give an indication that viable *Ampelomyces* sp. was present on the plant. Sampling was concentrated on plants that had been sprayed with AQ 10, with some “check” samples taken from a treatment that was not sprayed with the bioprotectant.

The central plant in the plot (pot 5) was sampled from all six of the replicates of AQ 10 (T2) and all six of AQ 10 plus wetter (T3). Only three replicate plants were sampled on the water only (T1), taking from Replicates 1, 3 and 5. No samples were taken from plants sprayed with Silwet L-77 only (T4). Leaf discs were sampled halfway down the plant stems (Figure 15). Leaf discs of 0.97 cm in diameter were extracted using the top of a 1.5 ml Eppendorf tube to act as a punch, with the disc falling into the tube as the lid was shut. The tubes were filled with 1.5 ml of sterile distilled water. After the tubes were returned to the clean area a sterile pipette tip was used per tube to push the discs down and ensure they were fully submerged in the water.

For the two AQ 10 spray comparison plots at the edge of the main trial, leaf samples were taken from the central three plants of each plot of the Hebe and Rosemary plants. The same

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size and number of leaf discs were taken from the Hebe as for the main trial and the same number of whole leaves of Rosemary as of Hebe leaf discs were sampled. Individual Rosemary leaves were measured after washing to obtain their surface areas (which were around 20 mm x 2 mm), as they were not a consistent area like the Hebe leaf discs.

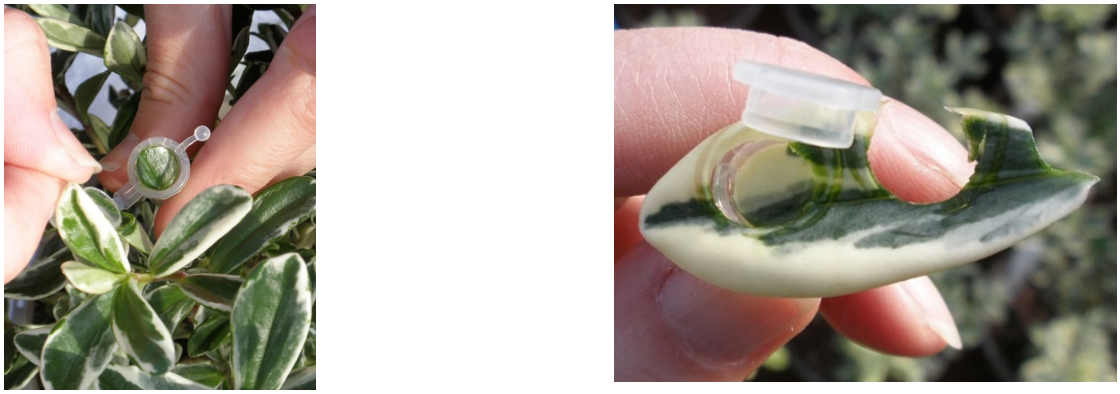

Figure 15. Leaf discs cut using the lid of an Eppendorf tube

On Day 0 (23 October) one leaf disc was taken from one leaf on the central plant. However, because examination of the incubated agar plates made from these leaves showed fewer than expected *A. quisqualis* colonies, for the 7 dpi samples on 30 October and 6 November the leaf surface area sampled was increased by taking two leaf discs into one Eppendorf tube from one leaf (Leaf x). However, in order to have a match to the leaf area washed for 0 dpi on the 23 October, one leaf disc continued to be taken on another leaf (Leaf Y). Different leaves on the same plant were used for leaf disc sampling on the different dates.

Timing of samples

Leaves fully expanded at the time of spraying were sampled at four times to equate to 0 and 7 days post inoculation (dpi) with AQ 10:

1) Day 0: as soon as the first spray on 23 October was nearly dry (0 dpi for first spray).

2) Day 7: just before the second spray on 30 October, 7 days after the first spray using the label recommended 7 - 10 day spray interval (7 dpi for first spray).

3) Day 7: as soon as the second spray on 30 October was nearly dry (0 dpi for second spray).

4) Day 14: on 6 November, 7 days after the second spray (7 dpi for second spray).

Removal of *A. quisqualis* colonies from leaf discs and production of colony count plates to assess AQ 10 viability

A “field laboratory” was set up in the nursery office to provide a clean area for putting *A. quisqualis* samples from leaf washings and the sprayer onto agar plates within a short space

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of time after they were taken as *A. quisqualis* spores were likely to germinate once hydrated and could become clumped together and so be counted on plates as a single colony and/or potentially lose viability.

On product application day, in addition to the leaf sampling, a 30 ml sample of the AQ 10 suspension was collected into a sterile universal tube from the spray tank. 10 µl of the spray suspension was pipetted and then spread over a 90 mm diameter potato dextrose agar plate supplemented with the antibiotics ampicillin (200 µg per L agar), streptomycin (200 µg per L agar) and chlortetracycline (20 µg per L agar).

Within about half an hour of sampling, the samples were vortexed to dislodge the *A. quisqualis.* 100 µl from each sample was pipetted onto a 90 mm diameter potato dextrose agar plate supplemented with the antibiotics ampicillin (200 µg per L agar), streptomycin (200 µg per L agar) and chlortetracycline (20 µg per L agar). Each 100 µl droplet of leaf washing was spread over the agar plate and individually sealed with “Parafilm”, All agar plates were incubated, inverted, at 21°C in 16h of light and 8hr of dark for eight days until the *A. quisqualis* colonies were big enough to be seen and counted. A record was also made of any microorganisms washed off the leaves and the approximate combined area of the agar plate that their colonies covered, obscuring / preventing any *A. quisqualis* colonisation. Checking for colonies was repeated two weeks after plating, as by then they were more-obviously pigmented and a little larger, but a further record of the spread of contaminant / leaf colonising micro-organisms was not made.

Environmental monitoring

Two Zensie screens were set up on 2 October 2019, at canopy height in the crop to record temperature and relative humidity every five minutes, one near the edge of the crop and the other centrally. Each had a flexible arm that was positioned to point onto the foliage surface to record leaf surface temperature. Records were logged every five minutes and transmitted via a relay to the nursery office and then via the telephone port to a file area on “The Cloud” managed by the Dutch company 30 MHz. It was possible for researchers to access the live information and all previous records using a “smart phone” and to gain an overview of the information using the “dashboard”. Records are held by 30 MHz on “The Cloud” for a year but were downloaded for local storage.

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Data analysis

Data analysis was not carried out because too few instances of powdery mildew infection were recorded on the plants to enable treatment comparison, and similarly there were insufficient records of *A. quisqualis* colonies from the leaf washings.

Results

Procedures and weather on spraying and leaf disc sampling days

On 23 October 2019, following a powdery mildew assessment the AQ 10 was soaked at 13:15 and 13:30 for T2 and T3, respectively. Applications to the Hebe commenced at 14:00 with the water sprays and were completed by 15:30. The air temperature at spray operator height was 19°C. As the spray started to dry, single leaf discs were sampled from one leaf of the central plant in the nominated pots of T1, T2 and T3 (none in T4) for 0 dpi, with vortexing to remove the spores commencing at 16:25 within two hours of AQ 10 application.

On 30 October 2019, the powdery mildew assessment was completed in the morning. The weather was cool, with weak sun and the air circulation fan was on. The leaves were dry when leaf discs were taken for 7 dpi from two leaves of the nominated central pots of T1, T2 and T3, (not T4), with one leaf per pot having two leaf discs removed, another leaf just one disc. Two leaf discs were roughly equivalent to two thirds of the leaf surface (Figure 15). The plots were then re-sprayed following the same procedure as the previous week, commencing at 14:30 and completing by 16:00. The weather had become cloudy with an air temperature in the glasshouse at spray operator height of 16.5°C, and RH 71.4%. The leaf disc per plant for 0 dpi was sampled from a single leaf in the nominated pots when wet specks of spray deposit were still visible on the leaves, the droplets too small to run off, half an hour after applying AQ 10 in T2 and shortly after spraying T3.

On 6 November, the plants were growing only slowly, with two new leaves per branch, but the plant canopies were starting to touch between some pots. The leaves were dry and there was weak sunlight with an air temperature of 12.5°C.

The results of powdery mildew assessments on Hebe are shown in Tables 14-16, for only those plants which showed powdery mildew at the weekly assessments, starting seven days after artificial inoculation, on the day of the first spray application. Mildew and Ampelomyces in mycelium were given Index 3 when most obvious. Little mildew developed, and for all but plant 504.6, it was on a green stem. The green stems were very infrequent across the pots and grew up above the variegated canopy of plants. There was the opportunity for 54 plants

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per treatment (six plots of nine plants) to develop powdery mildew, but in T1 (water) and T2 (AQ 10) it only developed on two plants in each of two plots of these treatments (Table 13; 14). In T4 (Silwet L-77), three plants in separate plots showed mildew (Table 17), although the single tiny lesion on plant 203.2 became darkened and grew no further a fortnight after first observation. No plant in T3 (AQ 10 + Silwet L-77) developed powdery mildew, but with such low infection incidence in the trial this could be a random occurrence (Table 14). As so few plants developed powdery mildew, no analysis to compare treatments was carried out.

Table 13. Hebe plants of T1 (water) on which mildew developed out of the nine plants per plot and six plots per treatment over six, weekly, assessment dates from 23 October to 5 December 2019. The mildew incidence on leaves and the severity per plant, and the incidence and discolouration intensity from parasitism by *A. quisqualis* (& lesion number).

Assessment date	T1 Plot.plant number	Total number of mildewed leaves / plant	% Mildew per plant	Mildew index 0-3 (mode)	Ampelo-myces index 0-3 (no.)
23.10.19	404.4	1	0.03	2	0
	503.2	2	0.4	2	0
30.10.19	404.4	1	0.03	2	0
	503.2	2	0.2	3	0
06.11.19	404.4	6	0.3	2	0
	503.2	4	0.1	3	0
13.11.19	404.4	7	0.75	3	0
	503.2	4	0.1	3	0
27.11.19	404.4	7	0.79	2	0
	503.2	5	0.05	3	0
05.12.19	404.4	9	0.85	3	1 (single)
	503.2	5	0.05	3	0

Table 14. Hebe plants of T2 (AQ 10) on which mildew developed out of the nine plants per plot and six plots per treatment over six, weekly, assessment dates from 23 October to 5 December 2019. The mildew incidence on leaves and the severity per plant, and the incidence and discolouration intensity from parasitism by *A. quisqualis* (& lesion number).

Assessment date	T2 Plot.plant number	Total number of mildewed leaves / plant	% Mildew per plant	Mildew index 0-3 (mode)	Ampelo-myces index 0-3 (no.)
23.10.19	304.5	0	0	0	0
	504.6	0	0	0	0
30.10.19	304.5	7	0.001	2	0

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	504.6	0	0	0	0
06.11.19	304.5	7	0.5	3	0
	504.6	1	0.25	3	0
13.11.19	304.5	12	0.3	3	0
	504.6	1	0.1	3	0
27.11.19	304.5	12	0.6	3	0
	504.6	2	0.55	3	3 (single)
05.12.19	304.5	19 (12 tiny)	1.0	3	2 (single)
	504.6	7	0.75	3	3 (single)

Table 15. No Hebe plants of T3 (AQ 10 + Silwet L-77) developed mildew out of the nine plants per plot and six plots per treatment over six, weekly, assessment dates from 23 October to 5 December 2019.

Assessment date	T3 Pots	Total number of mildewed leaves / plant	% Mildew per plant	Mildew index 0-3 (mode)	Ampelo-myces index 0-3
23.10.19	all	0	0	0	0
30.10.19	all	0	0	0	0
06.11.19	all	0	0	0	0
13.11.19	all	0	0	0	0
27.11.19	all	0	0	0	0
05.12.19	all	0	0	0	0

Table 16. Hebe plants of T4 (Silwet L-77) on which mildew developed out of the nine plants per plot and six plots per treatment over six, weekly, assessment dates from 23 October to 5 December 2019. The mildew incidence on leaves and the severity per plant, and the incidence and discolouration intensity from parasitism by *A. quisqualis* (& lesion number).

Assessment date	T4 Plot. plant number	Total number of mildewed leaves	% Mildew per plant	Mildew index 0-3 (mode)	Ampelo-myces index 0-3 (no.)
23.10.19	104.3	0	0	0	0
	203.2	1	0.03	2	0
30.10.19	104.3	0	0	0	0
	203.2	1	0.001	2	2 (single)
06.11.19	104.3	6	0.01	2	0
	203.2	1	0.001 dead*	1	3 (single)
	601.4	0	0	0	0

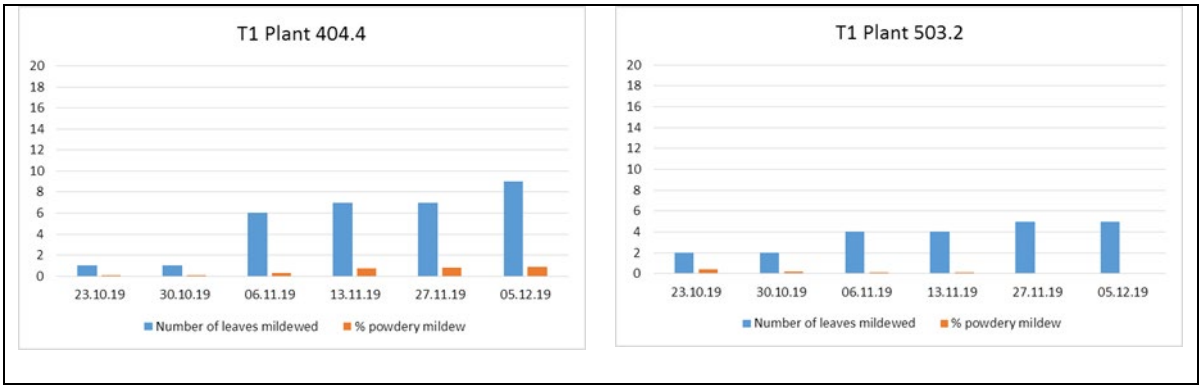
13.11.19	104.3	7	0.1	2	0
	203.2	1	0.01	0	1*(single)
	601.4	0	0	0	0
27.11.19	104.3	7	0.3	3	0
	203.2	1	0.001 dead*	0	1*(single)
	601.4	5	0.5	3	0
05.12.19	104.3	7	0.5	3	0
	203.2	1	0.001 dead*	0	1*(single)
	601.4	7	1.25	3	0

* The mildew colony speck became browner and ceased growth and finally disintegrated

Progress of powdery mildew and increase in severity on plants and individual leaves and evidence of *A. quisqualis* parasitism

Graphs for individual plants show the number of leaves affected by powdery mildew and the % cover mildew progress between 23 October and 5 December (Figure 16). The plants were quite uniform in growth, most commonly there were 10 leaves on each of 20 branches per plant, and therefore plants with greater coverage had more mildew colonies or wider colonies. Where % mildew cover on a plant increased over time this was principally through a combination of greater coverage on the same leaves and the mildew having colonised new leaves. New leaves were unfolding during the assessment period and so when mildew lesion diameters were increasing on the plants the overall mildew coverage was not always increased. A noticeable increase in lesion sizes (and some additional leaves with mildew on Plant 601.4) was recorded on 27 November, but healthy leaves had also grown, and so disease severity changed little.

A. quisqualis parasitism was only confirmed following AQ 10 application on plants 304.5 and 504.6, and only on one lesion each (Table 15), and its effect was to change the colour of the mildew colony not its size. Although the graphs chart the development of powdery mildew, too few plants per treatment were affected to highlight any potential treatment effects.



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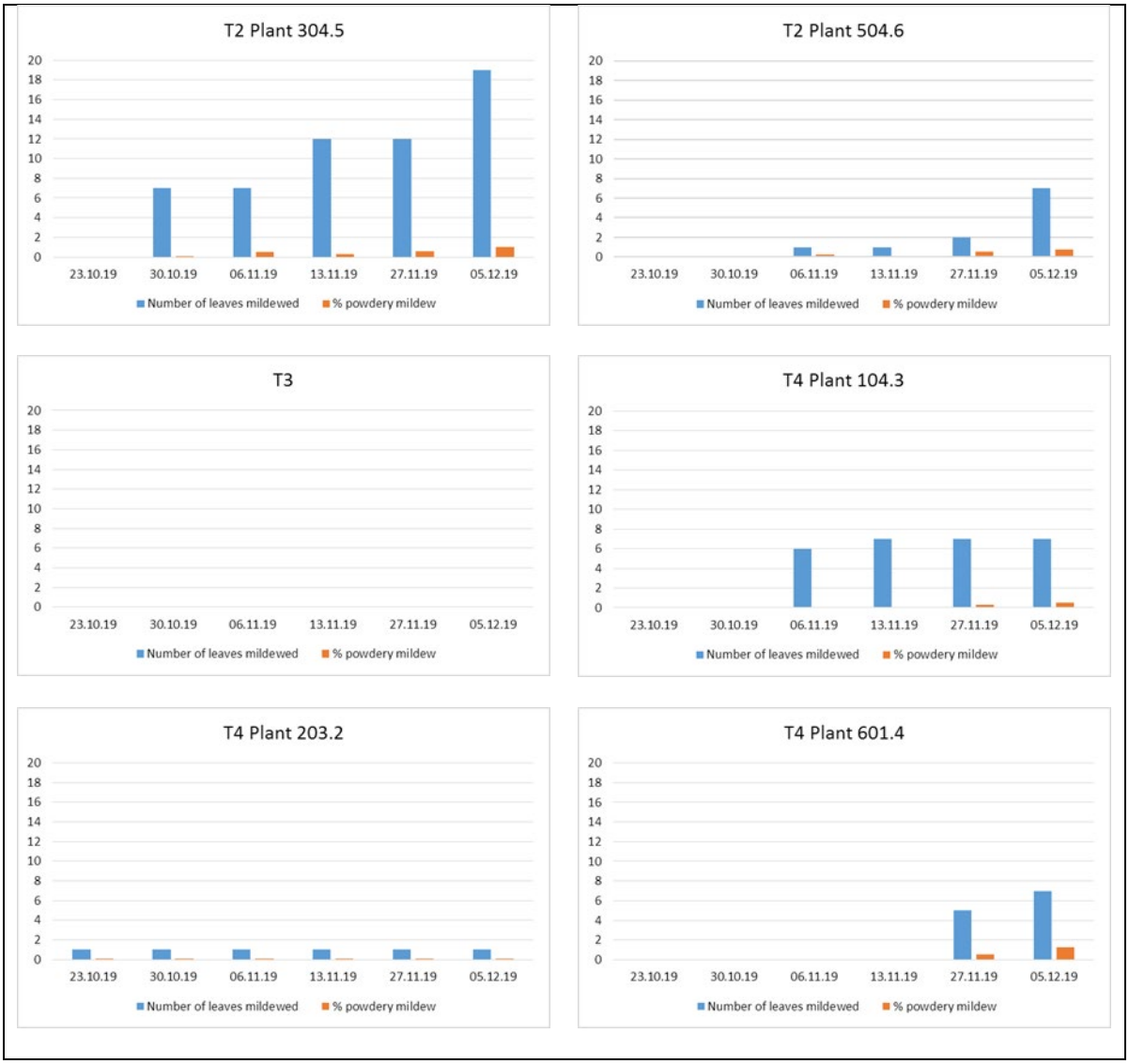

Figure 16. The weekly progress of powdery mildew on the seven Hebe plants affected; T1 (water), T2 (AQ 10) and T4 (wetter), plus a blank graph for T3 (AQ 10 + wetter).

Where mildew was seen, it was usually already white (Index 2) indicating that the mycelium had grown on the leaf surface and quickly thickened in the week after the previous assessment, and within a week most colonies then became very powdery with more spores (Index 3)(Tables 13;14;16)

On the first spray day, on 23 October a small colony of powdery mildew was recorded on a single (Figure 17) and a pair of leaves on plants 404.4 and 503.2 in T1 (water) and on one leaf on plant 203.2 in T4 (Silwet L-77) (Table 13; Table 16). By the second spray day, on 30 October, plant 3014.5 in T2 (AQ 10) (Table 14) had seven mildewed leaves, with no further leaves affected in the other treatments.

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Figure 17. Powdery mildew colony covering about 7% of the only affected leaf on plant 4 of plot 404 on 30 October 2019. A mildew index of 2 indicating sporulation, but not very thick. The lesion had virtually doubled in size by the 6 November when five other leaves on the same stem also then showed mildew colonies. Water sprayed plot (T1). Leaf ~20 mm long.

By 6 November, mildew was mainly comprised of initial starter colony specks, for example with 13 colonies covering 20% of a leaf on 404.4, but earlier colonies had also increased in size (Figure 17). Mildew cover on individual leaves ranged from 1% to 50%. The spot of mildew on Silwet L-77 sprayed plant 203.2 was grey and looked dead. The only variegated shoot with mildew was on AQ 10 treated Hebe plant 504.6 which had not shown mildew previously but was next to a guard plant with several badly mildewed shoots (the only guard plant with powdery mildew). There was still no mildew on the Rosemary trial at this date, but Botrytis was developing and due to be sprayed with a chemical fungicide by the nursery and so the experiment on this crop was terminated.

On 13 November, with weak sunlight when assessments were carried out two weeks after the second AQ 10 spray, no further branches had developed mildew, but some had more leaves with lesions and lesions had enlarged. The single mildew lesion on 203.2 (Silwet L-77) was dead. Overall, there was still little mildew and no parasitism by *A. quisqualis* had developed. Single branches of around 10 leaves were affected by mildew, mostly below 10% cover on individual leaves, although more than half of the leaves were usually mildewed. The only plant badly affected on more than one branch was in the discard.

There was a sudden fall in air temperature to below 6°C and falling humidity in the period 17 to 21 November. By 27 November 2019, the branches had grown to 30 cm tall and there were still no flowers. The weather outside was dry and at 09:30 around 9°C, but it was noted that condensation had formed on the plants under the then shut roof vent, causing water spots on the leaves of plots 104 and 302.

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The first record of *A. quisqualis* was on 27 November, with a single lesion (about 7 mm diameter) on the AQ 10 sprayed plant, 504.6 on a leaf about 75% covered by mildew (Figure 18). This was 36 days after the first AQ 10 application and had turned the mycelium obviously grey over about 10% of the mildew area on the leaf in the week since the previous observation. However, the other part of this mildew lesion and all other lesions on the plant were smaller and still sporulating well. Plant 504.6 in T2 was the only instance when “true” variegated leaves of this Hebe variety were mildewed.

By 27 November, new mildew lesions had appeared on a short green branch on plant 601.4 and had already covered leaves over a range of 5% to 60% mildew on their upper leaf surface. Across the pots with affected branches some smaller leaves were totally covered in mildew and 20% cover of a leaf was common. In pot 104.3 (Silwet L-77) the mildew spots were noted to have enlarged and the coverage on pot 304.5 (AQ 10) was much worse on the previously affected leaves.

On 5 December, the weather was cold and icy with weak sunshine. At the final assessment only seven branches, each on a separate plant, had become mildewed. The mildew was still only on the single green branch of each plant that had grown around 200 mm out above the variegated foliage of the plant canopy. The mildew had spread to most of the leaves (out of around 10) on the affected branch of each of the seven pots. Infection on individual leaves ranged from 1 to 80% mildew cover resulting in between 10% to 20% of the affected branch being covered in sporulating (Index 3) mildew. On pot 601.4 (Silwet L-77) in particular the mildew was noticeably worse than the previous week. On pot 404.5 (water control) an area of the mildew looked a little brown. On pot 504.6 (AQ 10 treated) the parasitised proportion of the mildew lesion was now 30% of the colony. Darkening of an area of the sporulating mildew was also seen on a leaf of pot 304.5 (AQ 10 treated). The original brown speck on plant 203.2 (Silwet L-77) had disintegrated and was not examined further.

No powdery mildew developed on the Hebe and Rosemary plots at the edge of the main trial and so *A. quisqualis* parasitism was unable to be observed.

Microscope examination of potentially parasitised mildew colonies

Leaves with discoloured powdery mildew mycelium were sampled on 5 December for laboratory examination. When scrapes of mycelium were taken onto a glass slide for high power microscope examinations, in places darker mycelium was found under the white sporulating mycelium. The brown / grey areas visible in the crop did not have floccose aerial mycelium, tending instead to be velvety. Confirmation of *A. quisqualis* presence was recorded from the partial-grey lesion of AQ 10 treated pot 504.6 (Figure 18). Mildew conidiophores

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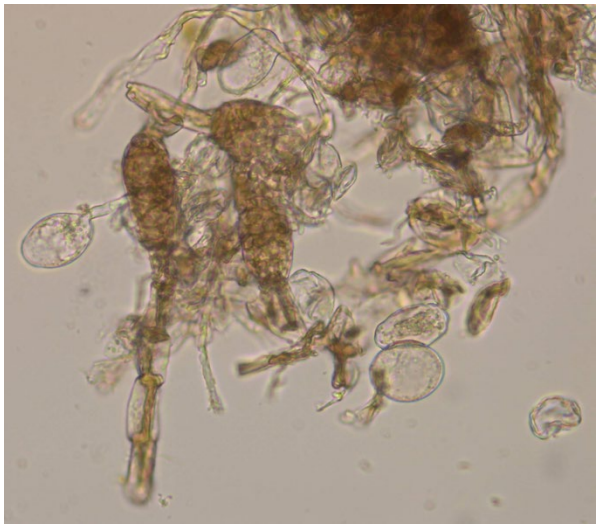
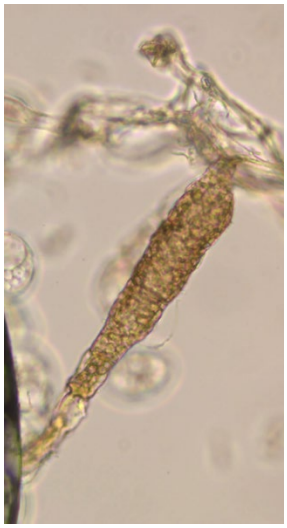
were seen with agglomerations of brown hyphae inside (much like the clusters seen on *A. quisqualis* colony count agar plates) or brown strands passing along the conidiophore with brown “plates” at cell junctions looking “dog-bone / dumbbell shaped”. There were swelling conidiophore heads were brown pigmented material inside that was probably the parasite. Swellings produced in the mildew mycelium were packed with *A. quisqualis* conidia and a spherical pycnidia ruptured under examination to release hundreds of the small elongated-oval spores of the parasite (Figure 19). There were also abundant powdery mildew spores that had no evidence of parasitism, having a colourless cytoplasm.

Figure 18. Hebe leaf of plant 6 in plot 504 (AQ 10 sprayed) with dense powdery mildew sporulation (index 3) covering most of the leaf, with a grey / brown approx. 7mm wide area of parasitised mildew mycelium (*Ampelomyces* sp. index 3).





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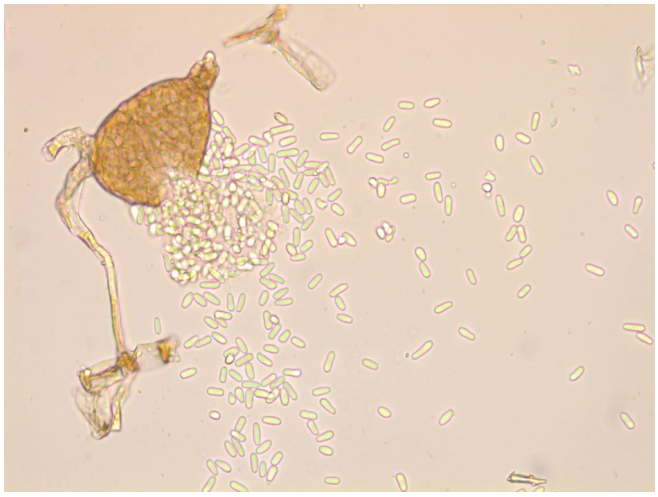

Figure 19. Brown pycnidia of *A. quisqualis* formed within the mycelium and conidiophores of powdery mildew on a leaf of Hebe 504.6. Oval mildew spores present. *A. quisqualis* spores are visible inside the pycnidia and in the lower photograph parallel-sided spores have been ruptured out. Examples shown of “dog-bone” shaped pigmented cytoplasm within destroyed mildew mycelium.

The leaf sampled from the water sprayed plant 404.4 (Figure 20) and the AQ 10 sprayed plant 304.4 (Figure 21) had specks of mildew that were very powdery and grey / brown under the high-power microscope. They both had a desiccated brown hyphal “dog-bone shaped” structure within a mildew conidiophore similar to that seen for plant 504.6. However, in the current cases the brown spheres associated with spore production by *A. quisqualis* were not also present and so *A. quisqualis* cannot confidently be confirmed (and would not be expected in plot 404, since this was a water sprayed plot). Powdery mildew spores filled with the colourless cytoplasm that indicated viable spores were abundant, confirming that powdery mildew spore production was continuing.

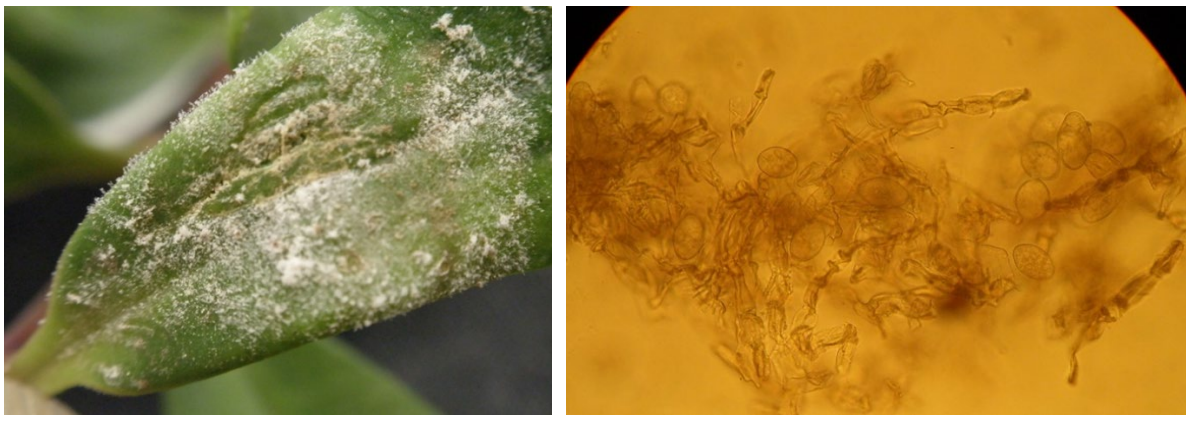

Figure 20. Hebe leaf of plant 4 of plot 404 (water spray T1) with diffuse discolouration of the powdery mildew (photograph taken after mycelium was sampled).

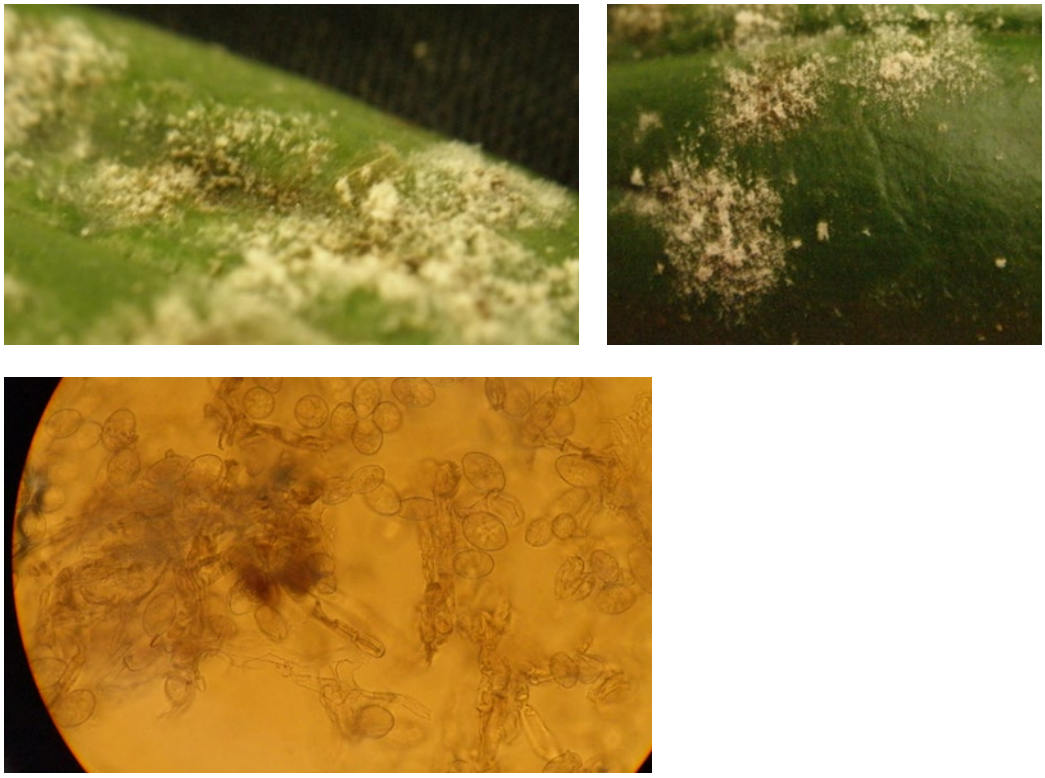

Figure 21. Hebe leaf of plant 5 of plot 304 (AQ 10 spray, T2) with grey / brown discolouration of the powdery mildew within a number of colonies. Viable (cytoplasm containing) powdery mildew spores were present, but no *A. quisqualis* spores.

Environmental conditions for powdery mildew and *A. quisqualis* development

Read-outs were similar for the plot edge and centrally placed Zensie loggers (about 1.5 m apart) and so data is presented for just the central logger (Figure 22-26). Canopy temperature was similar to the readings from the sensor arm focussed on a leaf surface (Figure 25). The logger readings from within the canopy (Figures 22-24) showed the temperature and humidity conditions for growth of both the powdery mildew and the *A. quisqualis* were most favourable earlier in October, as maximum (daytime) temperatures fell from 18.7°C on 5 October to 14.8°C on the 5 November to 5.3°C by the final assessment on the 5 December 2019. Throughout this period the minimum temperature was below 12°C, except on 11 October. The optimum temperature range of *A. quisqualis* is 12°C to 30°C and high humidity.

When the powdery mildew was inoculated on 16 October the weather was warm, but the records show within the following 10 days that maximum temperatures dropped steeply on the 20 October and 24 October to 13°C and minimum of 4 to 5°C. Humidity was mainly high (above 85% RH) around the plants during the two month observation period.

At the first spray on 23 October, the maximum temperature was 16.9°C, but minimum temperature then dropped for a day to 4.3°C, the humidity ranged from 93.8 to 75.2% RH on

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the 23 October. There was a steep dip in minimum temperature and humidity around the period of the second spray applications (Figures 22 -26) and may not reflect the conditions in the trial, because this data was taken from another logger in the same glasshouse after the connection to “the Cloud” was interrupted on the trial site logger. Other than in this period, in late October / early November, mean humidity records were on average above 88% RH, with minimum humidity rising in general for 63% RH on 5 November to 94% on the 4 December 2019.

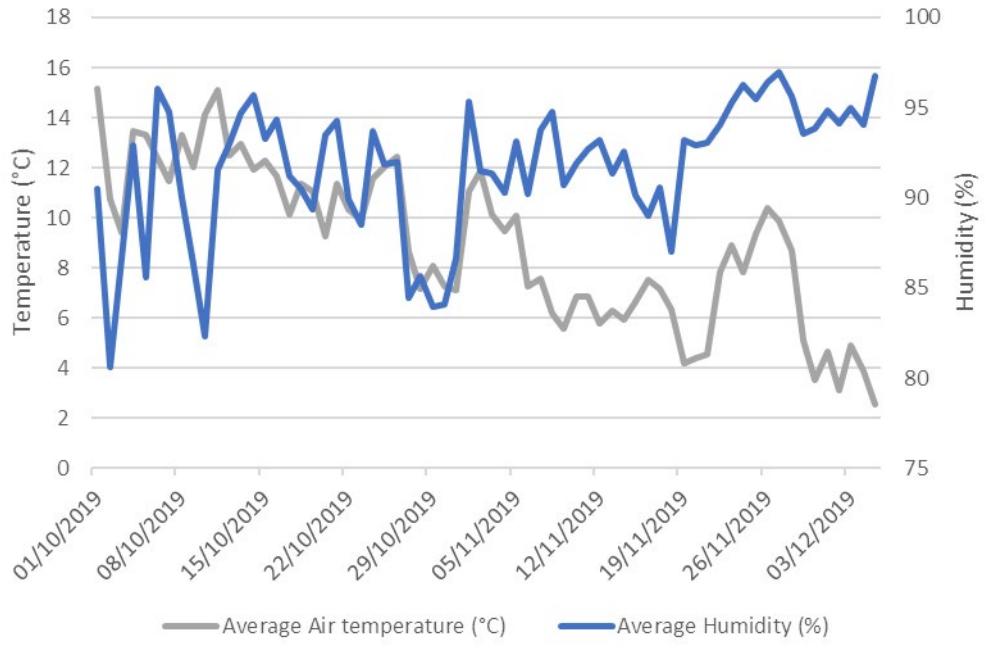

Figure 22. Mean daily air temperature (grey line) and relative humidity (blue line) between 1 October and 5 December taken from Zensie Sensor 08C3 in the centre of the Hebe trial. The data between 23/10/19 – 4/11/19 and 14/11/20 – 18/11/20 has been taken from another Zensie sensor in a crop in the same glasshouse due to data break transmission from the trial.

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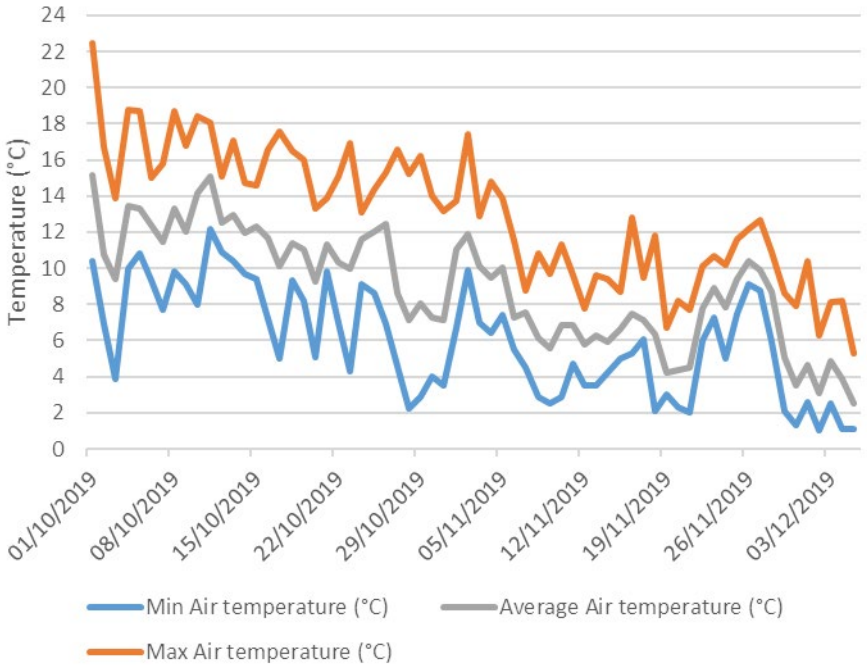

Figure 23. Minimum, mean and maximum daily air temperature between 1 October and 5 December taken from Zensie Sensor 08C3 in the centre of the Hebe trial. The data between 23/10/19 – 4/11/19 and 14/11/20 – 18/11/20 has been taken from another Zensie sensor in a crop in the same glasshouse as the trial to substitute for a data transmission break.

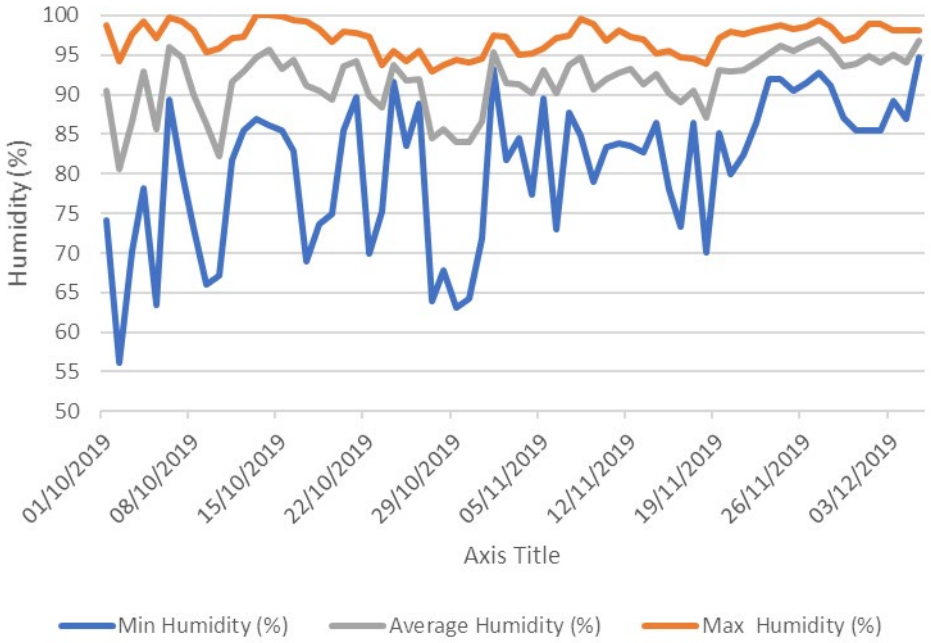

Figure 24. Minimum, maximum and mean daily relative humidity between 1 October and 5 December in the Hebe trial centre. The data between 23/10/19 – 4/11/19 and 14/11/20 – 18/11/20 has been substituted by that from another Zensie sensor in the same glasshouse, to fill gaps in data transmission from the trial.

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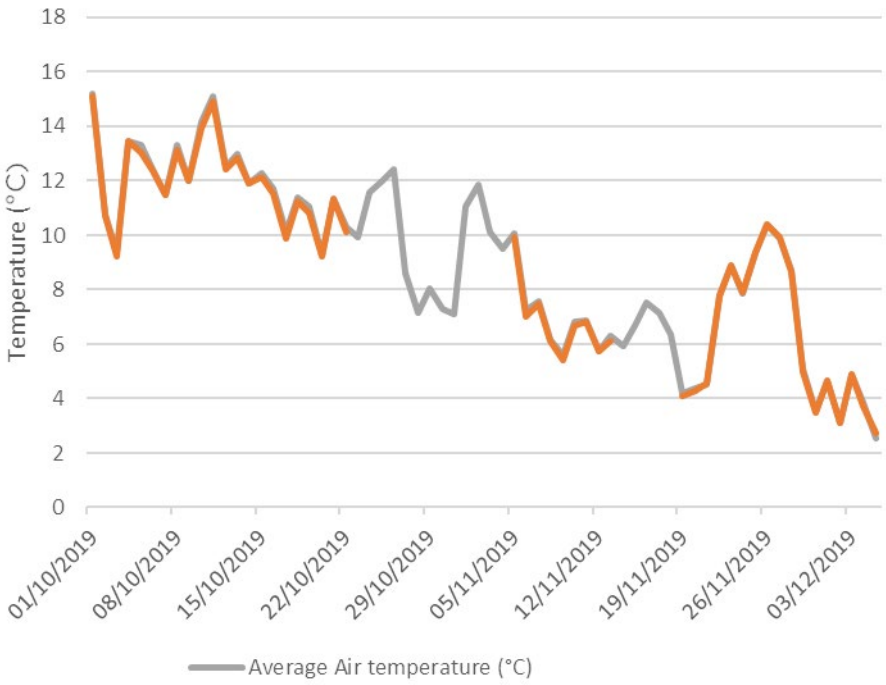

Figure 25. Daily mean air temperature and mean leaf surface temperature between 1 October and 5 December taken from Zensie Sensor 08C3 in the centre of the Hebe trial. The data between 23/10/19 – 4/11/19 and 14/11/20 – 18/11/20 has been taken from another Zensie sensor in a crop in the same glasshouse as the trial in order to fill in a gap in data transmission from the trial. Surface temperature has not been taken from the other sensor to fill the data gap since this might not be the same as for the trial crop.

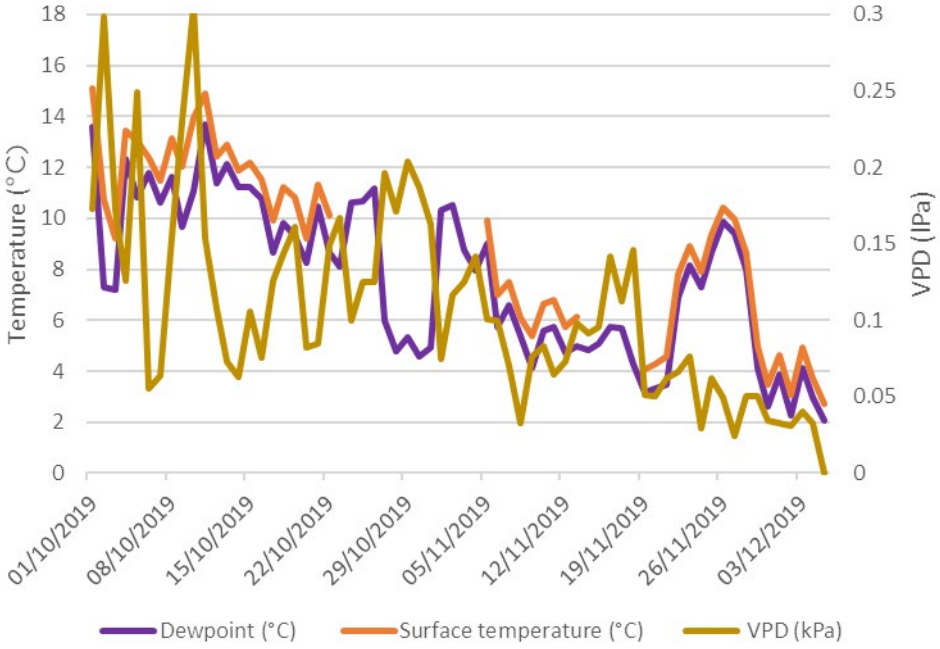

Figure 26. Mean daily leaf surface temperature and the calculated dewpoint and vapour pressure deficit between 1 October and 5 December taken from Zensie Sensor 08C3 in the centre of the Hebe trial. The data between 23/10/19 – 4/11/19 and 14/11/20 – 18/11/20 was taken from another Zensie sensor in a crop in the same glasshouse in order to fill in a gap in data transmission from the trial, except for the surface temperature of the other crop.

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Colony counts of *A. quisqualis* from leaf disc washing.

Good viability of the AQ 10 was shown from the 10 µl sample from the spray tank on 23 October, with 458 *A. quisqualis* colonies counted. The use of nursery non-mains water had not resulted in contamination problems, as only 5% of the plate area grew a white colony that was possibly *Trichoderma* sp.

The full tables of colony counts carried out to record the presence of viable *A. quisqualis* immediately after application of AQ 10 and seven days later are given in the Appendix (Tables 26-31). *A. quisqualis* colonies were readily identifiable provided they were left to grow for eight days until they started to become pigmented and about 1 mm diameter (half that of a pin head). Under the microscope the *A. quisqualis* colonies had a typical morphology of spherical clusters of hyphae at intervals along mycelial threads (Figure 27). Re-examination of the plates after another eight days produced no additional colonies, showing there was no delay to the start of the *A. quisqualis* growth once on the agar.

A range of fungi were washed off the leaf discs, and these are listed in order of dominance alongside each leaf disc washing record for the plot. The fungi were leaf saprophytes and potential pathogens (incubation of empty plates confirmed they were not from air-borne contamination of plates). Some fungi, particularly a fast-spreading, initially white, fungus that was later identified as a *Trichoderma* spp., *Botrytis* sp. and a white yeast, grew from a few starter-colonies to cover a significant area of the agar plate in the eight days required for the very slow growing colonies of *A. quisqualis* to able to be seen for counting. Bacteria are also likely to have been present on the leaves, but the antibiotics in the agar would have stopped them growing. In many plates “contaminants” left 75% of agar surface clean for *A. quisqualis* to grow on, but no *A. quisqualis* colonies were found. Where the agar was e.g. 33% “contaminated” there might have been other colonies washed off the leaf disc but hidden under the other fungi.

A single eight-day old colony of *A. quisqualis* on an agar plate, with mycelial strands (encircled by the blue ring). Salmon colonies were a yeast also washed off the Hebe leaf disc.

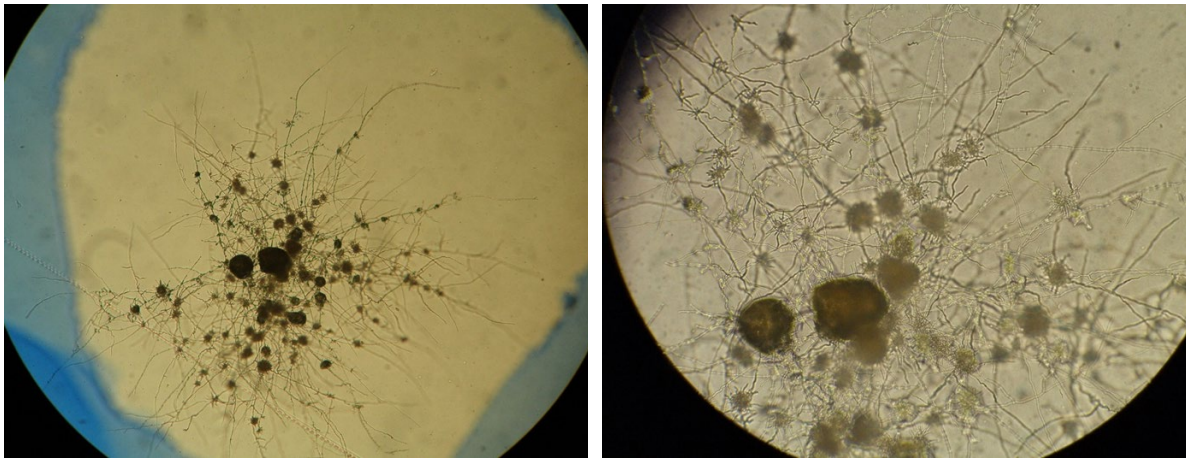

Figure 27. Colony of A. quisqualis washed from a leaf of plot 303 after growing on agar for eight days. Upper picture shows a colony as visible at counting without magnification. Lower pictures show high (left) and higher (right) magnification of the same colony. Hyphal clusters are formed at intervals along the mycelial threads growing from the propagule washed off the Hebe at 0 dpi after the second application of AQ 10 on 30 October 2019.

Only a total of five plates of T2 (AQ 10) and five T3 (AQ 10 + Silwet L-77) had any *A. quisqualis,* all but a T2 record seven days after the first spray coming from 0 dpi leaf disc washing on the two application days. This was out of plates made from 72 leaves (two sample dates each with 12 plates for 0 dpi and the two sample dates each with 24 plates for 7 dpi). None of the T1 (water) check plates grew any *A. quisqualis* (Table 17).

Table 17. Colony counts after eight days incubation of agar plates of *A. quisqualis* from 100 µl of Hebe leaf disc washings at 0 and 7 days (dpi) after 1st spray on 23 October & 2nd spray on 30 October 2019. Listed in order of sampling. Six plots of T2 = AQ 10, six plots of T3 = AQ 10 + Silwet L-77 and three plots of T1 = water sampled. Samples X & Y differed in leaf disc content. The area of each agar plate covered by other fungi washed from the leaves that reduced the area assessment that could be assessed, and the type of "contaminants" is given (key below). Full records for all samples are given in the Appendix.

Leaf sample date & time 2019	No. of leaf discs per sample. Treatment spray timing	dpi	Plot with colony count	Colony count *A. quisqualis* per 100 µl from 1.5 ml wash water	% of plate with "contam-inants"	Colony-type of "contaminants" (see key) ~. Given in dominance order
23 Oct.	One disc					
pm	of 1st T2	0	201	5	36	-
	of 1st T2	0	504	1	8	S
	of 1st T2	0	604	2	30	bacteria
	of 1st T3	0	101	3	40	-
	of 1st T3	0	202	1	35	S
	of 1st T3	0	303	5	30	P
30 Oct.	Two discs (X)	7		All 0	Mean 47	Include S,P,W,B
am	of 1st spray					

30 Oct. am	One disc (Y) of 1st T2	7	201	1	60	B,P
	of 1st T2	7	403	5	50	P
30 Oct. pm	One disc (Y) of 2nd T3	0	303	2	10	S
	of 2nd T3	0	602	1	22	S,P
6 Nov. am	Two discs (X) of 2nd spray	7		All 0	Mean 54	Include F,P,B,S
6 Nov. am	One disc (Y) of 2nd spray	7		All 0	Mean 25	Include S,W,P,F

plots where no *A. quisqualis* colonies were counted have been omitted from the table but are given in full in Appendix Tables 26-27 for 0dpi, Tables 28-29 for 7 dpi after the 1st spray and Tables 30-31 for 7 dpi after the 2nd spray.

~Key to colony types of fungal propagules other than *A. quisqualis* washed from leaf discs onto agar plates: B Botrytis, F Fusarium, P Penicillium, FW Fast white mycelium possibly Trichoderma, W White yeast, S Salmon yeast. Not recorded - .

Survival of *A. quisqualis* to seven days on Hebe in a glasshouse in Autumn was shown, but only from the washing on 30 October of the 23 October sprayed leaves, not the 7 November leaf washing (Table 18). This was in the absence of any visible powdery mildew on the sampled plants following mildew inoculation on 16 October. The number of colonies in the 1.5 ml of water in Eppendorf tube could have been 15 times the number put on the agar plate in the 100 µl sample (6.6% of the tube volume). Therefore, for e.g. five colonies there could have been 75 colonies on the 0.97 cm diameter leaf disc after AQ 10 application at the commercial rate, even after seven days.

From the Hebe and Rosemary edge plots, set up as preliminary work to compare *A. quisqualis* colony viability on the different hosts, only four *A. quisqualis* colonies from one Hebe leaf disc washed directly after the 23 October AQ 10 spray were obtained (full results at the bottom of Appendix Tables 26-31). It was noted that the leaf washings from the Rosemary leaves more frequently than on the Hebe resulted in Botrytis colonies on the agar that left little area of the agar plate uncontaminated. Botrytis was later seen on the Rosemary trial from which the plants were taken and required a chemical fungicide application because some branches started to rot.

Discussion

Low powdery mildew incidence and severity

When the experiment was set up at the nursery in August 2019 this period was one where the grower was anticipating the need for fungicides against powdery mildew on the Hebe and Rosemary crops, with control programmes for this disease on perennials usually continuing into the autumn (Hewson, 2010). Two beds with the required number of pots were selected for the application of AQ 10 once mildew was detected, but it is possible that their location in the house adjacent to the air circulation unit was less favourable to the disease because smaller batches of Hebe and Rosemary elsewhere in the house became severely infected (in the region of 30% powdery mildew cover). However, the relative humidity recorded within the canopy should have been high enough for mycelial growth on the leaf surface. Powdery mildews tend to be most active in warm conditions around 20°C, but spore germination and mycelial growth can occur between around 5°C to 30°C (Sall, 1980), and in the current work mildew colonies did continue to expand and sporulate on the Hebe into December when daytime temperatures had fallen to between 5°C and 8°C

It is also possible that these batches had infected plants amongst them which released spores over time to adjacent plants. One such spreader plant was seen in the Hebe trial and in commercial crops removal of such plants should be carried out, with their presence being a reason to trigger a preventative AQ 10 application on the rest of the plants.

One point to note was that high coverage of powdery mildew developed on some leaves and was confined to one Hebe branch on plants, resulting in a low % cover score across the whole plant and even lower over the whole bed. The guidance published to apply AQ 10 at 3% powdery mildew or below can only sensibly be followed if there is a more-widespread low level of visible mildew across the whole crop canopy such as resulting from starter colonies. The AQ 10 was unable, in the current experiment, to “catch up” with established mildew colonies and so in such situations it might instead be used to protect other leaves from mildew spores spread from the affected branch and the branch ideally be cut out.

Low incidence of *A. quisqualis* in colony counts and parasitism

The low colony counts soon after AQ 10 application were unexpected following much higher counts in the previous growth cabinet work using a standardised spore dilution. However, the commercial dose rate would still have given a reasonable density of around 75 colonies within a 10 mm diameter area of the leaf surface using extrapolation of count in 100 µl of leaf washing taken out of the 1.5 ml of washing-water. The volume of water in the Eppendorf could be reduced if the leaf discs were pushed further down before vortexing and so increase the

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spore concentration and increase the probability of colonies being sucked up for plating. A detergent, such as Tween 20, could be used in the vortexing, but as the washing at 0 dpi was done shortly after application the spores should have been easily dislodged without its use.

There was a possibility that leaf discs which produced no colonies were sampled from leaves with lower spray deposition than those from which they were counted. Observation of the spray pattern directly after spraying using the standard volume of water used on the nursery (700 L / ha) showed speckled droplets across the upper surface of leaves (Figure 28). Silwet L-77 use was expected to spread out or break the surface tension on the droplets so the AQ 10 spore suspension covered the leaf better, but no greater number of colonies were detected from these leaves (nor any greater mildew parasitism).

Figure 28. Spray droplets on a Hebe shoot directly after the first application of AQ 10 on 23 October 2019. Although the leaf surface was shiny the droplets did not run off the leaf.

There was a low incidence of *A. quisqualis* parasitism, but there was too small a powdery mildew incidence across the Hebe trial to be able to give any weight to comments on efficacy. However, where discolouration of the powdery mildew colonies was seen this was not until a month after the first applications of AQ 10. The same delay in visible evidence of parasitism was seen in the work earlier in this project on tomato plants in ideal conditions of high humidity at 25°C. This rate of destruction of the powdery mildew colony is too slow to stop the pathogen spreading, and in both the Hebe and tomato work partial colonisation of a colony arose. The sporulation of the mildew is stopped by colonisation of the conidiophores of the mildew (with the colony becoming grey / brown with velvety aerial mycelium). As seen by the *A. quisqualis* grown on agar it is not a fast-growing fungus compared with many foliar pathogens. The conditions in unheated glasshouses going into winter will at times be below the 12°C lower end of its optimum growth requirements (as given in the Technical Notes, 2018) and so it is even less likely to be able to keep up with powdery mildew growth. However, some autumns in the UK are mild and so AQ 10 could be effective, but consultation of a weather forecast prior to application is advisable. The use by growers of in-crop temperature and humidity

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recorders, with examination of their records in relation to the control level achieved, should provide a better understanding of the conditions required for satisfactory control of powdery mildews and aid decision making on fungicide product selection and timing.

Conclusions

- The Hebe plants in the trial did not develop powdery mildew naturally between 28 August and 16 October 2019, but another batch of variegated plants potted-up at the same time in the same glasshouse became severely infected and was used to inoculate the trial plants. The influence of very localised environmental and inoculum source conditions on disease development are likely to be important and impact on spray decisions.
- The Rosemary trial kept under observation through September 2019 also did not develop powdery mildew up to the 6 November, but plants in another bed in the same glasshouse had become badly infested by this date, highlighting the difficulty in being able to know when to apply AQ 10 based on detecting early disease presence or likelihood of infection.
- Powdery mildew failed to develop on the majority of plants following inoculation, and where it developed it was principally on green shoots that grew up from some of the variegated plants. The green tissue may have been more susceptible to the mildew than the variegated, or the above-canopy environment favoured the disease development.
- AQ 10 was applied when powdery mildew was starting to be seen on plants, however, most plants, whether or not treated, showed little increase in mildew in the two months following inoculation and cooler temperatures towards winter may have been less conducive to both *A. quisqualis* development, and powdery mildew colony enlargement with the development of new colonies from the sporulating lesions.
- Only one incidence of *A. quisqualis* parasitism was recorded on the Hebe from repeat application of AQ 10 at a seven-day interval. The addition of Silwet L-77 to the AQ 10 did not increase the incidence of parasitism, but more parasitism needs to be seen for valid comparison.
- One plant sprayed with just Silwet L-77 developed a small powdery mildew lesion that then darkened and died. It is not possible to say if this was the effect of the product.
- The single *A. quisqualis* parasitised lesion of a powdery mildew lesion recorded at the end of November took six weeks to become visible after the first AQ 10 application, and only colonised part of the lesion
- Similar, small numbers of viable *A. quisqualis* were retrieved from leaves either straight after AQ 10 application or seven days later, but no colonies were obtained from the

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majority of leaves. It is possible that viable colonies may have been washed off, but the amount of water needed for washing caused too great a dilution for detection in the small sample volume required for spreading on an agar plate.

- Retrieval of viable colonies of *A. quisqualis* after seven days showed that the fungus was able to survive on the Hebe leaves in the absence of any visible powdery mildew mycelium. It was not clear whether their presence at the end of October, but not after seven days in early November, was a reflection of different conditions at the later time causing early death or because fewer viable colonies were present and not detected.
- Too few Hebe plants became infected by powdery mildew to determine the efficacy of AQ 10 against this disease.
- The proportion of leaves with powdery mildew that showed parasitism by *A. quisqualis* was low which could have resulted from the cool conditions in the unheated glasshouse leading into winter, in which case the benefit of application of AQ 10 in late October in the UK remains to be determined.

Experiment 2: Rosemary

An area of rosemary cv. White Water in 3 L pots, with irrigation using a sand bed, in an unheated glasshouse was reported by the grower to be starting to show powdery mildew. The commonest powdery mildew species infecting rosemary (*Rosemarinus officinalis*) is *Neoërysiphe galeopsidis* (formerly *Erysiphe galeopsidis*), which has the conidial chains and surface mycelium seen on the nursery plants, but three others (all formerly *Erysiphe* spp.) have been recognised by German mycologists (Wichura *et al*., 2012). The glaucous foliage of rosemary means that the initial lesions of powdery mildew are less easily spotted during crop walking, and so it can be missed pre-sporulation. Instead of a chemical fungicide application it was agreed that AQ 10 could be trialled. Part of the knowledge gained from this trial was to be what level of control might be obtained from application of AQ 10 at this time of year. Only application in high humidity is advised on the product label, but the technical notes indicate application should be when temperatures are between 12 to 30°C, so application to the plots would be at the lower end of this range. Within the bed some plants had obvious mildew (at least 10% of the foliage was white with mildew), others had mildew that could be seen without lifting the pot up (3% or less of the foliage mildewed) and others had no clearly visible mildew if the pot was lifted for inspection.

The label for AQ 10 directs that application should be either before powdery mildew is seen or before 3% is visible. What 3% looks like in crops is not easy to interpret as it may refer to a low density coverage of mildew colonies across each plant, or a smaller number of larger

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mildew colonies across each plant. Alternatively, 3% could mean an average over a crop with hot-spots of infestation, or an average over plants with a part of the canopy clean.

In the following experiment the aim was to observe any level of control achieved by AQ 10 when applied to plants with three different mildew severity ranges, to see how disease development progressed on individual plants following AQ 10 application. The number of plants available on the nursery bed for the experiment meant that only one plant per severity range was able to be set up per plot.

Materials and methods

Experiment set-up

On 6 November 2019, eight rosemary plants were selected from the bed from each of the three severity levels then present;

a) High mildew (at least 10%, as an average across the whole plant),
b) Medium mildew (3% or less visible without picking up the plant for inspection)
c) Low mildew (no mildew symptoms visible when the pot was briefly picked up)

A plant of each category was arranged to form eight lines between other pots left *in situ* in the bed (Figure 29). Any severely mildewed plants flanking the test plants were swapped for those with less obvious symptoms. Plots of three pots wide by three pots deep were created with the test plants in the central strip. Eight plots were laid out, with half the plots to be sprayed with AQ 10 and the other four to be left untreated. Rather than completely randomise the AQ 10 plots across the bed, they were blocked together because of the potential for *A. quisqualis* parasitised mildew to produce *A. quisqualis* spores that could spread to the untreated plots.

On 21 November it became necessary for the nursery to use a chemical fungicide on the Rosemary bed to get a quick knock-back of the mildew which had increased in the week since AQ 10 was applied to test plants. The 24 observation plants were therefore taken out of the area of “discard pots” to be sprayed and put back flanking the edge of the bed beside where replicates 4 and 8 had been. The plants were arranged in two lines keeping their original plot order, with one line holding pots 101 to 403 and the other adjacent parallel line with pots 501 to 803. Half the bed away from the pots was sprayed by the nursery. No further nursery fungicide applications were used nearby on the bed during the observation period.

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Figure 29. Rosemary observation pots on 13 November (left) and 27 November 2019 (right). The lines of three labels mark high (orange), medium (blue) and low (white) powdery mildew affected plants assessed individually in the positions sprayed on 13 November. On 21 November pots were moved in plot order to one side of the bed.

Assessments

Assessments of the mildew levels on individual plants were commenced just before the plants were sprayed on 13 November. The second assessment was left until 27 November by which time *A. quisqualis* was expected to have caused pigmentation in the mildew colonies. Further assessments were made on 5 and 20 December 2019, keeping a record of the changes for each plant.

Assessments were made per plant of:

- % powdery mildew cover
- Number of shoot tips mildewed out of the total present per plant (average 30 shoots)
- Powdery mildew index, the commonest stage of infestation; 0 = none, 1 = mycelium just visible, 2 = white, 3 = intense white usually because of spore production
- % of mildew colonies grey through parasitism by *A. quisqualis*
- *Ampelomyces* sp. index to record that potential parasitism had been seen and the commonest development stage of such mildew parasitism; 0 = none, 1 = slight greying / uncertain, 2 = grey, 3 = obviously grey or grey / brown

When the final assessment was done an additional record was made per plant:

- Number of shoot tips heavily sporulating (powdery mildew index 3)

On 27 November some mildewed leaf samples were taken from the four high and four medium mildew category plants that had been sprayed with AQ 10 and pots 301, 303, 501 and 503 of the water sprayed. They were sampled with sterile forceps and placed in empty

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Eppendorf tubes. In addition, in case sampling dislodged parasitised mycelium some sections of transparent adhesive tape were placed on leaves to sample the surface. These tapes and the leaves were examined under a high-power microscope using either direct mounting of leaf sections, or transparent adhesive tape sampling of the leaf surface. Evidence of *A. quisqualis* parasitism of the powdery mildew mycelium was sought.

Treatment application

On 13 November, between 13:30 and 14:14h, spray application was made using the equipment used on the nursery for treating small areas of crop as was described fro the Hebe trial above. At the same time as the Rosemary plants were sprayed with AQ 10, two Hebe plants were moved beside the trial and sprayed. Two leaf discs 0.97cm were punched into an Eppendorf tube of 1.5 ml sterile distilled water from a leaf on each plant for spore washing and colony counting to compare with the deposition on the Rosemary leaves.

Samples to assess AQ 10 viability

On 13 November product application day, 30 ml samples of the AQ 10 suspension were collected into sterile universal tubes from four sources. These were from the sprayer tank before and after spraying, and from the spray lance at the start and finish of AQ 10 application. to the crop. All samples were treated as described previously for the Hebe trial. Two replicate plates were made per sample source (a total of eight plates). In addition, another couple of the agar plates were sprayed directly with a pass of the lance at the same 0.3 m height as had been used over the plants.

Two mature leaves were taken from a Rosemary plant in two of the replicates (plots 101 and 701) that had just been sprayed with AQ 10 and each leaf pair placed together in an Eppendorf tube containing 1.5 ml of sterile distilled water. These were vortexed to dislodge the *A. quisqualis* and 100 µl from each sample pipetted onto each of two potato dextrose agar plates, supplemented with the antibiotics ampicillin (200 µg per L agar), streptomycin (200µg per L agar) and chlortetracycline (20µg per L agar). Each droplet of leaf washing was spread over the agar plate before returning the plates to the laboratory. A record was made of the length and maximum width of each leaf so that the surface area sampled could be approximated.

All agar plates were incubated at 21°C in 16h of light and 8hr of dark for eight days until the *A. quisqualis* colonies were big enough to be seen and counted. A record was also made of any other microorganisms washed off the leaves.

Environmental monitoring

Two Zensie screens were set up at canopy height in the crop to record temperature and relative humidity, one near the edge of the crop and the other centrally. Each had a flexible arm that was positioned to point onto the foliage surface to record temperature. Records were logged every five minutes and transmitted via a relay to the nursery office and then via the telephone port to a file area on “the Cloud” managed by the Dutch company 30 MHz. It was possible for researchers to access the live information and all previous records using a “smart phone” and to gain an overview of the information using the “dashboard”.

Results

When the trial was laid out, on 6 November 2019, the temperature at crop height recorded by the Zensie logger reached a maximum of 11°C (Figures 29-30). After this week mean temperatures began to fall and humidity rise except for a warmer week in late November. At the time of AQ 10 application, on 13 November, there was weak sunshine in the glasshouse and the temperature at the start of spraying at 13:30 h was 11.6°C and 66.3% relative humidity, and when all the spraying was complete 45 minutes later it was 12.9°C and 67.8% relative humidity at spray operator height. The Zensie logger in the crop canopy gave readings of 10.1°C and a higher, 82.1%, relative humidity than the hand-held monitor (Figures 30-31). In the month after application, temperatures ranged from a minimum 2.1°C to a maximum 12.8°C with humidity ranging from 76% to 100%. Leaf surface temperature was mainly the same as the air temperature measured within the plant canopy (Figure 32). Dewpoint and vapour pressure deficit were calculated by the sensor programme (Figure 33). Data for a week after application, sent wirelessly by the logger in the trial, did not reach “The Cloud” storage because the connector in the site office telephone socket was knocked loose.

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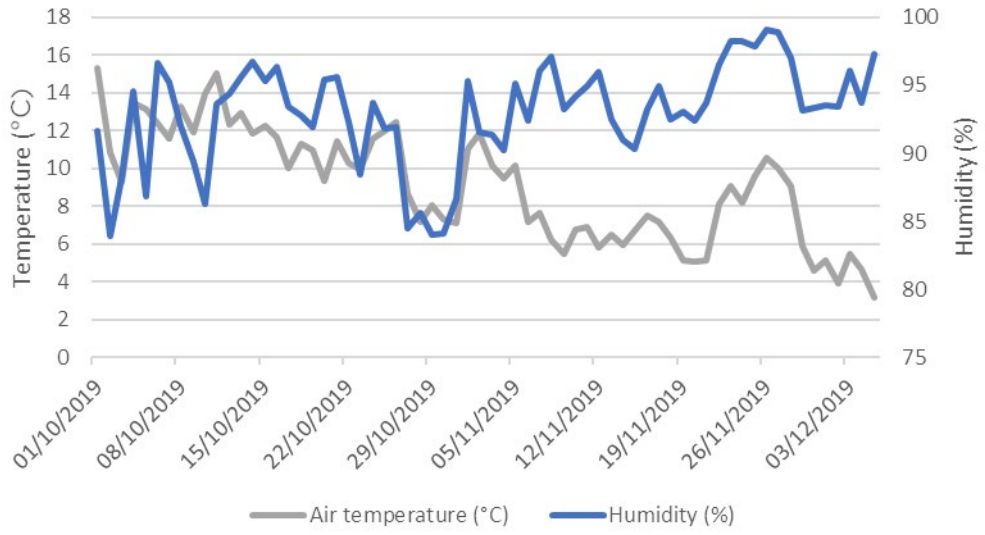

Figure 29. Mean daily air temperature (grey line) and relative humidity (blue line) between 1 October and 5 December taken from Zensie Sensor CA11 at the edge of the Rosemary trial. The data between 23/10/19 – 4/11/19 and 14/11/20 – 18/11/20 has been taken from another Zensie sensor in a crop in the same glasshouse as the trial to substitute for a break in data transmission from the trial sensors.

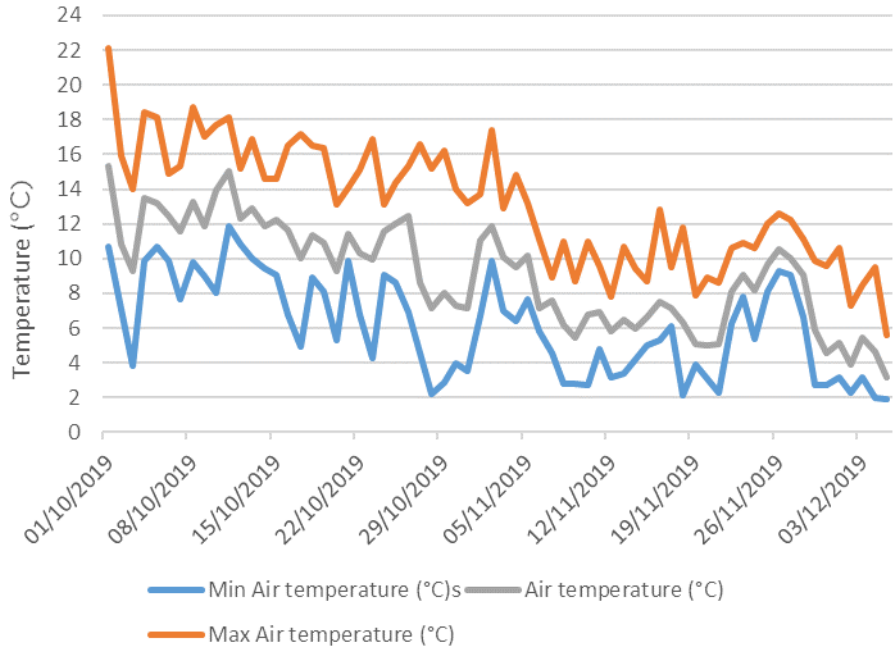

Figure 30. Minimum, mean (grey line) and maximum daily air temperature between 1 October and 5 December taken from Zensie Sensor CA11 at the edge of the Rosemary trial. The data between 23/10/19 – 4/11/19 and 14/11/20 – 18/11/20 has been taken from another Zensie sensor in a crop in the same glasshouse as the trial to substitute for a break in data transmission from the trial sensors.

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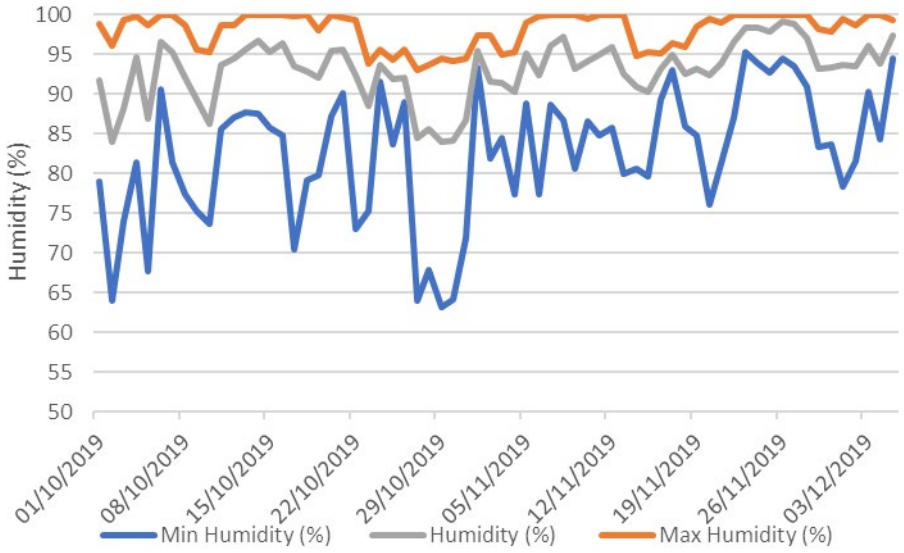

Figure 31. Minimum, maximum and mean daily relative humidity between 1 October and 5 December taken from Sensor CA11 at the edge of the Rosemary trial. The data between 23/10/19 – 4/11/19 and 14/11/20 – 18/11/20 has been substituted by that from another Zensie sensor in a crop in the same glasshouse as the trial, to fill the gaps in data transmission from the trial.

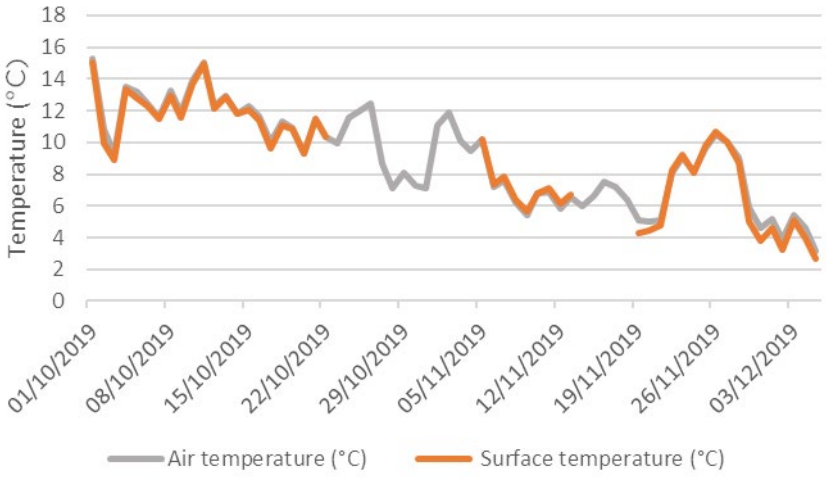

Figure 32. Daily mean air temperature and mean leaf surface temperature between 1 October and 5 December taken from Zensie Sensor CA11 at the edge of the Rosemary trial. The data between 23/10/19 – 4/11/19 and 14/11/20 – 18/11/20 has been taken from another Zensie sensor in a crop in the same glasshouse as the trial in order to fill in a gap in data transmission from the trial. Surface temperature has not been taken from the other sensor to fill the data gap since this might not be the same as for the trial crop.

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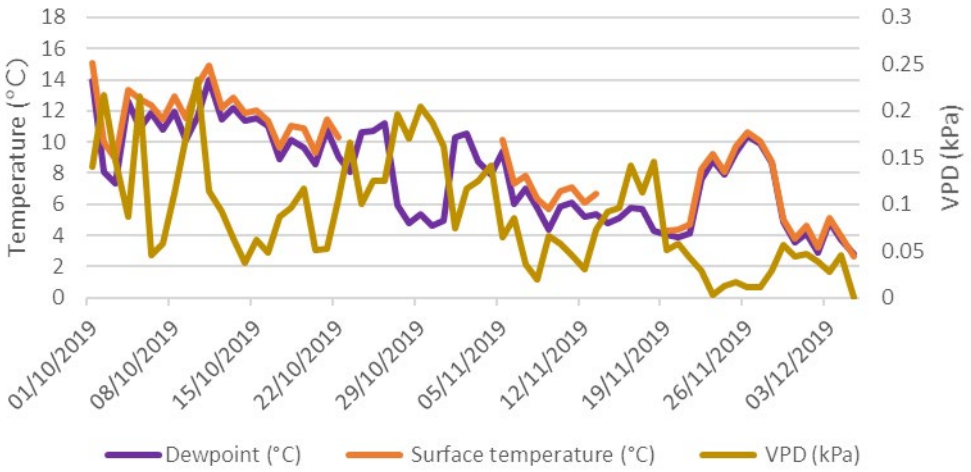

Figure 33. Mean daily leaf surface temperature and the calculated dewpoint and vapour pressure deficit between 1 October and 5 December taken from Zensie Sensor CA11 at the edge of the Rosemary trial. The data between 23/10/19 – 4/11/19 and 14/11/20 – 18/11/20 has been taken from another Zensie sensor in a crop in the same glasshouse as the trial in order to fill in a gap in data transmission from the trial. Surface temperature (red-brown line) was not taken from the other crop sensor to fill this data gap since the crop may have differed.

A. quisqualis colony counting on agar plates

The agar plates examined on 21 November, after these were sprayed with AQ 10 at the same time as the plants, confirmed a high number of viable *A. quisqualis* spores in the spray suspension. Samples of the product taken from both the tank and the lance, before starting to spray and on completion, spread on agar resulted in colony densities within a quite close range of between 296 and 468 in 10 µl (Table 18). The mean counts of viable colonies equate to around 3.5 x 10^4 colonies per ml of spray suspension i.e. 35,000 colonies in 1000 µl (based on 100 x 350 spores in 10 µl). The colony morphology on agar was similar in all samples, with hyphae spreading out from colony centres with intermittent hyphal clusters along them and an average 1.5 mm colony diameter (Figure 34). This indicated that *A. quisqualis* colony viability was not lost between the spore suspension in the tank and after passing through the lance, nor was there any obvious difference between the full tank and what was left after completing the application (Figure 35). Only a few colonies of other fungi developed on the plates, possibly originating during plate spreading.

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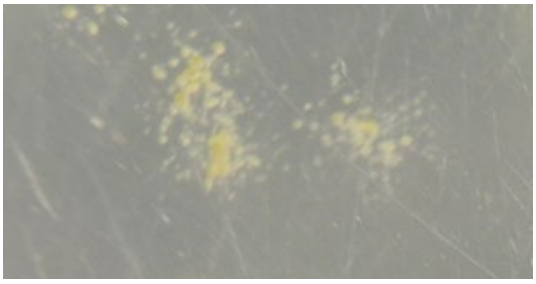
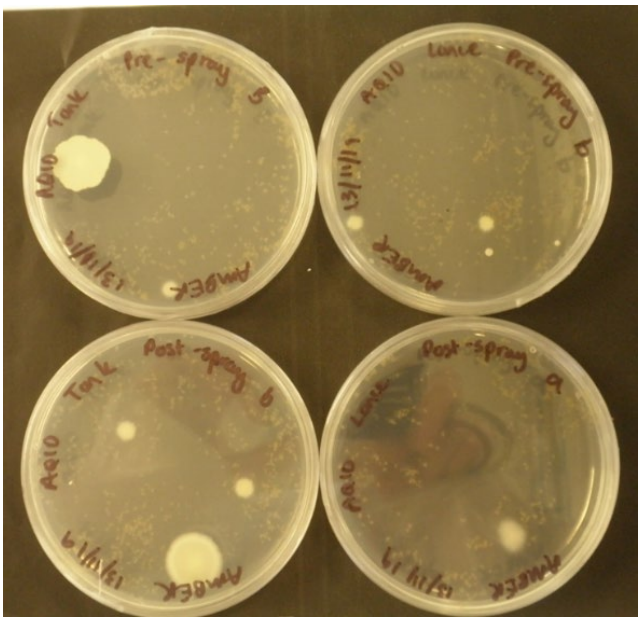


Figure 34. *A. quisqualis* colonies on agar after eight day's growth.

Table 18. Colony counts of *A. quisqualis* on replicate agar plates of AQ 10 from the day of spray application, 13 November 2019

Source of AQ 10 sample	Colony count plate a in 10 µl	Colony count plate b in 10 µl	Mean colony count in 10 µl
Spray tank pre-application	304	468	386
Lance pre-application	357	376	366
Spray tank post-application	347	456	401
Lance post-application	296	357	326



AQ10 sample from sprayer tank (left) and lance (right) just before spraying pots

AQ10 sample from sprayer tank (left) and lance (right) just after spraying pots

Figure 35. Undersides of 90 mm diameter agar plates made on 13 November from 10 µl samples taken from the spray tank and the lance at the start and on completion of spraying rosemary plants with AQ 10. Similar densities of *A. quisqualis* colonies (hundreds of tiny cream-coloured specks) on each plate visible after eight days.

The pair of rosemary leaves sampled into each tube directly after spraying AQ 10 on 13 November were 27mm to 31 mm long and 4 mm wide. On 21 November, of the pair of agar plates made from the washing of each tube, only a single colony of *A. quisqualis* was present

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on a plate from plot 701, but none from plot 101. However, there was a very high level of fungal leaf flora contaminants (covering 35%, 60%, 65% and 75% of the plates), which was likely to have obscured some of the *A. quisqualis* colonies. There were multiple colonies (25, 16, 17 and 12 colonies / plate) of an unidentified fungus with creamy white mycelium (3 mm wide colonies, irregular margined, but without hyphal clumps) in addition to *Penicillium* spp., and some less frequent *Botrytis* spp., *Fusarium* spp. and yeast colonies. The pair of Hebe leaf discs, taken for comparison from two plants, produced plates that were generally less contaminated (covering 0%, 98%, 18% and 18% of plates mainly with *Botrytis* spp. and *Penicillium* spp.) but there was again little retrieval of *A. quisqualis*; two plates produced one colony, another two colonies and the last had zero.

Powdery mildew severity on Rosemary plants

Powdery mildew severity was recorded for individual plants before they were treated on 13 November, and the main difference in severity was related to the number of growing points infested by the powdery mildew, as shown in the photographs of examples (Figure 36 Low, Figure 37 Medium & Figure 38 High). Infestation was concentrated around these shoot tips.

Figure 36. Plot 701 before treatment with AQ 10 on 13 November. Low powdery mildew severity recorded: 1% mildew cover with 5 growing points affected, and sporulation index 3.

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Figure 37. Plot 702 before treatment with AQ 10 on 13 November. Medium mildew severity recorded: 10% mildew cover with 21 growing points affected, and sporulation index 3.

Figure 38. Before treatment with AQ 10 on 13 November. High powdery mildew severity recorded as 50% mildew cover with 35 (all) growing points affected, and sporulation index 3.

By 13 November, in the week after the plants were arranged according to their mildew severity categories, more mildew had become visible in each, doubling in most plants. Two rosemary plants due to be treated with AQ 10 to the rear of the bed (away from the path) had more mildew (mean 45%) than the other two (mean 22%) in the high category, but infestation was more similar across the medium (mean 6.8%) and low (mean 0.5%). In the plants due to be water sprayed the % mildew cover on plants within each of the categories was more similar; high (28.8%), medium (9.7%) and low (0.8%) (Figure 39).

On 27 November, more leaf tips were affected however these were only a very small proportion of the total canopy, particularly as the leaves unaffected by mildew were four times the size of the affected. The mildew was mainly on the growing points, but when present on the older leaves it was towards the leaf base. Examination of leaves from the AQ 10 high severity plants confirmed the presence of powdery mildew spores and also those of the saprophyte *Cladosporium* sp., but there was no evidence of any *Ampelomyces* sp..

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In the month following AQ 10 application the powdery mildew coverage changed very little on water sprayed plants (Figure 39). Therefore, the virtually static mildew coverage over time also seen on the AQ 10 plants was unable to be attributed to the activity of the product. No slowing or reduction in coverage following AQ 10 use was shown by averaging the % mildew cover of the four plants per category compared with those of the untreated (Figure 40).

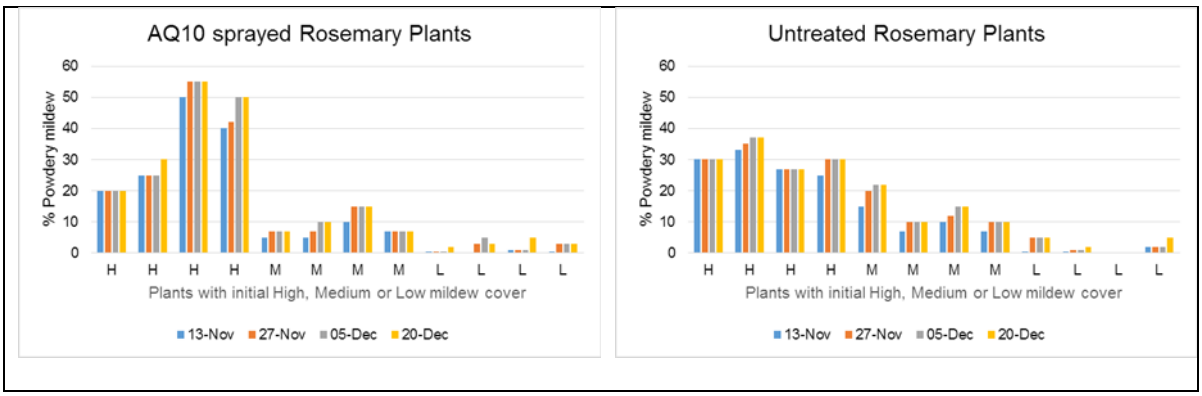

Figure 39. % powdery mildew on four assessment dates of 24 Rosemary, six AQ 10 and six water sprayed, with four plants each of High, Medium and Low mildew severity. The first bar (13 November) is just before the plants were sprayed. Bar colours show assessment dates.

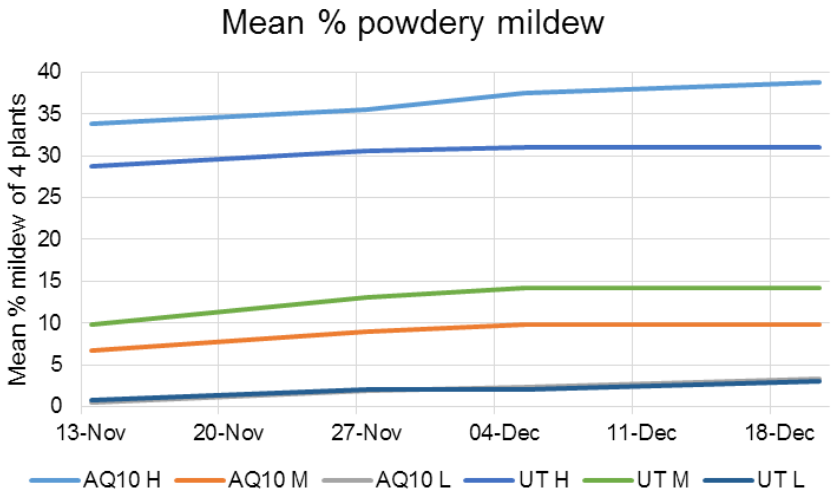

Figure 40. Mean % powdery mildew over time across the four plants in each treatment (AQ 10 or Untreated) and initial mildew severity categories (High - H, Medium - M or Low - L). Observations made on 13 November, 27 November, 5 December and 20 December 2019.

Parasitism by *A. quisqualis* of powdery mildew colonies

When AQ 10 parasitises mildew colonies it disrupts the mildew spore production by producing its own spores inside the spore-producing mildew conidiophores. In most plants the mildew was heavily sporulating at the start of the trial (Index 3) as shown by the lowest, blue-colour, bar on Figure 41. On 27 November, a fortnight after AQ 10 application, the mildew in six AQ 10 treated plants and similarly five untreated looked less powdery (Index 2). By the final

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assessment, on 20 December, for both treatments, the high and medium severity plants were all sporulating abundantly (Index 3). One AQ 10 and three untreated low mildew severity plants still had less sporulation (Index 2), as shown by the shorter yellow bars on Figure 41.

At no time was any greying of the mildew indicative of *A. quisqualis* parasitism seen in the crop. Mildewed leaves from eight plants that had been collected on 27 November, which had been sprayed with AQ 10 a fortnight before, showed no evidence of *A. quisqualis* when the mycelium was examined in water mounts under a high power microscope (nor was any seen on the water-sprayed leaves). Powdery mildew spores were confirmed present. The sticky tape mounts contained lots of leaf hairs and were harder to examine than mycelium scrapes.

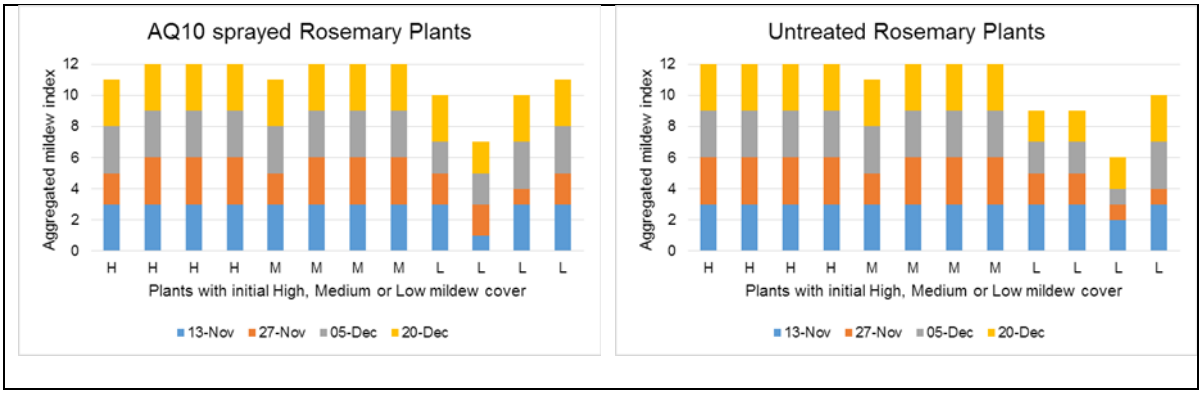

Figure 41. Each date-record different-colour block shows the mildew index of 1, 2 or 3, recorded on each Rosemary plant at each of the four assessment dates. The taller each block the whiter and more powdery the mildew on that date for that plant. Shown for 12 plants sprayed with AQ 10 and 12 untreated plants, comprising four plants of each of three mildew severity ranges. The first block (13 November) was just before plants were sprayed.

Incidence of powdery mildew across Rosemary branches on each plant

The rosemary plants were very bushy, and variation was seen from the start in the number of shoot tips showing mildew. Some new mildew became visible on additional shoot tips, particularly in the second half of December for both treatments (Figure 42), but as the tips were small their mildew did not add greatly to the total % coverage of the whole plant. By 27 November, six AQ 10 treated plants and nine untreated plants gained more mildewed tips. Between this date and 5 December, the number of infected tips increased on three AQ 10 treated and three untreated plants. The mildew was causing stunting of some shoots (Figure 43). By 20 December, eight AQ 10 treated and seven untreated had gained further mildewed tips. Overall, the AQ 10 treated plants developed no fewer mildewed tips than the untreated after 13 November.

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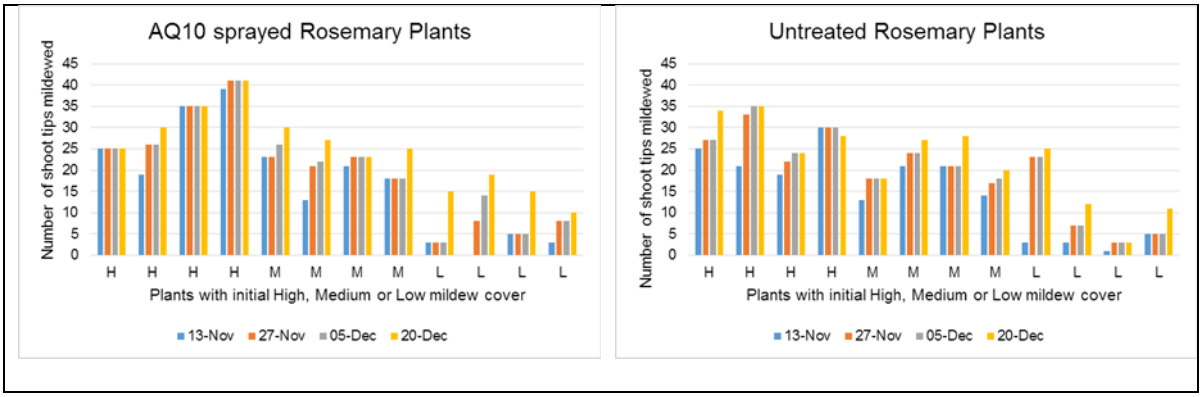

Figure 42. The number of shoot tips per plant with mildew at each of four assessment dates, of 24 Rosemary, six AQ 10 and six water sprayed, with four plants each of High, Medium and Low mildew severity. The first bar (13 November) is just before the plants were sprayed. Bar colours show assessment dates.

Figure 43. Rosemary plant with heavily sporulating powdery mildew at the shoot tips, causing stunting. No visible parasitism (greying) by *A. quisqualis*.

At the 20 December assessment, some variation was noted in the number of shoot tips that were heavily sporulating (index 3). Few tips were mildewed at all in the low severity plants (Figure 44), and so low numbers were recorded as heavily sporulating. In the high and medium severity plants the proportion of index 3 tips was a mean 40% of the total mildewed on AQ 10 treated plants and 48% of the Untreated, so little different.

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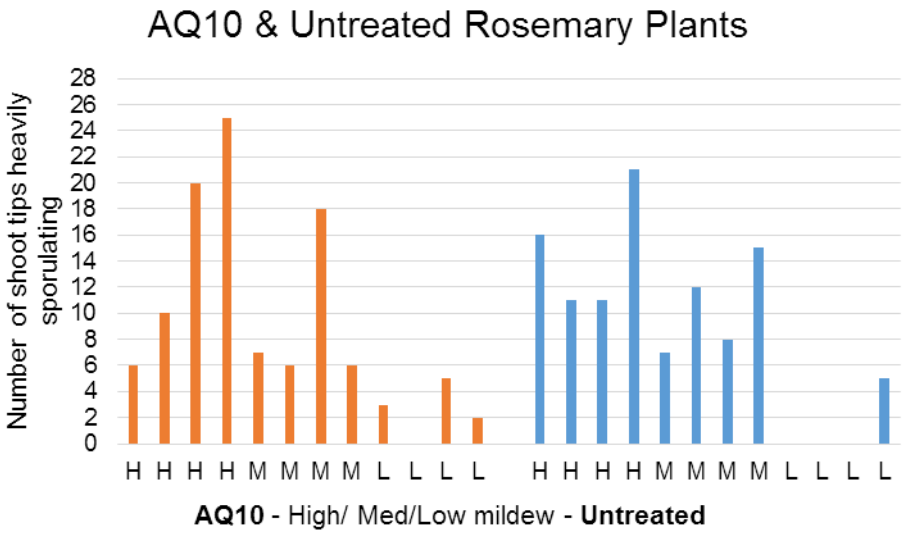

Figure 44. The number of heavily sporulating shoot tips on 20 December 2019 for 24 rosemary plants that received either AQ 10 (left hand, 12 orange bars) or remained Untreated (right-hand 12 bars) on 13 November, comprising four plants of each of High, Medium and Low mildew severity ranges.

Discussion

Observations were completed on the efficacy of AQ 10 application to plants at differing mildew levels, including those within the technical guidance of 3% or below. The powdery mildew coverage on the rosemary was unable to be shown to be slowed by the AQ 10 because the conditions in the glasshouse cooled during November and December and so the mildew on the older leaves progressed only minimally across the trial. There was a small increase in the number of shoot tips showing mildew, but this varied across the trial. When the plants were sprayed most plants were above the 3% coverage, but regardless of coverage the AQ 10 was expected to be able to cause some reduction in mildew sporulation, even if unable to "catch up" with the mildew colony growth and prevent further mildew coverage. However, no parasitism was detected in the mildew.

The testing of the spray suspension for viable spores in the tank and then in the lance showed that there was no filtering out of the *A. quisqualis* spores by the lance, but fewer viable colonies were counted on the agar plates from both tank and lance than anticipated from the spore content stated on the product. From culturing on agar, the mean count of *A. quisqualis* applied equated to around 3.5×10^4 colonies per ml of spray suspension. A dose rate of 1 g of AQ 10 per 10 L water was made up. The label states that AQ 10 contains a minimum of 5×10^9 spores per g (i.e. 5 000,000,000) so adding 1g to 10 L (10,000 ml) should give 500,000 spores per ml = 5×10^5. There is a possibility that colonies seen on agar from the product in suspension may equate to spore clumps rather than individual spores, as irregular shaped

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colonies develop without a definitive central origin. The poor recovery of viable *A. quisqualis* spores from the leaves was seen in other experiments in 2019, with fewer colonies than anticipated being recovered the same day from tomato leaves sprayed with the higher than commercial rate of 1 x 10^6 spores / ml (the rate determined to be used experimentally from work in 2018). With the commercial rate used in the nursery trials, then it could follow that the leaf washings were too diluted in 1.5 ml of water used to be able to pick up spores within the 100 µl samples plated-out. Use of a surfactant, such as Tween, might have released more spores rather than relying on vortex vibration shaking. *A. quisqualis* spores where confirmed to have been capable of germination when the diluted product from the sprayer was plated on agar.

The rough type of foliage of rosemary with upright whorls of leaves and many branches does not make an optimum spray target. The mildew was mainly on the growing points, but when present on the older leaves it was towards the leaf base. It is hard for the spray to penetrate into the plant centre and within the leaf whorls and in particular the leaves of the shoot tips are held close together. When droplets land on the plant the texture was likely to reduce the ability of the spray to spread out over the leaf. The product technical notes indicate that some trials have been done using wetters, but for this project it was agreed that the product would be used as supplied as there was a range of potential wetters and concentrations. When wetters are used with chemical fungicides the pesticide dose has to be reduced, but with AQ 10 this would reduce the colony numbers.

The UK label recommends applying AQ 10 at temperatures between 12°C to 30°C, and to typically apply in early morning or late evening. The temperature was within the lower end of the indicated range for *A. quisqualis* application when it was applied, although temperatures fell over the month of the experiment. In a study by Legler *et al.* (2016) colony growth of *A. quisqualis* decreased from 25°C (0.38 mm / day on average), to 20°C (0.34 mm / day on average), and to 15°C (0.26 mm / day on average). However, information is lacking on efficacy at lower temperatures because most work on this fungal parasite has been carried out on protected edibles in hot glasshouses or on grapes in hot seasons. The parasite should have spread to kill the mildew by the first post-spray assessment, as this is said to take place within 7 to 10 days of parasite entry into the mildew host.

The recorded mean 95.5% RH in the canopy (with no record falling below 80% RH after the first spray on 13 November) should have been ideal for the product (Figure 31). The UK label for AQ 10 indicates that the product works best if applied when the humidity is increasing or high, such as early morning or late evening. Application early or late in the day is less possible where there is no artificial lighting over crops in winter, and the 30 to 45 minute granule soaking period necessary for the product to disperse at mixing acts to delay application. High

relative humidity has been observed to enhance the growth and sporulation of *A. quisqualis*: in a study by Romero *et al.* (2007) which examined the effects of multiple biopesticides in controlling cucurbit powdery mildew on greenhouse melon, AQ 10 was most efficient at 90–95% relative humidity, achieving disease reduction values ranging from 60 to 90%. This is in line with other work where efficacy of *A. quisqualis* has been reported to decrease rapidly when the relative humidity is below 90-95% (Philipp *et al,* 1984; Philipp and Hellstern, 1986; Verhaar *et al.* 1999).

With current technology allowing growers and advisors to look at previous and actual environmental records in their crops remotely, and to have them displayed graphically in various ways (Figure 45), this opens up huge opportunities for understanding the conditions beneficial for both pathogens and beneficial microbes. Growers can see when conditions have been right to cause condensation / dew on the leaves, which can have favoured pathogen infection. Dew forms on upper and lower leaf surfaces when the leaf cools to the dew point temperature of the surrounding air and the air immediately next to the leaf is saturated with water vapour. Saturation (100% relative humidity) can occur close to the leaf, even if not recorded further away (Rowlandson *et al.,* 2015). They are also able to intervene to change settings in their glasshouses to improve the growing environment if they are witnessing unfavourable conditions developing. Further work in this project will provide growers with information on the conditions favourable for the microbes present in particular bioprotectants. Growers can check their crop environments to decide when in the day the best conditions to spray normally arise, then can check what is actually happening before filling up the sprayer, and finally they can see the conditions after spraying and record these against how successful the control measures were eventually. As knowledge is gained this will support future decisions on products and timings.

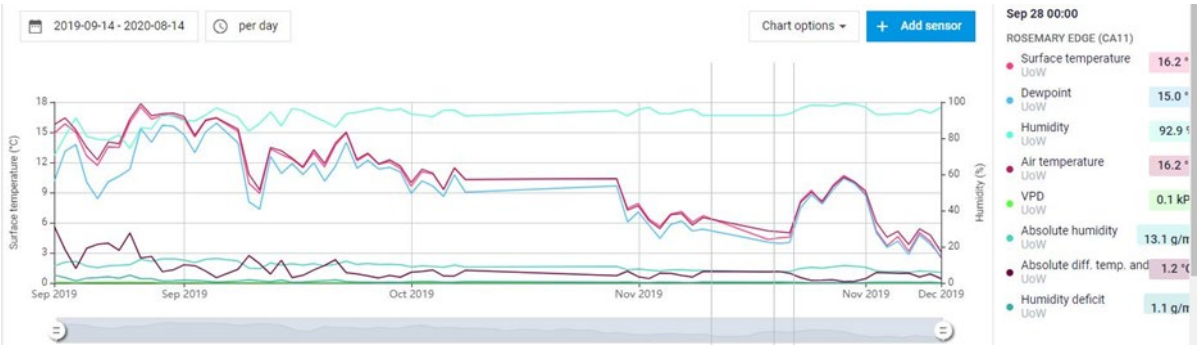

Figure 45. A screen shot from the Rosemary trial in the nursery glasshouse, showing the records of humidity (pale blue upper line) and air temperature (dark pink line) from inside a Zensie screen over three months. Leaf surface temperature (red line) from an arm probe close to a leaf surface at times differed from the air temperature. Values of dew point, vapour pressure deficit (VPD), absolute humidity and humidity deficit are calculated by the system.

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The current AQ 10 technical notes (Anon, 2018) indicate that applications should be repeated every 7-10 days, with at least two successive applications. Only one application was made in the current work because it was intended to seek information on how long it took for an application to take effect, without confounding the situation by giving a repeat application. The repeat application would perhaps give activity against powdery mildew mycelium developing from spores after the 7-day persistence of the AQ 10, after the initial application attacked the powdery mildew mycelium present prior to this.

A. quisqualis destroys the invaded powdery mildew colonies only slowly, depending on the ambient temperature, relative humidity and other abiotic factors (Gaudrey, no date). Infection is favoured by warm temperatures (20 - 30° C) and under favourable conditions, infection can occur in less than 24 hours. The mycoparasite (fungal feeder) directly penetrates the walls of hyphae, conidiophores (spore producing hyphal filaments), and immature chasmothecia / cleistothecia (sexually produced resting spores) but may be unable to infect mature chasmothecia. For approximately 7-10 days, the mycoparasite spreads within the hyphae of the mildew colony without killing it. Thereafter, the process of pycnidial (spore containing bodies) formation begins and is then completed within 2 - 4 days. Infected cells generally die soon after pycnidial formation begins. During that period of time, some of the conidiophores of the invaded mycelium still produce fresh conidia, although these might contain intracellular hyphae of *A. quisqualis*. It has been reported that powdery mildew epidemics can reach damaging levels before their growth and sporulation are arrested by *A. quisqualis* in the field (Falk *et al.,* 1995; Gadoury *et al*. 2012). The effect of *A. quisqualis* in the control of powdery mildew infections is slow, but it suppresses their sporulation rate and infected plants regain vigour after the parasite has killed the powdery mildew (Kiss *et al.,* 2004). Where leaf quality across the whole plant is important, then AQ 10 may not be a suitable fungicide choice.

Conclusions

- A single AQ 10 application at 70 g / ha to Rosemary plants with 0% to 50% powdery mildew coverage did not result in visible *A. quisqualis* parasitism on the plants within six weeks of application.
- Rosemary plants treated with AQ 10 continued to develop powdery mildew on new shoots and abundant powdery mildew sporulation continued.
- Application of AQ 10 in early winter in an unheated glasshouse was unable to give any control of powdery mildew on Rosemary.

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2.2 Biopesticides and pest population modelling

Objective 2, Work Package WP2.2.7 Insights into biopesticide performance using pest population modelling

Introduction

The optimal use of biopesticides can differ markedly to that for conventional pesticides. For instance, the lag between the application of a biopesticide and control of the pest can often take longer than for conventional pesticides. Identifying optimal application programmes for biopesticides experimentally would be onerous and expensive. Using a modelling approach to identify aspects of an application programme that are likely to produce greatest improvements in control (e.g. reducing or increasing the interval between applications) and testing this experimentally would provide quicker results and be more cost effective.

The objective of this work package was to develop a deterministic model to predict pest population increase over time and the effect of biopesticides on pest population development and management. The model allows the impact of different initial pest population sizes, biopesticide attributes and spray frequency and timing on pest control to be determined. Glasshouse whitefly and entomopathogenic fungi have been modelled initially and other pests, e.g. aphids, will be added later. Data for the model have been obtained initially from the scientific literature and will be supplemented with data from our own experiments where there are gaps in knowledge or where validation is needed. By evaluating methods for making biopesticides more effective the model will increase grower confidence and the uptake of biopesticides within their IPM programmes. Further, by providing data on likely optimal biopesticide control strategies and biopesticide research priorities the model will be a useful tool for researchers and biopesticide manufacturers. Key messages for improving the efficacy of biopesticides will be given to growers.

Methods

Model structure

A discrete time, stage-structure population dynamics model was developed. The pest population increase over time was simulated using a ‘boxcar train’-type model in which each individual in a pest population transitions from one development stage to the next until it reaches adulthood and reproduces. The rate of population growth depends on the number of life stages, the development time of each life stage, the natural mortality occurring in each life stage, the adult pre-reproductive period (the time between becoming an adult and producing offspring), reproductive rate (number of offspring produced per female adult), and adult sex ratio.

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The efficacy of a biopesticide control programme is simulated by altering the initial pest population size, infection efficacy (the percent of the population killed by the biopesticide), the time from exposure until death, the persistence of the biopesticide (the length of time the biopesticide remains effective following application), programme start date (the time between pest arrival and the first biopesticide application), the number applications and the interval between applications. Differences between developmental stages of the pest in terms of the infection efficacy and time until death can also be included in the model. Additional details on the activity of the biopesticide can also be integrated into the model, for example biopesticide 'escape', whereby an individual exposed to an entomopathogenic fungus can avoid infection by the disease by shedding its cuticle during transition to the next developmental stage, can be included by setting infection efficacy to zero for any individuals that develop to the next stage before the time until kill period is complete.

The effect of climatic conditions and host plant can also be integrated into the model, however these depend on the availability of suitable data.

Model parameterisation

A literature review was done to identify parameter values for the development time and natural mortality of each stage of the pest, the adult pre-reproductive period, reproductive rate and sex ratio, and the biopesticide infection efficacy, time until kill and persistence. The literature review included peer reviewed literature, conference papers, relevant reports, 'grey' material and relevant expert knowledge. The Web of Science and Google Scholar search engines were used to carry out the literature review. Any gaps in the literature review were then filled by carrying out bioassay experiments. To ensure maximum relevance of data taken from different sources, it was decided that, where possible, parameter values would be chosen from papers using the same host plant (tomato) and similar environmental conditions.

The model was initially parameterised for the glasshouse whitefly (*Trialeurodes vaporariorum*) and the tobacco whitefly (*Bemisia tabaci*) and control with the entomopathogenic fungi (EPF) *Lecanicillium* spp. and *Beauveria bassiana*. Once this model was complete, a further literature search to parameterize the model for peach-potato aphid (*Myzus persicae*) was started.

Identifying optimal control programmes

The completed model was used to explore the effect of altering biopesticide control programmes (e.g. changing the programme start date, number of applications and application interval) on control efficacy. This would allow the rank order (in terms of control efficacy) of different spray programmes to be determined and compared experimentally.

Results

Model parameterisation

Relevant parameter values for *T. vaporariorum* and *B. tabaci* were identified in 49 papers. The parameter values chosen for *T. vaporariorum* are shown in Table 19. Parameter values were primarily chosen from papers in which experiments used tomato as the host plant. The effect of temperature is available for the majority of parameters.

Table 19. *T. vaporariorum* parameter values (at 21°C) and whether a model is available for temperature-dependent effects.

Parameter	Value	Temperature-dependent model available?	Reference
Egg development time	8.1	Yes	Roermund & van Lenteren (1992)
1st instar development time	4.5	Yes	Roermund & van Lenteren (1992)
2nd instar development time	3.3	Yes	Roermund & van Lenteren (1992)
3rd instar development time	3.5	Yes	Roermund & van Lenteren (1992)
4 instar + prepupa + pupa development time	8.7	Yes	Roermund & van Lenteren (1992)
Adult longevity	39.2	Yes	Burnett (1949)
Egg survival (%)	96.3	No but can be adjusted for extremes	Roermund & van Lenteren (1992)
1st instar survival (%)	95.8	No but can be adjusted for extremes	Roermund & van Lenteren (1992)
2nd instar survival (%)	97.4	No but can be adjusted for extremes	Roermund & van Lenteren (1992)
3rd instar survival (%)	96.3	No but can be adjusted for extremes	Roermund & van Lenteren (1992)
4 instar + prepupa + pupa survival (%)	92.7	No but can be adjusted for extremes	Roermund & van Lenteren (1992)
Adult survival (%)	96.4	No	Burnett (1949)
♀ sex ratio	0.48	No	Roermund & van Lenteren (1992)
Pre-oviposition period (days)	1.3	Yes	Roermund & van Lenteren (1992)
Reproductive rate (eggs/day/female)	6.7	Yes	Roermund & van Lenteren (1992)

The parameter values chosen for *B. tabaci* are shown in Table 20. For *B. tabaci* the literature review focused on papers using the *B. tabaci* 'Mediterranean' species (formerly known as the 'Q biotype') as this is the sub-species most commonly imported into the UK. Parameter

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values were primarily chosen from papers in which experiments used tomato as the host plant.

Table 20. *B. tabaci* parameter values (at 21°C) and whether a model is available for temperature-dependent effects.

Parameter	Value	Temperature-dependent model available?	Reference
Egg development time	14	No but data available to fit model.	Bonato *et al*. (2006)
1st instar development time	7.1	No but data available to fit model.	Bonato *et al*. (2006)
2nd instar development time	4.1	No but data available to fit model.	Bonato *et al*. (2006)
3rd instar development time	8.8	No but data available to fit model.	Bonato *et al*. (2006)
4 instar + prepupa + pupa development time	5.6	No but data available to fit model.	Bonato *et al*. (2006)
Adult longevity	28.8	Yes	Bonato *et al*. (2006)
Egg survival (%)	98.7	No but data available to fit model.	Bonato *et al*. (2006)
1st instar survival (%)	98.7	No but data available to fit model.	Bonato *et al*. (2006)
2nd instar survival (%)	93.4	No but data available to fit model.	Bonato *et al*. (2006)
3rd instar survival (%)	93	No but data available to fit model.	Bonato *et al*. (2006)
4 instar + prepupa + pupa survival (%)	97	No but data available to fit model.	Bonato *et al*. (2006)
Adult survival (%)	64	No but data available to fit model.	Bonato *et al*. (2006)
♀ sex ratio	0.5	No	Bonato *et al*. (2006)
Pre-oviposition period (days)	<1	No but data available to fit model.	Bonato *et al*. (2006)
Reproductive rate (eggs/day/female)	3.9	Yes	Bonato *et al*. (2006)

The literature review found 26 papers with relevant information on the effect of *Lecanicillium* spp. or *B. bassiana* against *T. vaporariorum* or *B. tabaci*. This found important data on most parameter values but revealed some knowledge gaps, including infection efficacy for specific pest stages, time until kill, and the effect of temperature on EPF performance. The parameter values are still to be chosen by the project team and so will not be shown here but will be available in future project reports. Bioassays are being undertaken by Warwick in 2020 to fill in knowledge gaps.

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The development of the *M. persicae* model has begun with 47 papers containing relevant information identified. The parameter values are still to be selected by the project team and so will not be shown here but will be available in future project reports.

Identifying optimal control programmes

The *T. vaporariorum* and *B. tabaci* population models were used to explore control strategies using dummy data for the biopesticide. Once biopesticide parameter values are chosen for *Lecanicillim* spp. and *B. bassiana* these can be included in the model. The below indicates the model output under a range of scenarios.

Effect of initial pest population size:

The time taken for a pest population to reach 10 million individuals was 53% faster when the initial pest population consisted of 1000 adults (63 days) compared an initial pest population of 10 adults (133 days) (Figure 46).

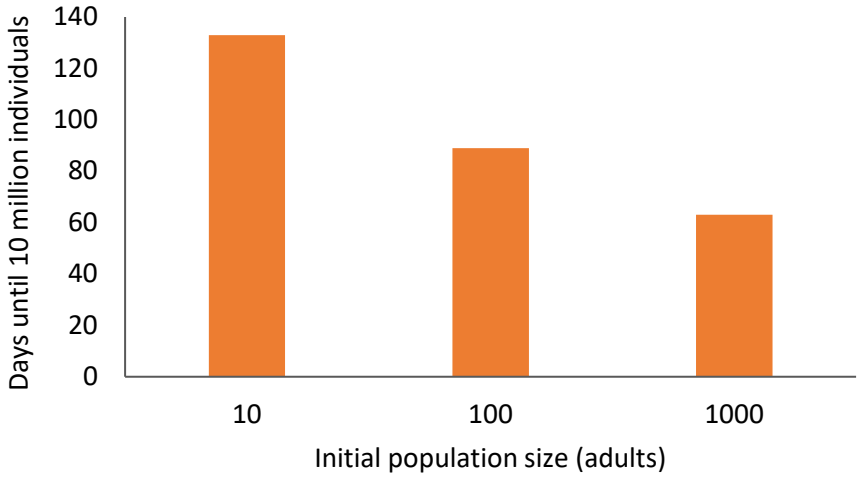

Figure 46. The time taken for a *T. vaporariorum* population to reach 10 million individuals from different starting populations at 21°C.

Effect of EPF infection efficacy on pest control:

The time taken to eradicate a pest population was 37% faster when EPF infection efficacy was 90% (19 days) compared to when EPF infection efficacy was 60% (30 days). The pest population was not controlled when infection efficacy was 30% (Figure 47).

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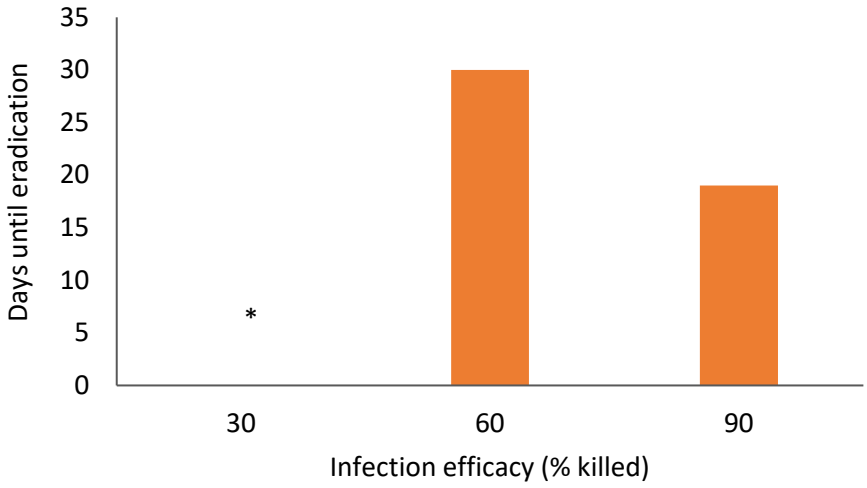

Figure 47. The time taken to control a *T. vaporariorum* population at different EPF infection efficacies at 21°C. * = no control achieved and pest population reached 10 million individuals after 92 days. Initial pest population = 1000 adults, all pest stages effected, time until kill = 3 days, persistence = 5 days, applications on days 7, 14, 21, 28 and 35.

Effect of EPF persistence on pest control:

The time taken to eradicate a pest population was 10% faster when the EPF persisted for 10 days (27 days) compared to EPF persistence was five days (30 days). The pest population was not controlled when EPF persistence was two days (Figure 48).

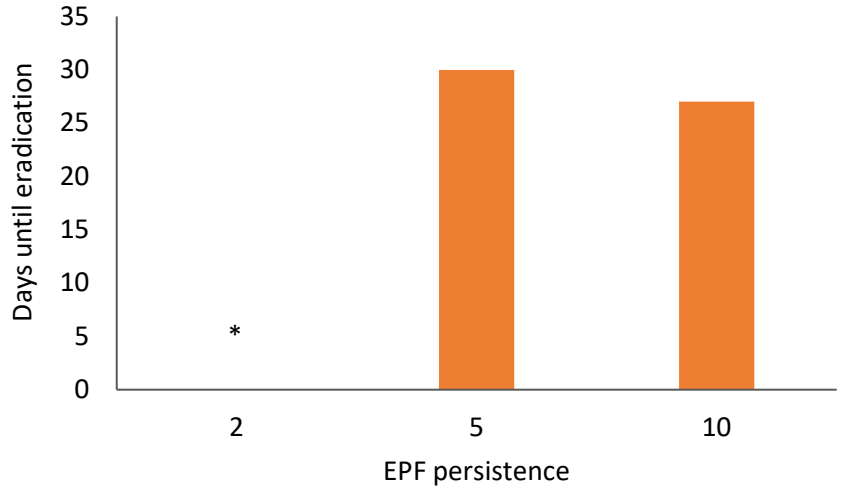

Figure 48. The time taken to control a *T. vaporariorum* population at different durations of EPF persistence at 21°C. * = no control achieved and pest population reached 10 million individuals after 97 days. Initial pest population = 1000 adults, all pest stages effected, infection efficacy = 60%, time until kill = 3 days, applications on days 7, 14, 21, 28 and 35.

Effect of EPF time until kill on pest control:

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The time taken to eradicate a pest population was 61% faster when the EPF took two days to kill an exposed individual (17 days) compared when the EPF took 6 days to kill an exposed individual (44 days) (Figure 49).

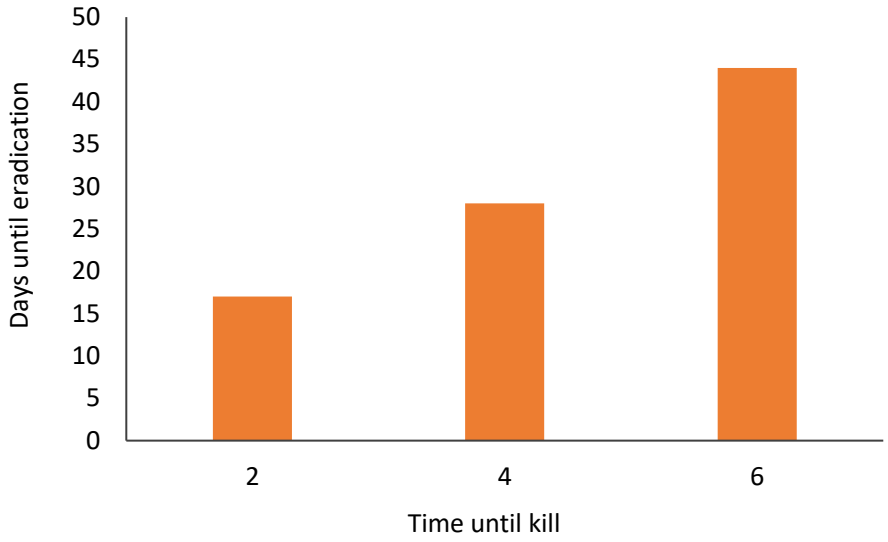

Figure 49. The time taken to control a *T. vaporariorum* population at different durations of EPF time until kill at 21°C. Initial pest population = 1000 adults, all pest stages effected, infection efficacy = 90%, persistence = 5 days, applications on days 7, 14, 21, 28 and 35.

The effect of initial pest population size on pest control:

The time taken to eradicate a pest population was 57% faster when the initial pest population consisted of 10 adults (13 days) compared to an initial pest population of 1000 adults (30 days) (Figure 50).

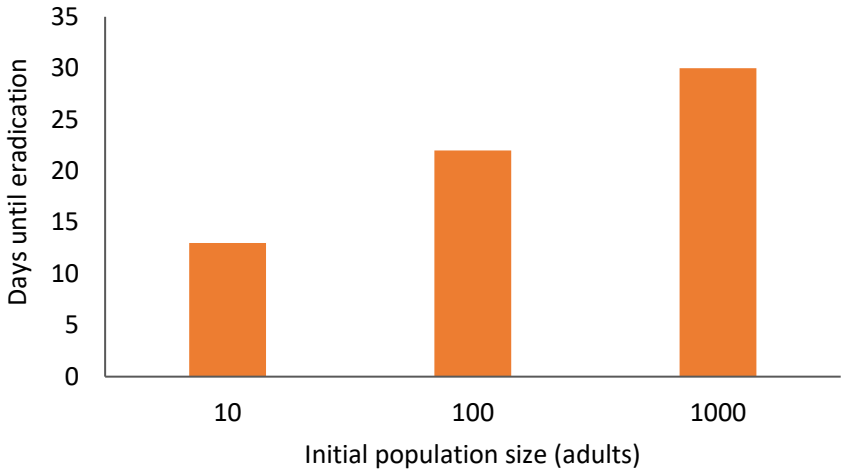

Figure 50. The time taken to control a *T. vaporariorum* population at different initial pest population sizes at 21°C. All pest stages affected, infection efficacy = 90%, persistence = 5 days, time until kill = 3 days, applications on days 7, 14 and 21.

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The effect of control programme start date on pest control:

The time taken to eradicate a pest population was 45% faster when the EPF was first applied five days after the pest arrived (26 days) compared when the EPF was first applied 20 days after the pest arrived (47 days) (Figure 51).

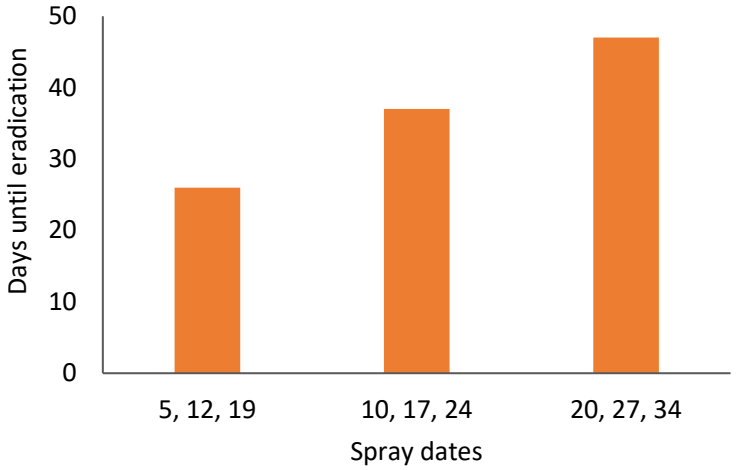

Figure 51. The time taken to control a *T. vaporariorum* population at different control programme start dates at 21°C. All pest stages effected, infection efficacy = 60%, persistence = 5 days, time until kill = 3 days, three applications at seven day intervals.

The effect of application frequency on pest control:

The time taken to eradicate a pest population was 39% faster when the EPF was applied at five day intervals (23 days) compared 10 day intervals (38 days). No control was achieved when the EPF was applied at 20 day intervals (Figure 52).

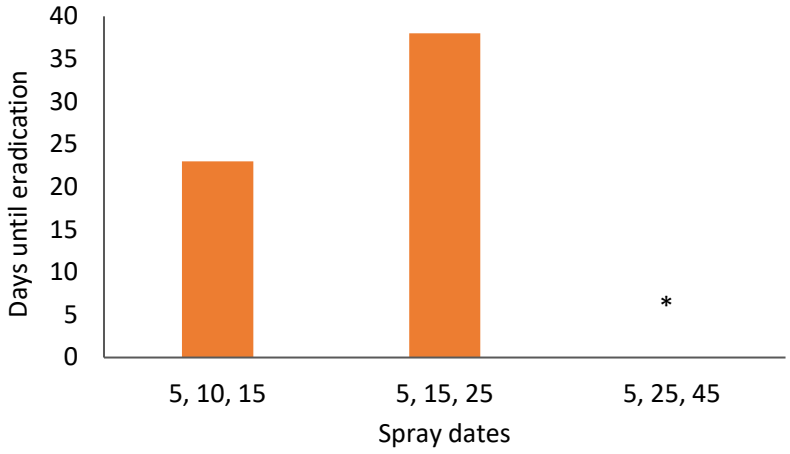

Figure 52. The time taken to control a *T. vaporariorum* population at different EPF application frequencies at 21°C. * = no control achieved and pest population reached 10 million individuals after 143 days. All pest stages effected, infection efficacy = 60%, persistence = 5 days, time until kill = 3 days, three applications starting five days after the pest arrived.

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Discussion

This work developed a mathematical model to compare biopesticide control strategies. The model was constructed for both *T. vaporariorum* and *B. tabaci*, and control with *Lecanicillium* spp. and *B.bassiana*. The model can be used to identify the ways in which a control programme can be improved and the aspects of the control programme that provide the greatest improvement in control when changed. Ultimately the model will be used to rank the order of biopesticide control programmes in order of control efficacy. The model will be validated experimentally in the next year of the project. A further model is currently being developed for *M. persicae* control with biopesticides.

2.3 Spray application

Objective 2, WP 2.2.1 Better delivery of biopesticide to the target

Investigations into improving spray application have focused on optimising water volumes, using a range of different crops as examples for experimental work.

The rationale for this focus is that it is important to identify the optimum volume range to be used before appropriate equipment and techniques can be explored. Previously in the project, we have identified that:

(a) Growers are using relatively high volumes, particularly those using manual spray equipment;
(b) Label recommendations for biopesticides often specify relatively high volumes, possibly because such labels need to cover a wide range of crop structures;
(c) Data supporting the recommended volumes does not appear to be available.

It is known from work with a wide range of crops, particularly in arable situations, that lower volumes result in higher quantities of active substance on the crop, when applied at constant dose. Thus low volumes are a more efficient method of transferring biopesticide to a crop and result in less waste. However, in the case of biopesticides in particular, a minimum quantity of water may be needed to ensure it performs adequately (e.g. so that it does not dry out too quickly), but there is no information available relating to this.

We have therefore undertaken studies in three areas:

1) To identify the relationship between applied volume and the quantity of spray or active substance deposited on plants for a relatively small pot-grown plant that could be treated with a horizontal boom. This involved a specific laboratory experiment using a track sprayer;

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2) To explore the relationship between applied volume and the quantity of spray or active substance deposited on plant for large plants with a vertical structure that could be treated with a vertical boom. This involved a trial in an experimental glasshouse combined with a re-analysis of previously obtained data;
3) An initial exploration of how applied volume could influence the biological efficacy of an application through a laboratory experiment with real insects and an example biopesticide.

2.3.1. Investigation of the relationship between spray deposit and application volume for pot-grown basil plants

Methods

Using the wind tunnel with a track sprayer as a spray chamber, a range of volumes can be applied to a crop through changing nozzle and forward speed. Because changing nozzle also changes droplet size, which influences the quantity retained on the plant, we chose to use the speed of the track sprayer to manipulate volume. Most growers we observed used relatively small nozzle sizes – '02' and '03' sizes which deliver a fine or medium/fine quality spray. In protected crops, drift is not an issue and therefore fine sprays from conventional nozzles can be used. We also observed that nozzle pressure was often relatively low – 2 bar being a typical maximum achievable, particularly with manual boom systems.

A three-nozzle boom was set up on the track sprayer with pot-grown basil plants placed underneath. Volumes between 100 and 1000 L/ha were tested, using a flat fan '02' nozzle operated at 2.0 bar (Table 21). The spray liquid was tap water, 0.1% tracer (Green S), and 0.1% non-ionic wetting agent (Activator 90, DeSangosse Ltd).

Table 21: Speed of the track sprayer and volumes delivered with an 02 nozzle at 2.0 bar

Speed, km/h	8	4	2	1.3	0.97	0.78
Volume, L/ha	98	196	392	603	808	1005

Six plants were sprayed to evaluate the total quantity on the plant. All above-ground plant material was sampled. Plastic discs were also placed on the soil surface to evaluate the quantity of spray reaching the soil (Figure 53). Additional plants were sprayed to determine the quantity deposited on upper leaf surface and under leaf surface, for individual sampled leaves. For chrysanthemum plants, and additional treatment was undertaken with a larger

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nozzle producing a coarse spray (an 08 flat fan nozzle at 2.0 bar and 4 km/h, delivering 784 L/ha).

The quantities of spray liquid deposited on the different parts of the plant and on the soil surface discs were determined by washing in a known volume of water and the rinsate evaluated using spectrophotometry, based on a calibration curve created from samples of the original tank mix, according to standard protocols.

The weight of the plant material in each sample was determined so that results can be presented as quantity of spray liquid per unit mass of plant material. It is then also normalised for the applied volume, and presented as quantity of spray liquid per mass of plant material per 100 L/ha applied volume. This allows the quantity of active substance to be estimated on the assumption that concentration increases as volume reduces.

Figure 53. Example basil plant, sprayed at 603 L/ha. White plastic discs can be seen under the plant to collect spray reaching the soil surface

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Results

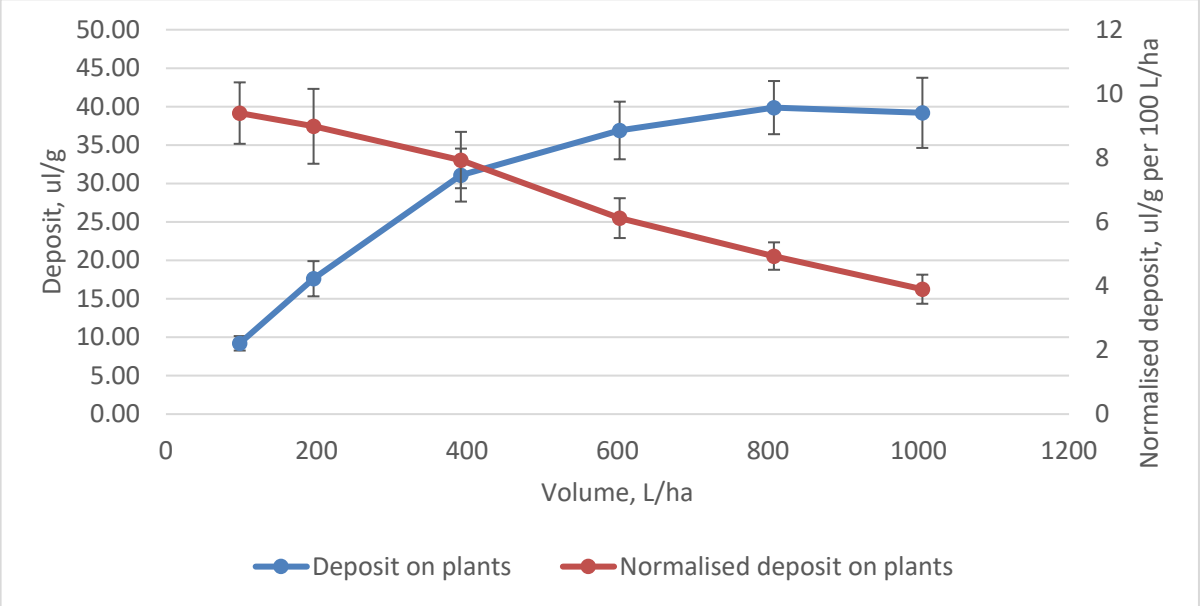

Figure 54. The quantity of spray liquid deposited on basil plants

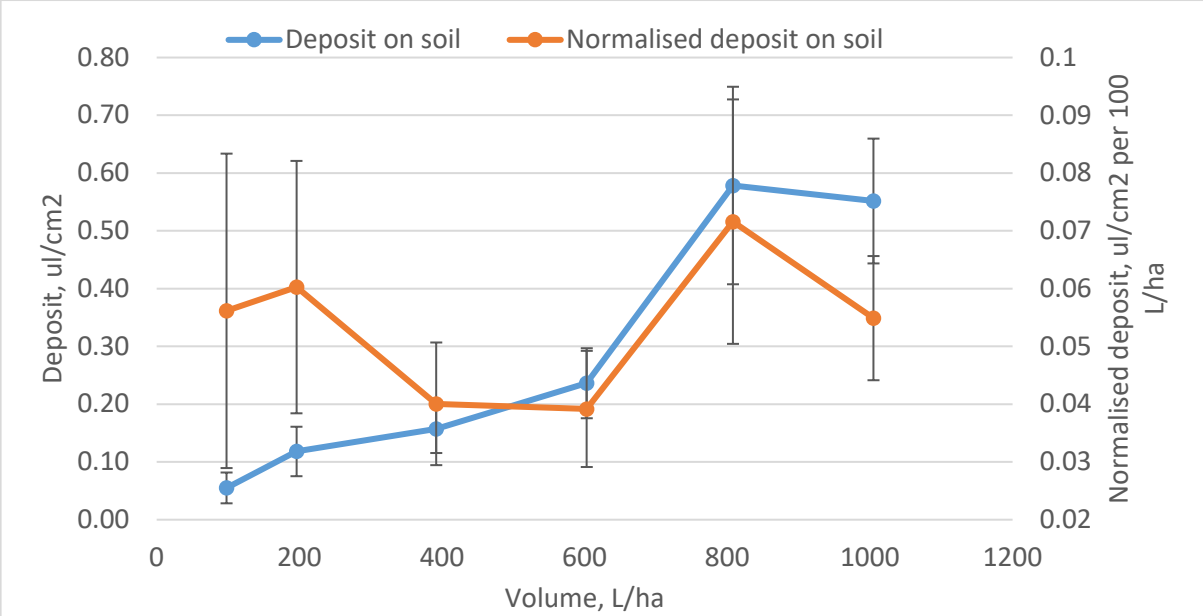

Figure 55. The quantity of spray liquid reaching the soil of basil plants

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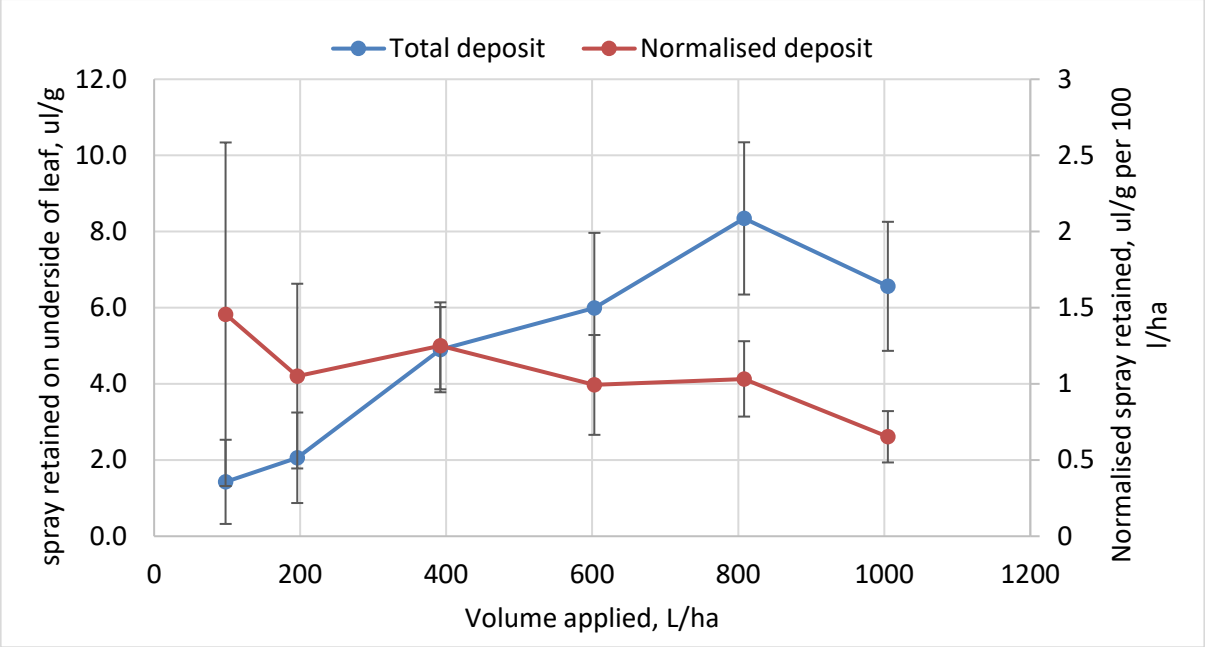

Figure 56. Quantity of spray liquid deposited on the underside of basil leaves

The data suggest that the maximum quantity of spray that could be retained on the two plant types under investigation is likely to occur at an application volume of around 800 L/ha.

If biopesticides are to be applied at a constant concentration, this volume should give the maximum deposit of active substance on the plant surface. However, if they are to be applied at a constant dose, and concentration can be increased, the highest levels of deposit will be achieved at the lowest volume, and increasing water volumes results in higher levels of wasted biopesticide.

Figures 55 and 56 show that increasing volume above 800 L/ha did not increase either the quantity of spray liquid deposited on the soil or the quantity reaching the underside of leaves. The highest quantity of active substance to reach either the soil or the underside of the leaves is achieved with the lowest volume when applied at constant dose.

2.3.2. Investigation of the relationship between spray deposit and application volume for tomato plants

Because it is not possible to transport plants to the laboratory to re-create a realistic spraying scenario, a different approach was required to look at how volume influences the quantity of spray liquid and active substance deposited on large plants with a vertical structure, such as tomatoes.

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A previous study, funded by HDC (PC136) looked in some detail at optimising application volumes and equipment for tomatoes and cucumbers. This was a much larger project than the application component of Amber, and therefore provides a useful baseline. The approach taken, however, and the way the results were reported, were not directed towards answering the specific questions we have relating to biopesticides. However, a re-analysis and re-interpretation of the data was possible to a limited extent, and so we have done this.

In addition, a small experiment was also undertaken to supplement this data. The resources were not available in this project to conduct a full-scale trial with a range of volumes, but two volumes, applied with the same equipment at two speeds, were compared with an experimental crop in the glasshouse at Wellesbourne.

In comparing different data sets, it is important that we compare like-with-like. Because the traditional method of defining applied volume for vertical crops is to define quantity per unit floor area, which is dependent on crop height and row spacing, it is possible that two treatments that appear to have similar volumes are actually delivering very different volumes to the crop. We therefore have done all our analysis based on *the volume delivered to the crop, per unit vertical crop area*. The terminology we have used below – volume applied to the crop – relates to this measure, whereas volume applied relative to the ground area relates to the traditional application volume measure.

Methods

PC136 identified an optimum equipment configuration (Figure 57) and this was therefore used as the baseline equipment for the experiment. A single-sided spray boom was constructed based on a pressurised canister, which ran on rails in the glasshouse (Figure 58), which sprayed over a 1 m vertical height.

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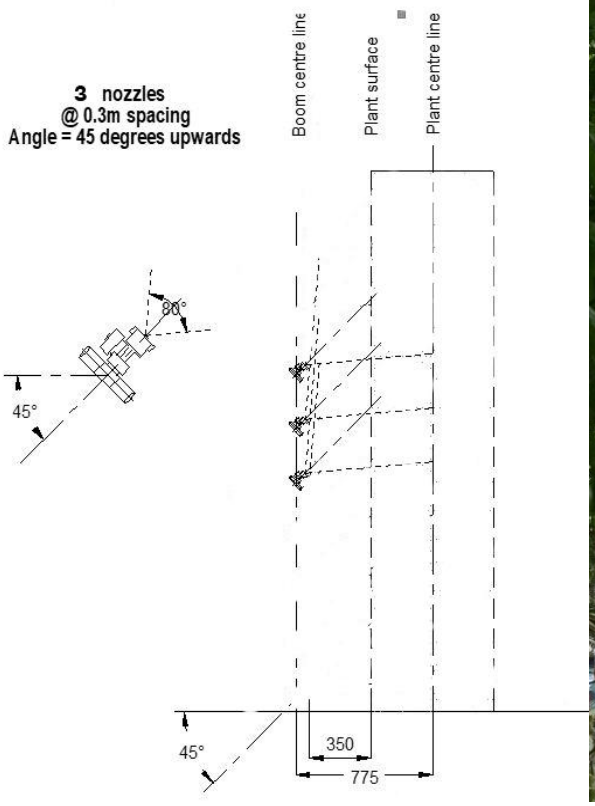

Figure 57. Sprayer design from HDC project.

Figure 58. Experimental spray equipment

The spray boom was moved by manually winding a rope on a drum (Figure 59). Two speeds were achieved, approximately 1.2 and 0.6 m/s, delivering around 500 and 1000 L/ha to the crop. This would be approximately equivalent to an applied volume of 1,200 and 2,400 L/ha relative to the ground area, for a 1.5 m tall crop and a 1.5 m row spacing.

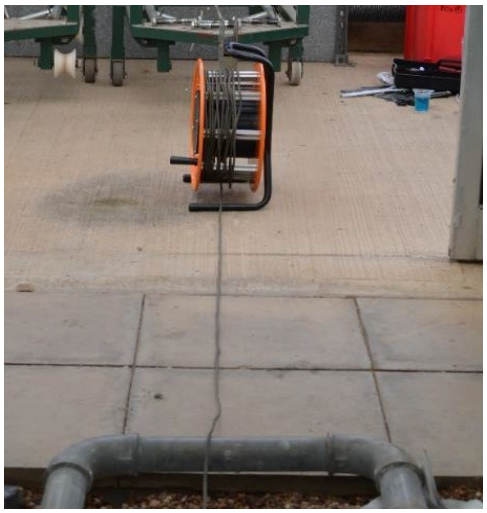

Figure 59. Winding mechanism for moving the boom sprayer

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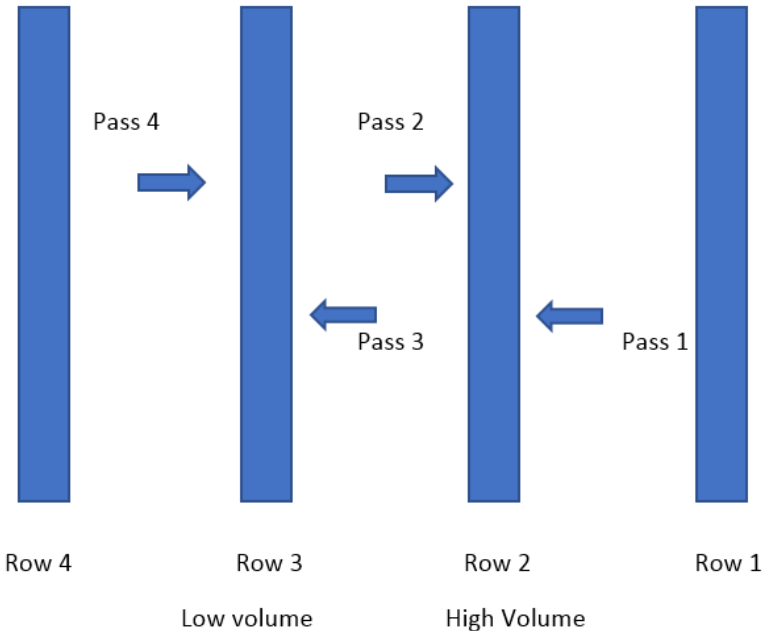

Figure 60. Experimental layout

Two rows of crop were sprayed, one at high volume, one at low volume. Each row was 4.5m total length. Speed measurements were made over a measured 2m length in the middle of the row.

A 3 nozzle vertical boom was fitted with FF80-03 @ 45 degrees upwards on 0.3m spacing at 2.5bar pressure. The lowest nozzle was 1.5 m above the pipe rail. The spray liquid was 0.1% green s (dye tracer) with 0.1% Tween 20 (non-ionic surfactant).

The sampling area was 2.0 m long by 0.6 m height, in the central area of each row. All leaves were allowed to dry completely before being sampled to enable any run-off to take place and ensuring that all retained spray was captured.

Five samples of three whole leaves were taken from each side of the row for both inner and outer canopy. The average weight of each sample was 21 g. Samples were placed into pre-weighed and labelled bags, weighed and collected dye washed off in 50ml of de-ionised water.

3 whole leaves were sampled from both sides (inner and outer) of each row. From each leaf, the terminal and 2 compound leaflets were removed and then weighed and recorded, with the upper and lower surfaces being washed separately with de-ionised water using pipettes.

Control samples were taken to establish background readings

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Results

Figure 61 shows the quantity of spray liquid deposited on upper and lower surfaces of leaves, and Figure 62 shows the normalised quantity of spray liquid on the underside of leaves.

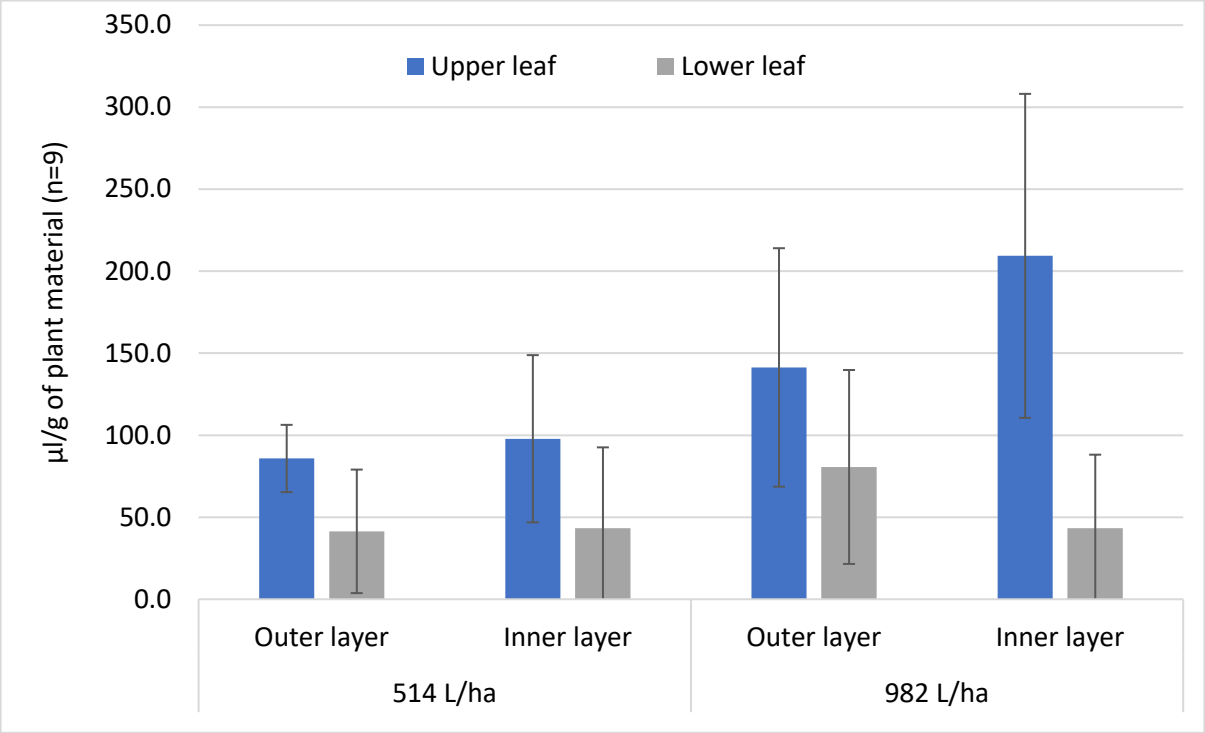

Figure 61. Mean spray liquid deposited on upper and lower surfaces of individual leaves for two volumes applied to the crop.

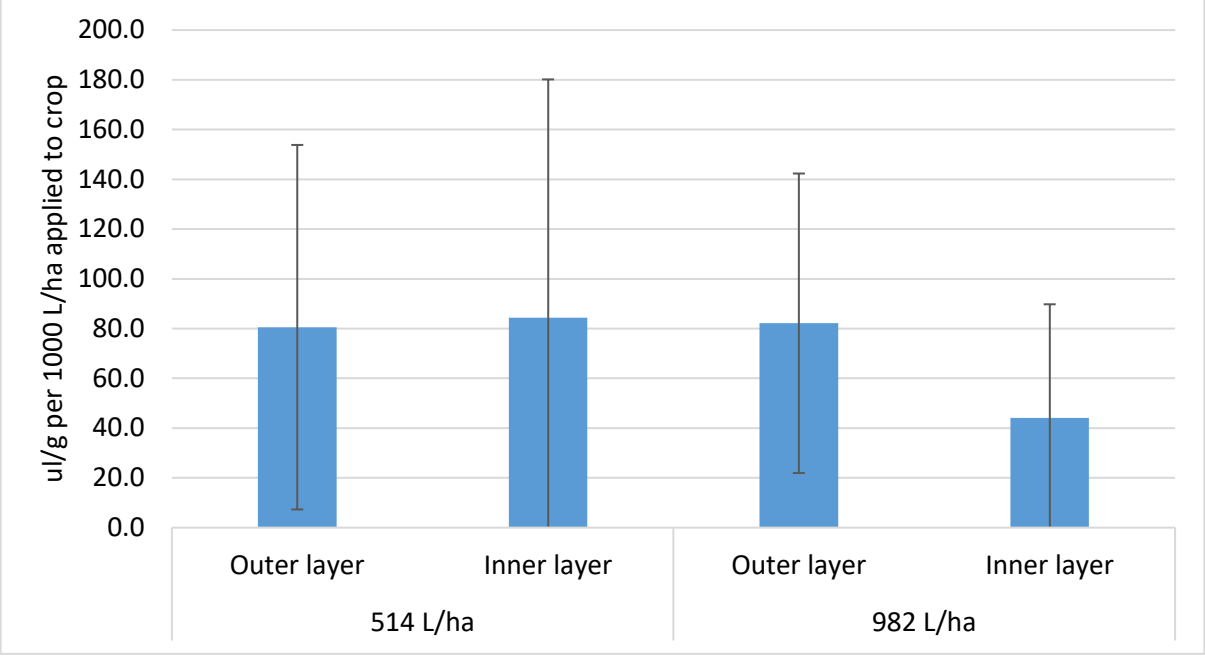

Figure 62. Total normalised deposit on the underside of leaves - µl spray per gram of leaf per 1000 L/ha applied to the crop.

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Figure 61 shows that a much higher percentage of the total spray retained on the crop is on the underside than for a crop sprayed from above with a horizontal boom. This is because we are able to spray from both sides, and the nozzles are oriented to spray slightly upwards. However, gravity still has a very strong effect, ensuring that there is still more applied to the upper leaf surfaces. Figure 62 shows that the higher volume did not increase the quantity of active substance on the leaf underside.

The total amount of spray liquid deposited on leaves is given in Table 22, and the normalised quantity is given in Table 23.

Table 22. Quantity of spray liquid deposited on leaves, µl/g

	514 L/ha		982 L/ha	
	Mean	St dev	Mean	St dev
Outer	35.98	9.20	84.18	15.52
Inner	42.41	18.21	95.45	18.66
All	39.20	14.42	89.82	17.67

Table 23. Normalised quantity of spray liquid deposited on leaves, µl/g per 1000 L/ha applied to crop

	514 L/ha		982 L/ha	
	Mean	St dev	Mean	St dev
Outer	69.99	17.89	85.73	15.80
Inner	82.52	35.43	97.20	19.00
All	76.26	28.06	91.46	18.00

The higher volume gives a slightly higher normalised deposit, which is contrary to the results we consistently get with crops sprayed with a horizontal boom, but is not statistically significant, and it needs to be put into the context of other data, since it was a small experiment. Analysing the data obtained with the leaves that were sampled for assessing the quantity on upper and lower surfaces suggests that the higher volume does not give a higher normalised deposit. It should be noted that the variability, as shown by the standard deviation of each data point and represented by the error bars in the charts, is much higher than for a crop sprayed with a horizontal boom.

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Review of previous data from HDC project PC136

SSAU staff were involved in the HDC project that took place between 1997 and 2000, and therefore we have some access to the original data. However, much of it was retained electronically which was then archived when Silsoe Research Institute closed, so we have only access to the physical records that transferred ultimately to SSAU Ltd, and the final report.

The objective of the project was to determine the most effective spray practices for long season tomato crops and provide growers with cost-effective and robust recommendations. The guidelines reported were developed over a three year period of detailed trialling.

The results were reported related to an application volume expressed as L/ha relative to the ground area, so were specific to the row spacing and crop height, which were not always reported clearly.

The study concluded that the maximum deposit of the spray liquid on the plants occurred at around 2000 L/ha relative to the ground area. Normalised deposits were maximum at around 1300 L/ha relative to the ground area, so unlike for short crops sprayed with a horizontal boom, the lowest volume did not appear to deliver the highest deposit of active substance.

We reviewed all the data where deposit on leaves were measured for different applied volume rates and converted those volume rates to be relative to the crop area rather than the ground area. Some guess-work was needed to do this, and we identified some potential reporting errors, so there are limitations on the accuracy of this analysis. We also had only mean values available – no information on the variability within each experiment was given in the reports. There was also quite a wide range of crop sizes and densities within the data.

Figure 63 shows all the data available, including the two new datapoints from the Amber project (averaged of both inner and outer canopy). The Amber data are consistent with previous data, although at the higher end which is likely to be because the Amber crop was of relatively low density.

While there are insufficient data to identify an applied volume for maximum spray liquid deposit, there is a suggestion from Figure 63 that it might occur at around 1,000 - 1,500 L/ha applied to the crop. Clearly, the quantity retained on the crop is greater at 1,000 L/ha than at 500 L/ha, which is consistent with what we found with the new data. We have a measure of variability only from the new Amber data, which suggests that statistically significant differences are going to be difficult to identify.

When the data are normalised, the relationship between applied volume and deposit is probably – on average – flat (Figure 64). Thus the data suggests that if a given dose of a

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product can be applied at a lower volume, there will be no reduction in quantity of active substance on the plant, but there may not be any increase either. So if the quantity of active substance is insensitive to volume within the range of data that we have, we can choose volume for other purposes, e.g. for logistical reasons.

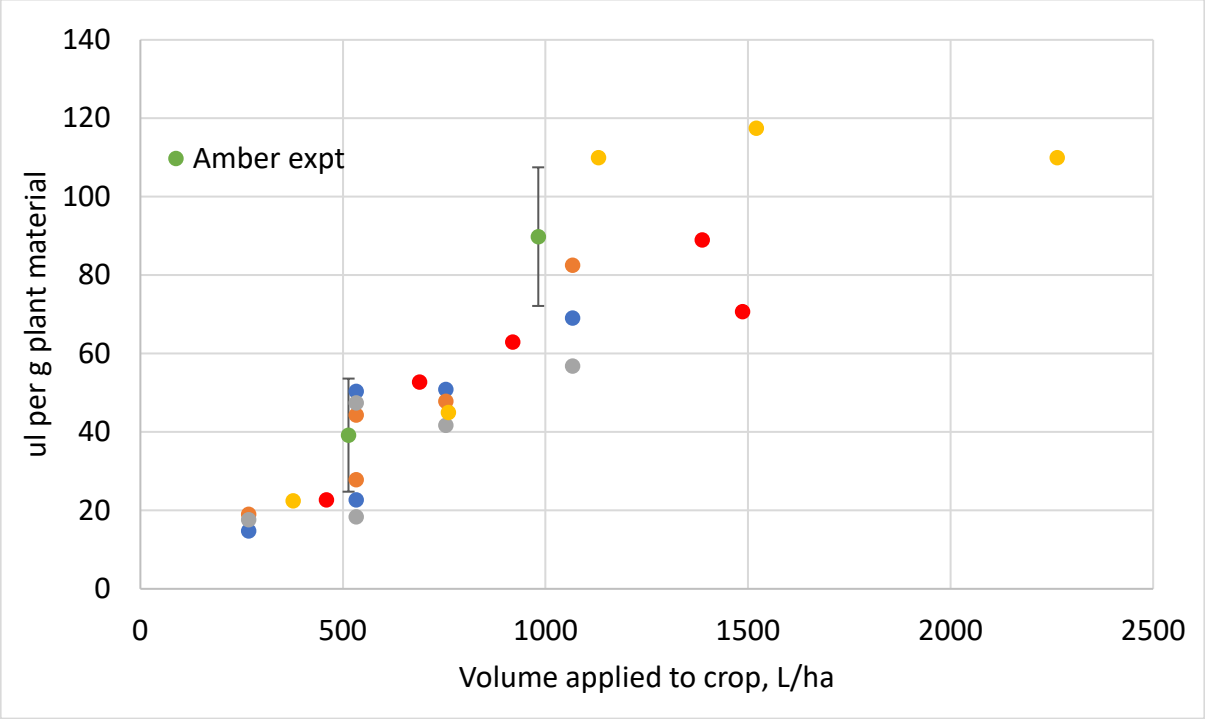

Figure 63. Mean quantity of spray liquid deposited on tomato leaves for a range of volumes applied to the crop. Data from a range of experiments from PC136 and Amber – each colour represents a different experiment.

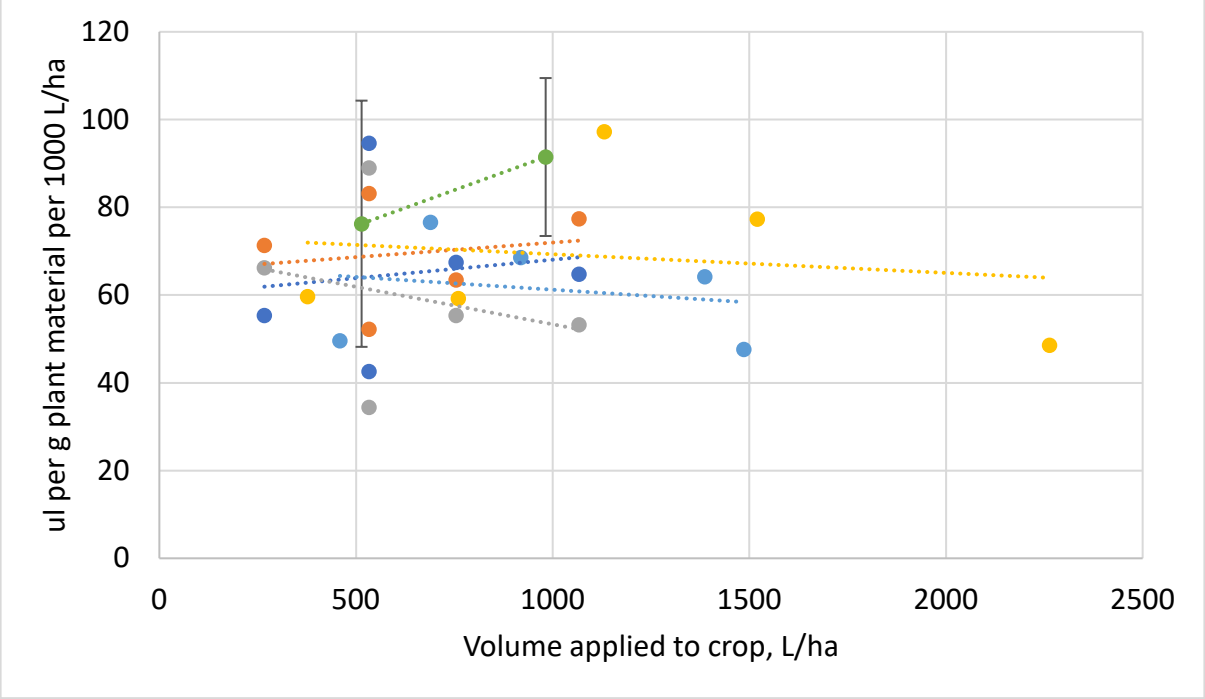

Figure 64. Normalised quantity of spray liquid deposited on tomato leaves for a range of volumes applied to the crop. Data from a range of experiments from PC136 and Amber – each colour represents a different experiment. Dotted lines are a linear fit to each experiment (although a linear fit might not be appropriate for all data).

2.3.3. Investigation of the efficacy of varying the volume of applied biopesticide on the control of a target organism

The objectives of this experiment were to determine the efficacy of lower volume applications of biopesticides on the control of target insects inoculated onto metabolising plants, under controlled conditions. This was a novel experiment and a very difficult one to design, with several iterations and many compromises. However, while it was probably not the perfect experiment, we have learned a lot about undertaking such investigations and can be developed and improved upon in future studies.

Methods

Tomato plants were grown at Wellesbourne and brought to SSAU for treatment (Figure 65). The plants were sprayed with one of five different volumes of either water alone or water plus Botanigard. The spray was delivered with a three-nozzle boom mounted on a track sprayer with FF110-02 nozzles at 2.5 - 3 bar pressure spraying downwards. Forward speed was varied to give different volume rates between 250 and 1500 L/ha. Eight replicate plants were sprayed per treatment.

Figure 65. Example tomato plant brought to SSAU.

The distribution of spores on leaves throughout the canopy were assessed by excising up to 6 leaves from each plant. The upper or lower leaf surfaces were pressed onto selective media and incubated to assess the number and distribution of spores on each leaf surface. Further

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leaves were removed from a different set of plants sampling from the upper, middle and lower canopy to compare application characteristics throughout the canopy, for upper and lower leaves.

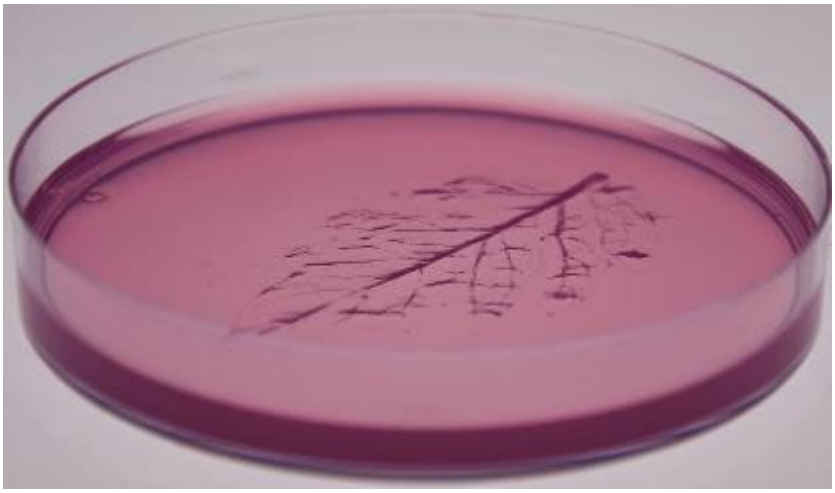

Figure 66. Leaf imprint on selective media before incubation

The culture media used to quantify the *B. bassiana* GHA was Sabourand Dextrose Agar (SDA), with rose bengal and chloramphenicol antibiotic to ensure selectivity. Agar leaf prints (Figure 66) were incubated at 20ºC +/22ºC for 4-5 days until colonies could be enumerated. Untreated controls were included to assess any background populations.

Spider mites had been cultured as a synchronised colony of fixed age females by Warwick University. A known number of spider mites were applied to the upper side of a set of excised tomato leaves, within 24 hrs after whole plant treatments had been applied. These were then incubated for up to 7 days and efficacy assessed.

Leaf area was assessed using photographic images of the excised leaves and processing the images using ImageJ.

The full analysis of data from this experiment was not completed until 2020 and will therefore be included in the next report.

Discussion

While we have been unable to look at the full range of crop structures and sizes that are within the remit of this project, a clear picture is emerging that will enable us to better define optimum volumes to achieve maximum quantity of the active substance on plants.

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For small plants that are sprayed with a horizontal boom:

Where concentration can be varied to deliver a defined dose at different volumes, the maximum active substance will be applied using the lowest volume – ensuring that the maximum concentration is not exceeded.

Where products are to be applied at a fixed concentration, the maximum volume that should be used is 1000 L/ha, and there may be benefits for smaller plants of reducing this down to around 500 L/ha.

For tall plants that are sprayed with a vertical boom:

Where concentration can be varied to deliver a defined dose at different volumes, the quantity of active substance deposited on the plant is probably relatively insensitive to volume, so volume can be chosen to suit other requirements.

Where products are to be applied at a fixed concentration, the maximum volume that should be used is 1000 - 1500 L/ha applied to the crop.

These volumes can be converted into volume applied per floor area, using the specific row spacing and crop height, to ensure label recommendations are met. The Excel spreadsheet calculator we have developed allows this to be done easily. See the example in Table 24 below.

Table 24. Example of the relationship between volume applied to the crop and volume applied relative to the ground for a given crop and application scenario.

Row spacing = 1.5 m. '02' size nozzles mounted 0.3 m apart operated at 2.5 bar with a speed of 1.4 km/h

Number of nozzles on boom	Approx crop height, m	Volume applied to crop, L/ha	Volume applied relative to the ground, L/ha
6	0.9	1043	1669
10	1.5	1043	2087
16	2.4	1043	3338

The volume of water applied to the crop can also influence other parameters relevant to the performance of the biopesticide, such as rate of drying and distribution of spores over a leaf. It should be noted that the maximum leaf area covered by spray liquid is likely to be at around 500 – 600 L/ha applied to the crop, although this depends on both the product applied and the crop itself and has not been investigated in this project. The third experiment has begun

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to explore the relationship between volume and efficacy although further work will be needed to turn this into recommendations for growers.

3. Overall conclusions and future work

The aim of AMBER is to develop improved management practices for biopesticides used in the protected edibles, and protected and outdoor ornamentals sectors. The project is driven by the increasing availability of biopesticides as alternatives to synthetic toxicant pesticides in commercial horticulture. Biopesticides have a range of attractive properties from the grower / spray operator perspective: they pose minimal risk to human safety and most produce little or no toxic residue, meaning that many biopesticides have a zero or low re-entry and handling interval (an issue that is becoming increasingly important when selecting a plant protection product for use, especially in protected crops). In addition, biopesticides are generally considered to have a lower impact on the environment than conventional pesticides. Some of the microbial biopesticides reproduce within the target pest zone, which gives a certain amount of self-perpetuating control, and in this respect, they can be used similarly to other biological control agents such as insect pathogenic nematodes or arthropod predators and parasitoids. The costs of developing a biopesticide are significantly lower than those of a conventional chemical pesticide, which should encourage companies to develop a wide range of products. However, the downsides of biopesticides compared to conventional chemical pesticides include a slower rate of control and lower efficacy, shorter persistence, and greater susceptibility to adverse environmental conditions. Biopesticide products can only be sold in the UK if they have gone through the official authorisation procedure which includes providing detailed evidence on their efficacy using standardised protocols. Growers are keen to incorporate biopesticides into their crop protection systems because they recognize the benefits in terms of human and environmental safety, but they have often reported poor or inconsistent results when first using them. This is an important issue because growers need products that are consistent and reliable. The exact reasons for poor performance will vary from situation to situation, but in overall terms it usually comes down to the fact that biopesticide performance is very dependent on good management practice. Biopesticides tend to be much less ‘forgiving’ than synthetic chemical pesticides, which can give good levels of control even if environmental conditions are not ideal or poor spray equipment is used. Accurate delivery of biopesticides to the target pest or disease at the right time is a key issue, because most biopesticides are contact acting (in contrast, synthetic chemical pesticides that have systemic or translaminar activity can control pests or disease in locations that are hard to reach, such as the underside of leaves). Loss of the biopesticide

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by run-off, or inactivation by UV light or other environmental conditions may also mean that the pest or disease does not receive an effective dose.

For these reasons, it is essential to understand the conditions under which biopesticides work best, and also to make application as effective as possible so that a workable dose reaches the target pest or disease. The challenge is to identify improved management practices that apply to a wide range of biopesticides, and hence will have greatest impact. In AMBER, we are approaching this partly by developing new fundamental knowledge of how biopesticides operate, which we can then apply across the board to different crops, pests and diseases, and biopesticide products. We are also focusing on systems to help targeted application of biopesticides. One of keys to success with biopesticides is to be able to deliver an effective dose of product to the target at the right time, with repeat applications done so that the effective dose is maintained at the target site. This has to be done as part of a wider IPM programme that considers the application process within the context of the economic action threshold for the pest / disease, alongside knowledge of how rapidly the pest / disease can multiply over time, and how the biopesticide can be used in complementary ways with other pest / disease management tools. Biopesticide manufacturers provide label guidance on application strategy, with regulations on application frequency and timing, and guidance on spray application including water volumes. However, because label guidance has to apply for the wide range of crops for which the biopesticide is registered, it tends to be generalised. It is also clear from some of our conversations with companies that some of the application recommendations for particular products have not been developed systematically. There is also a lack of data in the public domain that can help crop protection specialists in the industry to refine biopesticide application strategies.

A small number of microbial biofungicides are currently being used commercially. Products based on the fungus *Gliocladium catenulatum* and the bacterium *Bacillus subtilis,* which are both used against botrytis, are robust preventative treatments, which is related to their ability to readily grow, colonise and persist on leaf surfaces and prevent the establishment of the plant pathogen. In the case of *B. subtilis*, the production and secretion of antifungal secondary metabolites can also be an important mechanism of disease control depending on the strain used. In contrast, biofungicides based on obligate mycoparasites, such as *Ampelomyces quisqualis* (used against powdery mildew) appear to be harder to use, since they do not persist for very long in the absence of their powdery mildew host. The *A.quisqualis* - powdery mildew system has proved experimentally challenging to work with: issues include delivering controlled levels of disease inoculum to test plants in ways that reflect the colonisation process that occurs in whole crops, as well as issues about monitoring *A. quisqualis* levels on tomato plants. Mycoparasites have a lot of potential as biological control

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agents of fungal plant pathogens but given that the conditions for successful use are not as broad as for other biofungicides, they will require careful monitoring of environmental conditions and reliable host detection systems. It will also be valuable to have common data recording standards and tools so that results and information from different end users of these products can be pooled together to widen the evidence base on reasons that contribute to their efficacy and reliability.

The research done in WP 2.2.7 (Insights into biopesticide performance using pest population modelling) is driven by the fact that many bio-insecticides (particularly those based on living microorganisms such as insect pathogenic fungi) take several days to kill the target pest. In addition, they may not persist for long on the plant surface, and they may not affect all life stages of a pest equally. Therefore, their effectiveness is sensitive (i) to 'pest' variables such as population size, reproductive rate, the presence of different proportions of larvae and adults, as well as (ii) 'bioinsecticide' variables such as application timing and frequency (which are in the grower's control) as well as things like inherent virulence, the effective dose needed, and persistence on the plant surface (these are variables that are generally outside the grower's control, although things like persistence can be improved by careful manipulation of environmental conditions in some cases). Until now there hasn't been a way of quantifying how these variables interact to affect the performance of a biopesticide, including how to optimise the use of the biopesticide through management interventions such as the timing and frequency of biopesticide applications, other than by doing traditional multifactorial experiments, which in nearly all cases would be too expensive and time consuming to put into effect. The deterministic model developed in WP 2.2.7 allows us to test out different bioinsecticide application scenarios in a much faster way than by using traditional experiments. It can be used to identify the best options for using biopesticides in IPM against a particular pest species by adjusting timing and frequency of applications matched to the population biology of the pest. Modelling the effect of fungal bioinsecticides on glasshouse whitefly has highlighted the importance of early application (i.e. making applications when populations are low, suggesting that fungal bioinsecticides are not best suited for use as a knock down treatment), and increasing spray application frequency to once per five days (product application guidelines are usually based on one application every seven days). The model also suggests that the time required to eradicate or control a whitefly population is particularly sensitive to infection efficacy (= the percentage of whitefly killed by each spray application), which itself is dependent upon good spray application (i.e. delivering an effective dose to susceptible stages of the pest). The plan going forward is to adapt the whitefly model to study population development of the peach potato aphid, *M. persicae*. Aphids are notoriously difficult to control in IPM programmes because of their high fecundity and short

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generation times, and hence the insights from modelling are likely to be particularly useful. Comparing the two different pest species (whitefly vs. aphids) will enable us to start answering a range of questions that cannot be addressed if just using a single pest species; for example, is there a bioinsecticide application scenario that works equally well for whitefly and aphids, or does each pest need to have its own specific application strategy? An important part of the work going forwards will be to validate the model by testing predictions made for a specific bioinsecticide using carefully targeted cage experiments. Once validated, we could then generate input data for different types of bioinsecticide, identify the most promising application scenarios for them using the model, and make comparisons between the different biopesticides. The main benefit of this work package will be a fast track system for optimising application strategies for bioinsecticides that can be applied to a wide range of different crops and pests. This approach has not been used before with bioinsecticides, although similar modelling systems have been used previously to investigate the efficacy of insect biocontrol with natural enemies (predators and parasitoids) and to optimise their application rates, which are now used widely in the protected crops sector.

It has become increasingly apparent through AMBER that spray application of biopesticides to horticultural crops could be made significantly more effective than at present. We can divide the problem into three main areas: (1) there is a severe lack of knowledge and guidance available to growers about the sets of conditions (water volume, pressure, speed, nozzle type, angle etc.) needed to deliver an effective dose to the target in the most efficient way for different types of biopesticides; (2) growers and spray operators need to make sure they are following general best practice (e.g. storing and mixing correctly, checking and changing nozzles, correctly calibrating sprayers, preventing contamination of biopesticides by tank residues of incompatible chemical pesticides etc.); (3) generally, the equipment used for spray application to horticultural crops by many growers does not give efficient, reliable and consistent application, and investment in new equipment would be worthwhile. The work in AMBER is addressing point (1) - where we are seeking to develop some basic principles that can be used by growers and by biopesticide producers - as well as point (2) through our biopesticide application workshops for growers. Our main focus has been on identifying the optimum volume range to be used, as this needs to be in place before appropriate equipment and other techniques can be explored. As stated earlier in this report, growers are using relatively high volumes as set out by the product label recommendations, possibly because such labels need to cover a wide range of crop structures. Unfortunately, data is not available from biopesticide companies to support the volumes being recommended.

At present, the application of most biopesticide products follows a constant dose model, where the total amount of product applied per unit area of crop remains constant but the

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volume of water used as a carrier for the product can vary within the upper and lower limits required by the product label. This means that the concentration of the product varies in proportion to the water volume. The alternative is to apply at a constant concentration, in which the total amount of product applied to the crop varies in accordance to the area of crop sprayed provided that it does not exceed the upper limit as required by the label. Growers tend to believe that higher water volumes give better coverage and penetration into the crop canopy although there is no evidence that this is the case. The data generated for application to small plants, that are sprayed with a horizontal boom, using a tracer dye system, suggests that, where the biopesticide is applied at a constant dose, the maximum active substance will be applied using the lowest water volume providing that the maximum label concentration is not exceeded. Where biopesticide products are used at a constant concentration, the maximum volume that should be used is 1000 L/ha, but there are likely to be benefits for smaller plants of reducing this down to around 500 L/ha. This is considerably less than the upper water volume allowed for most biopesticides on the label (which is typically 1500 L/ha). In contrast, for tall plants such as tomato that are sprayed with a vertical boom, the quantity of active substance deposited on the plant appears to be relatively insensitive to volume. Thus, for biopesticide products applied at a constant dose, water volume can be chosen to suit other needs (e.g. use a low water volume to reduce the time needed to spray the crop). Where products are to be applied to tall crops at a fixed concentration, the maximum volume that should be used is 1000 - 1500 L/ha applied to the crop. The findings are consistent with the fact that the efficacy of most contact acting biopesticides is dependent upon the concentration of active substance deposited in the target zone. For example, in the case of microbial biopesticides, efficacy is reliant on depositing sufficient number of microbial cells per unit area of the plant surface. When used according to a constant dose model, this means that biopesticide concentration reduces as the volume of water increases. Therefore, high water volumes are likely to reduce biopesticide efficacy by reducing the concentration. They will also increase the risk of losing product through run-off.

Prior to AMBER, the whole issue of developing targeted biopesticide spray application in horticulture had largely been overlooked. Based on our observations we believe that spray application is likely to be a major factor in explaining lack of consistency in biopesticide performance. At present, there is no information available on the optimum spray conditions needed for different crops, biopesticides, pests and diseases apart from that coming out of the AMBER project.

The next phase in the spray application work has been to investigate how control of water volume translates into effects on biopesticide efficacy. This was investigated by setting up a pilot system using a fungal biopesticide sprayed against spider mite on tomato. The system

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was difficult to develop, since it required application of the biopesticide in a track sprayer (allowing control of a range of spray variables including water volume) followed by maintenance of spider-mite infested leaves under highly controlled conditions to allow mite survival to be monitored. At the same time, we used a selective microbiological medium to allow us to count the number of fungal spores deposited per unit leaf area. The data will be analysed in 2020 although the indications from the raw data are in keeping with our tracer dye experiments, i.e. that the best strategy for optimising control is to manipulate the water volume to achieve the highest concentration of biopesticide on the leaf surface (i.e amount of active substance per unit leaf area). The experiment shows significant promise as a cost-effective technique that can begin to explore the relationship between efficacy and application method without the need for costly field trials. By evaluating a range of variables under controlled conditions on a whole plant scale, it will enable optimum spray conditions for different crops, pests and biopesticides to be narrowed down. It would far too costly to attempt to do this 'narrowing down' on a crop scale. We need to gain further experience with this and improve some elements of the protocol so that the science is as robust as it can be, and therefore extrapolation to crop scale in the glasshouse or outdoors is possible. One significant "knowledge gap" that we frequently encounter with biopesticides is the lack of information about the effective dose, expressed as amount of active substance per unit leaf area or per unit volume of soil, that growers need to achieve in order to make the product work. Data is not available from biopesticide companies on the effective dose, which is a serious omission. If the effective dose is known, then we would have a target to work towards in terms of controlling the spray conditions by adjusting water volume etc in order to optimise delivery of the biopesticide to the target.

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4. References

Anon 2018. Technical Notes AQ 10. Publication version 12/18. Fargro Limited, Arundel Road, Poling, Arundel, West Sussex. Notes can be downloaded from www.fargro.co.uk.

Bonato, O., Lurette, A., Vidal, C. & Fargues, J. (2006). Modelling temperature-dependent bionomics of *Bemisia tabaci* (Q-biotype). Physiological Entomology, DOI: 10.1111/j.1365-3032.2006.00540.x.

Burnett. T. (1949). The Effect of temperature on an insect host-parasite population. Ecology, 30 (2), 113-134.

CRD (2008). Registration Report-AQ 10 WG, *Ampelomyces quisqualis*, isolate M-10.

Falk, S. P., Gadoury D. M., Pearson, R. C. & Seem, R. C. (1995). Partial control of grape powdery mildew by the mycoparasite *Ampelomyces quisqualis*. Plant Disease, 79**:** 483-490.

Gadoury, D. (no date given, online). *Ampelomyces quisqualis*. In: A guide to natural enemies in North America. Anthony Shelton, Professor at Cornell University.

Gadoury, D. M., Cadle-Davidson, L., Wilcox, W. F., Dry, I. B., Seem, R. C. & Milgroom, M. G. (2012). Grapevine powdery mildew (*Erysiphe necator*): a fascinating system for the study of the biology, ecology and epidemiology of an obligate biotroph. Molecular Plant Pathology*,* 13**:** 1-16.

Hewson, A. (2010). Herbaceous perennials. A guide to the production of container grown plants. AHDB 11 pp.

Jacob, D, David D.R., Sztjenberg, A. and Elad, Y. (2008). Conditions for development of powdery mildew of tomato caused by *Oidium neolycopersici*. Phytopathology 98: 270-281.

Kiss, L., Pintye, A., Zséli, G., Jankovics, T., Szentiványi, O., Hafez, Y. M. & Cook, R. T. (2010). Microcyclic conidiogenesis in powdery mildews and its association with intracellular parasitism by Ampelomyces. European Journal of Plant Pathology 126**:** 445-451.

Kiss, L., Russell, J., Szentiványi, O., Xu, X. & Jeffries, P. (2004). Biology and biocontrol potential of Ampelomyces mycoparasites, natural antagonists of powdery mildew fungi. Biocontrol Science and Technology, 14**:** 635-651.

Legler, S. E., Pintye, A., Caffi, T., Gulyás, S., Bohár, G., Rossi, V. & Kiss, L. (2016). Sporulation rate in culture and mycoparasitic activity, but not mycohost specificity, are the key factors for selecting Ampelomyces strains for biocontrol of grapevine powdery mildew (*Erysiphe necator*). European Journal of Plant Pathology, 144**:** 723.

Philipp, W.-D. & Hellstern, A. (1986). Biological control of powdery mildew by *Ampelomyces quisqualis* under reduced air humidity conditions. Biologische Mehltaubekämpfung mit Ampelomyces quisqualis bei reduzierter Luftfeuchtigkeit / Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz / Journal of Plant Diseases and Protection**,** 93: 384-391.

Philipp, W.-D., Grauer, U. & Grossmann, F. (1984). Additional investigations on biological and integrated control of cucumber powdery mildew in greenhouses by *Ampelomyces quisqualis*. Ergänzende Untersuchungen zur biologischen und integrierten Bekämpfung von Gurkenmehltau unter Glas durch *Ampelomyces quisqualis* Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz / Journal of Plant Diseases and Protection**,** 91: 438-443.

Roermund, H.J.W. & van Lenteren, J.C (1992). The parasite-host relationship between *Encarsia formosa* (Hymenoptera: Aphelinidae) and *Trialeurodes vaporariorum* (Homoptera: Aleyrodidae) XXXIV. Life-history parameters of the greenhouse whitefly, *Trialeurodes vaporariorum* as a function of host plant and temperature. Wageningen Agricultural University Papers.

Romero, D., De Vicente, A., Zeriouh, H., Cazorla, F., Fernández-Ortuño, D., Torés, J. & Pérez-García, A. (2007). Evaluation of biological control agents for managing cucurbit powdery mildew on greenhouse-grown melon. Plant Pathology, 56: 976-986.

Rowlandson, T., Gleason, M., Sentelhas, P., Gillespie, T., Thomas, C. & Hornbuckle, B. (2015). Reconsidering leaf wetness duration determination for plant disease management. Plant Disease, 99: 310-319.

Sall M. A., (1980). Epidemiology of grape powdery mildew: A model. Phytopathology 70: 338-342.

Tomatoes and cucumbers: Evaluation of effective use of pipe-rail boom sprayers. Final Report PC136, HDC 2000.

Verhaar, M. A., Kerssies, A. & Hijwegen, T. (1999). Effect of relative humidity on mycoparasitism of rose powdery mildew with and without treatments with mycoparasites / Der Einfluss von relativer Luftfeuchtigkeit auf die Bekämpfung von Echtem Mehltau an Rosen mit und ohne Behandlungen mit Mykoparasiten. Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz/Journal of Plant Diseases and Protection, 106: 158-165.

White, S. D. (2012). Implications of new sustainable greenhouse systems for pests, diseases and biological control: A modelling approach to using *Oidium neolycopersici* and *Tetrancychus urticae*. PhD thesis for University of Warwick May 2013. http://go.warwick.ac.uk/wrap/50453 .

Wichura, A., Braun, U., Weber, R.W.S. & Hildebrands, A. (2012). *Golovinomyces orontii* and other powdery mildews on *Rosemarinus officinalis*. Plant Pathology and Quarantine 2: 162-166.

5. Knowledge and Technology Transfer

Presentations

Chandler, D. The use of biopesticides in IPM: the UK AMBER project. Presentation at AHDB biopesticides conference, Stoneleigh, Warwickshire, 26 March 2019.

Chandler, D. The use of biopesticides in IPM: the UK AMBER project. Presentation at AHDB vine crops growers meeting, Stoneleigh, Warwickshire, 27 March 2019.

Chandler, D. Helping growers get the best out of biopesticides. Presentation to Biopesticides Summit 2019, Swansea UK 2 July 2019.

Chandler D. Progress with AMBER project, presentation to AHDB Horticulture Board, 10 July 2019 Stoneleigh Warwickshire.

Chandler, D. Getting the best from biopesticides –AMBER project. Presentation to UK Herb Growers Association technical meeting, Spalding, Lincolnshire 5 September 2019.

Chandler, D. Use of biopesticides in IPM. Presentation to International Plant Propagators' International Meeting, Stratford upon Avon, Warwickshire 9 October 2019.

Chandler, D. Optimising biopesticide use for field vegetable production. Presentation to IOBC Integrating Protection of Field Vegetables working group meeting, Stratford upon Avon, Warwickshire, 16 October 2019.

Chandler, D. Potential for biopesticides in field crops. Pea and Bean Conference, Peterborough UK 5 November 2019.

Chandler, D. Lessons learnt from monitoring biopesticide use on nurseries. Presentation to Protected Edibles and Soft Fruit AMBER day, 10 December 2019, Wellesbourne, Warwickshire.

Gwynn, R. Biopesticides - an industry perspective. Presentation to Protected Edibles and Soft Fruit AMBER day, 10 December 2019, Wellesbourne, Warwickshire.

Bennison, J. Improving biopesticide performance. Presentation to Protected Edibles and Soft Fruit AMBER day, 10 December 2019, Wellesbourne, Warwickshire.

Lane, A. & O'Sullivan, C. Improving the application of biopesticides. Presentation to Protected Edibles and Soft Fruit AMBER day, 10 December 2019, Wellesbourne, Warwickshire.

Jacobson, R. Integrating biopesticides into current control programmes for protected edible and soft fruit crops. Presentation to Protected Edibles and Soft Fruit AMBER day, 10 December 2019, Wellesbourne, Warwickshire.

Articles

Time for a biopesticide revolution? The Grower, Issue 243, Dec/Jan 2019

Workshops

Ornamentals AMBER Day, 26th February 2019, Kenilworth, Warwickshire

Protected Edibles and Soft Fruit AMBER day, 10 December 2019, Wellesbourne, Warwickshire.

Website

The website went live in June 2017.

Table 25: Website summary statistics for 2019

Page	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sept	Oct	Nov	Dec	Total
Amber project	915	1013	990	914	953	612	599	607	870	792	967	984	10216
What are biopesticides?	373	449	431	354	431	340	250	278	351	398	508	483	4646
Biopesticides- pros & cons	296	323	267	293	302	138	192	186	180	182	264	243	2866
Project details	96	120	138	145	118	45	62	57	53	72	61	74	1041
Research plan	26	16	26	19	8	10	14	10	9	15	15	11	179
Project team	25	21	32	19	15	14	17	11	19	20	14	20	227
Links	16	3	12	9	2	6	8	10	1	11	7	5	90
Total	1747	1945	1896	1753	1829	1165	1142	1159	1483	1490	1836	1820	19265

6. Appendices

Colony counts of *A. quisqualis* from leaf discs of Hebe experiment sample 0 and 7 days after application of AQ 10 on 23 and 30 October 2019 at a nursery site Tables 26-31).

Table 26. Colonies of *A. quisqualis* washed from the single leaf disc of Hebe taken on 23 October 2019 afternoon from the leaf of a central pot directly following applications that day the 23 October 2019 (0dpi), and the extent of spread of other fungi across the agar plates and an indication of what they were. Six pots for AQ 10 (T2) and AQ 10 + Silwet L-77 (T3) and three for water (T1) check.
One sample per pot taken from each of the three supplementary pots of Hebe and Rosemary.

Treat-ment	dpi	Plot No. (23 Oct. Sample)	Colony count *A. quisqualis* per plate	Conta-minant area 0-2%	Contam-inant area 3-25%	Contam-inant area 26-50%	Contam-inant area 50-99%	Colony type of contam-inants (see key)~
T2	0	103	*				100	-
	0	201	5			36		-
	0	304	0				85	B?
	0	403	*				95	B?
	0	504	1		8			S
	0	604	2			30		bacteria
T3	0	101	3			40		-
	0	202	1			35		S
	0	303	5			30		P
	0	401	*				100	S, P, FW
	0	501	*				99	FW/B
	0	602	*				100	B
T1	0	102	*				100	B
	0	302	*				100	FW/B
	0	503	0	2				P
T2	0	Hebe 1	4		24			S, P, FW
	0	Hebe 2	0			30		P
	0	Hebe 3	0			45		P, S, F
T2	0	Rosm.1	*				100	B
	0	Rosm.2	0				80	B
	0	Rosm.3	0			45		F
AQ 10 10 µl from tank			458		5			FW

~Key: B Botrytis, F Fusarium, P Penicillium, FW Fast white mycelium possibly Trichoderma, W White yeast, S Salmon yeast. * missing count where contaminants cover >80% of plate.

One whole leaf of Rosemary was sampled in each of the three supplementary plots.

Table 27. Colonies of *A. quisqualis* washed from the single leaf disc of Hebe taken on 30 October 2019 afternoon from the leaf of a central pot directly following second applications on that day 30 October 2019 (0dpi), and the extent of spread of other fungi across the agar plates and an indication of what they were. Six pots for AQ 10 (T2) and AQ 10 + Silwet L-77 (T3) and three for water (T1) check.
One sample per pot taken from each of the three supplementary pots of Hebe and Rosemary.

Treat-ment	dpi	Plot No. (30 Oct. Sample)	Colony count *A. quisqualis* per plate	Contam-inant area 0-2%	Contam-inant area 3-25%	Contam-inant area 26-50%	Contam-inant area 50-99%	Colony type of contam-inants (see key)~
T2	0	103	0		6			S
	0	201	0				55	B,F,P,S
	0	304	0		25			F
	0	403	0				85	B,P,F,S,W
	0	504	0		17			P
	0	604	0		4			P
T3	0	101	0				95	B
	0	202	0			55		B
	0	303	2		10			S
	0	401	0			55		B
	0	501	0				80	B
	0	602	1		22			S,P
T1	0	102	0				80	B
	0	302	0				100	T
	0	503	0			50		P,W,S
T2	0	Hebe 1	0		25			B, S
	0	Hebe 2	0				98	B, T
	0	Hebe 3	0				56	B
T2	0	Rosm.1	0				100	B
	0	Rosm.2	0		25			P
	0	Rosm.3	0			27		P
AQ 10 10 µl from tank			Not done					

~Key: B Botrytis, F Fusarium, P Penicillium, T Trichoderma, S Salmon yeast, W White yeast.

* missing count where contaminants cover >80% of plate

One whole leaf of Rosemary was sampled in each of the three supplementary plots.

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Table 28. Colonies of *A. quisqualis* washed from two leaf discs of Hebe taken on 30 October 2019 morning from the leaf X of a central pot seven days after first applications on 23 October 2019 (7dpi) before re-applications. The extent of spread of other fungi across the agar plates and an indication of what they were. Six pots were sampled for AQ 10 (T2) and AQ 10 + Silwet L-77 (T3) and three for water (T1) check.
Two samples / pot taken from each of the three supplementary pots of Hebe and Rosemary.

Treat-ment	dpi	Plot No. (30 Oct. sample Leaf X)	Colony count *A. quisqualis* per plate	Contam-inant area 0-2%	Contam-inant area 3-25%	Contam-inant area 26-50%	Contam-inant area 50-99%	Colony type of contam-inants (see key)~
T2	7	103	0		7			S
	7	201	0		25			S,P
	7	304	0			30		W,S,P
	7	403	*				100	B
	7	504	*				95	B
	7	604	0			27		B,P
T3	7	101	0		7			B,W
	7	202	0		10			P,S,W
	7	303	*				97	B
	7	401	0			27		F,P
	7	501	0		7			P,W
	7	602	0		16			P,S
T1	7	102	0				55	B
	7	302	0			30		B,P
	7	503	0				60	B
T2	7	Hebe 1	*				99	B
	7	Hebe 2	*				100	B
	7	Hebe 3	0				78	B,F
T2	7	Rosm.1	*				100	B
	7	Rosm.2	0				80	B
	7	Rosm.3	0	10				W,S,P

~Key: B Botrytis, F Fusarium, P Penicillium, T Trichoderma, S Salmon yeast, W White yeast

* missing count where contaminants cover >80% of plate

Two whole leaves of Rosemary were sampled in each of the three supplementary plots.

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Table 29. Colonies of *A. quisqualis* washed from the single leaf disc of Hebe taken on 30 October 2019 morning from leaf Y of a central pot seven days after applications on 23 October 2019 (7dpi) before re-applications. The extent of spread of other fungi across the agar plates and an indication of what they were. Six pots were sampled for AQ 10 (T2) and AQ 10 + Silwet L-77 (T3) and three for water (T1) check.
One sample per pot taken from each of the three supplementary pots of Hebe and Rosemary.

Treat-ment		dpi	Plot No. (30 Oct. Sample Leaf Y)	Colony count *A. quisqualis* per plate	Contam-inant area 0-2%	Contam-inant area 3-25%	Contam-inant area 26-50%	Contam-inant area 50-99%	Colony type of contam-inants (see key)~
T2		7	103	0				55	B,F,S
		7	201	1				60	B,P
		7	304	0				60	F,P, S
		7	403	5				50	P
		7	504	0		25			P,S
		7	604	0		25			S,W
T3		7	101	0				75	B,S,W,P
		7	202	0		15			S,W,P
		7	303	0		10			S,W,P
		7	401	0		25			B
		7	501	*				90	B
		7	602	0		5			S
T1		7	102	*				90	B
		7	302	*				90	B
		7	503	0			50		P,W
T2		7	Hebe 1	0				80	B,W
		7	Hebe 2	0		10			B,S,W
		7	Hebe 3	0		25			S,W,P
T2		7	Rosm.1	*				98	B
		7	Rosm.2	0				60	B,F,P,S
		7	Rosm.3	0				80	B,P
	AQ 10 10 µl from tank			Not done					

~Key: B Botrytis, F Fusarium, P Penicillium, T Trichoderma, S Salmon yeast, W White yeast

* missing count where contaminants cover >80% of plate

One whole leaf of Rosemary was sampled in each of the three supplementary plots.

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Table 30. Colonies of *A. quisqualis* washed from two leaf discs of Hebe taken on 6 November 2019 morning from leaf X of a central pot seven days after applications on 30 October 2019 (7dpi) and with no further sprays. The extent of spread of other fungi across the agar plates and an indication of what they were. Six pots were sampled for AQ 10 (T2) and AQ 10 + Silwet L-77 (T3) and three for water (T1) check.
Two samples / pot taken from each of the three supplementary pots of Hebe and Rosemary.

Treat-ment	dpi	Plot No. (6 Nov. Sample Leaf X)	Colony count *A. quisqualis* per plate	Contam-inant area 0-2%	Contam-inant area 3-25%	Contam-inant area 26-50%	Contam-inant area 50-99%	Colony type of contam-inants (see key)~
T2	7	103	*				100	B
	7	201	0				52	F,P
	7	304	0			50		P
	7	403	0				60	B
	7	504	0			30		P,S
	7	604	0			35		P,S
T3	7	101	0			37		P,S
	7	202	0		25			P,S
	7	303	*				95	B
	7	401	0				70	F,B
	7	501	0			45		P
	7	602	*				85	B,P
T1	7	102	0				60	B,S
	7	302	*				85	B
	7	503	*				100	B
T2	7	Hebe 1	0				60	B,P,W
	7	Hebe 2	*				100	B
	7	Hebe 3	*				100	B,P,W
T2	7	Rosm.1	*				100	B
	7	Rosm.2	*				85	B,P
	7	Rosm.3	*				100	B

~Key: B Botrytis, F Fusarium, P Penicillium, T Trichoderma, S Salmon yeast, W White yeast

* missing count where contaminants cover >80% of plate

Two whole leaves of Rosemary were sampled in each of the three supplementary plots.

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Table 31. Colonies of *A. quisqualis* washed from the single leaf disc of Hebe taken on 6 November 2019 morning from the leaf Y of a central pot seven days after applications on 30 October 2019 (7dpi) and with no further sprays. The extent of spread of other fungi across the agar plates and an indication of what they were. Six pots sampled for AQ 10 (T2) and AQ 10 + Silwet L-77 (T3) and three for water (T1) check.
One sample per pot taken from each of the three supplementary pots of Hebe and Rosemary.

Treat-ment	dpi	Plot No. (6 Nov. Sample Leaf Y)	Colony count *A. quisqualis* per plate	Contam-inant area 0-2%	Contam-inant area 3-25%	Contam-inant area 26-50%	Contam-inant area 50-99%	Colony type of contam-inants (see key)~
T2	7	103	0		9			P,S
	7	201	0		11			S,W
	7	304	0		20			S,W
	7	403	0			30		S,W
	7	504	0			30		S,F
	7	604	0		25			S,W,P
T3	7	101	0				75	B,P,S,W
	7	202	0			30		F, S,W,P
	7	303	0		22			S,W
	7	401	0				60	B,P,W
	7	501	0			30		P,S
	7	602	0		5			S,W
T1	7	102	0		25			S,W
	7	302	0		5			W
	7	503	0				70	P,S
T2	7	Hebe 1	0		15			W
	7	Hebe 2	0		25			P,S
	7	Hebe 3	0		25			P
T2	7	Rosm.1	*				100	B
	7	Rosm.2	0		5			S,P,W
	7	Rosm.3	*				99	B

~Key: B Botrytis, F Fusarium, P Penicillium, T Trichoderma, S Salmon yeast, W White yeast

* missing count where contaminants cover >80% of plate

One whole leaf of Rosemary was sampled in each of the three supplementary plots.

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