Grower Summary

CP 120

Understanding the impact of phylloplane biocontrol agents on insects

Final Report 2019

Project title:	**Understanding the impact of phylloplane biocontrol agents on insects**
Project number:	CP120
Project leader:	Prof Rob Jackson, University of Reading
Report:	Annual report, October 2019
Key staff:	Dr Louise Johnson, Dr Alice Mauchline, Prof Mark Fellowes, Dr Georgina Key
Location of project:	University of Reading
Industry Representative:	Martin Emmett, Tristram Plants, Binstead and Walberton, West Sussex
Date project commenced:	3rd October 2016
Date project completed (or expected completion date):	1st April 2020

DISCLAIMER

[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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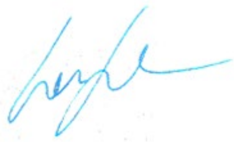
Signature ....  .... Date 07/01/2018

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GROWER SUMMARY

Headline

Reduce cost of chemical pesticide use in glasshouses by using bacterial biological control alternatives against aphids in an IPM system.

Background

The control of insect pests in glasshouse systems is a major challenge. Aphids in particular thrive in controlled environmental conditions, damaging crops by direct feeding and transmitting plant diseases. Due to their vast range in host plants and rapid reproductive cycle, they are particularly hard to eradicate once they have become established in a glasshouse system.

Chemical insecticides are commonly employed against aphids but growers are under increasing pressure from supermarkets and consumers to find alternative, environmentally friendly, non-chemical methods of control. Intensive and indiscriminate use of chemical pesticides has resulted in certain aphid populations to develop resistance to some chemical-based treatments, limiting effective products available to growers. Additionally, chemical pesticides have significant detrimental effects on beneficial insects used in glasshouses, such as natural enemies and pollinators. The use of microbial agents as biocontrols is a rapidly developing field and work conducted by a previous AHDB-funded student, Dr Amanda Hamilton, investigated the potential for bacteria naturally occurring on plants to act as biocontrol agents against aphids and thrips.

In a process known as 'bioprospecting,' 140 bacterial isolates were taken from the rhizosphere (area around plant roots) and phyllosphere (above ground portion of plants) of a variety of plants and tested for virulence against aphids (Hamilton, 2015). Three isolates were found to be most virulent to aphids when inoculated in artificial set ups: *Pseudomonas fluorescens*, *Citrobacter werkmanii* and *Pseudomonas poae* (*P. poae*). *P. poae* was found to have plant growth promoting properties and no noticeable effect on non-target insects (caterpillars and ground beetles). Further investigations found that of the three isolates, *P. poae* PpR24 had the highest success rate in killing aphids, probably because it reproduced in the insect gut and produced aphicidal toxins. These resulted in a 70% decrease in aphid populations when applied on plants as a foliar spray. Foliar applications also deterred aphids from a plant. *P. poae* PpR24 also proved to be effective at killing aphid clones known to be resistant to chemical pesticide treatments; therefore it has great potential for future use in an IPM system.

This project aims to take the next steps in investigating the potential for using *P. poae* as a biological control in glasshouses.

Summary

Many bacteria and microbial organisms in the natural world play an important role in regulating insects and other microbial populations. Some inadvertently have these beneficial properties and there has been an increase in research in harnessing their abilities as biological controls. Microbial-based biological controls offer many benefits to growers. Compared to chemical pesticides, microbial controls are more cost-effective and safer to use for humans and non-target organisms as they are generally highly specific (only dangerous to a few organisms). They also have less of an environmental impact and pose little or no threat to biodiversity as they are naturally present in the ecosystem. They can also be applied to crops by conventional means, making use of systems in place, such as foliar sprays or soil drenching systems. There is also the potential for bacterial-based treatments to become self-sufficient in the crop, offering protection against target pests without the need to be regularly applied. They may also be a solution to the issue of treatment resistance in pests. As bacteria have a rapid reproduction time, they are quick to evolve and so may be able to evolve alongside the pest species, such as aphids, and prevent them becoming tolerant to the treatment.

The bacteria investigated for use as a biological control, *P. poae* PpR24, was originally found on the roots of *Brassica oleracea* and found to cause disease (pathogenic) in the green peach-potato aphid (*Myzus persicae*), lettuce aphid (*Nasonovia ribisnigri*), glasshouse potato aphid (*Aulacorthum solani*), cabbage aphid (*Brevicoryne brassicae*), lupin aphid (*Macrosiphum albifrons*) and pea aphid (*Aphis fabae*). It worked most effectively as a foliar spray or by soil drenching. For this study, foliar spray application was used and the green peach-potato aphid (*Myzus persicae*) was the target pest on sweet pepper plants (*Capsicum annuum*).

The overall purpose of this project was to investigate whether we can improve the wild version of *P. poae* PpR24 as a potential biocontrol agent and to assess whether it can be used in a glasshouse environment. The project was done in three parts, described below in the next three sections.

Experimental evolution to improve Pseudomonas poae PpR24

Experimental evolution is a well-established method for examining the underlying mechanisms of evolution, such as natural selection. ‘Passaging’ is when bacterial cells are grown in a petri dish, and then some of those cells are transferred to a new petri dish to grow

and multiply again as a different generation (or isolate). This can also be done in insects like aphids. By passaging an organism such as *P. poae* PpR24 in a controlled environment for multiple generations, random mutations and adaptations can appear and the different populations can be tracked. Experimental evolution was used to see whether the ability of *P. poae* PpR24 to kill aphids could be improved. This was done in two ways:

1. *P. poae* PpR24 was passaged through aphids via an artificial diet for ten cycles (ten lineages) to try and evolve the *P. poae* and improve aphid killing.
2. Successive passages of *P. poae* PpR24 were also done in a broth environment in an attempt to evolve biofilm formation. Biofilms are clumps of bacteria that are able to stick to surfaces and form communities, held together by substances produced by the bacteria.

The phylloplane can be a harsh environment for bacteria, with challenges such as UV radiation, nutrient limitations and competition from other microbes, therefore biofilms may improve bacterial survival. Biofilm formation may be beneficial for a biocontrol agent as it may lead to bacteria surviving on the plant for longer, reducing the number of applications and possibly remove other, non-desirable microbes from the plant. Unfortunately, the ability of the bacteria to kill aphids was not significantly improved, but out of the ten lineages, one isolate evolved the ability to form strong biofilms. However, there seems to be a trade-off between being able to form a biofilm (for survival) and killing aphids. There was also no improvement in bacterial colonisation and growth on the host plant.

The effects of Pseudomonas poae PpR24 semiochemicals on Myzus persicae behaviour

The ability to repel pests from crops to minimise damage is especially useful in a biocontrol agent. The wild-type *P. poae* PpR24 has a deterrent effect on aphids when sprayed on a plant. In this project, we identified the volatiles (gases) produced by the *P. poae* PpR24 which may explain the aphid-deterrent properties. In addition, volatiles of the aphid-passaged and biofilm-forming isolates were also studied as, although not as lethal to aphids as the wild-type, they may still have deterrent properties. Mulitple volatile organic compounds (VOCs) emitted by the bacteria were found.

When presented with a choice to settle on either a control plant (no VOCs) or a wild-type spray plant, more aphids settled on the control plant which corroborates what was previously found. No significant difference was seen in aphid host plant choice when aphids were presented with a control sprayed plant and plants either sprayed with the biofilm or aphid-passaged isolates. Different VOC levels were detected between the biofilm isolates to the wild-type which may account for the loss of repellency.

Non-target effects of Pseudomonas poae PpR24 on commercial aphid natural enemies

Natural enemies are often a key component in IPM management systems where they are introduced or encouraged into crop systems as part of augmentative and conservation biocontrol programs. *Aphidius colemani*, *Orius laevigatus* and *Macrolophus pygmaeus* are three commercially produced aphid natural enemies commonly used to control *Myzus persicae* in glasshouse cropping systems. To investigate potential lethal effects the bacteria may have on the insects, three experimental set-ups were devised to simulate likely routes of exposure to *P. poae* if the bacteria is applied as a foliar spray.

Topical application of *P. poae* had no significant effect on *M. pygmaeus* mortality after 72 hours or *A. colemani* mummy emergence. However, an effect was observed for adult *O. laevigatus* and *A. colemani*, implying that *P. poae* does affect their survival. Exposure to spray residues did not have a significant effect on the generalist predators but the bacteria appeared to have a damaging effect on *A. colemani* survival. Finally, when left to feed on *M. persicae* that had ingested the bacteria, no significant lethal effect was observed in *M. pygmaeus* but a significant change in mortality was seen in *O. laevigatus.*

Financial Benefits

The annual cost of crops lost to aphids and the viruses they transmit, including the control methods put in place to fight them, is over £100 million. The annual loss to the UK potato industry alone is estimated at £12 million. In an average protected pepper crop, the focal plant of this study, the cost of everyday aphid control is estimated at £5800 per hectare per season. However, this dramatically increases when serious aphid outbreaks occur due to increased applications of biocontrol and insecticide treatments and cleaning the crop of honeydew.

Action Points

- This microbial-based product could be used in a glasshouse integrated pest management system as a foliar spray alongside other biocontrol agents, such as natural enemies. *Pseudomonas poae* PpR24 may be applicable in both preventative and corrective biocontrol strategies to manage aphid infestations.
- *P. poae*'s aphid deterrent properties may make it suitable to deter aphids onto banker plants. Pre-emptive spraying of crops before serious infestations occur may 'push' pests onto sink banker plants, minimising crop losses. Such a method could be combined with natural enemies established in the banker plant to feed on the displaced aphids.

- There is potential for *P. poae* to be used in a management system alongside aphid parasitoids and predators to ensure maximum aphid control. *Macrolophus pygmaeus* may be the most applicable aphid predator for use together with *P. poae*. Carefully timed spray applications may also mean *P. poae* is applicable for use with other parasitoids and predators. It may also be possible to spray crops when parasitoids are developing as mummies. Juvenile *O. laevigatus* may avoid direct contact with *P. poae* spray as they spend early life-cycle stages in more concealed areas of the plant (e.g. in the flowers) and as such are less likely to directly encounter the bacteria.
- Finally, as this microbial, environmentally friendly form of control is meant to be used instead of chemical based pesticides, a reduction/total loss of chemical based products would also be advised to get the full environmental benefit.

