AHDB
HORTICULTURE

Grower Summary

CP120

Understanding the impact of phylloplane biocontrol agents on insects

Annual Report 2018

Disclaimer

The results and conclusions in this report may be based on an investigation conducted over one year. Therefore, care must be taken with the interpretation of the results.

Use of pesticides

Only officially approved pesticides may be used in the UK. Approvals are normally granted only in relation to individual products and for specified uses. It is an offence to use non-approved products or to use approved products in a manner that does not comply with the statutory conditions of use, except where the crop or situation is the subject of an off-label extension of use.
Before using all pesticides check the approval status and conditions of use.
Read the label before use: use pesticides safely.

Further information

If you would like a copy of the full report, please email the AHDB Horticulture office (hort.info.@ahdb.org.uk), quoting your AHDB Horticulture number, alternatively contact AHDB Horticulture at the address below.

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Project title:	**Understanding the impact of phylloplane biocontrol agents on insects**
Project number:	CP120
Project leader:	Prof Rob Jackson, University of Reading
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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):	19th September 2019

GROWER SUMMARY

Headline

Applying experimental evolution to improve naturally aphid-killing bacteria, *Pseudomonas poae*, resulted in a strong biofilm-forming mutant but failed to improve the efficacy of aphid killing.

Background

The control of insect pests in glasshouse systems is a major challenge. Aphids in particular thrive in controlled environmental conditions, causing damage to crops by feeding and by transmission of 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 used against aphids but growers are under increasing pressure from supermarkets and consumers to find alternative, environmentally friendly, non-chemical methods of control. Also, indiscriminate use of chemical pesticides can increase the chance of resistance developing in the aphids and also kills off other 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, particularly against aphids and thrips. 140 bacterial isolates from a variety of plants were tested for virulence against aphids (Hamilton, 2015) and three were found to be most effective: *Pseudomonas fluorescens*, *Citrobacter werkmanii* and *Pseudomonas poae*. Further investigations (Paliwal, 2017) found *Pseudomonas poae* (*P. poae*) to have the highest success rate in killing aphids, with a 70% reduction in aphid populations when treated on plants as well as appearing to deter aphids from going on the plant. Furthermore, application did not have any negative effects on the plants. Not only were they effective at killing a range of aphid species but these bacteria also proved to have no noticeable effect on non-target insects that they may come into contact with, such as species of lepidopterans and ground beetles.

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 to harness 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. Additionally, they have less of an environmental impact and pose little or no threat to biodiversity as they are naturally present in the ecosystem (Lacey *et al*., 2001). 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 that we are investigating for use as a biological control, *Pseudomonas poae* PpR24 (*P. poae*), was originally found on the roots of *Brassica oleracea* and found to be pathogenic to 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*). Previous work investigated its success for a range of application methods and found it to be most effective as a foliar spray or by soil drenching; therefore these are the application methods we intend to use for this project.

So far this project has mainly focused on improving the bacteria to become more efficient as a biological control by experimental evolution and examining the trade-offs between traits that arise. The process of experimental evolution involves identifying beneficial trait of the bacteria we want to enhance or develop, and selecting for it in 'passaging' situations over several weeks. At the end of this 'passaging' process we examined trade-offs between the evolved strains. This involved comparing whether improving one trait of the bacteria was at a cost to another, for instance improving bacterial toxicity may have caused bacterial growth on a plant to become less efficient. Due to time constraints, we focused on evolving two traits.

Toxicity to aphids

A key outcome of the evolutionary passages was to improve the toxicity of the bacteria. Currently, 70% of aphids are killed by *P. poae* in 42 hours; we hoped to improve this by increasing the overall mortality and reducing the time it takes for the bacteria to be effective. This would be beneficial to growers as it would significantly reduce the time taken to combat aphid infestations as well as reduce the need for subsequent applications.

Formation of biofilms

We investigated whether the bacteria possess the ability to form biofilms. Biofilms are aggregations of bacteria that are able to adhere to surfaces and form communities. Such an adaptation offers numerous benefits to bacteria which would also be relevant as a biocontrol. Biofilms offer bacteria more protection from the environment, allowing the bacteria to survive longer on the plant, and help create space for the bacteria to grow and move. Not only would this aid in colonisation of plants when it has been applied but it may also remove other, non-desirable microbes from the plant. Furthermore, testing whether *P. poae* can form such a structure may provide insight as to how it kills the aphids, as one theory suggests it coats the insides of the aphids in a biofilm which ultimately may cause the pest to starve to death.

Trade-offs

We explored how the two traits performed in each other's selective environment, as well as investigated how the bacteria performed against the wild-type *Pseudomonas poae* in situations relevant to crop protection, notably how well evolved isolates could survive on a crop plant. An improvement to the colonisation of plant leaves and how long the bacteria can last on the plant would reduce how often it would have to be applied to the crop. This would also provide further insight as to whether the bacteria can sustain itself in the crop environment and the possibility of a single spray solution to aphid infestations

Each property of the bacteria was investigated over 10 passages. We succeeded in evolving biofilm-forming isolates of *P. poae* in a broth environment, with one isolate in particular forming significantly strong biofilms. However, this ability came at the cost of aphid virulence and survival on the crop plant, proving to be significantly poorer than aphid-passaged isolates and the wild-type *Pseudomonas poae.*

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 (Harris and Maramorosch, 1997). 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

Research is still ongoing therefore it is not yet feasible to make well defined action points. However, we would expect to use this microbial based product in an integrated pest management system as a foliar spray alongside other biocontrol agents, such as natural enemies. 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.