Project title:	**Understanding the impact of phylloplane biocontrol agents on insects**
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Project leader:	Prof Rob Jackson, University of Reading
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Location of project:	University of Reading
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Date project commenced:	3rd October 2016
Date project completed **(or expected completion date):**	19th September 2019

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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GROWER SUMMARY

Headline

Initial results indicate that *Pseudomonas poae* is capable of forming biofilms in a broth environment and so may be able to survive for longer on the plant. There are also significant changes to attachment strength over time, indicating we are able to improve this trait over time via experimental evolution.

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 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 employed 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. Of the 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 it 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 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. Furthermore, 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 methods of application we intend to use for this project.

The first year of this project has focussed on improving the bacteria to become more efficient as a biological control. We intend to do this by experimental evolution, where the bacteria's beneficial trait we want to enhance is focused on and selected for over several weeks. At the end of this 'passaging' process, we will compare if there have been any trade-offs between the evolved strains. This involves comparing whether improving one trait of the bacteria will be at a cost to another, for instance improving bacterial toxicity may cause bacterial growth on plant to become less efficient. There are four traits that are the focus of our evolution experiment.

Toxicity to aphids

A key outcome of the evolutionary passages would be to improve the toxicity of the bacteria. Currently, 70% of aphids are killed by *P. poae* in 42 hours, we hope 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.

Growth and persistence on plants

We will attempt to improve the colonisation of bacteria on plant leaves and how long the bacteria can last on the plant, thus reducing 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.

Formation of biofilms

Finally, we intend to investigate 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, thus 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 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.

Each property of the bacteria will be investigated over 10 passages. Only the biofilm passages have been conducted thus far and although the dataset is incomplete, there are promising results. *P. poae* is capable of forming biofilms in a broth environment and there are significant changes in attachment strength (how well the bacteria can adhere to a surface) over the passages, indicating that we are able to improve this over time.

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.

This bacterial biological control has the potential to significantly reduce costs of aphid crop protection as it would remove the need for chemical treatments and the improvements we are working on should increase the efficacy of this approach; therefore decreasing application costs. As the bacteria may be self-sustaining in the crop system, a reduction of applications would be likely. However, it is still very early in the project for definitive figures.

Action Points

As this is the first year of this project, 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.

