Australian Government
Cotton Research and Development Corporation

SUMMER and HONOURS SCHOLARSHIP APPLICATION 2013-14 SEASON

1.	**Project Title** (Maximum 85 char)	:	Interaction between beneficial insects, aphids & biopesticides.
2.	**Proposed Start Date**	:	18th November 2013
	Proposed Cease Date	:	**30th January 2014 (includes Christmas holiday)**
	Scholarship Type (summer or honours): Summer		
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SUMMER SCHOLARSHIP REPORT

1. Executive Summary:

Commercial biopesticides based on entomopathogenic fungi are expected to be registered for use as microbial biological control of aphids in Australia in the near future. Field trials of prototype biopesticides based on entomopathogenic fungi have shown that while aphids are highly susceptible, natural enemies are not affected in the field, even though they are susceptible in the laboratory (DAQ111C). Based on the work of Roy *et al.* (2008), the hypothesis tested was that beneficial insects have behavioural responses to the biopesticides that allow them to avoid infection. The project observed the behaviour of predators of aphids (green lacewings) and both infected aphids and cotton leaf surfaces treated with biopesticides. Most of these studies were conducted on leaves taken from cotton plants grown in temperature controlled growth cabinets at QUT. The biopesticides used are based on a commercial formulation of the fungus *Metarhizium (Met 52, Novozymes Ltd)*, and Australian isolates of *Beauveria*, both held at QUT.

2. Background:

The series of experiments examined the ability of beneficial insects to detect and avoid microbial biological control agents in infected prey and when applied to plants. Microbial biological control is where naturally occurring pathogens (Ormond *et al.* 2010) of a crop pest are produced on a commercial scale to manage an extensive variety of arthropod agricultural pests (New, 2002). Microbial agents include entomopathogenic fungi and viruses**.** Normally these control agents have a lesser ecological impact risk attached to the use compared to releasing non-native anthropoid predators, which in some cases have become agricultural pests themselves and lead to the extinction of native species (Kenis *et al.* 2008, Evans 2004 and Pell *et al*.2008).

The main reason for using microbial biological control is because agricultural pests are becoming increasingly resistant to chemicals. It is very hard to acquire resistance to naturally occurring pathogens, as the pathogen is in an evolution arms race with the host (Cook, 1993). This is particularly true with host specific pathogens such as viruses and gut bacteria. Host specific pathogens are important as natural enemies of the pest are not targeted by the pathogen and therefore the predator instils another selection pressure which reduces the pest population's ability to develop resistance to the pathogen.

Research on the interactions of the biopesticide and beneficial insects has to be conducted, as many fungal biological controls are not selective in host infections (Roy & Pell, 2000). However, field trials of fungal pathogens in Australian cotton have shown that, though susceptible in laboratory conditions, fungal biopesticides applied to cotton have no measurable impact on populations of beneficial insects (Hauxwell et al. DAQ111C final report to CRDC)

The use of microbial biological control in conjunction with an integrated pest management plan and with good agricultural practices; such as, crop rotation, fallow periods, weed control within the crop, and a monitoring program (consisting of sticky traps, spot inspections of the crop and making sure under the leaves are checked), will allow pest populations to remain within manageable levels and significantly reduce the occurrence of resistance within pest populations.

3. Aims and Objectives:

The purpose of this study is to better understand the ecological relationship and behaviours between two particular natural enemies of the cotton pest *Aphis Gossypii* (Cotton Aphid); *Mallada signatus* (Green Lacewing) (supplied by Bugs for Bugs) and *M. anisopliae*. The goal of the study is to be able to improve management of insect pests in the future. More specifically this study aims to identify if *M. signatus* larvae can detect the presence of insect pathogenic fungi *M. anisopliae*. *M. signatus* is a non-selective or generalist predator. The lack of a specific prey make this insect extremely useful as a biological control because once the pest population has been brought to low levels the predator will then move on to a different more abundant prey in the area which means that the population of pest insects will be slower to recover as there is ongoing predation in the area keeping numbers of pests low.

This study used two different experiments to assess if *M. signatus* can detect and avoid a surface containing fungus, such as would occur following spray application of a biopesticide, with and without the presence of aphids as bait.

4. Materials and Methods:

The environment detection experiment designed used methods set out by Meyling & Pell (2006), Roy *et al.* (2008), Ormond *et al.* (2011) and Bahar *et al.* (2012). Preparatory work was conducted to learn techniques and to establish and maintain colonies of lacewing, aphids and cotton plant, and cultivate *Metarhizium* spores.
Preliminary experiments were run to test the experimental plan, particularly in design of an effective experimental arena in which insects could be contained and observed. Maximum does challenge experiments were also completed to ensure that *Metarhizium* would infect lacewing larvae and kill the lacewing larvae, which resulted in a a 75% death rate within 7 days from direct contact spray of a stock solution *Metarhizium* and a 0.02% solution of tween.

An initial phase of the project was spent designing and testing a functional arena for behaviour experiments. The initial experiment on cotton leaves found that lacewing larvae move consistently to the underside of the cotton leaf, avoiding the upper test surfaces. The second design involved a small circle of filter paper treated and placed in larger glass petri dish. In this case the lacewing larvae moved to the glass petri dish around the edge and not the paper arena. The third design used 90mm filter paper that fitted exactly into the glass petri, but resulted in the similar results as the second attempt: the larvae moved to the upright edges of the petri dish and avoided the paper. Finally a design was developed to avoid these difficulties: the petri dish was placed upside down on a glass ring and covered with the paper arena. The assembly was placed in a water bath in which the water was almost level with the upper surface of the Petri dish to insure the larvae remained within the arena.

24 hours before the experiment, second instar *M. signatus* larvae (10 days old) were separated into solo cups with or without food. Spore concentration was counted in a stock solution of *Metarhizium* spores in 0.02% Tween 80 and percentage spore viability was confirmed by germination for 24 hours on water agar. One hour prior to experiment commencement, 100mm glass petridishes were sterilised by autoclaving to form a base for the experimental arena, labelled to identify treatment, and turned upside down and placed on top of glass rings in a water bath.

Half an hour prior to the commencement of the experiment, an airbrush was used to apply treatments to the paper: the control (0.02% tween 80 solution) to one half of the moist filter paper and the stock solution(1:100) of *Metarhizium* isolate 251 to the other half of the filter paper. The paper was then placed on the Petri dish at and the dish placed in a randomly selected orientation to avoid environmental factors such as sunlight to cause bias in the results. The *M. signatus* larva was then placed in the centre of the arena. The larva was observed for 10 minutes and the location of the larva was recorded every 30 seconds.

A second treatment placed uninfected bait aphids on the half of the filter paper treated with fungus to see if the presence of prey would induce the predator to enter the fungus-treated area. Bait aphids were killed by freezing, and removed from the freezer fifteen minutes prior to experiment. Immediately prior to commencement of the experiment, 5 aphids were placed 2cm away from the centre of the dish and 2cm away from each other in the fungus-treated areas only. The *M. signatus* larvae were then placed in the centre of the dish. The arena was observed for 10 minutes and the location of lacewing larvae was recorded every 30 seconds.

Finally, the experiment was repeated using lacewing larvae starved for 24 hours to test if the presence of prey combined with starvation would induce them to enter the fungus-treated area.

5. Results:

Tables 1 and 2 display the average time (in seconds) that larvae spent on each of the treatment areas.

Treatment	Mean time spent on control (seconds)	Mean time spent on *M. anisopliae* treatment (seconds)	Difference between treatment means (seconds)
Starved, choice without bait	481	119	362
Starved, choice with bait	353	314	39

Table 1: Average time spent on treatment – lacewing larvae starved 24 hours prior to experiment

Treatment	Mean time spent on control (seconds)	Mean time spent on *M. anisopliae* treatment (seconds)	Difference between treatment means (seconds)
Fed, choice without bait	451	149	302
Fed, choice with bait	461	194	267

Table 2: Average time spent on treatment – Larvae kept with food until 15 minutes prior to the experiment

First the data was transformed (arc sine transformation) to allow for statistical analysis, then a Shapiro-Wilks normality test was performed on the data to insure normalcy (table 3).

TREATMENT	Control		Standard Deviation	*M. anisopilae*		Standard Deviation
	W VALUE	P VALUE		W Value	P Value	
Starved, choice without bait	0.9469	0.5925	0.29784249	0.9469	0.5925	0.29784249
Starved, choice with bait	0.8255*	0.01394*	0.431907894	0.8288*	0.01532*	0.429093358
Fed, choice without bait	0.9283	0.2892	0.371304403	0.9548	0.6381	0.370203234
Fed, choice with bait	0.9474	0.5593	0.344943402	0.9474	0.5593	0.344943402

Table 3: Shapiro-Wilks Normality test of time spent in control versus the *Metarhizium* treated area. Treatment marked with '*' is not normally distributed.

This shows that one of the results (starved larvae with bait) not normally distributed (* table 3). A non-parametric test, the Kruskal-Wallace rank sum test, was therefor performed. The null hypothesis was that the lacewing has no preference between control and *Metarhizium* areas. The Kruskal-Wallis chi-squared value was 27.7, (on 7 degrees freedom), p-value = 0.0002456. Thus the null hypothesis was rejected, i.e. insects showed a highly significant difference in preference.

In all treatments, the lacewing larvae preferred to spend time on the control area than on any of the *Metarhizium* treatments, even in the presence of bait aphids. The greatest difference was observed in both starved and fed larvae where no bait was offered as an inducement to cross into the *Metarhizium*-treated area. Where bait was offered only on the *Metarhizium*-treated area, larvae fed until 15 minutes before the test still preferred to spend time on the control than on the treated area. Lacewings larvae showed no clear preference only in the case where starved larvae were offered aphid bait on the *Metarhizium*-treated area.

Discussion and Conclusions:

The results show that lacewing predators avoid surfaces treated with *Metarhizium* even in the presence of prey, and that only a combination of starving and the presence of bait aphids will induce them to spend almost equal time on the treated area. This discovery leads to some potentially very exciting news: that there is a mechanism to explain the observation that commercial products can be applied with minimum disruption to the beneficial predatory insect, even if those insects may be susceptible to infection in laboratory conditions. This supports the observations reported under DAQ111C, that it is possible to use biopesticides within an IPM framework to assist in controlling pest species such as aphids or mirids without disrupting beneficial insects.

6. Highlights:

This discovery leads to some potentially very exciting news: that there is a mechanism to explain the observation that commercial products can be applied with minimum disruption to the beneficial predatory insect, even if those insects may

be susceptible to infection in laboratory conditions. This supports the observations reported under DAQ111C, that it is possible to use biopesticides within an IPM framework to assist in controlling pest species such as aphids or mirids without disrupting beneficial insects.

This project supplied many challenges that I had never faced prior in my two years of undergraduate study, from designing experiment to creating protocols to establish colonies of lacewings and aphids not previously maintained at QUT. The project also allowed me to meet new, interesting, and extremely knowledgeable people: Jamie Hopkinson at DAFF who supplied the cotton seeds and explained aphid culturing techniques, and Mark Kinkema and the staff at Ag BiTech (Toowoomba), who supplied the large amount of food (in the form of *Helicoverpa* eggs) that young, perpetually hungry lacewing larvae need. The lacewings in turn were supplied by Wes at Bugs for Bugs. I was able to visit the AgBiTech production facility in Toowoomba and the DAFF laboratories and glasshouses at Tor St, Toowoomba to see how their processes worked.

The colony work involved was time consuming but rewarding. With this project came the knowledge of exactly how much work is needed and how many intricate facets there is in managing four very different biological organism, all with protocols that needed to be ironed out before even commencing pilot studies. Being able to create a protocol for successfully rearing and maintaining a healthy lacewing colony is an achievement I am still proud of.

During the course of the experiment I experienced firsthand how rewarding and frustrating running experiment involving biological components can be. This experience has given me vital understanding of the importance of solid groundwork to ensure the proposed experimental plan is as robust as possible as well as the knowledge that what may appear to be a failed experiment can in fact, with careful observation and recording, show something of keen importance that otherwise would never have been discovered. Speaking to some of the academics this understanding is key to the ability to becoming a good biologist, as with most biological experiment 'what can go wrong will go wrong' and it take a lot of perseverance on the part of the scientist to be able to cope with these constant speed bumps on the road to discovery. So in my opinion this is the most vital lesson I have learnt from the huge increase in knowledge and ability that this opportunity has provided. This understanding and the additional skills I gained during this summer project has allowed me to better understand and better participate in my continuing undergraduate studies. It has also allowed me to secure a part time job at QUT working in insect pathology. So saying that this has been the most rewarding projects I have completed in my degree and probably will remain so for many years to come through out my further career.

7. Future Research:

QUT have continued the work over the past year beyond this initial scholarship to look at questions regarding the lacewing's behaviour. I have been heavily involved with these and have lead some pilot studies looking at prey preference (aphids and cotton boll worm), time-to-death and dose range challenges for infection rate of aphids, cotton boll worms and lacewings, but most excitingly I have led a study to determine if lacewings can identify and avoid prey that has been infected by entomopathogenic fungi before the infected aphids show over symptoms such as sporulation or mycelium, and prior to death, and found that they do. Future research would involve expanding these pilot studies into full studies and repeating the current work with more virulent strains of *Beauveria* or *Metarhizium* now available. Also an exciting avenue would be to examine the chemical queues the lacewing uses to identify infected prey.

8. Presentations and Public Relations:

Currently there has been no public presentation or publication in regards to this work, but with addition of some experimental work planned for this year we expect to publish this work this year. With the exciting development I have made in studying the behaviour of the seldom studied green lacewing and its ability to detect and avoid areas of the plant treated as well as the potential found within our pilot studies it is very possible that a publication can be made if not yet to a peer reviewed journal in and industry focused magazine such as the CRDC's Spotlight on cotton R&D.

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