January, August & Final Reports

REPORTS

Part 1 - Summary Details

Please use your TAB key to complete part 1 & 2.

CRDC Project Number: **DAQ 95C**

January Report: ☐ Due 29-Jan-01

August Report: ☐ Due 03-Aug-01

Final Report: ☒ Due within 3 months of project completion

Project Title: In Field Development of Novel Options for Helicoverpa spp. Control in Central Queensland

Project Commencement Date: Jan 2000 **Project Completion Date:** June 2002

Research Program: Insect Management

Part 2 – Contact Details

Administrator: Ms Vicki Battaglia, Senior Planning Projects Officer

Organisation: QDPI - Farming Systems Institute

Postal Address: PO Box 2282, QDPI - Farming Systems Institute
Toowoomba, Qld 4350

Ph: 07 4639 8886 **Fx:** 07 4639 8881 **E-mail:** vicki.battaglia@dpi.qld.gov.au

Principal Researcher: Dr Paul Grundy

Organisation: QDPI - Farming Systems Institute

Postal Address: LMB 1 QDPI, Farming Systems Institute
Biloela Qld 4715

Ph: 07 4992 9172 **Fx:** 07 4992 3468 **E-mail:** paul.grundy@dpi.qld.gov.au

Supervisor: Dr Ian Titmarsh

Organisation: QDPI - Farming Systems Institute

Postal Address: LMB 6 QDPI, Farming Systems Institute
Emerald Qld 4720

Ph: 07 4982 7409 **Fx:** 07 4983 7459 **E-mail:** ian.titmarsh@dpi.qld.gov.au

Researcher 2 (Name & position of additional researcher or supervisor).

Organisation:

Postal Address:

Ph: **Fx:** **E-mail:**

Signature of Research Provider Representative: David Hamilton
15/10/02

Cotton Research & Development Corporation

" In Field Development of Novel Options for *Helicoverpa* spp. Control in Central Queensland "

(DAQ 95C)

(January 2000 to June 2002)

Paul Grundy and Sherree Short

Queensland Department of Primary Industries Tel: (07) 4992 9172
LMB 1, BILOELA, QLD 4715 Fax: (07) 4992 3468

"A Final Report prepared for the Cotton Research and Development Corporation"

Table Of Contents

Project Personnel & Key Collaborators

Paul Grundy is the Project Entomologist and was appointed to the DPI during the early stages of this project. Before joining the DPI in 2000, Paul was involved with cotton and grain research using applied biological controls as part of his postgraduate program.

Sherree Short is the Project Experimentalist. Sherree has been working with the DPI on Entomology and Pulse Agronomy research for over ten years before joining this project.

Caroline Hauxwell is a Principal Scientist in QDPI and leads the Biopesticides Unit at Indooroopilly. Carrie led the field-testing research with *Nomuraea rileyi* during this project.

David Holdom is a Scientist with QDPI who has specialised in the development of fungal pathogens as biopesticides such as *Nomuraea rileyi*. David has also contributed to the field-testing research conducted with *Nomuraea rileyi* during this project.

Alex Glauerdt is a Technical Officer with the QDPI Biopesticides Unit who provided key assistance with the conduct of Nomuraea rileyi field experiments during the project.

Glenn Graham is the Team Leader for the Centre for Identification and Diagnostics (CID) at the Uni of QLD. This group are investigating the use of micro-satellites to determine population structure of *Helicoverpa armigera*.

Kendle Wilkinson is a Post Doctoral Fellow with CID and has had a major contribution in the analysis and interpretation of the micro-satellite results.

Kirsten Scott is a Senior Research Officer and Program Leader – Insect Genetics with CID. Kirsten plays a significant role in co-ordinating the collection and processing of samples as well as reporting the results of this project.

Plain English Summary

Helicoverpa spp. continue to present significant challenges for the sustainable production of cotton in Central Queensland. As part of on-going Integrated Pest Management and Area Wide Management programs this project focussed on identifying and testing several novel approaches for *Helicoverpa* spp. management each of which aim to reduce dependence on traditional insecticide controls.

The fungal pathogen *Nomuraea rileyi* was assessed for its suitability as a prototype biopesticide against *Helicoverpa* spp. in cotton under field conditions. The results suggest that the spores of *N. rileyi* persist well upon cotton plants in the field and can cause comparable levels of larvae mortality when compared with NPV products. The performance of this pathogen was variable, with observations suggesting that a factors such as environment, canopy coverage and plant chemistry may influence efficacy. A significant response to increased rate of application was observed during a final experiment, suggesting that efficacy may be significantly improved with an increased application rate. At this stage *N. rileyi* has shown promise as a prototype biopesticide.

Several liquid additives were investigated for their potential to improve the field performance of NPV's under CQ conditions. The results from a number of experiments suggested that the addition of Aminofeed® and Aminofeed UV® significantly enhanced the field efficacy (by 20%) of NPV's and prolonged the period for which NPV was infective on cotton foliage. It was also demonstrated that virus infectivity was similar for morning, noon and evening applications, suggesting that priority should be placed on gaining adequate coverage and targeting of small larvae. A field study on the effects of food sprays (Aminofeed®, Predfeed® and Envirofeast®) on *Helicoverpa* spp. oviposition also suggested that these products have no significant effect on oviposition rates in cotton.

Pulse Industry concerns that ascochyta leaf blight may gain entry into CQ through the current use of chickpea for trap cropping was the impetus for an experiment that compared alternative legume species. The main focus for the experiment was to record *Helicoverpa* spp. oviposition on trap crops of chickpea, popani vetch, namoi vetch and field peas. Results suggested that field peas were significantly more attractive to ovipositing moths that laid 60-70 eggs per m/row compared to 10-20 egg per m/row in chickpeas for the whole month of September, the period for which peak attractancy is desirable. Field peas were further advantageous in that 73±3.5% of the eggs were observed to perish before hatching as opposed to only 27±2.9% on chickpeas. The egg losses on field peas were due largely to the waxy leaf surface and presence of beneficial insects such as ladybirds. Research on the use of field peas as an alternative trap crop is ongoing.

Area Wide Management (AWM) can only succeed if *Helicoverpa armigera* populations are indeed locally recruited within the farming system. The Dawson and Callide region is geographically isolated from other cotton growing areas and compared to southern districts lacks the conditions nesesary to induce a regular diapause in *Helicoverpa* spp. populations. The continuity in cropping within this

region between summer cotton and grains and winter chickpeas provides in an ideal situation for *Helicoverpa* spp. to cycle locally within the farming system without significant interruption. With the use of microsatelite biotechnology in collaboration with the University of Queensland's *Centre for Identification & Diagnostics* (CID) (Lead Agency) we have been conducting a sampling program to determine the extent of local recruitment within the Dawson Callide Farming system. Preliminary results from these studies is suggesting clear differentiation between *Helicoverpa armigera* populations from the Dawson Callide region and populations from the Narrabri, and Darling Downs regions and the genetics of Heliothis are not crop dependent: e.g. the Heliothis found on cotton are not different from those found on commercial chickpea, or trap crops.

The information compiled in this study is providing an improved basis on which to build and effective area wide and resistance management program for *Helicoverpa armigera* in the Dawson Callide region. Many of the novel options addressed in this project have been subject to basic research that has formed the foundation for future research that is currently being continued in other CRDC and GRDC funded projects. It is likely that many of the novel options assessed during this project will go on to be developed and implemented as tools that reduce insecticide dependence within future area wide and integrated pest management programs.

1. Project Background

Helicoverpa spp. moths continue to pose significant pest management problems for Queensland cotton growers. To address the *Helicoverpa* spp. problem the Department of Primary Industries (DPI) was successful in securing funding from the Queensland Treasury for a new initiative entitled *Supporting Green Industries*. The objective of this new initiative was to develop new tools and techniques that could be used to better manage *Helicoverpa* spp. more sustainably and reduce the cotton and grains industries dependence on insecticides.

A significant feature of the new initiative was the establishment of Biopesticide and Chemical Ecology Units led by Dr's Caroline Hauxwell and Chris Moore respectively. These units were chartered to investigate and develop new biopesticides and chemical compounds that have potential for *Helicoverpa* spp. management.

The purpose for this project (DAQ95C) was two fold. A major objective was to field test and evaluate the technologies developed by the Biopesticide and Chemical Ecology units. The second objective was to develop and test novel field control options for *Helicoverpa* spp. that could be incorporated into an Area Wide Management program for Central Queensland. A brief background for each of these technologies is given below.

1.1. New Biopesticides

Nucleopolyhedrosis viruses and *Bacillus thuringiensis* bacterial pathogens have been successfully adopted as insecticides in the cotton industry. Likewise fungal biopesticides have the potential to play a significant role in pest management programs, offering several advantages compared to viral and bacterial products. Unlike virus and bacterial biopesticides that act via ingestion, fungal pathogens infect through contact with the endospore stage of the pathogen. This infection pathway may offer a significant advantage in crops such as cotton where it is often difficult to target application to protected larval feeding sites such as squares, flowers and bolls.

Nomuraea rileyi (Farlow) Samson (Deuteromycotina: Hypomycetes) is a fungal pathogen that infects the larvae of Noctuid moths. The QDPI biopesticide unit have developed mass-production, stabilisation and formulation techniques for *N. rileyi*. Preliminary research has previously demonstrated efficacy on grain crops under glasshouse and field conditions. In this project we conducted basic field efficacy experiments with *N. rileyi* formulated at various rates and volumes in oil on cotton.

1.2. Additives To Improve Biopesticide Performance

A difficulty faced by growers using nucleopolyhedrovirus (NPV) products such as Gemstar® or Vivus® can be inconsistency of the products' performance for *Helicoverpa* spp. control. This problem has lead to the advent of additives developed to improve biopesticide performance. The use of additives to improve the performance of NPVs has been the subject of considerable research. The objective for using an additive with biopesticides such as NPV has been to improve field performance by increasing larval intake, improving the microbes' virulence or protecting the microbes and enabling them to persist for longer periods of time in the external environment (Cunningham *et al.* 1997; Morales *et al.* 2001). A number of liquid additives for use with NPVs have recently entered the Australian marketplace. Aminofeed® was one of the first products available and was subject to testing by Murray *et al.* (2000). In these tests Aminofeed® gave an equivalent response to the calf feeding supplement Denkavit® which had been widely used as an additive after initial work conducted by Teakle and Monsour (unpublished data). Due to a liquid formulation which eliminates the application difficulties associated with milk powders, Aminofeed® has become a popular choice as an additive for NPVs.

Since the successful introduction of Aminofeed®, several new liquid IPM tank mix products for use with biopesticides have also entered the Australian market. Aminofeed UV® by Agrichem is a new product that incorporates sunscreening agents with the original Aminofeed® formulation. Coaton ILP® by Biostarch Australia has been designed as a liquid alternative for calf milk powders and Mobait® by Nufarm has been promoted as a feeding attractant suited for addition to a variety of insecticides. In this project we evaluated the efficacy of these liquid additives and their ability to enhance the performance of NPVs on cotton under central Queensland conditions.

1.3. New Winter Trap Crops for *Helicoverpa* spp.

Chickpeas are currently grown by CQ cotton growers as a spring trap crop for *Helicoverpa* spp. (Sequeira 2001). At present CQ is free of ascochyta blight, a disease that has seriously reduced the profitability of chickpea production in southern regions. Members from the Pulse industry have expressed concern that the use of chickpea as a spring trap crop in CQ may inadvertently lead to the introduction of ascochyta. In response to these concerns a number of winter active legumes were evaluated for their potential to be substituted for chickpea as a winter trap crop under CQ conditions and thus alleviate any cross-industry conflict. Field peas and vetch were chosen for this purpose as feedback from pulse agronomists in other regions had suggested that these legumes may be suitable candidates for trap cropping in the CQ environment. A preliminary assessment of these legumes for their suitability for trap cropping in CQ were made during the last year of this project.

1.4. Heliothis Movement and Local Recruitment Ecology In CQ

Area Wide Management (AWM) can only succeed if *Helicoverpa armigera* (Hübner) populations are indeed locally recruited within the farming system. If *H. armigera* are localised in their origin, management programs can then be implemented to target these populations as they occur within the cropping cycle of each region. The Dawson and Callide region is geographically isolated from other cotton growing areas and compared to southern regions lack, the conditions nesesary to induce a regular diapause in *Helicoverpa* spp. populations. The continuity of cropping within this region between summer cotton and grains and winter chickpeas provides an opportunity for *Helicoverpa* spp. to cycle locally within the farming system without significant interruption. Pupae sampling studies by Sequeira (2001), strongly suggested that there are significant correlations between *Helicoverpa* spp. population in winter chickpeas and summer cotton and grains.

The advent of microsatelite biotechnology has provided a tool whereby the correlations in *Helicoverpa* spp. movement and recruitment as observed by Sequiera (2001) to be further verified. These genetic markers are short DNA sequences in which the nucleotides (the building blocks of DNA) repeat themselves. The University of Queensland's *Centre for Identification & Diagnostics* (CID) have for the past few years built a catalogue of these microsatelites for *H. armigera.* Since microsatelites occur widely across the genome, the analysis of their frequencies and sizes are extremely useful for determining population structure and can be used to answer questions as to the movement and origin of *Helicoverpa* spp. populations within the farming system.

Successful Area Wide Management depends on a knowledge of *H. armigera* recruitment patterns so that populations can be better targeted with appropriate controls. To gain a better understanding of the population dynamics of *H. armigera* in the Dawson Callide regions an extensive sampling program of moths and larvae was conducted in both cotton and grain crops throughout the region. In collaboration with the team at CID, the microsatelites in these samples have been identified and used to better explain the pattern of *H. armigera* activity in the local region. The findings from these studies are generating considerable interest amongst cotton and grain growers in adopting and implementing better pest management strategies as part of a larger AWM program in CQ.

2. Project objectives and the extent to which they have been achieved.

2.1. Develop New Biopesticides and Test Novel Compounds.

A series of experiments to test application rates, spore persistence/viability after application and efficacy of *N. rileyi* when compared with existing biopesticides were conducted in collaboration with Dr Caroline Hauxwell and Dr David Holdom (QDPI). Results from these experiments suggest that spores of *N. rileyi* persist well on cotton foliage, that *N. rileyi* can give similar control compared to NPV's and that optimum application rates have yet to be determined.

A number of novel larvae behaviour modifying compounds were to be investigated during this project. This research was to be conducted in collaboration with Dr Chris Moore at QDPI as part of the QDPI's Heliothis New Initiative. We were unable to conduct this research during the project due to the un-availability of plant extracts that had been developed sufficiently to the point at which field-testing could commence.

2.2. Improve Activity and Field Life of Current Biopesticides.

A series of experiments were conducted during 2000/01 and 2001/02 seasons to assess the benefits of using additives with NPV biopesticides. The experiments compared the ability of a range of liquid additives (Aminofeed®, Aminofeed UV®, Mobait® & Coaton®) to enhance NPV performance on cotton in the field. Aspects such as application timing was also investigated.

Results from these experiments strongly suggest that the addition of Aminofeed and Aminofeed UV to NPV's during application reliably increase the field performance of NPV's in CQ cotton. Mobait® and Coaton® were less successful additives for use with NPV products. An experiment looking at the effects of Aminofeed® on Heliothis oviposition in cotton suggested that this product has no significant effect on Helicoverpa behaviour as recorded by field oviposition rates.

2.3. Develop Novel Approaches to Heliothis Management.

In-Season Trap Crops

Strips of mungbeans, chickpea, niger and cotton were planted during the 2000/01 season adjacent to cotton to test their potential as an in-season trap crop. Heliothis egg laying in both the trap crops and adjacent cotton was recorded during the experiment. The trap crop rows were only effective in causing a reduction in egg numbers for the first adjacent 8 rows of cotton. The sink effect of the trap rows was minimal beyond this distance.

A second experiment that investigated an in-season trap crop in the form of a chickpea-cotton mix planted in every fourth row was conducted during the 2001/02 season. The concept was to condense Heliothis egg laying onto the trap rows and then band spray them as part of an IPM program. If successful, this approach may be used to reduce the total volume of early season insecticide (by 75%) that would otherwise be applied over an entire area. Unfortunately the trial site was destroyed by a hail storm before meaningful measurements could be made.

Alternative Winter Trap Crops

Field peas and vetch were evaluated for their potential to be substituted for chickpeas as a spring trap crop in CQ. The main focus for the experiment was to record Heliothis oviposition on trap crops of chickpea, popani vetch, namoi vetch and field peas. Results suggested that Heliothis laid significantly more eggs on field peas (60-70 eggs per m/row) than chickpeas (10-20 egg per m/row) for the whole month of September, the period for which peak attractancy is desirable. The field peas were further advantageous in that 73±3.5% of the eggs were observed to perish before hatching as opposed to only 27±2.9% on chickpeas. The egg losses on field peas were due largely to the waxy leaf surface and presence of beneficial insects such as ladybirds. Field peas in this experiment were vastly superior to chickpeas.

2.4. Identify Opportunities Specific to Local Conditions for Novel Control Options which will Contribute Towards Building an IPM Program for Heliothis in CQ.

At the commencement of this project we had an opportunity to collaborate with Mr Glenn Graham and the *Centre for Identifications and Diagnostics* (CID) at the University of Queensland. Mr Graham's CRDC funded project (UQ32C) which is investigating the use of micro-satellites to determine Heliothis movement and local recruitment provided a method to identify opportunities specific to local conditions for building an Area Wide Management program for the Dawson/Callide valleys.

H. armigera sampling has been conducted in the Dawson and Callide valleys for 2 years with the aim of identifying local recruitment patterns and to address questions such as whether or not trap crops are effectively attracting heliothis generated from winter chickpeas and how much "exchange" occurs between cotton and grain crops within the local region. Samples of *H. armigera* have been taken from host crops such as chickpeas cotton and mungbeans as well as trap crops from across the region throughout each season.

The *H. armigera* samples collected from the Dawson/Callide region have been sent to the University of Queensland's CID for micro-satellite identification and population analysis.

Preliminary results from these analyses are strongly suggesting that a large percentage of the *H. armigera* population sampled during the first year of the project were locally recruited. Processing of the second seasons samples is underway, with early indications suggesting a continuation of this trend. Information from this research will provide a platform for the development of a more effective local area-wide management strategy for the Dawson and Callide valleys.

2.5. Promote 'Best Practice' in Insect Pest Management Among CQ Cotton Growers.

Best management practices have been promoted to the industry through a number of avenues. Most of the research conducted under this project has been reported in brief to CQ cotton growers through the Cotton Tales series coordinated by Mr David Kelly (QDPI). Reports from most of our trials have also been published in the CQ Cotton Trial and Yearbooks compiled by David Kelly.

Several meetings and field days that have focused on our research were held with both the Theodore and Emerald cotton growers. As well as these activities, DAQ95C results have been presented at various industry meetings including the 11th Cotton Conference and annual CCA meetings. Various articles detailing the scope of our research have been published in the Rural Weekly section of a number of regional Queensland newspapers. Several radio interviews with the ABC have also been recorded and broadcast during the last two years. We have also facilitated the Theodore cotton growers with an Area Wide Management practices. This group meets informally, in conjunction with association meetings.

3. How this research has addressed the Corporations three outputs: Sustainability, profitability and international competitiveness, and/or people and community?

The current dependence on insecticides for *Helicoverpa* spp. management in the cotton industries poses significant risks in terms of sustainability and profitability. The research conducted during this project aimed to develop and test a range of novel approaches to *Helicoverpa* spp. management that would provide increased 'soft' alternatives to traditional insecticides in a pest management program. This project was an integral part of QDPI's new initiative for fostering cleaner and greener industries through the development of more environmentally sound Heliothis management practices.

Research on potential new biopesticides such as *N. rileyi* has provided significant preliminary data on how this fungal pathogen may be developed and used as a biopesticide in cotton. If successful the future development of *N. rileyi* would provide an alternative control option for *Helicoverpa* spp.

Research on additives for NPV biopesticides has demonstrated that the performance of NPV's can be reliably increased resulting in better *Helicoverpa* spp. control efficacy. The increased performance of these biopesticides through the addition of additives has resulted in their increased uptake as a cost effective replacement for synthetic insecticides that has improved the sustainability of cotton IPM programs and resistance management.

The development of field peas as an alternative trap crop will also serve to limit the opportunity for ascochyta leaf blight to enter CQ and thus support the chickpea industry of which many cotton growers are a part in the local region. The greater effectiveness of field peas as a trap crop would also serve to further reduce *Helicoverpa* spp. populations and thus lower insecticide control costs.

The microsatelite studies of which this project was a part also serve to greatly extend our knowledge of *Helicoverpa* spp. ecology in the local region. The knowledge developed through this research will be used to greatly enhance local area wide management programs with such strategies offering to manage *Helicoverpa* spp. populations in a more sustainable and cost effective manner.

4. Research Methodology & Results

4.1 New Biopesticides – *Nomuraea rileyi*

4.1.1 Methodology

A series of experiments were conducted at various locations throughout central Queensland on irrigated cotton grown on 1 metre rows to investigate the performance and persistence of *Nomuraea rileyi* on field populations of *Helicoverpa* spp. on cotton.

N. rileyi isolate EFD45 was used in all experiments as follows. The fungus was produced by fermentation and the spores were harvested, processed and formulated according to DPI Biopesticides Unit protocols. In each experiment, *N. rileyi* spores were mixed with Propar 12 oil (Caltex-Ampol) immediately prior to application and the suspension applied with a hand-held spinning disk applicator (Micron, Bromyard England).

In efficacy trials, larvae were collected after application. Larval samples were taken first from the control plots to reduce the risk of cross-contamination and each person conducting the sampling sterilised their hands with a hand crème between treatments. Small and small/medium larvae were collected from each plot, transferred to individual artificial diet in ventilated 28 mL plastic portion cups and held at 25 ± 1°C, 65% RH and 12:12 photoperiod to determine their fate (survival to pupation, NPV, *N. rileyi*, parasitised or unknown cause of death).

Experiment 1. Persistence of N. rileyi on cotton

A laboratory assay was used to determine the persistence of *N. rileyi* spores when applied to cotton (Sicot 189) in the field. *N. rileyi* spores were applied to cotton plots as a suspension of 1 x 10^{13} conidia/mL in 4.0 L/ha of Propar 12 oil. Twelve leaves were then sampled from each treatment plot at 12 hourly intervals from 0-96 hours after application. Upon return to the laboratory, each sample of leaves was divided and 4 leaves were placed into individual disposable plastic containers (750 mL) and 30-40 *Helicoverpa armigera* neonates introduced into each container. The larvae were left to feed at 25 ± 1°C, 65% RH and 12:12 photoperiod for 24 hours. 30 larvae were then transferred from the leaves for each treatment replicate and reared on artificial diet in individual cup and their fate determined as above.

Experiment 2. Field Test Number 1

This experiment was conducted at the QDPI Biloela Research Station. The treatments were, 1 x 10^{13} conidia/mL *N. rileyi* spores in Propar 12 at 4L/ha, Gemstar® at 500mL/ha with Aminofeed® at 1L/ha applied with a hydraulic boom at 150Lwater/ha, and a control of Aminofeed® at 1L/ha applied with a hydraulic boom at 150Lwater/ha.

Plots were arranged in a randomised complete-block design with four 25m^2 replicates of each treatment separated by a 5m buffer. No pesticides were used on the surrounding crop either prior or during the experiment.

Pre-treatment counts determined the larval population density and size structure. Ten sampling sites were randomly selected across the trial area and at each site 1 m of cotton were visually assessed for larvae. For each sample, larvae were recorded as very small (VS) less than 3 mm in length, small (S) 3 to 7 mm, small medium (SM) 7 to 13 mm, medium large (ML) 13 to 23 mm and large (L) greater than 23 mm. These sizes approximated instars I, II, III, IV, and V and greater respectively. A collection of 50 larvae representative of S, and SM size categories was taken to determine species composition and pre-treatment pathogen infection levels.

The treatments were applied early in the morning before 7:00h on December 18, 2000. A north-easterly crosswind of 1-3 m/sec was present during the application of the various treatments. 24 larvae (small and small/medium, 3-13mm) were collected from each plot two days after treatment (DAT) and returned to the laboratory to determine their fate.

Daily maximum and minimum temperatures in a Stephenson screen together with rainfall were recorded for the duration of the trial.

Experiment 3. Field Test Number 2

This experiment was conducted on irrigated cotton (Sicot 189) sown on 1 m rows situated near Alton Downs near Rockhampton. The treatments used were the same as those in experiment 2.

Plots were arranged in a randomised complete-block design with four 25m^2 replicates of each treatment separated by a 5m buffer. No pesticides were used on the surrounding crop either prior or during the experiment.

Pre-treatment counts to determine the larval population density and size structure were made using the same procedure as for experiment 2. A collection of 50 larvae representative of S, and SM size categories was taken to determine species composition and pre-treatment NPV infection levels.

The treatments were applied mid-morning between 9.00 h and 10:30 h on January 11, 2001. A north-easterly crosswind of 2-4 m/sec was experienced during the application of the various treatments. 30 larvae (small and small/medium 3-13mm) were collected from each plot two days after treatment and returned to the laboratory for assessment.

Daily maximum and minimum temperatures in a Stephenson screen located at the Rockhampton airport located 20km away. Rainfall was recorded for the duration of the trial at the property location.

Experiment 4. Test of Oil Volume

This experiment was conducted in a field of cotton (Sicot 189) at the same Alton Downs site used for the second field test. On this occasion *N. rileyi* was applied using two different volumes of oil at 4 & 8 L/ha. These treatments were again compared to Gemstar® (500mL/ha) together with Aminofeed® (1L/ha) applied with a hydraulic boom at 150Lwater/ha and a second control of oil applied at 4L/ha.

Plots were arranged in a randomised complete-block design with four 25m^2 replicates of each treatment separated by a 5m buffer. No pesticides were used on the surrounding crop either prior or during the experiment.

The treatments were applied late in the afternoon between 17:00 h and 18:30 h on February 11, 2001. A south-easterly crosswind of 3-5 m/sec was experienced during the application of the various treatments. 30 larvae (small and small/medium 3-13mm) were collected from each plot two days after treatment and returned to the laboratory for assessment.

Daily maximum and minimum temperatures in a Stephenson screen located at the Rockhampton airport located 20km away. Rainfall was recorded for the duration of the trial at the property location.

Experiment 5. Test of Spore Rates

This experiment was conducted in a field of cotton (Sicot 189) at the QDPI Biloela Research Station. The treatments were, *Nomuraea* mixed with Propar 12 (Caltex-Ampol) to make a suspension of 1 x 10^{13} conidia/mL and 2 x 10^{13} conidia/mL both applied at 4.0 L/ha, Gemstar® (500mL/ha) together with Aminofeed® (1L/ha) applied with a hydraulic boom at 150Lwater/ha and a control of Propar oil applied at 4L/ha.

Plots were arranged in a randomised complete-block design with four 75m^2 replicates of each treatment separated by a 5m buffer. No pesticides were used on the surrounding crop either prior or during the experiment.

Pre-treatment counts determined the larval population density and size structure. A collection of 50 larvae representative of S, and SM size categories was taken to determine species composition and pre-treatment NPV infection levels.

The treatments were applied early in the morning before 7:00h on January 4, 2002. A easterly crosswind of 1-2 m/sec was present during the application of the various treatments. 30 larvae (small larvae 3-7mm) were collected from each plot two days after treatment and returned to the laboratory as described above. Larvae were only collected out of the first, third and fifth row of each plot, leaving the second and fourth rows for field assessments of larvae densities.

The proportion killed was corrected for control mortality using Abbots equation. The treatments were then compared using Genstat version 5. Data was transformed to logits of proportion dead with binomial errors. Significant differences were tested using linear regression and pairwise tests.

The plots were re-treated again four days after the initial application on 8 January.

Visual counts of *Helicoverpa* spp. larvae on the cotton plants were made on 4 randomly selected 1 m lengths of row of cotton plants in each treatment replicate 10 days after the first application/ 6 days after second application. The growing points and squares of the upper two thirds of the plants canopy were searched for small larvae and the flowers and bolls throughout the plants were also inspected for larger larvae. Larvae were recorded as small 2-10mm, medium 11-20mm and large >20mm.

Daily maximum and minimum temperatures in a Stephenson screen together with rainfall were recorded for the duration of the trial.

4.1.2 Results

Experiment 1. Persistence of N. rileyi on cotton

N. rileyi spores were reasonably persistent on cotton foliage under field conditions and caused mortality for up to 72 hours after application (Fig 4.1.1).

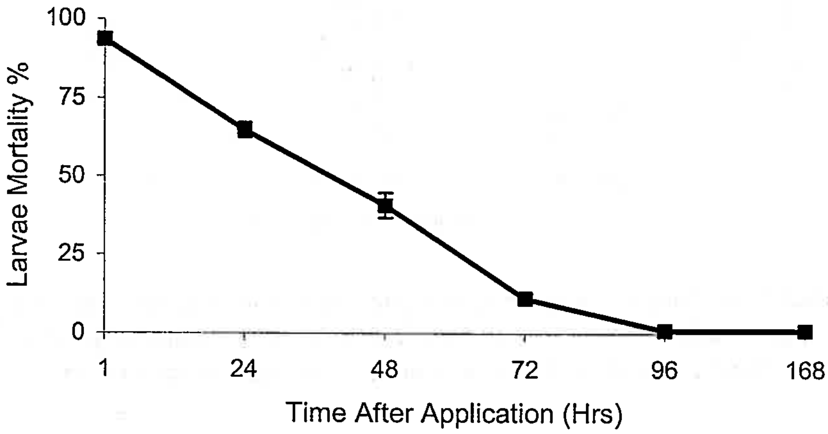

Figure 4.1.1 Residual activity of *Nomuraea riley* on cotton under field conditions.

Experiment 2. Field Test Number 1

At the time of treatment, *Helicoverpa* spp. infestation levels across the trial site averaged 0.9 ± 0.23 larvae/m^2 with 100% of larvae in the VS - S size range. *H. armigera* made up 98% of the *Helicoverpa* spp. present during the pre-treatment collection of larvae on 17 October 2000 and no NPV or *N. rileyi* was recorded. By the time of collection 48 hours after application the larvae were small (7mm or under). No *N. rileyi* was recorded in pre-treatment collections, nor at 2 DAT in any plots not treated with *N. rileyi*. A small amount of rainfall was recorded approximately 12 hours after treatment application which was followed by high ambient relative humidity of >90% and heavy morning dews (Table 4.1.2).

The percentage infection rates of the different treatments in collected *Helicoverpa* larvae are given in figure 4.1.2 The application of *N. rileyi* resulted in moderate infection levels in the larvae collected and grown out on artificial diet. The mean mortality caused by *N. rileyi* was significantly lower (*P<0.05*, LSD 5.50) than that caused by Gemstar®. The application of Gemstar® also caused moderate mortality levels within the larvae collected and grown out on artificial diet. A higher proportion of larvae infected with NPV died during the third instar compared with those infected with *N. rileyi* whose mortality levels peaked during the fourth instar (Table 4.1.1).

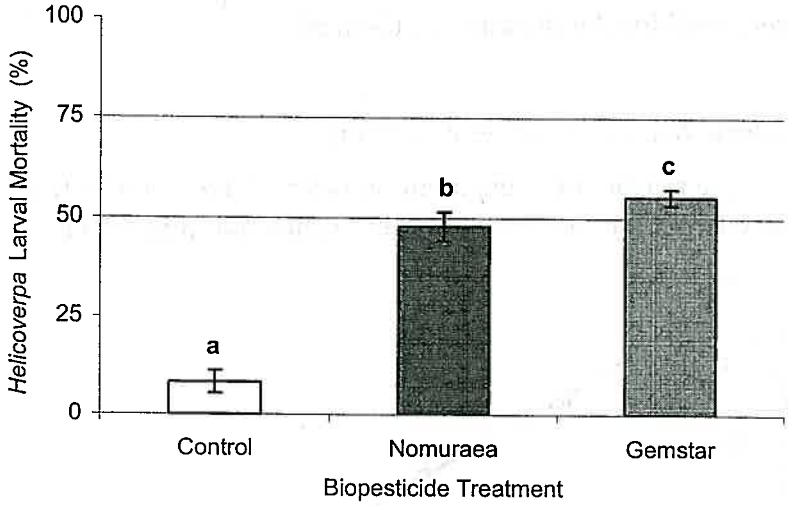

Fig. 4.1.2. Mortality of *Helicoverpa* spp. larvae collected from treatment plots sprayed with Gemstar/Aminofeed® & *Nomuraea rileyi*. No NPV or *Nomuraea* infected grubs were found in the control plots. Treatments with the same letter are not significantly different.

Table 4.1.1. The percentage larvae that died during each development stage.

Treatment	% died as 2nd Instar	% died as 3rd Instar	% died as 4th Instar	% died as 5th Instar
Nomuraea	4.5	32.0	59.0	4.5
Gemstar	24.0	74.0	2.0	0

Table 4.1.2. Daily maximum, minimum, average temperatures (°C) and rainfall (mm) at Biloela Research Station during the trial.

Date	18 Dec	19 Dec	20 Dec
Maximum	29.6	33.2	34.4
Minimum	14.2	14.4	17.7
Average	21.9	23.8	26.0
Evap RH%	100	97	96
Rainfall	0.6	0	0

Experiment 3. Field Test Number 2

At the time of treatment, *Helicoverpa* spp. infestation levels across the trial site averaged 5.7 ± 0.42 larvae/m^2 with 44% of larvae in the VS - S size range. *H. armigera* made up 72% of the *Helicoverpa* spp. present during the pre-treatment collection of larvae on 11 January 2001. All of the larvae collected 48 hours after application were of a small medium size (5-13mm or under). No rainfall was recorded between when the treatments were applied and the field collections of larvae were made (Table 4.1.3).

Table 4.1.3. Daily maximum, minimum, average temperatures (°C) and rainfall (mm) at Alton Downs during the trial.

Date	11 Jan	12 Jan	13 Jan
Maximum	30.8	33.9	32.9
Minimum	19.8	17.4	15.9
Average	25.3	24.7	23.9
Evap RH%	73	69	73
Rainfall	0.0	0.0	0.0

Larvae mortality due to the two treatments and control are given figure 4.1.3. No NPV or *N. rileyi* was recorded in pre-treatment collections. The application of *N. rileyi* resulted in very low infection levels in the larvae collected and maintained on artificial diet. The application of Gemstar® gave moderate infection levels within the larvae collected and grown out on artificial diet. The majority of larvae infected with both NPV and fungus died during the third instar.

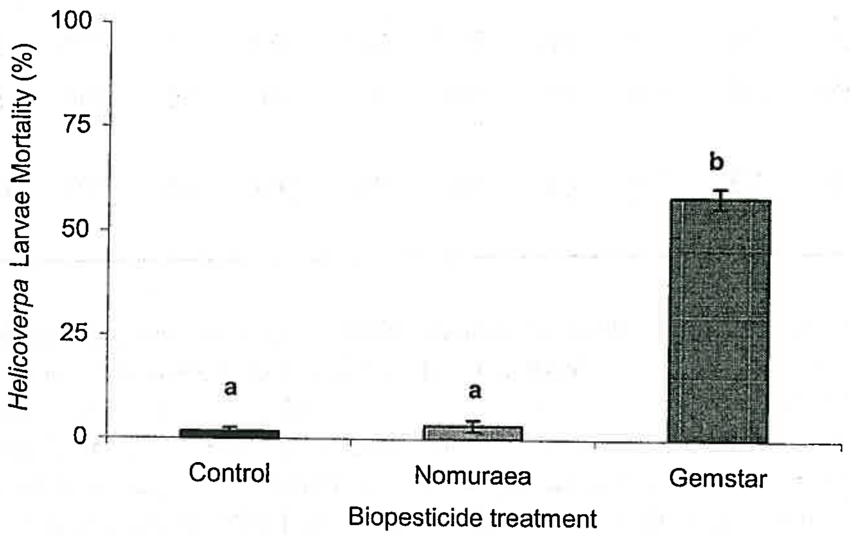

Fig. 4.1.3 Mortality of *Helicoverpa* spp. larvae collected from treatment plots sprayed with Gemstar/Aminofeed® & *Nomuraea rileyi*. No NPV or *Nomuraea* infected grubs were found in the control plots. Treatments with the same letter are not significantly different.

Experiment 4. Test of Oil Volume

The results from this trial were inconclusive. Treatment effects were highly variable across each replicate which suggests significant cross-contamination between treatments either during application or larvae collection. Overall, treatments infection levels were low NPV (>30%) and *Nomuraea rileyi* (>10%). The crop used for the trial was also overgrown and conditions were hot and dry. This may have contributed to the poor treatment responses recorded.

Experiment 5. Test of Spore Rates

At the time of treatment, *Helicoverpa* spp. infestation levels across the trial site averaged 5.5 ± 0.52 larvae/m^2 with 75% of larvae in the VS - S size range. *H. armigera* made up 96% of the *Helicoverpa* spp. present during the pre-treatment collection of larvae on 4 January 2002. No NPV or *N. rileyi* was recorded in pre-treatment collections. All of the larvae collected 48 hours after each application were of a small medium size (7-13mm).

Table 4.1.4 Daily maximum, minimum, average temperatures (°C) and rainfall (mm) at Biloela Research Station during the trial.

Date	4/1	5/1	6/1	7/1	8/1	9/1	10/1	11/1	12/1	13/1	14/1
Max °C	34	30	32	32	31	33	34	35	35	36	38
Min °C	18	18	21	21	20	20	19	21	20	22	22
Mean	26	24	26.5	26.5	25.5	26.5	26.5	28	27.5	29	30
Evap RH%	100	100	100	98	90	85	84	91	86	83	84
Rain (mm)	3.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Larvae mortality due to the three treatments after correction for control mortality are given in figure 4.1.4. The time to death differed between larvae collected from plots treated with NPV and those treated with *Nomuraea rileyi*. Larvae collected from virus treated plots began to die at 4 days after treatment, with most larvae dying between 4 and 8 days after treatment. Larvae collected from *Nomuraea rileyi* treated plots began to die at 6 days after treatment, with the majority dying between 7 and 10 days after treatment.

There was a significant treatment effect on total mortality of larvae collected from different treatments. All treatments were significantly different from each other (fig 4.1.4). The most effective treatment was Gemstar, with a mean of 73% mortality. This was significantly better than either *Nomuraea rileyi* treatments, with a mean of 51% and 32% mortality at $2x10^{13}$ and $1x10^{13}$ spores/ha respectively.

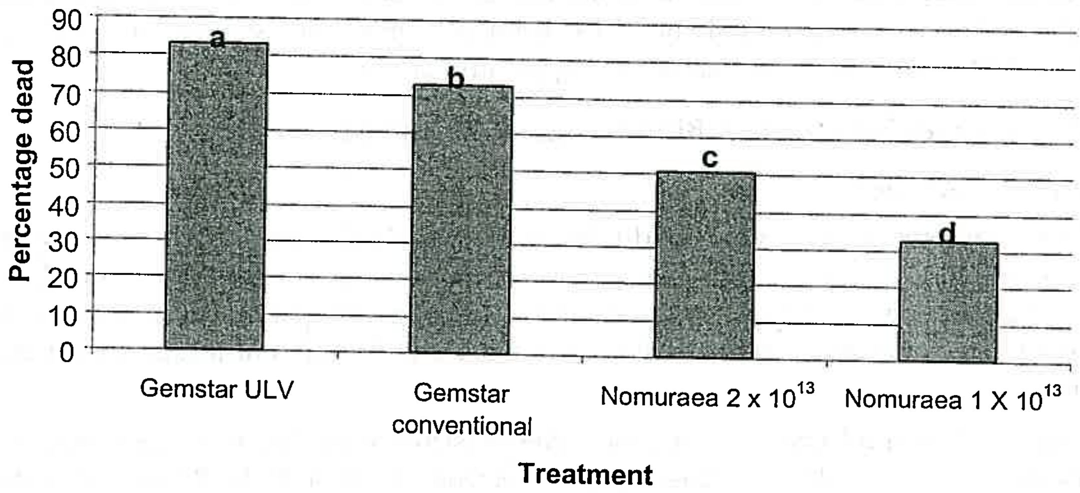

Figure 4.1.4. Mean total number of larvae per metre row with different treatments. Treatments with the same letter are not significantly different.

There was no significant difference between any treatments and controls in number of larvae in each of the separate size classes (small, medium, large) counted at 10 days after first treatment/ 6 days after second application (table 4.1.5). However, analysis of variance of total number of larvae in all size classes showed that NPV had a significant effect, reducing the mean number of larvae from 3 per metre in control plots to 2 per metre in the NPV plots (fig. 4.1.5).

Table 4.1.5. Mean number of larvae per metre row by treatment, 10 days after first application and 6 days after second application. Treatments with the same letter are not significantly different.

Treatment	**Mean number of larvae/m**			
	Small	Medium	Large	All sizes
Control	0.56c	1.32d	1.2e	3.08a
Gemstar	0.28c	0.88d	0.76e	1.92b
Nomuraea 2 x 10^{13}	0.88c	1.36d	1.24e	3.48a
Nomuraea 1 x 10^{13}	0.48c	1.32d	1e	2.8a

Neither of the *Nomuraea* treatments showed a significant difference in total larval numbers from control. This may have been due to poor control, possibly due to climate, or dose, or coverage, but may also be affected by the date of recording. Counts of larval numbers were taken at 10 days after first treatment and 6 days after repeat application. Records of time to death show that larvae collected from *Nomuraea* treated plots only began to die at 6 days, with the majority dying between 7 and 10 days after treatment. In comparison, viral deaths began at 4 days after treatment and most larvae died between 4 and 8 days after treatment. It is thus possible that counts of larval numbers at 6 days after second application underestimated the eventual mortality from *Nomuraea,* but would have included some of the larvae killed by the second application of NPV.

4.2. Additives To Improve Biopesticide Performance

4.2.1 Methodology

A series of experiments were conducted at the Biloela Research Station on irrigated cotton (Sicot 189) grown on 1 metre rows with sub-surface drip irrigation during 2000-02. A series of assays were conducted during this period to investigate the performance and persistence of NPVs when used with different liquid additives on cotton.

A standard method was used to assess the persistence of Gemstar when used with different additives. This method will be referred to as a Field Based Laboratory Assay. This assay was used to assess the presence of virus on sprayed leaf surfaces for increasing time periods after an initial application. For each assay, twelve leaves were sampled from each treatment plot at 12 hourly intervals from 0-96 hours after application. The leaves for these assays were sampled from the terminals of the plants as this canopy section was fully exposed to sunlight throughout the day. Upon return to the laboratory, each sample of leaves was divided and 4 leaves were placed into individual disposable plastic containers (750 mL). 30-40 *Helicoverpa armigera* neonates were then introduced into each container. The larvae were left to feed at 25 ± 1°C, 65% RH and 12:12 photoperiod until they had become satiated (24-36 hours later). 30 larvae were then transferred from the leaves for each treatment replicate and placed onto artificial diet in 28 mL plastic portion cups with 8 pin holes in each lid for ventilation. Only larvae that had fed on the upper leaf surface were transferred to artificial diet as this leaf surface had been exposed to the maximum amount of sunlight whilst in the field. Larvae were then grown out and their fate determined :- healthy, NPV, or unknown.

All treatment results were corrected for control mortality using Abbott's formulae. The data was then converted to probits and the values of each slope and intercept were checked in Genstat 5 (Payne *et al.* 1989) after pooling the replicates for each experiment.

Experiment 1. Aminofeed® As An Additive For NPV

This first experiment was conducted to quantify the potential benefit of using Aminofeed as an additive for NPV under central Queensland conditions. Two treatments of Gemstar® (500 mL/ha) together with Aminofeed® (1 L/ha) and Gemstar® (500 mL/ha) alone, were applied to cotton plots (20 m long x 3 rows wide) at 6:00a.m on 15 November 2000 together with an untreated control. Treatments were applied with hand held hydraulic equipment equipped with 0.02 110 flat fan nozzles operated at 3 bar delivering 150 L water/ha. Each treatment was replicated three times. Leaves were then collected from the plots for 0-96 hours and exposed to larvae as per the Field Based Laboratory Assay protocol outlined earlier. Weather conditions during the trial period were predominantly sunny with daily maximums varying between 32-36°C.

Experiment 2. Aminofeed®, NPV And Application Time Of Day

A common concern for NPVs usage is their rapid breakdown due to sunlight and alkaloids on leaf surfaces. Given the intensity of central Queensland's summer season, this experiment was conducted to test whether or not the time of day that an application takes place makes a difference to the performance of Gemstar® when used with Aminofeed® on cotton.

Gemstar® (500 mL/ha) together with Aminofeed® (1 L/ha) was applied to cotton plots (20 m long x 3 rows wide) at 6:00 a.m., 12:00 p.m. and 6:00 p.m on 10 December 2000 using the same application method and equipment outlined earlier. Leaves were again sampled from the plots and exposed to larvae using the Field Based Laboratory Assay protocol. Weather conditions during the trial period were predominantly sunny with daily maximums varying between 30-34°C.

Experiment 3. Aminofeed® and Silica Sharps as additives for NPV

Liquid Silica Sharps is a product currently being developed by Envirocare Technologies Australia Pty Ltd as a potential additive for their range of organic spray products and as an additive for conventional insecticides. The product aims to create a microscopic layer of razor sharp pieces of silicate material on the leaf surface. This product serves to irritate target insects by damaging the cuticle (skin) as they move about the plant. The product also serves to damage the mouthparts and stomach linings of the insect upon ingestion. We tested the potential for including this product with Gemstar® and Aminofeed® in the hope that the abrasive qualities of the product may increase infection through increased laceration to the gut lining of larvae.

The treatments were:

1. Gemstar® (500 mL/ha) & Aminofeed® (1 L/ha)
2. Gemstar® (500 mL/ha), Aminofeed® (1 L/ha) &Liquid Silica Sharps (1L/ha)
3. Water/Aminofeed® control.

Plots were arranged in a randomised complete-block design with four 25 m^2 replicates of each treatment separated by a 5 m buffer. No pesticides were used on the surrounding crop either prior or during the experiment.

Pre-treatment counts determined the larval population density and size structure. A collection of 50 larvae representative of small and small-medium size categories was taken to determine species composition and pre-treatment NPV infection levels. The treatments were applied before 9:30 a.m. on January 11, 2001. A north-easterly crosswind of 1-3 m/sec was experienced during the application of the various treatments. Two days after treatment (DAT), 30 larvae (small medium 7-13 mm) were collected from each plot and transferred singularly to artificial diet. Larvae were returned to the laboratory and held at 25 ± 1°C, 65% RH and 12:12 photoperiod to determine their fate - healthy, NPV, parasitised or unknown.

Experiment 4. Laboratory Assessment of Aminofeed®, Aminofeed UV®, Mobait® and Coaton ILP® as additives for NPV

This experiment was conducted to compare Aminofeed with Aminofeed UV, Coaton and Mobait as additives for NPV under central Queensland conditions. Treatments of Aminofeed® (1 L/ha), Aminofeed UV® (1 L/ha), Coaton ILP® (2 L/ha) and Mobait (250 mL/ha) were combined with Gemstar® (500 mL/ha) and applied to cotton plots (Sicot 189) at 6:00 a.m using 100 L water per hectare on 27 November 2001. An un-sprayed control and treatment of Gemstar® (500 mL/ha) alone were also applied for comparison. Each of the treatments were replicated four times within a randomised block experimental design. Leaves were sampled from the plots and exposed to larvae using the Field Based Laboratory Assay protocol. Weather conditions during the trial period were predominantly sunny with daily maximums varying between 31-35°C.

Experiment 5. Field Mortality assessment of Aminofeed®, Aminofeed UV®, Mobait® and Coaton ILP® as additives for NPV

Treatments of Aminofeed® (1 L/ha), Aminofeed UV® (1 L/ha), Coaton ILP® (2 L/ha) and Mobait (250 mL/ha) were combined with Gemstar® (500 mL/ha) and applied to cotton plots at 5:00 a.m using 120 L water per hectare on 6 December 2002. An un-sprayed control and treatment of Gemstar® (500 mL/ha) alone were also applied for comparison. Each of the treatments were replicated four times within a randomised block experimental design. 4-6 small (>3 mm) *Helicoverpa armigera* larvae were present in the crop at the time of application.

30 larvae were field collected from each treatment plot 48 hours after application and transferred onto artificial diet in 28 mL plastic portion cups with 8 pin holes in the lid for ventilation. Larvae were then grown out in a constant climate laboratory at 25 ± 1°C and their fate determined. Data was corrected for control mortality using Abbots (1925) formulae and subject to ANOVA using the Genstat 5 computer program (Payne *et al.* 1989) with LSDs calculated to determine treatment differences at $P<0.05$. Weather conditions during the trial period were predominantly sunny with daily maximums varying between 31-35°C An evening shower of rain in which 4 mm fell occurred 36 hours after application.

Experiment 6. Effects of Aminofeed®, Envirofeast® and Predfeed ® on Helicoverpa spp. oviposition.

The experiment was conducted in the centre of a 2 ha field of subsurface drip irrigated cotton (Sicot 80) sown on 1 m rows. No pesticides were used on the surrounding crop either prior to or during the experiment.

The treatments were Aminofeed® applied at 1 and 3 L per hectare and Predfeed & Envirofeast applied at 2.5Kg per hectare respectively. An untreated control was used for comparison. The treatments were applied to plot 10 rows wide by 40m in length. Each treatment and control was replicated 4 times.

On each occasion the treatments were applied either early in the morning before 09:00 or late in the afternoon after 16:00. The treatments were applied on 6, 13, 19, 24, 30 March and 4 April 2002.

The density of *Helicoverpa* spp. eggs and larvae were checked every several days throughout the trial period. To ascertain *Helicoverpa* spp. densities in each treatment, four 1 m lengths of row were selected randomly per plot (16 samples per treatment per sample interval) and at each site the plants where visually searched for *Helicoverpa* eggs and larvae.

Beat sheet sampling was used to assess the presence of beneficial insects at weekly intervals during the trial. The sheet used was 1.5m wide by 2m long and made from yellow canvas. A 25mm diameter piece of timber dowel (1.5 m long) was fixed to each end of the sheet to prevent the ends lifting in the breeze. Samples were taken by placing the sheet behind the cotton plants to be sampled, along the inter-row and up over the adjacent row of cotton to create a 'wall' to catch flying insects. A one metre long stick made of plastic conduit was then used to shake the predators of 1 m of row onto the sheet for assessment. The cotton bushes were then shaken several times from the base of the plants to the top. Insects were then assessed quickly before flying off the beat sheet. Four 1 m lengths of row were selected randomly per plot for beat cloth sampling (16 samples per treatment per sample interval) every 6-8 days during the experiment.

Insect count data for each sampling date were subject to ANOVAs using a Genstat computer program. Least significant differences were calculated to determine treatment differences at $P<0.05$.

4.2.2 Results

Experiment 1. Aminofeed® As An Additive For NPV

The effect of Gemstar® with and without Aminofeed® on larvae mortality over time is given in Figure 4.2.1. Regressions for the data (excluding data for 0 hrs) and a comparison of slopes indicated no significant differences between treatment slopes but a significant difference ($P<0.03$) for slope intercepts. What this suggests is that Aminofeed® delayed the onset of the viruses decline on the cotton foliage for a short period of time which in this case was 24 hours, before then proceeding to decay at a similar rate as for Gemstar® applied alone. The net result of this process was greater persistence of virus as indicated by higher larvae mortality over time (Fig 4.2.1).

Experiment 2. Aminofeed®, NPV And Application Time Of Day

The effect of each treatment being Gemstar® and Aminofeed® applied at 06:00, 12:00 and 18:00 hours on the rate of larvae mortality over time is given in Figure 4.2.2. Regressions for the data (excluding data for 0 hrs) and a comparison of treatment slopes and their intercepts suggested that time of day that the treatments were applied had no significant effect on the persistence of Gemstar® whether it had been applied during the morning, noon or night. Figure 4.2.2 shows that the virus was viable on the leaves for a considerable period of time and that mortality dropped off more rapidly after the first 36 hours.

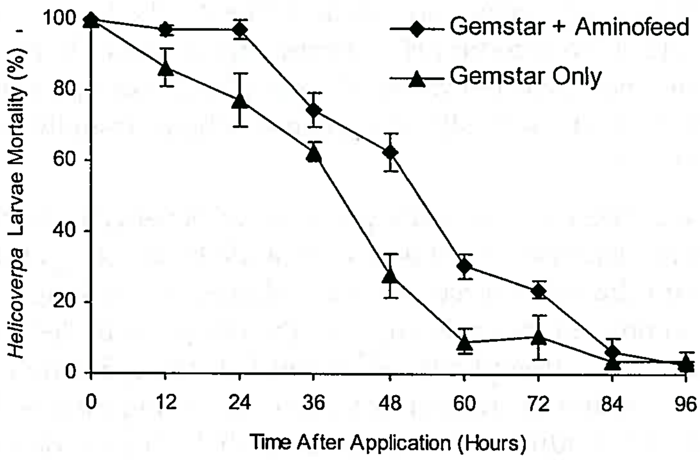

Figure 4.2.1. Mortality of *Helicoverpa* neonates exposed to NPV-treated foliage weathered in the field for increasing periods of time. There was a significant increase in larvae mortality when Aminofeed® was used with Gemstar®.

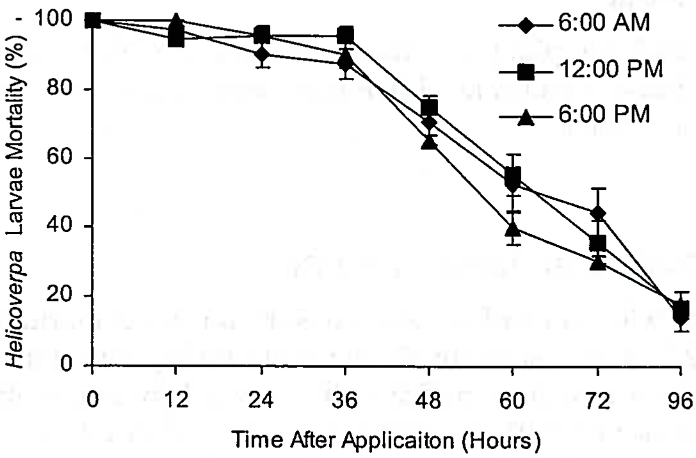

Figure 4.2.2. Mortality of *Helicoverpa* neonates exposed to NPV-treated foliage weathered in the field for increasing periods of time. There no significant difference between applications made at 6:00 a.m., 12:00 p.m. and 6:00 p.m.

Experiment 3. Aminofeed® and Silica Sharps as additives for NPV

The *Helicoverpa* complex present in the field at the time of application consisted of 88% *Helicoverpa armigera*. The effect of the treatments on larvae mortality is given in Figure 4.2.3. In this experiment the addition of silica sharps with Aminofeed® resulted in significantly reduced ($P<0.05$) infection levels compared to Aminofeed® and Gemstar® alone.

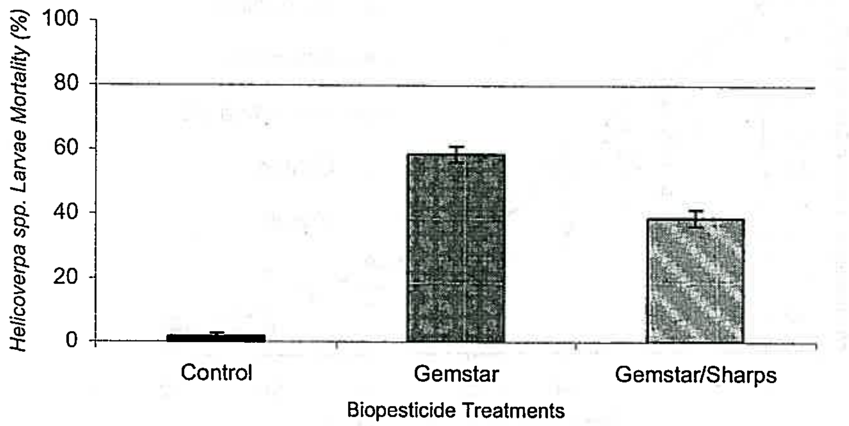

Figure 4.2.3. The percentage larvae mortality of *Helicoverpa* collected from cotton plots sprayed with Water/Aminofeed (control), Gemstar/Aminofeed (Gemstar) and Gemstar/Aminofeed/Liquid Silica Sharps (Gemstar/Sharps).

Experiment 4. Laboratory Assessment of Aminofeed®, Aminofeed UV®, Mobait® and Coaton ILP® as additives for NPV

The effect of each of the additive treatments on larvae mortality over time is given in Figure 4.2.4. Regressions for the data (excluding data for 0 hrs) and a comparison of slopes suggested no significant differences between each of the treatments. However, a comparison of treatment slope intercepts suggested that Aminofeed UV® had a significantly different ($P<0.01$) intercept to all of the other treatments. In this experiment the addition of Aminofeed® did not provide a significant response ($P>0.11$) compared to Gemstar® alone. What the results suggest is that Aminofeed UV® initially delayed the onset of the viruses decline on the cotton foliage before then proceeding to decay at a similar rate as for Gemstar® applied alone. This resulted in an overall increase in persistence as indicated by larvae mortality (Fig 4.2.4). The original formulation of Aminofeed® in this experiment did not provide the same level of persistence as recorded in earlier experiments. However, conditions during this experiment were warmer and dryer than in previous tests. Coaton® and Mobait® did not provide a response that was different to Gemstar® applied alone in this experiment.

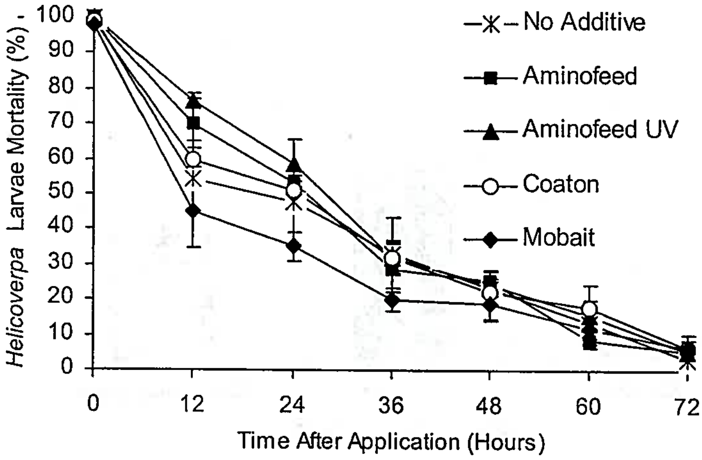

Figure 4.2.4. Mortality of *Helicoverpa* neonates exposed to foliage treated with Gemstar® and various additives that have been weathered in the field for increasing periods of time.

Experiment 5. Field Mortality assessment of Aminofeed®, Aminofeed UV®, Mobait® and Coaton ILP® as additives for NPV

The effect of the treatments on larvae mortality is given in Figure 4.2.5. In this experiment the addition of Aminofeed® resulted in significantly higher levels of infection (P<0.05) in field collected larvae than did Gemstar® alone. The addition of Aminofeed UV® produced the highest infection levels which were significantly better (P<0.05) than Aminofeed® and Gemstar® alone. In this experiment the addition of Coaton ILP® and Mobait® did not improve the level of infection by NPV compared to the application of Gemstar® alone. However, Mobait® was applied at 250mL/Ha in 120L of water which according to the label (that recommends a 2.5% application concentration) would have been 17% lower than the recommended rate. This reduction in the applied rate may have affected the efficacy of Mobait®.

A comparison of the rate at which larvae succumbed to viral infection after spraying suggests that mortality occurred more rapidly in the Aminofeed treatments than for Mobait®, Coaton ILP® and Gemstar® alone (Fig 4.2.6).

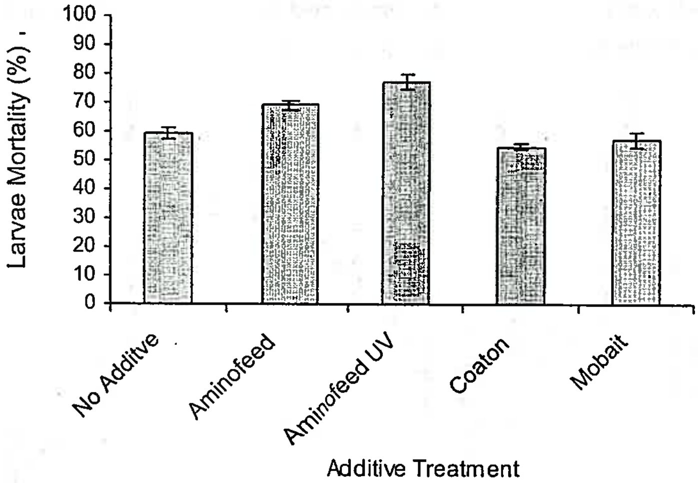

Figure 4.2.5. The percentage of field collected *Helicoverpa* spp. larvae that died from NPV infection where different additives were used.

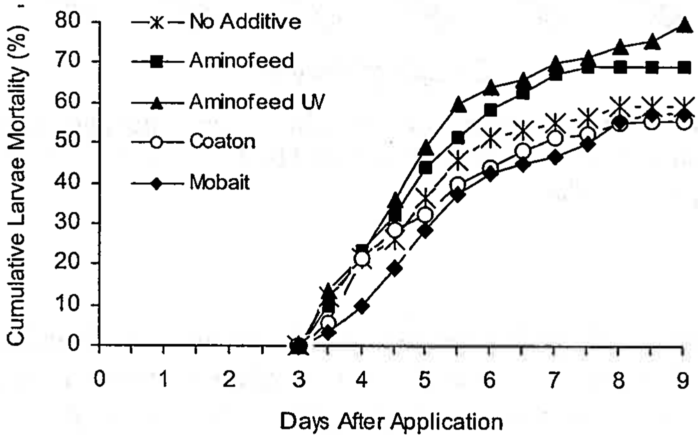

Figure 4.2.6. The time taken for field collected *Helicoverpa* spp. larvae to die from NPV infection after spraying (day 0) where different additives were used.

Experiment 6. Effects of Aminofeed®, Envirofeast® and Predfeed ® on Helicoverpa spp. oviposition.

Trends in the number of *Helicoverpa* spp eggs is given in figure 4.2.7 which suggests little difference in the mean number of eggs per metre row in each of the treatments; and indeed, ANOVA's for egg numbers on each sampling date showed no significant difference ($P>0.05$) in egg numbers between each treatment and the control throughout the experiment. *Helicoverpa* spp. larvae were present in insufficient numbers to make meaningful assessments of their densities in response to each of the treatments.

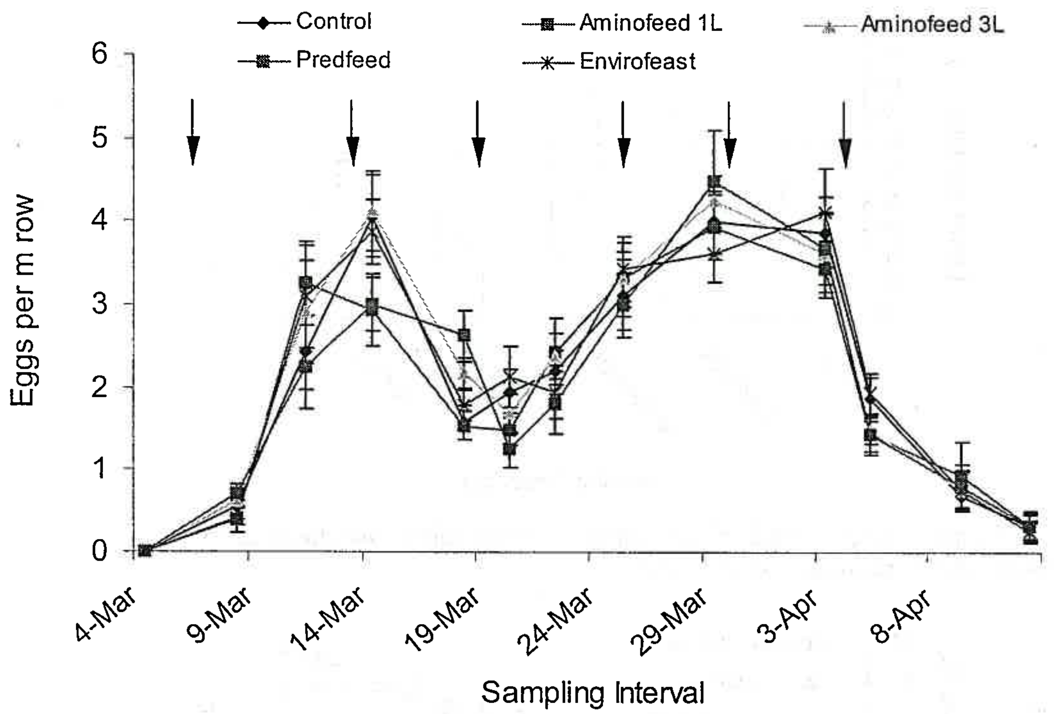

Fig. 4.2.7. Time series showing numbers per m row of *Helicoverpa* spp. eggs in cotton plots for the four food spray treatments and unsprayed control. The bars denote ± SE and arrows represent treatment application dates.

Damsel bugs, ladybirds and spiders were the only frequently abundant beneficial insects found during the experiment and trends in their numbers is given in figure 4.2.8. ANOVA for damsel bug numbers suggested that each of the food spray treatments increased the number of predators significantly ($P<0.05$) compared to the unsprayed control during the first half of the experiment (Fig 4.2.8). However, there were no significant differences ($P<0.05$) between each of the treatments and the control for ladybird and spider densities (Fig 4.2.8).

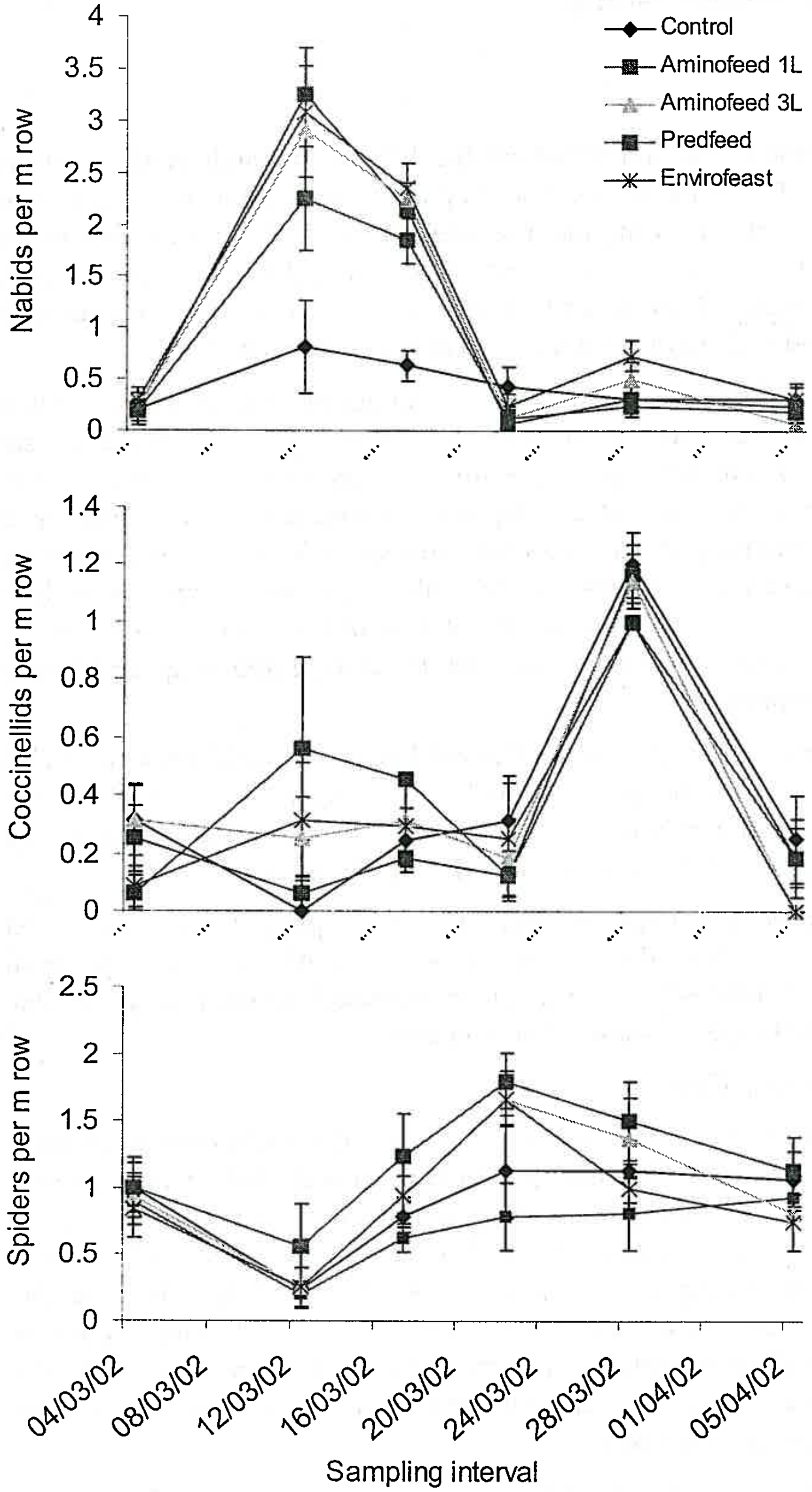

Fig. 4.2.8. Time series showing numbers per m row of Damsel bugs (Nabids), Ladybirds (Coccinelids) and Spiders in cotton plots for the four food spray treatments and unsprayed control. The bars denote ± SE.

4.3. Trap Crops for *Helicoverpa* spp.

4.3.1 Methodology

Spring Trap Crops

A replicated experiment was conducted on the Biloela Research Station, central Queensland (24°22'S, 150°06'E). The legumes Popani vetch, Namoi vetch, Field peas and Chickpeas were planted during the first week of July 2001 on 1 m rows in the centre of a 10 ha field of wheat. The treatments were arranged in a randomised block design with four replicates. Treatment plots were 400m^2 (20 rows x 20 m) with each plot being surrounded with 10 m of buffer sown to wheat on each side.

Helicoverpa spp. were abundant during the experiments. *H. armigera* (Hübner) was the dominant species, with only low numbers (<30%) of *H. punctigera* (Wallengren) observed. Counts of *Helicoverpa* spp. eggs were made on 4 randomly selected 1 m lengths of row of foliage in each treatment replicate. The foliage for each sample was destructively taken from the plots and searched thoroughly for *Helicoverpa* spp. eggs. *Helicoverpa* spp. larvae were also assessed during the experiment using a beat sheet method on 4 randomly selected 1 m lengths of row of foliage in each treatment replicate. The plots were sampled regularly for *Helicoverpa* spp. eggs and larvae throughout the experiment.

The fate of *Helicoverpa* spp. eggs laid on the chickpeas and field peas were also investigated during the experiment. This involved tagging 110 newly laid eggs in each treatment replicate and then re-visiting the eggs every day to determine if they had hatched or disappeared (presumed eaten or fallen off the plants).

Count data for *Helicoverpa* spp. eggs and larvae at each sampling date were analysed using a repeated measures ANOVA with the Genstat version 5.0 computer program (Payne *et al.* 1989). Differences between treatments ($P<0.05$) on each sampling date were determined with the least significant differences.

In-Field Early Season Trap Crops

A replicated experiment was conducted to investigate the usefulness of an early season in-field trap crops in cotton. The experiment was conducted on a commercial cotton property near the township of Biloela, central Queensland. Trap crops of chickpea, chickpea mixed with mungbean, chickpea mixed with niger and a control of cotton were planted during the first week of October 2000 on 1 m rows. The treatments were arranged in a randomised block design with four replicates. Treatment plots were 420m^2 (6 rows x 70 m) and were planted down the centre of a 40ha field of commercial cotton. The adjacent cotton crop on each side was planted at the same time as the trap crop rows.

The crop was checked for *Helicoverpa* spp. oviposition activity during the first ten weeks of the crop. The aim of sampling the cotton crop adjacent to the trap crop rows was to try and detect a gradient in *Helicoverpa* spp. egg numbers that might suggest a sink effect associated with the trap crop rows.

Helicoverpa spp. were scarce during the experiment. Only one major oviposition event occurred during the experiment from which data could be collected. On this occasion, three 1m samples of cotton row were checked for eggs at increasing distances (adjacent rows 1, 2, 4, 8, 16, 32, 64 & 130) from the trap rows for each treatment replicate (96 samples per treatment). The data was collated and entered into a spread sheet and used to create a chart. No statistical analysis was applied to the data as no obvious trends were apparent within the data gathered during the experiment.

4.3.2 Results

Spring Trap Crops

Chickpeas had significantly ($P<0.05$) more larvae from late August through until the end of September (Fig 4.3.1) that would suggest that chickpeas attracted significantly higher numbers of *Helicoverpa* spp than the other three species. However, the number of eggs found suggested a different response with field peas attracting far greater ($P<0.05$) egg-laying activity than chickpeas (Fig 4.3.2). The field peas carried on average 50-70 eggs per metre row for most of September, the period for which a trap crop needs to be most attractive for CQ conditions.

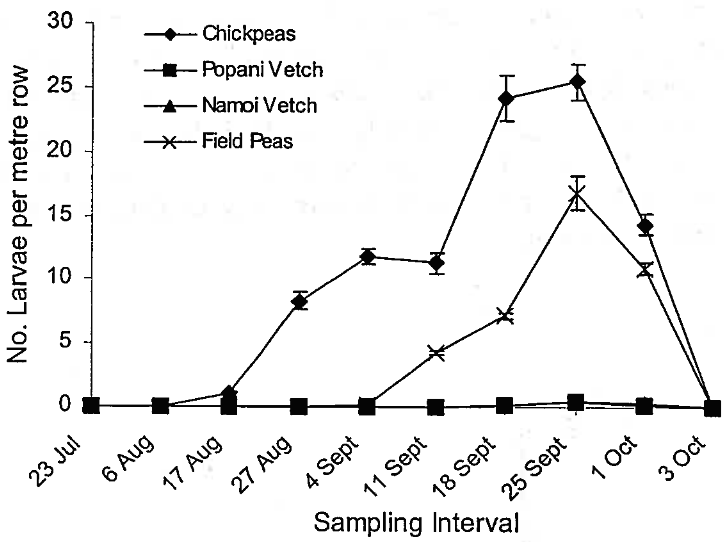

Figure 4.3.1. The number of *Helicoverpa* larvae found per metre row. The bars denote ± SE

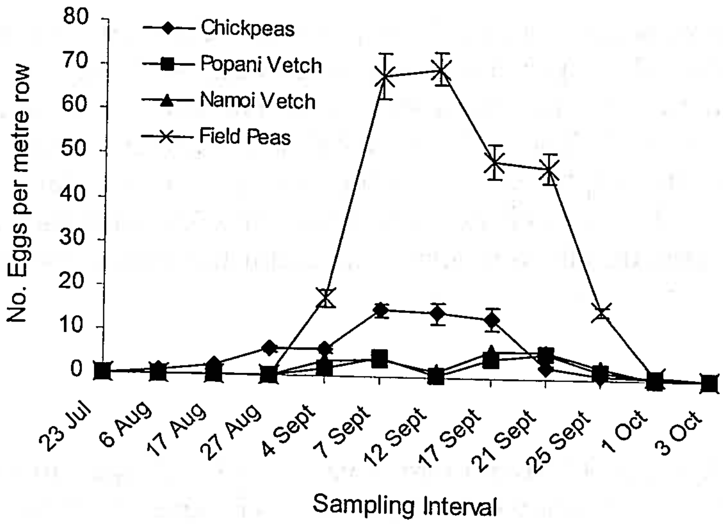

Figure 4.3.2. The number of *Helicoverpa* eggs laid per metre of crop row. The bars denote ± SE.

It could be expected that in having the highest densities of eggs that field peas would have also had the highest larvae numbers. However, the fate of eggs laid on the two trap crops suggested that only 27 ± 3.5% of the tagged eggs on field peas survived to hatching as opposed to 73 ± 2.9% egg survival on chickpeas (Fig 3). The losses observed in the field peas may have been due in part to predation by ladybirds and lacewings that were abundant in the field pea treatments. The waxy surface of the field pea leaves may have also contributed significantly to the observed losses by causing eggs or newly hatched larvae to fall off plants particularly during windy conditions. In contrast chickpeas had neither the beneficial insects or waxy leaf surfaces which may have contributed to greater egg survival to larvae in this treatment.

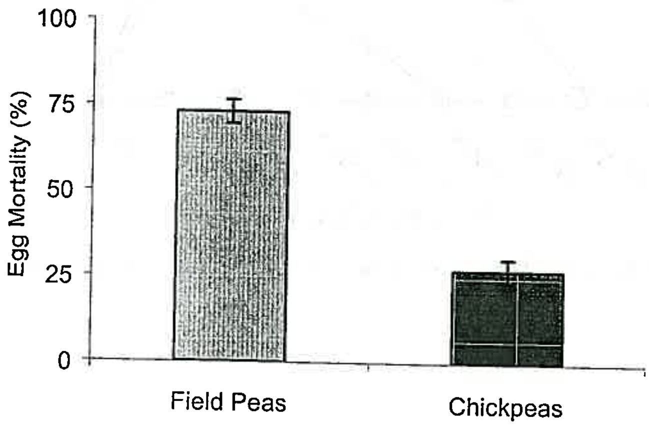

Figure 4.3.3. The percentage mortality of *Helicoverpa* eggs laid on field peas and chickpeas.

In-Field Early Season Trap Crops

Figure 4.3.4. suggests that the trap rows had little effect on *Helicoverpa* spp. egg numbers in the adjacent cotton crop. In the three treatments of chickpea, chickpea

mix mungbeans and chickpea mix niger, the trap rows of each treatment appeared to have increased egg density on the directly adjacent first row of cotton, before declining in density by rows 4-8 and then increasing by row 16. There appeared to be no additional effects beyond row 16 rows of cotton. The rows beside the cotton control had elevated numbers of eggs for the first 4 rows before plateuing for the remaining rows.

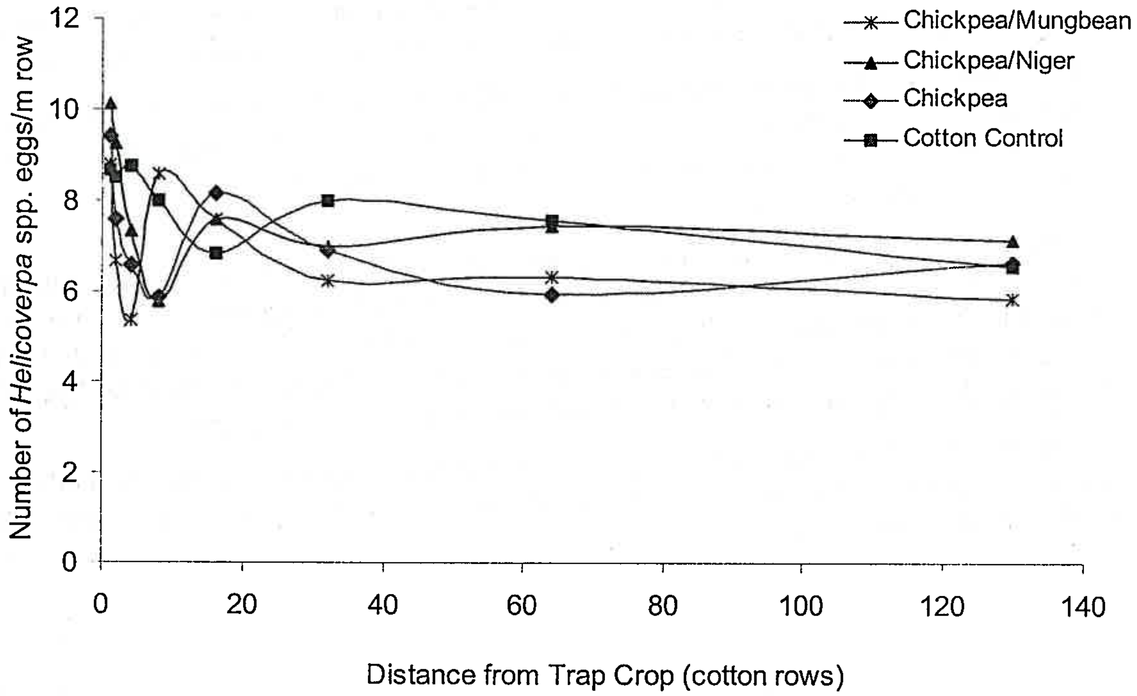

Figure 4.3.4. Egg density (eggs/m row) recorded on cotton rows at increasing distances from the trap crop treatments of chickpea, chickpea mix mungbean, chickpea mix niger and cotton control.

4.4. Heliothis Movement and Local Recruitment Ecology In CQ

4.4.1 Sampling Methodology

The sowing of chickpeas from May onwards during winter and growing of cotton and grains such as sorghum and mungbeans from September through to April provides a significant opportunity for *Helicoverpa armigera* to cycle continuously within the farming system. In an effort to try and measure the extent to which such cycling may be occurring an extensive sampling program was commenced in July 2000 in collaboration with the *Centre for Identification and Diagnostics* at the University of Queensland. The main aim of the sampling strategy was to sample *H. armigera* moth and larvae from as many host crops as possible across the region throughout the year to try and ascertain the profile of local populations. This involved collecting *H. armigera* from commercial dryland chickpea crops between the months of May and September, chickpea trap crops in August and September, Cotton from

September to March and summer grains from October to April. The intent of the sampling program was to capture *H. armigera* populations that may have been cycling between these crops in the local region.

H. armigera samples stored in 100% ethanol until transported to the University of Queensland for processing and analysis. The date of collection, crop type and GPS location for each sample was recorded.

4.4.2 Results

A comprehensive set of results will be reported in the final report of the lead project UQ32C currently being conducted by the *Centre for Identification and Diagnostics*.

Over 3000 samples from the Dawson Callide region have been processed and analysed using a molecular diagnostic test to distinguish *H. punctigera* individuals from *H. armigera,* and using microsatelite markers to determine the genetic structure of *H. armigera* populations.

In the seasons examined to date, data analysis is clearly indicating differentiation between Heliothis populations from the Dawson Callide region and populations from the Narrabri, and Darling Downs regions. This result suggests that the *H. armigera* populations in the Dawson Callide region are highly localised and not the likely result of regular mass immigration from neighbouring regions.

The genetics of *H. armigera* also appear to be non-crop dependent: e.g. the Heliothis found on winter chickpeas are not different from those found on cotton and chickpea trap crops.

5. General Discussion.

5.1 New Biopesticides – *Nomuraea rileyi*

N. rileyi, spores were found to be reasonably persistent on cotton foliage and caused mortality for up to 72 hours after application. This result compared well with NPV's that degrade more rapidly.

The application of *N. rileyi* in oil to *Helicoverpa* spp. in cotton resulted in significant levels of larvae infection in most experiments, giving comparable responses to NPV (Figs 4.1.2 & 4.1.3). However, not dissimilarly to NPV's the response from *N. rileyi* varied considerably and in some experiments resulted in lower infection levels.

It should be emphasised that each of the experiments reported were preliminary. The main aim was to provide preliminary data on efficacy in cotton part of development and there is significant potential to improve performance through improved coverage and increased rates. However, the experiments reported here have highlighted a number of considerations that require further investigation concerning the use of *N. rileyi* as a biopesticide.

The cause of variable responses observed in some experiments may have been due to dosage, coverage or environmental factors. Free moisture from rainfall and high ambient relative humidities were recorded during the occasions where *N. rileyi* performed well in field tests. Alternatively dry conditions with low ambient humidities were present during the less successful experiments with *N. rileyi*. This suggests that moisture and relative humidity may have an impact the post-application activity of *N. rileyi* in cotton. The application of fungal spores in oil has been shown to overcome the effects of relative humidity with Metarhizium on locusts with significant infection being recorded at humidities as low as 35% (Lomer *et al.*, 1992). Further research is needed to address gaps in the knowledge with regard to humidity and to perhaps identify better oils than Propar 12 for formulation.

The last field experiment showed a significant dose response with the increased application of spores resulting in improved efficacy. This result suggests that the performance of *N. rileyi* may be improved with increased application rates of spores per hectare. A higher rate of *N. rileyi* should be tested in further trials.

Larvae collected from the field took a several days to die from infection by *N. rileyi* (6-10 days), with larvae reaching later instars before death in some trials. Trials in mungbeans have shown that increased rates can decrease mean time to death (Hauxwell, Holdom and Grundy, unpublished). However, time to death is likely to be longer than with NPV, and must be considered when considering control.

N. rileyi has shown considerable promise as a biopesticide in the experiments reported here. Future research will focus on improving the coverage and rates of application as well as investigating environmental effects such as canopy closure, humidity and rainfall.

5.2. Additives To Improve Biopesticide Performance

The data from these experiments strongly suggest that the use of additives is beneficial for NPV applications in cotton. When added to Gemstar®, Aminofeed® and Aminofeed UV® were both found to consistently increase the rate of larvae mortality compared to when Gemstar® was used alone. It is not entirely clear how these additives improve the performance of Gemstar® although it is likely that they serve to both protect the virus from the field environment as well as encourage rapid ingestion by larvae after application.

Applications of NPV and Aminofeed® at morning, noon and night also strongly suggested that there is little advantage in making evening applications on the basis of avoiding exposure to U.V light. Instead application should focus on ensuring adequate coverage and targeting of small larvae to maximise the performance of NPV's in cotton.

Research on the effects that Aminofeed® may have on *Helicoverpa* spp. oviposition in cotton suggested that neither Aminofeed®, Predfeed® or Envirofeast® have a significant effect on the rate of oviposition recorded in field plots. The level of egg pressure was low during the experiment. However, it may be expected that if a food spray treatment was attractive to *Helicoverpa* spp. during a period where moths were less abundant, that a treatment effect would have been more pronounced compared to a situation where very high numbers of ovipositing moths were present and competing for a limited food source which in this case was a relatively small field of cotton.

Each of the food spray treatments did increase the number of damsel bugs in the plots compared with the un-treated control during the first half of the experiment suggesting that each product may be useful for encouraging the presence and activity of this predator. Ladybird and spiders tended to be more abundant in the Predfeed treatments although this trend was not significantly different to the other treatments.

5.3. Trap Crops for *Helicoverpa* spp.

As a trap crop is intended to attract and divert egg laying Heliothis moths, the field peas were found to outperform chickpeas in this experiment by attracting more eggs. As an added bonus, in contrast to chickpeas, field peas supported high populations of predators such as lady beetles and lacewings. The use of field peas would overcome the ascochyta disease risk currently associated with chickpeas in CQ. The lack of *Helicoverpa* spp. activity in the two vetch varieties suggests that these are poor trap crop candidates, however it does bode well from a pest management perspective for this legume's use as a green manure crop during winter.

Research on the use of field peas is continuing under a new project.

The attempt to use chickpea, chickpea mixed with mungbean and chickpea mixed with niger as an in-field trap crop in cotton had little effect on *Helicoverpa* spp oviposition. During the one significant oviposition event, the trap crops were found to have a negligible effect on the adjacent cotton rows from an economic perspective. The only reduction observed was restricted to within the first 16 rows and even within this area, the actual reduction achieved was not sufficient to gain economic control. Such an approach is unlikely to succeed under current agronomic conditions and hence no further experiments were conducted.

5.4. Heliothis Movement and Local Recruitment Ecology In CQ

Although preliminary in that the results are only based on the first 12 months of samples, the micro-satellite data is strongly suggesting that the *Helicoverpa armigera* population within the Dawson Callide region is largely locally recruited. This result has several implications for the management of *H. armigera* if continued sampling within the Dawson Callide demonstrates a consistent trend towards local recruitment.

Insecticide resistance management within the Dawson Callide would be of greater importance as part of an area wide management program where a *H. armigera* population is locally recruited. Greater emphasis should be given to a resistance management strategy for both cotton and grains particularly for newer chemistry such as Steward® (indoxacarb) that may come under increasing pressure due to widespread use in chickpeas, cotton and mungbeans. Such products deserve careful consideration within future resistance management strategies.

Increasing *H. armigera* mortality in both cotton and grains using sustainable soft options such as NPV's should be a second consideration for an area wide management program in the Dawson Callide region. NPV's would be best targeted on *H. armigera* populations in winter chickpeas, summer grains and early season cotton where the efficacy of these products is often adequate for control. Early season control thresholds should perhaps be evaluated and NPV's instead used on what would be considered to be sub-threshold populations that would otherwise survive and contribute to later populations. Early season *H. armigera* thresholds perhaps need to be more dynamic with respect to considering potential future generations rather than dealing with this pest as a series of un-related discrete outbreak events.

The third implication relates to the management of the farming system so as to disadvantage *H. armigera* populations via Area Wide Management. Samples taken from both chickpea and pigeon pea trap crops for micro-satellite analysis have indicated that the populations are the same as those collected form cotton and commercial chickpea. This suggests that the trap crops are indeed attracting a proportion of the populations that are emerging from winter chickpeas and late summer cotton. With further sampling it may be possible to ascertain the relative proportions that are being attracted to and destroyed in the trap crops currently grown.

Future research at CID will determine the extent of migration within the Dawson Callide valley. Since current collections have been limited to a short time period, it essential that further analysis and increased sampling be continued to further identify the genetic structure of populations with the Dawson Callide valley in comparison with other regions. Several more years of data will be required to determine whether migration trends observed to date are typical. It is also unclear whether the rates of genetic change over time are normal, and the result of evolutionary pressure. The following year's data will help to determined what is driving this change.

6. The likely impact of the results and conclusions of the research project for the cotton industry.

The novel options investigated during this project offer to provide alternative control options for *Helicoverpa* spp in the future and reduce insecticide dependence. Some of the research reported here is of a developmental nature and thus is not yet ready for immediate implementation within the cotton industry. However, the current study has shown that

- *Nomuraea rileyi* has the potential to be further developed as a biopesticide for use in cotton. Further research and development is being conducted by QDPI and GRDC.
- The use of additives such as Aminofeed® have been shown to consistently improve the performance of NPV biopesticides by 10-20%. Given that the cost of this additive is $3.50 per hectare, it represents an increase of 10-12% to the application cost of an NPV. This is favourable given that the corresponding increase in mortality is often greater than this cost but more importantly the additional increase in efficacy is often enough to make the selection of an NPV a viable option for a wider range of control situations. The demonstration of additives and NPV's on cotton has increased grower confidence within the industry concerning the use of NPV's. The use of NPV's is useful from both sustainability and resistance management viewpoints.
- New winter trap crops such as field pea offer to significantly improve the effectiveness of CQ area wide management strategies whilst also eliminating friction between the cotton and grains industries within CQ over ascochyta leaf blight concerns. Research to ensure the results are replicable under both experimental and commercial conditions is being continued.
- Data from the micro-satellite studies on *H. armigera* local recruitment will strengthen the case for implanting area wide management and more judicious use of insecticides. The data from this study has already had a significant impact on the local cotton industry with the realisation that pest populations may be more localised than previously thought and therefore current management decisions may have further reaching implications on future pest population densities and resistance levels.

7. Detail a plan for the activities or other steps that may be taken;

(a) to further develop or to exploit the project technology.

Much of the research and development of novel options reported here is being continued under current and new projects. *N. rileyi* will continue to be developed as a potential biopesticide by the QDPI biopesticides unit at Indooroopilly. Further research with field peas and micro-satellite local recruitment studies will be continued in the new CRDC funded project DAQ122C.

(b) for the future presentation and dissemination of the project outcomes.

The majority of project outcomes have already been disseminated to industry. As much of the research reported here is continuing in new and other projects, further outcomes will be reported in due course.

8. List the publications arising from the research project.

Conference Proceedings

Grundy P & Short S. 2002. Additives that enhance nucleopolyhedrovirus performance on central Queensland cotton. In: *Proceedings of the Eleventh Australian Cotton Conference.* Brisbane, Queensland. Pp 289-295. Australian Cotton Growers Research Association, Wee Waa.

Grundy P, Short S & White D. 2002. Field peas, A potential alternative to chickpeas for trap cropping in central Queensland. In: *Proceedings of the Eleventh Australian Cotton Conference.* Brisbane, Queensland. Pp 407-410. Australian Cotton Growers Research Association, Wee Waa.

Hauxwell C, Knight K, Grundy P & Holdom D. 2002. New biopesticides. In: *Proceedings of the Eleventh Australian Cotton Conference.* Brisbane, Queensland. Pp 791-795. Australian Cotton Growers Research Association, Wee Waa.

Cotton Trial and Year Booklets

Grundy P & Short S 2001. Central Queensland biopesticide research roundup. In: *Central Queensland Cotton Trial and Yearbook* (Ed D. Kelly) pp. 83-86. QDPI Emerald, Queensland.

Grundy P, Short S & Sequeira R. 2001. Ladybirds: A useful heliothis egg predator. In: *Central Queensland Cotton Trial and Yearbook* (Ed D. Kelly) pp. 73-76. QDPI Emerald, Queensland.

Grundy P, Short S & Graham G. 2001. Heliothis micro-satellite research - Dawson Callide. In: *Central Queensland Cotton Trial and Yearbook* (Ed D. Kelly) pp. 81-82. QDPI Emerald, Queensland.

Grundy P, Short S, Wilkinson K, Scott K, Merritt M, Scott L & Graham G. 2002. Heliothis micro-satellite research update - Dawson Callide. In: *Central Queensland Cotton Trial and Yearbook* (Ed D. Kelly). QDPI Emerald, Queensland In press.

Grundy P & Short S. 2002. Additives that enhance nucleopolyhedrovirus performance on central Queensland cotton update. In: *Central Queensland Cotton Trial and Yearbook* (Ed D. Kelly). QDPI Emerald, Queensland In press.

Grundy P & Short S. 2002. Field peas, A potential alternative to chickpeas for trap cropping in central Queensland. In: *Central Queensland Cotton Trial and Yearbook* (Ed D. Kelly). QDPI Emerald, Queensland In press.

CQ Cotton Tales Series

Grundy P & Short S. 2000-01. Heliothis activity in the Theodore irrigation area. (Ed D. Kelly) QDPI Emerald. Issue 5, September.

Grundy P & Short S. 2000-01. Gemstar application and time of day. (Ed D. Kelly) QDPI Emerald. Issue 17, December.

Grundy P & Short S. 2000-01. First field results with *Nomuraea rileyi* - a fungal pathogen of *Helicoverpa* spp. (Ed D. Kelly) QDPI Emerald. Issue 19, January.

Grundy P & Short S. 2000-01. Field day report – Novel options for *Helicoverpa* control in CQ. (Ed D. Kelly) QDPI Emerald. Issue 25, March.

Grundy P & Short S. 2000-01. Assassin bugs – a new weapon for pest management. (Ed D. Kelly) QDPI Emerald. Issue 26, March.

Grundy P & Short S. 2000-01. Heliothis "Micro-satellite" DNA update for Dawson/Callide. (Ed D. Kelly) QDPI Emerald. Issue 28, March.

Grundy P & Short S. 2000-01. Use of Aminofeed as an additive with Gemstar® applications. (Ed D. Kelly) QDPI Emerald. Issue 29, March.

Grundy P & Short S. 2001-02. LadyBirds: A Useful Heliothis Egg Predator. (Ed D. Kelly) QDPI Emerald. Issue 7, October.

Grundy P, Short S & White D. 2001-02. Field Peas:- An Alternative Winter Trap Crop to Chickpeas in CQ? (Ed D. Kelly) QDPI Emerald. Issue 19, December.

Grundy P, Short S & Purvis-Smith N. 2001-02. Liquid additives for enhancing biopesticides in CQ. (Ed D. Kelly) QDPI Emerald. Issue 25, February.

9. References

Abbott WS. 1925. A method for computing the effectiveness of an insecticide. *Journal Economic Entomology* **18**, 265-267.

Cunningham JC, Brown KW, Payne NJ, Mickle RE, Grant GG, Fleming RA, Robinson A, Curry RD, Langevin D & Burns T. 1997. Aerial spray trials in 1992 and 1993 against gypsy moth, *Lymantria dispar* (Lepidoptera: Lymantriidae), using nuclear polyhedrosis virus with and without optical brightener compare to *Bacillus thuringiensis*. *Crop Protection* **16** 15-23.

Lomer CJ, Bateman RP, Godonou I, Kooyman C, Kpindu D, Prior C & Shah P. 1992. Field trials against *Zonocerus variegates* and *Schistocera gregaria* with amycopesticide formulation of *Metarhizium flavoviride*. *Abst. 25th Meeting, Soc. Invertebrate Pathology, Heidelberg, Germany 77.*

Morales L, Moscardi F, Sosa-Gomez DR, Paro FE & Soldorio IL. 2001. Fluorescent brighteners improve *Anticarsia gemmatalis* (Lepidoptera: Noctuidae) Nucleopolyhedrovirus (AgMNPV) activity on AgMNPV- susceptible and resistance strains of the insect. *Biological Control* **20** 247-253.

Murray D, Powell R & Hopkinson J. 2000. Additives to enhance biopesticides. In: *Proceedings of the Tenth Australian Cotton Conference, Brisbane, Queensland*, pp 207-211. Australian Cotton Growers Research Association Inc., Wee Waa.

Payne R, Lane PW, Ainsley AE, Bicknell KE, Digby PGN, Harding SA, Leech PR, Simpson R, Todd AD, Verrier PJ & White RP. 1989. Genstat 5 Reference Manual. Clarendon Press, Oxford.

Sequeira R. 2001. Inter-seasonal population dynamics and cultural management of *Helicoverpa* spp. in a central Queensland cropping system. *Australian Journal of Experimental Agriculture*. **41**, 249-259.

10. Are changes to the Intellectual Property register required?

N/A