

Part 1 - Summary Details

REPORTS

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

CRDC Project Number: UQ35C

Annual Report: Due 30-September

Progress Report: Due 31-January

Final Report: Due 30-September

(or within 3 months of completion of project)

Project Title: Population genetics of Helicoverpa migration, recruitment and origins.

Project Commencement Date: 1 July 2003 **Project Completion Date:** 30 June 2004

Research Program: Crop Protection

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1. Outline the background to the project.

The UQ35c twelve-month project continued on from the UQ32c research covering the *Helicoverpa armigera* microsatellite survey of field collections over twelve of the major cotton growing regions during the 2003-2004 season. This microsatellite survey provides information on population structure and movement of *H. armigera* at both the local and regional levels. Over the preceding three years of study, more and more collaborating groups have provided collections to the microsatellite research, and as such the work is reaching a national scale.

This studies primary object was to continue collecting data on the migration and recruitment of *H. armigera* and then to extend this research to include a description of (i.e. tracking) the movement of resistant and susceptible *H. armigera* across these regions. A secondary aim for the new project was to incorporate ecological data to provide a more comprehensive understanding of *H. armigera* movement. These combined outputs are intended to provide better and more specific information on the control for *H. armigera* into area wide management strategies for the cotton and grains industries.

2. List the project objectives and the extent to which these have been achieved.

The project had three primary objectives

A. Application of microsatellite analysis to evaluate population structure, migration and movement of H. armigera at the local, regional and national scales.

The 2003-2004 project was successful in achieving objective A, and was able to describe in detail the proportion of *H. armigera* that were local, the proportion which were immigrant, and the source of immigrant events to each of the major cotton growing regions of eastern Australia (see Fig. 2 for an example).

B. To relate the development of resistance in H. armigera to the genetic data.

This objective “to incorporate resistance information into the genetic data” in this twelve month project has been met, and was more effective than anticipated. Through collaboration with Dr. Louise Rossiter it became possible to include in the genetic analysis, *H. armigera* that had been directly bioassayed for resistance and susceptibility to Emamectin benzoate, Endosulfan, Fenvalerate, Indoxacarb, Methomyl, Profenofos and Spinosad. This development has permitted the extension of analysis to include the direct measurement of the movement or localness of “resistance”.

C. To correlate ecological based observations/data to the microsatellite results.

Objective C is met through ongoing publication of the microsatellite research with our collaborators (refer to list of collaborators in Q3). By reporting the projects outcomes in consultation with our collaborators, the ecological and observational information from each of the regional locations is integrated with the genetic analysis.

3. Detail the methodology and justify the methodology used.

Justification of molecular methodology for studying migration of H. armigera:

Previous studies which used direct measures of *Helicoverpa* movement have provided important information regarding the flight behaviour and dispersal of adult moths, but do not demonstrate whether successful reproduction has occurred after moth migration. Hence, the influence of *H. armigera* movements on the level of gene-flow between regions in these studies was unknown. Our molecular approach measures gene-flow both with-in and between

regions to estimate the movement of *H. armigera*. We use the term ‘migration’ to refer to an individual that has moved to a new location and successfully reproduced there.

Details of methodology applied:

Sample source:

H. armigera larvae and moths were collected via the network of dedicated collectors supporting the current project (David Murray, Melina Miles, Peter Gregg, Paul Grundy, Martin Dillon, Scott Hardwick, David Kelly, Hugh Brier, Carrie Hauxwell, Joanne Dawson, Cathy Mansfield, Iain Kay, Julie O’Halloran, Robert Dimsey, Ian Crosthwaite, Annie Spora, Annie Sullivan, Ingrid Christiansen, Macpherson Ag., Ag. Street Services, Andrew Ward, Lavinia Zirnsak, Slobodan Vujovic, Sean Boland, Nick Gillingham, Louise Rossitter, Colin Tann, Stephens Ginns, Jamie Hopkinson, K.Alexander, Fiona Tessmann, Angus Andrews, Stewart Leadbetter, and John Duff). These collections covered the major cotton growing regions (see Fig.1).

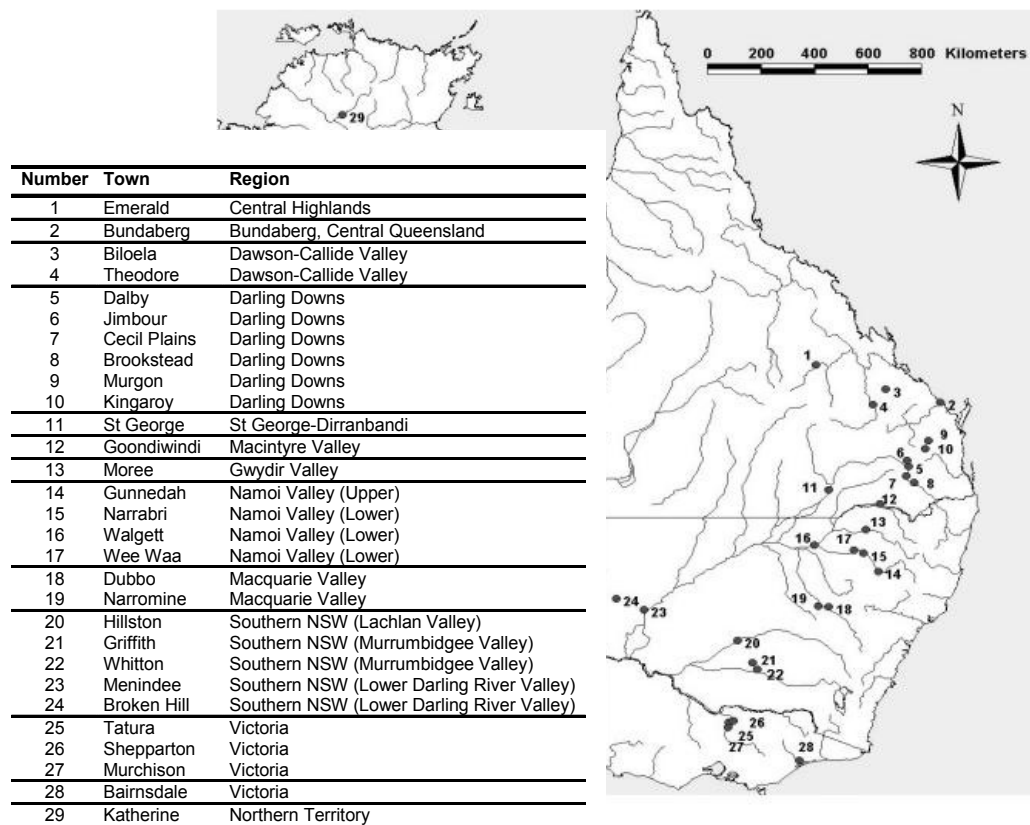


Fig.1 – Regional map showing the localities where *H. armigera* populations have been sampled and analysed.

Samples have been obtained from a variety of crops: cotton (*Gossypium hirsutum*), peanut (*Arachis hypogaea* L.), pigeon pea (*Cajanus cajan*), chickpea (*Cicer arietinum* L.), watermelon (*Citrullus vulgaris* L.), soy bean (*Glycine max*), sunflower (*Helianthus annuus*), barley (*Hordeum vulgare* L.), tomato (*Lycopersicon esculentum*), field pea (*Pisum sativum* L.), millet (*Panicum milliaceum*), sorghum (*Sorghum bicolour*), wheat (*Triticum aestivum*), faba bean (*Vicia faba*), mungbean (*Vigna radiata*), and corn (*Zea mays*).

DNA extraction:

DNA for microsatellite analysis was extracted from individual moth heads or larval posterior prolegs using a 96-well modification of the Miller *et al.* (1988) protocol. The

remaining insect was placed in an ethanol vial, and cross-referenced to the DNA extraction. A PCR-based diagnostic (developed during previous research at the School of Life Sciences) was utilised to determine whether each individual was *H. armigera* or *H. punctigera*. The species diagnostic ensured microsatellite analysis was performed on *H. armigera* individuals only, as morphological determination of species after storage in ethanol was problematic.

Microsatellite analysis

Five microsatellite loci were used to analyse *H. armigera* individuals collected (Scott *et al.* 2004). The loci were HaB60 (25 alleles, expected heterozygosity [H_e]=0.45, observed heterozygosity [H_o]=0.04), HaD25 (58 alleles, H_e =0.73, H_o =0.36), HaD47 (85 alleles, H_e =0.75, H_o =0.24), HaC87 (33 alleles, H_e =0.50, H_o =0.17) and HaC14 (32 alleles, H_e =0.65, H_o =0.48). Microsatellites were *Hex* labelled with PCR amplification conditions and gel separation as published in Scott *et al.* (2003).

Statistical analysis

Microsatellite alleles were scored using ONE-Dscan (Ver 2.05, Scanalytics Inc., Billerica, MA USA). Allele sizes were entered into Excel (Microsoft Corp., North Ryde, NSW Australia) and analysed using GenAlEx (Peakall & Smouse, 2001). Nei distance between collections was calculated using Peakall *et al.* (1995). Allele frequencies and heterozygosity calculations followed the formulae of Hartl & Clark (1997). Migration rate (Nm) was estimated for localities where there were three or more collections in a single month using the private allele method of Slatkin (1985) and Slatkin & Barton (1989). Analysis of Molecular Variance (AMOVA) analysis was as for Excoffier *et al.* (1992), Peakall *et al.* (1995) and Michalakis & Excoffier (1996). The correlation of geographic distance to genetic distance used the Mantel test of Smouse *et al.* (1986) and Smouse & Long (1992).

Assignment tests were performed in GeneClass2.3 (Piry *et al.*, submitted) with 1000 Monte-Carlo resampling of gametes to preserve linkage disequilibrium from recent immigrations (Paetkau *et al.*, 2004). Assignment criteria for populations of less than 40 individuals used a 1% error rate, and for populations with greater than 40 individuals an error rate of 5% was applied. This was to account for the increase in type 1 errors when using smaller sample sizes. The assignment test used in this study was that of Paetkau *et al.* (2004) which enables the identification of immigrant individuals in the current generation. This differs from many methods such as Wilson & Rannala (2003) that estimate migration over several generations. Other assignment test methods such as Rannala & Mountain (1997) and Cornuet *et al.* (1999) also resample alleles rather than gametes, randomly distributing migrant alleles across the population (Paetkau *et al.*, 2004). These later methods represent unrealistic approaches for a species such as *H. armigera* that has continuous migration, and would lead to an excess of “locals” being wrongly identified as immigrant (Type 1 error).

Analysis of insecticide resistant/susceptible *H. armigera*.

H. armigera individuals bioassayed for resistance or susceptibility to Emamectin benzoate, Endosulfan, Fenvalerate, Indoxacarb, Methomyl, Profenofos and Spinosad have been kindly provided to the project by Dr. Louise Rossiter. These *H. armigera* are analysed by the same methodology. This important extension of the project allows us to define whether collected *H. armigera* from a location are not only local versus migrant, and from which region its migration originated, but whether a subset of the migrating individuals are resistant or susceptible to the listed insecticides.

4. Detail and discuss the results including the statistical analysis of results.

A. Tracking Resistance in *H. armigera*

The “proof of concept” for the addition of insecticide resistance data into the microsatellite surveys was pursued as a new collaboration with Dr. Louise Rossiter. In this collaboration we included all bioassayed materials in microsatellite analysis. This

collaboration has been very successful as on completion of Dr Rossiter's assays, the material are forwarded to the microsatellite program. We are now getting the first results from the 2003-2004 season, and have tracked the movement of the first insecticide resistant and susceptible individuals. We can now define which resistant individuals are local, which are migrants, and from where the migration has originated. As this work proceeds through 2004-2005 we anticipate that we will be able to construct a detailed map of the movement of resistant and/or susceptible *H. armigera* both within and across the cotton growing regions in the coming seasons. This information will detail how resistance accumulates and moves as the season's progress.

B. Movement of *H. armigera* within a region: Darling Downs case study

The use of assignment testing on individual *H. armigera* has provided insight into the direction of migration and quantity of immigration events within the twelve major cotton-growing regions shown in Figure 1 over the last 4 years. Providing a detailed analysis of each region here would be too lengthy, so we refer to Q9 for a list of publications that provide information on the separate regions and present here one example i.e. the Darling Downs.

The level of migration determined by assignment testing correlated well the differentiation between regions determined by AMOVA analysis. In the year with high AMOVA inter-regional differentiation (2002-2003) the proportion of *H. armigera* immigrating into the Darling Downs was only 5.7-10.7% of the population. This low level of immigration into the region resulted in very significant differentiation between occurrences of *H. armigera* within the Darling Downs i.e. Toowoomba, Kingaroy, Murgon and Dalby each formed distinct populations of *H. armigera* with significant AMOVA supports.

In contrast in a year with low AMOVA inter-regional differentiation (2001-2002) the proportion of *H. armigera* immigrating into the Darling Downs was much higher at 22.2-29.4% of the populations, resulting in no differentiation of *H. armigera* populations across the Darling Downs i.e. effectively a single population for management.

C. Movement of *H. armigera* between regions

Over the period of this research, broad panmictic migration of *H. armigera* has not been normal, with results from three of the four seasons showing genetic distinctions between most regions. The direction and quantity of migration, and the resulting regional differentiation of *H. armigera* has significant impact on the management of the pest and are outlined in detail on a seasonal basis in Scott, Wilkinson, Lawrence *et al.* (from Q9 below). Some examples and major trends are:

- **EXAMPLE OF DIS-PROPORTIONAL CONTRIBUTIONS:** Assignment test results suggest that in consecutive seasons (eg. 2000-2001) there was a much larger contribution of Namoi Valley immigrants into the Darling Downs *H. armigera* populations (11.1% for Oct-Dec, and 8.7% for Jan-March) than from Dawson-Callide Valleys immigrants into the Darling Downs (1.6% for Oct-Dec, and 4.3% for Jan-March).
- **EXAMPLES OF BOTH SINGLE AND MULTIPLE IMMIGRATION SOURCES:** There are examples of multiple immigrant sources into a region, for example in Figure 2 below the Dawson-Callide had immigration from the Central Highlands, Darling Downs, Namoi Valley and the Murrumbidgee. In contrast other regions had immigration from only a single source eg. the Lower Namoi had contributions only from the Darling Downs.
- **ISOLATION BY DISTANCE:** Mantel analysis conducted on *H. armigera* in Australia is consistent with an "isolation by distance" model. The implications of isolation by distance are that immigrants are most likely to come from nearby regions

than from afar. This is contrary to the view of *H. armigera* being panmictic, or arising primarily through spring migrations from central Australia.

These results highlight the importance of studies in groups such as the Lepidoptera extending over consecutive years, as short-term work would be misleading where population structures and quantities of migration change so significantly from year to year.

D. Seasonal changes in *H. armigera* genotypes

Principal coordinate analysis between collections, for example in the Darling Downs, illustrates that *H. armigera* genotypes change every few months. This observed change in genotypes is in concordance with observations made for *H. armigera* populations in the Dawson-Callide Valleys (Scott *et al.* 2003), and findings in Monarch butterflies (Eanes & Koehn 1978). This may reflect a 'founder-like effect' due to non-uniform distribution of the pest in both space and/or time, and also as a result of fluctuating population sizes (Eanes & Koehn 1978). Differential immigration directions and quantities, insecticide usage, agricultural practices and seasonal variation causing bottlenecks are other likely contributors to the changes in *H. armigera* genotypes observed.

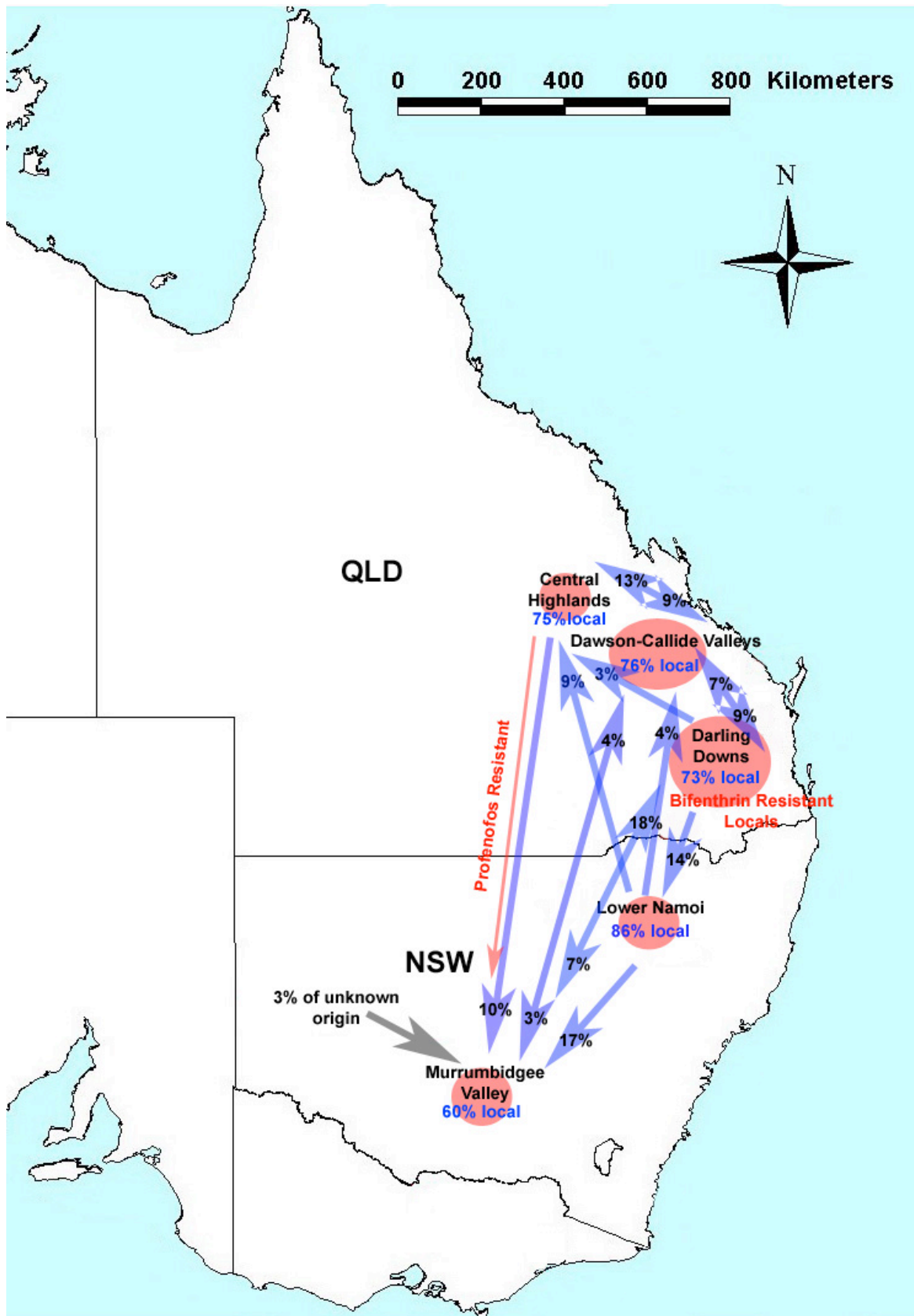


Figure 2. Demonstration of data collected in 3 month periods for 2003-2004. Estimates are provided for the percentage of *H. armigera* that are local in each region at each time (shown in blue text), and for the percentage and direction of *H. armigera* that immigrate (blue arrows). Red arrows indicate our new inclusion of *H. armigera* with known insecticide resistance and susceptibility.

5. Provide a conclusion as to research outcomes compared with objectives. What are the “take home messages”?

- The microsatellite research results for 2003-2004 fulfilled research objective A by demonstrating that *H. armigera* is in some years predominantly local and can thus be significantly influenced by local management practices - supporting the current area wide management strategies for *H. armigera*. However, regional or Australia-wide approaches need to remain co-ordinated to manage the pest in years where migration levels are high, as local management will not be an effective strategy in these years.
- Migration of *H. armigera* with known resistant/susceptible status are currently being tracked using our microsatellite methodology (objective B).
- The microsatellite results are correlating well with observed *H. armigera* occurrences (as made by the projects collaborators). The ongoing project is now attempting to extend this observational correlation via a collaboration with Prof. Myron Zalucki to allow integration of meteorological data with migrations detailed through the microsatellite data (objective C).

6. Detail how your research has addressed the Corporation’s three Outputs - Economic, Environmental and Social?

This project provides key information on the movement of insecticide resistance, and attempts to establish pre-emptive strategies for *Helicoverpa* management, to area wide management groups across a large portion of Australia’s Cotton growing regions. This has the potential to reduce pesticide usage, which simultaneously addresses the economic, environmental and social outputs for the corporation.

7. Provide a summary of the project ensuring the following areas are addressed:

a) technical advances achieved (eg commercially significant developments, patents applied for or granted licenses, etc.)

None of our technical achievements in this 12 month project are of commercial consequence.

b) other information developed from research (eg discoveries in methodology, equipment design, etc.)

In this 12 month project the primary technical advance has been the development of collaboration and methodologies for the inclusion of insecticide bioassayed materials, into our genetic migration analysis. At the initiation of this project, Louise Rossiter and myself negotiated a research proposal for the utilisation of pre-bioassayed material, for the microsatellite research. The *Helicoverpa* microsatellite team then developed methodologies for the utilisation of experimental material remaining from the bioassay program. Bioassayed *H. armigera* material is often in poorer condition than field collections, as a direct result of the frequent “lethal” effects of the bioassay testing, and as such methodologies needed to be established. After testing we were able to include in our study: material bioassayed for insecticides (Emamectin benzoate, Endosulfan, Fenvalerate, Indoxacarb, Methomyl, Profenofos and Spinosad) and include in our laboratory work material which had been dead >20 hours.

c) are changes to the Intellectual Property register required?

No changes are required for the Intellectual property register.

8. Detail a plan for the activities or other steps that may be taken:

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

Future prospects for this research technology are currently opening up down several interesting paths. We are establishing collaborative research projects on BT cotton verses refuge collected *H. armigera* in the upcoming twelve-month project (with Dr. Geoff Baker and Dr. Colin Tann), as well as new collaborations with Dr. Sharon Downes and Dr. Geoff Baker on sperm precedence and multiple paternity contributions to female egg lays. Both these projects may provide new insights into the effectiveness of refugia for diluting resistance to BT, and utilise the current molecular team and available methodologies.

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

Our strategies for communicating research outcomes during this twelve month project have included:

- **Local Reports:** We have provided a large number of specific local reports to collectors on request. This year it has included reports to the Dawson-Callide Valleys, Griffith, Darling Downs, Bundaberg, Central Highlands, and Victoria.
- **Industry Specific Conferences and Meetings:** National Helicoverpa Workshop June 2004, GRDC publication Groundcover, Direct CRDC and GRDC reports
- **Scientific Journals:** One new scientific paper has been published this year and a further three submitted for publication, with two others currently in preparation.
- **Community Publications:** A review of the *H. armigera* microsatellite research appeared in a community based Toowoomba publication.

This multi-strand approach taken by us last year to communication to all levels of the industry extends on the many and varied communication in the previous projects and will continue in the next twelve month project.

(c) for future research.

One particular avenue we are trying to progress in the next twelve-month project is the correlation of our migration measurements with meteorological data in collaboration with Prof. Myron Zalucki and Dr. Wayne Rochester. If it were possible to correlate observed genetic migration events with meteorological events, we would have vastly improved the predictive capacity for migration of *H. armigera*.

Our research group would also like to move into establishing two other lines of additional related research. We are looking for ways to fund equivalent research in *H. punctigera*, as we are interested in making a comparison in the migration frequencies and distances, and the movement of resistance in *H. punctigera* in comparison with *H. armigera*.

A second area research area that we see great potential in, is the implementation of the diagnostics we have for *Trichogramma* and NPV infection in *H. armigera*. We would like to establish funding and support for this technology.

9. List the publications arising from the research project and/or a publication plan. (NB: Where possible, please provide a copy of any publication/s)

PUBLISHED: (Copies included for CRDC records)

- Scott, K.D., Lange, C.L., Scott, L.J., Graham, G.C. (2004). Isolation and characterisation of microsatellite loci from *Helicoverpa armigera* Hübner (Lepidoptera: Noctuidae). *Molecular Ecology Notes*. **4(2)**: 204-205.
- Scott, K.D., Wilkinson, K.S., Merritt, M.A., Scott, L.J., Lange, C.L., Schutze, M.K., Kent, J.K., Merritt, D.J., Grundy, P.R., Graham, G.C. (2003) Genetic shifts in *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) over a year in the Dawson/Callide Valleys. *Australian Journal of Agricultural Research*. **54**: 739-744.

SUBMITTED:

- Scott, K.D., Wilkinson, K.S., Lawrence, N., Lange, C.L., Scott, L.J., Merritt, M.A., Lowe, A.J., Graham, G.C. (submitted). Gene-flow between populations of the economic pest Cotton bollworm *Helicoverpa armigera* Hübner (Lepidoptera: Noctuidae) are highly variable between years: Records from across Australia's major cotton and grain growing regions from November 1999 – March 2003. *Bulletin of Entomological Research*
- Scott, K.D., Lawrence, N., Lange, C.L., Scott, L.J., Wilkinson, K.S., Merritt, M.A., Miles, M., Murray, D., Graham, G.C. (submitted). Seasonal gene-flow and migration of the Cotton bollworm *Helicoverpa armigera* Hübner (Lepidoptera: Noctuidae) on the Darling Downs, Australia, from November 1999 – March 2003. *Journal of the Australian Entomological Society*
- M.A. Merritt, K.S. Wilkinson, K.D. Scott, M. Dillon, L.J. Scott, C.L. Lange, M.K. Schutze, J.K. Kent, D.J. Merritt and G.C. Graham (submitted) Gene flow in two consecutive collections of the Lepidopteran pest, *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) from the Narrabri region, New South Wales. *Journal of the Australian Entomological Society*

IN PREPARATION:

- Lawrence, N., Lange, C.L., Scott, L.J., Graham, G.C., Hardwick, S., Rossiter, L., Scott, K.D. (in prep). Gene-flow of *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) on cotton and grain crops in the Griffith region from October 2001 to January 2004. *Journal of the Australian Entomological Society*
- Lawrence, N., Lange, C.L., Scott, L.J., Graham, G.C., Dawson, J., Mansfield, C., Scott, K.D. (in prep). Gene-flow of *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) in Victoria from January 2003 to April 2004. *Journal of the Australian Entomological Society*

10. Provide an assessment of the likely impact of the results and conclusions of the research project for the cotton industry. Where possible include a statement of the costs and potential benefits to the Australian cotton industry or the Australian community.

This project's significance is its potential for reducing pest management costs by establishing the origin movements of *H. armigera* populations and tracking the occurrence and persistence of resistance in Australia. This will have an immediate effect on reducing cropping costs through reduction of unnecessary spraying and/or reduction in crop loss through unforeseen pest outbreaks. The results benefit the cotton industry's image by using information rich integrated pest management program. The likely follow on of a reduction in sprays is also an environmental achievement.

Part 4 – Final Report Executive Summary

The '*Helicoverpa armigera* population genetics, migration, recruitment and origins project' uses a genetic technology (microsatellites) to establish population structure, and the quantity, direction and timing of migration events of *H. armigera* across major cotton growing regions of eastern Australia. This twelve month project successfully proved the concept of incorporating *H. armigera* individuals bioassayed for insecticide resistance or susceptibility (to Emamectin benzoate, Endosulfan, Fenvalerate, Indoxacarb, Methomyl, Profenofos and Spinosad - kindly provided to the project by Dr. Louise Rossiter) into our migration analyses. This important development in the research allows us not only to define whether collected *H. armigera* are "local" or "immigrant" and from which region the immigration originated, but now also whether the migrating individuals are resistant or susceptible to the listed insecticides. This will provide the Cotton and Grains industry with an invaluable tool that supports decisions on pest management based on a fuller understanding of the pest and its movements, and provide detailed information on the occurrence, the speed, and the direction of movement of insecticide resistance in *H. armigera*. This will then enable refinement of management strategies for *H. armigera* through more effective resistance management strategies.

Current research ongoing in 2004-2005 is attempting to correlate the observed genetic migration events with meteorological data and events. With this inclusion we aim to provide vastly improved predictive information for migration behaviours of *H. armigera*.