Part 3.3 – Final Reports (due 3 months after completion of project)

1. Outline the background to the project.

This twelve-month project continued on from UQ35c and UQ32c research covering the *Helicoverpa armigera* microsatellite survey of field collections over twelve of the major cotton growing regions during the 2003-2005 seasons. This microsatellite survey provided information on population structure and movement of *H. armigera* at both the local and regional levels. The studies primary object was to continue collecting data on the migration and recruitment of *H. armigera* and to include a description of (i.e. tracking) the movement of resistant and susceptible *H. armigera* across these regions. These combined outputs were 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.

Obj No.	Objective	Achievement
1	Application of microsatellite analysis to evaluate population structure, and migration in *H. armigera* at the local, regional and national scales in 2005-2006.	Completed refer to Table 2 for all interpreted data from microsatellite data
2	To detail the movementof known insecticide resistant and known insecticide susceptible *H.armigera* to explain the occurrence and spread of insecticide resistance within and between cotton growing regions	Completed refer to Table 1 for details on data obtained
3	Reporting of the research to industry and scientific community	Several publications have come from the project refer below, as well as many conferences, seminars and industry reports

3. Detail the methodology and justify the methodology used.

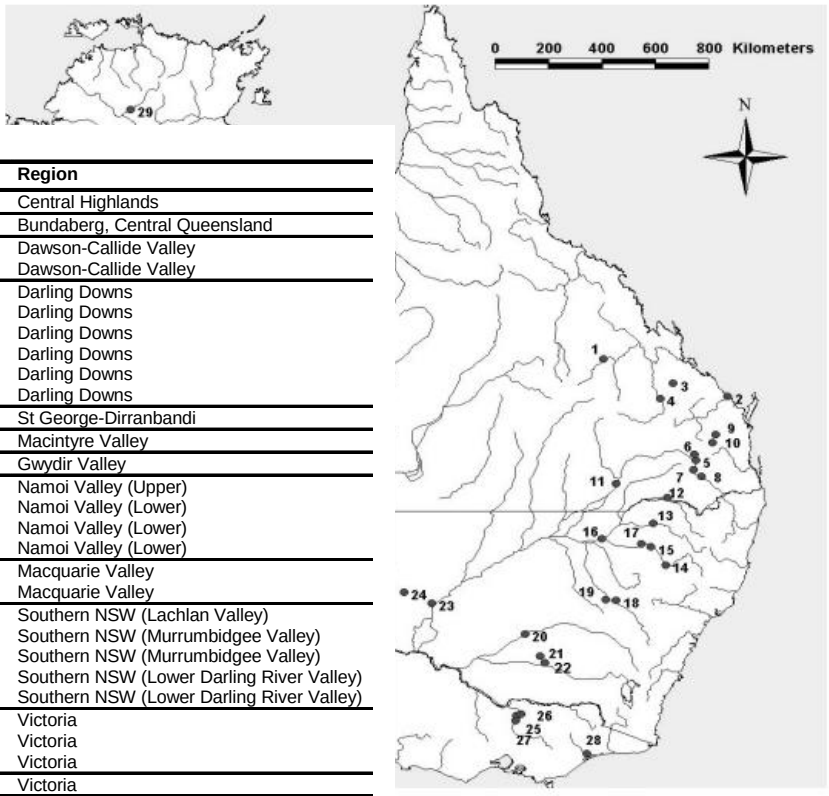
Justification of molecular methodology for studying migration of H. armigera:

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 measured 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.

Sample source:

H. armigera larvae and moths were collected via the network of dedicated collectors supporting the 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 Rossiter, 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).



Number	Town	Region
1	Emerald	Central Highlands
2	Bundaberg	Bundaberg, Central Queensland
3	Biloela	Dawson-Callide Valley
4	Theodore	Dawson-Callide Valley
5	Dalby	Darling Downs
6	Jimbour	Darling Downs
7	Cecil Plains	Darling Downs
8	Brookstead	Darling Downs
9	Murgon	Darling Downs
10	Kingaroy	Darling Downs
11	St George	St George-Dirranbandi
12	Goondiwindi	Macintyre Valley
13	Moree	Gwydir Valley
14	Gunnedah	Namoi Valley (Upper)
15	Narrabri	Namoi Valley (Lower)
16	Walgett	Namoi Valley (Lower)
17	Wee Waa	Namoi Valley (Lower)
18	Dubbo	Macquarie Valley
19	Narromine	Macquarie Valley
20	Hillston	Southern NSW (Lachlan Valley)
21	Griffith	Southern NSW (Murrumbidgee Valley)
22	Whitton	Southern NSW (Murrumbidgee Valley)
23	Menindee	Southern NSW (Lower Darling River Valley)
24	Broken Hill	Southern NSW (Lower Darling River Valley)
25	Tatura	Victoria
26	Shepparton	Victoria
27	Murchison	Victoria
28	Bairnsdale	Victoria
29	Katherine	Northern Territory

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 Integrative Biology) 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 and Statistical analysis

Five microsatellite loci were used to analyse *H. armigera* individuals collected (Scott *et al.* 2004). The loci were HaB60, HaD25, HaD47, HaC87 and HaC14, and it should be noted that null alleles were detected in HaD47. Computer modelling found that this null did not alter the results. All microsatellites were *Hex* labelled with PCR amplification conditions and gel separation as published in Scott *et al.* (2003).

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). Analysis of Molecular Variance (AMOVA) analysis was as for Excoffier *et al.* (1992), Peakall *et al.* (1995) and Michalakis & Excoffier (1996).

Assignment tests were performed in GeneClass2.3 (Piry *et al.*, 2004) 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 & Rananala (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 verses 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.

This projects aim was use microsatellites to describe *H. armigera* population movement while simultaneously integrating bioassay data to improve our understanding of the movement and accumulation of insecticide resistances within *H.armigera* populations. This project has achieved these objectives through the accumulation of an elegant data set (in collaboration with Dr Louise Rossiter) that includes *H. armigera* migration estimates, assignment analysis determining from which regions collected *H. armigera* have sourced, and knowledge of the resistance/susceptibility status of a proportion of the individuals.

Results from genotypiong bioassayed H. armigera:

This research's results clearly show that a significant proportion of insecticide resistance develops within a region in *H. armigera* (i.e. resistant individuals are local to the region and not emigrating in from another growing region), and insecticide resistant *H.*

armigera are in some cases immigrating between growing regions. One example from the data is that in the summer of 2004 (Dec03-Feb04 Table1), where the *H. armigera* assessed from the Gwydir (and were resistant to Methomyl and Profenofos) were all of local "Gwydir" origin i.e. the Methomyl and Profenofos resistance developed within the Gwydir region, and had not entered the Gwydir region from other growing regions. In contrast, *H. armigera* collected in the Macquire Valley in the summer of 2004 (and were resistant to Methomyl), had sourced from populations immigrating into the region from Central Queensland and the Lower Namoi. Full details of the resistance data are below in Table 1. In this case Methomyl resistance developed in both Central Queensland and in the lower Namoi and both regions supplied resistant *H. armigera* moths into the Macquire Valley.

Results from migration data:

Data from this research project cover the period from December 2002-May 2005 and verify earlier research data (November 1999-March 2001 in Scott *et al.* 2005b) in showing variable rates and patterns of migration of *H. armigera* across cropping regions in Australia. Some periods having higher levels of migration between regions, than other times. In December 2003-February 2004 the highest proportion of regionally "local" *H. armigera* were observed. In contrast, in March 2003-May 2003 there were higher levels of immigration between the regions, however still not as high as those recorded in previous research (i.e. April 2001-March 2002 in Scott et al 2005b).

Table 1: Assignment of insecticide bioassayed *H. armigera* individuals

(orange boxes highlight individuals which are local and resistant; Purple boxes highlight individuals which are immigrant and resistant)

	n insecticide resistant individuals	Source of resistant *H.armigera* determined through assignment testing							
		Central Queensland	Darling Downs	Dawson-Callide Valleys	Gwydir Valley	Lower Namoi	Macintyre Valley	Macquire Valley	Upper Namoi
Dec 03-Feb 04									
Central Queensland	6	2xBifenthrin 3xMethomyl						1xProfenofos	
Darling Downs	11		11xBifenthrin						
Dawson-Callide Valleys	2			1xMethomyl				1xMethomyl	
Gwydir Valley	4				2xProfenofos 2xMethomyl				
Lower Namoi	3					3xBifenthrin			
Macintyre Valley	4						1xBifenthrin 1xMethomyl 2xProfenofos		
Macquire Valley	2	1xMethomyl				1xMethomyl			
Mar 04-May 04									
Gwydir Valley	19				4xBifenthrin 11xMethomyl 4xProfenofos				
Lower Namoi	9	1xProfenofos				4xBifenthrin 2xMethomyl 1xProfenofos		1xProfenofos	
Macintyre Valley	1						1xProfenofos		
Upper Namoi	2	1xBifenthrin							1xBifenthrin
Sept 04-Nov 04									
Central Queensland	1	1xEndosulfan							
Dec 04-Feb 05									
Darling Downs	3		3xEndosulfan						
Gwydir Valley	5				2xProfenofos 3xEndosulfan				
Lower Namoi	2					1xProfenofos 1xBifenthrin			
Upper Namoi	2				1xBifenthrin				1xProfenofos

Table 2: Assignment of H. *armigera* migrants (Paetkau *et al*. 2004, Piry *et al*., 2004)

(Shaded areas indicate unavailability of samples, Values in bold indicate % local)

	n total	Biloela, Dawson-Callide Valleys	Brookstead, Darling Downs	Bundaberg, Central Queensland	Dalby, Darling Downs	Emerald, Central Queensland	Goondiwindi, Macintyre	Griffith, Murrumbidgee Valley	Gunnedah, upper Namoi	Kingaroy, Darling Downs	Moree, Gwydir Valley	Narrabri, lower Namoi	St George, Macintyre	Tatura, Victoria	Theodore, Dawson-Callide Valleys	Warren, Macquire Valley	Source Unknown
Dec 02-Feb 03																	
Griffith, Murrumbidgee Valley	191							**81.1**						4.2			14.7
Tatura, Victoria	84							7.1						**89.3**			3.6
Mar 03-May 03																	
Bundaberg, Central Queensland	51			**88.2**				2.0			0.0			3.9	2.0		3.9
Moree, Gwydir Valley	22			0.0				18.2			**72.7**			9.1	0.0		0.0
Griffith, Murrumbidgee Valley	169			2.4				**82.7**			1.8			1.8	2.4		8.9
Theodore, Dawson-Callide Valleys	49			0.0				8.1			0.0			0.0	**79.7**		12.2
Tatura, Victoria	54			1.9				5.6			3.7			**85.0**	1.9		1.9
Sept 03-Nov 03																	
Brookstead, Darling Downs	99		**78.8**	3.0						2.0					1.0		15.2
Bundaberg, Central Queensland	62		6.5	**80.6**						8.1					0.0		4.8
Kingaroy, Darling Downs	213		3.3	0.9						**88.8**					0.9		6.1
Theodore, Dawson-Callide Valleys	32		6.2	0.0						9.4					**78.1**		6.3
Dec 03-Feb 04																	
Dalby, Darling Downs	21				**95.5**	4.5	0.0		0.0	0.0	0.0	0.0		0.0	0.0	0.0	0.0
Emerald, Central Queensland	163				0.6	**90.3**	0.6		0.0	0.0	0.0	1.2		1.2	1.8	0.0	4.3
Goondiwindi, Macintyre	184				0.0	2.2	**89.7**		0.5	0.0	1.1	0.0		2.7	1.1	0.0	2.7
Gunnedah, upper Namoi	30				0.0	6.6	0.0		**83.5**	0.0	0.0	3.3		0.3	0.0	0.0	3.3
Kingaroy, Darling Downs	48				0.0	0.0	2.1		0.0	**91.6**	0.0	0.0		0.0	6.3	0.0	0.0
Moree, Gwydir Valley	155				1.9	0.6	0.6		0.6	0.0	**93.1**	0.0		6.0	26.0	0.0	0.0
Narrabri, lower Namoi	63				1.6	1.6	3.2		0.0	0.0	1.6	**90.0**		1.6	0.0	0.0	0.0
Tatura, Victoria	128				0.0	1.6	2.3		0.0	0.0	0.8	0.0		**88.3**	0.8	0.0	0.4
Theodore, Dawson-Callide Valleys	234				0.0	1.7	0.8		0.4	0.4	0.0	0.8		3.0	**92.5**	0.0	6.2
Warren, Macquire Valley	30				0.0	0.0	3.3		0.0	0.0	0.0	0.0		0.0	0.0	**96.7**	0.0

	n total	Biloela, Dawson-Callide Valleys	Brookstead, Darling Downs	Bundaberg, Central Queensland	Dalby, Darling Downs	Emerald, Central Queensland	Goondiwindi, Macintyre	Griffith, Murrumbidgee Valley	Gunnedah, upper Namoi	Kingaroy, Darling Downs	Moree, Gwydir Valley	Narrabri, lower Namoi	St George, Macintyre	Tatura, Victoria	Theodore, Dawson-Callide Valleys	Warren, Macquire Valley	Source Unknown
Mar 04-May 04																	
Bundaberg, Central Queensland	50			**80.8**					0.0	0.0	4.0	2.0	0.0	12.0			2.0
Gunnedah, upper Namoi	30			3.3					**90.1**	0.0	0.0	3.3	0.0	3.3			0.0
Kingaroy, Darling Downs	20			5.0					0.0	**85.0**	5.0	0.0	0.0	5.0			0.0
Moree, Gwydir Valley	102			1.0					2.9	0.0	**87.3**	0.0	1.0	2.9			4.9
Narrabri, lower Namoi	29			0.0					3.4	0.0	6.9	**82.8**	6.9	0.0			0.0
Mar 04-May 04																	
St George, Macintyre	30			10.0					0.0	0.0	3.3	0.0	**80.1**	3.3			3.3
Tatura, Victoria	471			1.9					0.6	0.6	0.4	0.0	0.8	**85.1**			10.6
Sept 04-Nov 04																	
Biloela, Dawson-Callide Valleys	21	**85.7**		9.5													4.8
Bundaberg, Central Queensland	35	8.6		**85.7**													5.7
Dec 04-Feb 05																	
Bundaberg, Central Queensland	29			**79.4**			0.0		0.0	0.0	0.0	0.0		20.6		0.0	0.0
Goondiwindi, Macintyre	22			0.0			**86.5**		0.0	4.5	0.0	4.5		0.0		0.0	4.5
Gunnedah, upper Namoi	63			0.0			0.0		**87.2**	0.0	0.0	4.8		1.6		3.2	3.2
Kingaroy, Darling Downs	64			0.0			0.0		1.6	**90.5**	0.0	3.1		1.6		1.6	1.6
Moree, Gwydir Valley	40			0.0			5.0		0.0	0.0	**90.0**	2.5		0.0		0.0	2.5
Narrabri, lower Namoi	79			0.0			1.3		3.8	1.3	0.0	**77.2**		7.6		5.0	3.8
Tatura, Victoria	119			2.5			0.0		0.0	0.8	0.8	0.0		**87.5**		0.8	7.6
Warren, Macquire Valley	76			0.0			1.3		3.9	0.0	1.3	3.9		0.0		**84.4**	5.2
Mar 05-May 05																	
Bundaberg, Central Queensland	27			**78.0**										22.0			0.0
Tatura, Victoria	306			1.3										**86.3**			12.4

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

This project has fulfilled its research outcomes and objectives and these migration patterns have significant implications for the management of insecticide resistance in *H. armigera*. During periods of low migration there are clear differentiations between populations from different growing regions, with each regions populations of *H. armigera* being relatively independent. Therefore, problems that arise from management practices, for example insecticide resistance, are likely under these circumstances to be derived locally. This is evidenced by the frequent "local" nature of the occurrence of resistance in the results described in Table 1. Development of resistance may also be exacerbated in periods of low migration as there is no influx of susceptible individuals to dilute the resistance that accumulates. In summary, our data shows migration patterns that are variable, periods of high migration, and that insecticide resistance is at times being transferred between cropping regions. This has been observed previously with the rapid spread of pyrethroid and endosulfan resistance in Australia (Forrester et al., 1993; Gunning & Easton, 1994). The national coordination of insecticide resistance management strategies is thus critical.

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.)

This projects primary discovery was the development of collaborations 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 *Heliocoverpa* 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.

This project was thus successful in the development of a new approach in pest management research that combines insecticide resistance bioassay information with population genetic data that can describe population differentiation and movement. This new approach has brought new insight into understanding the transfer of resistance within and between populations and can now be applied in other pest species of interest

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.

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

(c) for future research.

The approach taken in this work to combine bioassay data and population analysis with microsatellites can be applied to other species - thus exploiting the technology further. Dr Kirsten Scott is leaving the University of Queensland on 30th June 2006 when this project finishes and as such this project will not be continuing or extended upon by Dr Scott .

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)

Scott,L.J., Lawrence,N., Lange,C.L., Graham,G.C., Hardwick,S., Rossiter,L., Dillon,M.L., **Scott, K.D.** (2006). Population dynamics and gene flow of *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) on Cotton and Grain crops in the Murrumbidgee Valley, Australia. *Journal of Economic Entomology*. **99(1):** 155-163.

Scott,K.D., Lawrence,N., Lange,C.L., Scott,L.J., Wilkinson,K.S., Merritt,M.A., Miles,M., Murray,D., Graham,G.C. (2005a). Assessing moth migration and population structuring in the Cotton bollworm *Helicoverpa armigera* Hübner (Lepidoptera: Noctuidae) at the regional scale: Example from the Darling Downs, Australia. *Journal of Economic Entomology* **98(6):** 2210-2219.

Scott,K.D., Wilkinson,K.S., Lawrence,N., Lange,C.L., Scott,L.J., Merritt,M.A., Lowe,A.J., Graham,G.C. (2005b). Gene-flow between populations of cotton bollworm *Helicoverpa armigera* Hübner (Lepidoptera: Noctuidae) is highly variable between years. *Bulletin of Entomological Research* **95(4):** 381-392.

10. Have you developed any online resources and what is the website address?

No

11. 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 unforseen pest outbreaks. The results benefit the cotton industrys' 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

Provide a one page Summary of your research that is not commercial in confidence, and that can be published on the World Wide Web. Explain the main outcomes of the research and provide contact details for more information. It is important that the Executive Summary highlights concisely the key outputs from the project and, when they are adopted, what this will mean to the cotton industry.

Microsatellite data collected over many of the grain growing regions of eastern Australia from December 2002 through to May 2005, has detected and described variable gene-flow years (i.e. variable levels of migration). In December 2003-February 2004 the highest proportion of regionally "local" *H. armigera* were observed. In contrast, in March 2003-May 2003 there were higher levels of immigration between the regions, however still not as high as those recorded in previous research (i.e. April 2001-March 2002 in Scott *et al* 2005b). These data provide evidence that the direction of moth movement differs from season to season, and within a season, highlighting the importance of studies in groups such as the Lepidoptera extending over consecutive years, as short-term sampling may be misleading when population dynamics and migration change so significantly.

In some years, *H. armigera* populations may migrate very little and then be relatively independent within each region and thus significantly influenced by local management practices. This is shown by the significant proportion of insecticide resistance developing locally (i.e. within a single growing region) in *H. armigera* in this research. However, there are also periods with high migration across the cropping regions, and resistance may rapidly spread at these times. This research demonstrates that insecticide resistance to several chemistries is efficiently immigrating across growing regions in seasons of moderate "migration" levels. These research results thus reaffirm the critical importance of maintaining a nationally coordinated insecticide resistance management strategy.