Project Title: The role of IPM in sustainable cotton farming systems in the Northern Territory

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Cotton Catchment Communities CRC

Northern Territory Government
Department of Primary Industry, Fisheries and Mines

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Plain English Summary

The adoption of Integrated Pest Management (IPM) principles plays a crucial role in the future sustainability of a cotton industry based on transgenic cultivars in the Northern Territory (NT). Certain pest insects, some of which are present in the NT, have a demonstrable ability to develop resistance to conventional, and possibly novel, control tactics, and IPM utilises strategies to minimise that risk. Tactics employed during trials in Katherine included avoidance of high summer insect pest densities via a winter cropping system, reliance on natural enemies for biological control, provision of unsprayed refuges like companion crops for natural enemy proliferation, trap crops to attract insect pests away from cotton, understanding the ability of cotton plants to compensate for pest insect damage, and utilisation of target selective insecticides at lowest effective concentration only when absolutely necessary. The idea was to maintain a balance of insects, both good and bad, so principal insect pests were maintained below thresholds that cotton plants could tolerate without loss of yield. Transgenic cotton currently requires few insecticidal treatments in Katherine, allowing natural enemies to do their work, and IPM research is needed to ensure this system improves and remains sustainable.

Transgenic cotton trialled in Katherine produces proteins from a common bacteria (*Bt*) in its tissues that are poisonous to a select group of insect pests. Refuge crops play a pivotal role in prolonging the control efficacy of transgenic cotton by permitting development of relatively large numbers of pest individuals not exposed to these *Bt* proteins. Should a resistant individual successfully develop on transgenic cotton, its genes are dissipated when it mates within the local population dominated by non-resistant individuals from neighbouring companion crops. Irrigated lablab produces large numbers of *Helicoverpa armigera* moths, which are potentially resistant to *Bt* cotton, in the NT during the winter growing season, and was tested as a possible companion crop for a local transgenic cotton production system. Although lablab effectively maintains relatively large numbers of *H. armigera* season long, insect pest and natural enemy densities and lint yield and quality from cotton crops grown with and without lablab companions were not different. Companion crops with more discernible advantage to cotton production should be trialled to improve IPM in the future.

Insecticide use was kept to a minimum during cotton IPM trials in Katherine. The number of applications and products utilised per season steadily decreased as the IPM system was refined. In the final year of the project, an average of 2.8 sprays of mainly fipronil to selectively control sucking insects were applied to cotton crops which achieved record yields. The majority of insect pest suppression was achieved via *Bt* cotton's inherent control potential, which has not waned for the life of the project, and biological control with natural enemies. Many different insects make up the suite of effective natural enemies in Katherine cotton, most importantly ladybeetles, hover flies, spiders and parasitic wasps called *Trichogramma*. Ladybeetles and hover flies effectively control aphids and whitefly in Katherine cotton when not disrupted by insecticide application. Spiders are more robust and persist season long in Katherine cotton, but are less selective with prey, eating pests and natural enemies alike. *Trichogramma* parasitise a high percentage of *H. armigera* eggs early season in Katherine. They contribute to the management of possible resistance in *H. armigera* as parasitised eggs do not hatch so are never exposed to *Bt* proteins. Natural enemies are critical to the Katherine cotton IPM system, and their pest control efficacy governs the need, or lack there of, for judicious insecticide selection and application.

Sucking insects previously controlled by insecticides targeting *H. armigera* are not affected by *Bt* proteins in cotton. Insecticides that target sucking insects with minimal disruption to natural enemies were examined during Katherine cotton IPM trials. Mirids are the main sucking pests in Katherine cotton, and preliminary trials suggest they require control once a threshold of 0.5 per metre is reached. However, trials incorporated other sucking insects, such as redbanded shield bugs (RBSB) and green vegetable bugs (GVB), in threshold calculations despite their relative damage potentials not being quantified in Katherine. Further, the seasonal relative abundance of different mirids (green and brown) and their pest status in Katherine cotton has not been clarified. Consequently, large scale trials attempting to clarify control thresholds for this suite of sucking insects collectively were largely unsuccessful. The pest status and damage potential of RBSBs were examined in late season Katherine cotton in 2005 only, and more species specific research, including biological control possibilities, is required before trials examining sucking insect control can be further refined.

Managing potential resistance in moth pests requires a thorough understanding of their seasonal phenology. Four moth pests of cotton, including *H. armigera*, were monitored using pheromone traps in strategic locations throughout the Katherine region. *Helicoverpa armigera* persist year round with population peaks in both the wet and dry seasons in Katherine, hence their potential to develop resistance if continually exposed to repeated control measures. Closely related *H. punctigera* tend to migrate into crops early dry season so are less likely to develop resistance. *Pectinophora gossypiella* are a wet season phenomenon, so this potentially devastating cotton pest is effectively avoided by cropping through winter. *Spodoptera litura* are prevalent in the wet season but persist into cotton crops through the dry so, although they tend to be less damaging than the other three moths, their pest status and possible control measures require examination. To date, *H. armigera* resistance to *Bt* proteins has not caused problems in Katherine, although resistance to conventional insecticides is of concern and has been detected, often to products not utilised by the cotton project. Area wide management of *H. armigera* should be adopted to ensure potential resistance to insecticides does not become problematic in the Katherine region. Continued monitoring of *H. armigera*, *H. punctigera* and *S. litura* populations would be required should cotton production trials proceed.

Despite a lack of cotton production expertise and infrastructure at Katherine Research Station, the Katherine IPM project has laid the foundation for environmentally sound pest management in future local transgenic cotton production systems. Further development and refining of the IPM system is of course reliant on continued regionally specific research should cotton trials proceed. Rigorous adoption and application of the IPM principles outlined herein is critical to the future sustainability of transgenic cotton production in the NT.

Abstract

Critical components of proposed integrated pest management (IPM) systems designed to promote sustainability of a cotton (*Gossypium hirsutum* L.) industry based on transgenic cultivars that express *Bt* toxins (Bollgard II®, Monsanto) in the Northern Territory (NT), Australia, were investigated from 2000 to 2005. The noctuid pest, *Helicoverpa armigera* (Hübner) attacks cotton in the NT and has a demonstrable ability to develop resistance to conventional control tactics and possibly *Bt* cotton, so an IPM system to minimise the risk of resistance development was devised. Tactics employed during IPM trials in Katherine included a winter cropping system to avoid high pest densities in summer, reliance on natural enemies for biological control of pests, provision of unsprayed refuges for natural enemy proliferation, trap crops to attract insect pests away from cotton plants, incorporation of cotton's inherent ability to compensate for damage caused by sucking mirids (*Creontiades diltus* (Stål) and *Creontiades pacificus* (Stål)), and utilisation of target selective insecticides at lowest effective concentration only when absolutely necessary. The aim was to design a local cotton IPM system that avoids insecticidal control of pests where possible without loss of lint yield and quality. IPM tactics suggested from research results in previous years were implemented as a system in 2005 contributing to record yields with minimal (average 2.8 per field) insecticide applications.

Irrigated lablab (*Lablab purpureus* (L.)) produced relatively large numbers of *H. armigera* compared to other crops locally during the winter growing season, and so was tested as a possible companion crop in a Bollgard II® production system. Although lablab effectively maintains large numbers of *H. armigera* season long, insect pest and natural enemy densities and lint yield and quality from cotton crops grown with and without lablab companions were not different. Companion crops with more discernible advantage to cotton production should be trialled in the future.

Insecticide reliance steadily decreased as the IPM system was refined from 2000 to 2005. The majority of *Helicoverpa* suppression was achieved via Bollgard II®'s inherent control potential, which did not wane for the life of the project, and biological control with natural enemies. Coccinellids (ladybeetles) and syrphid (hover fly) larvae effectively maintain aphid (*Aphis gossypii* Glover) and whitefly (*Bemisia tabaci* (Gennadius)) densities below control thresholds in local cotton when not disrupted by insecticides. Spiders are more robust and persist in Katherine cotton regardless of insecticide usage, but are highly polyphagous, consuming insect pests and natural enemies alike, so their benefit in biological control is marginalised. *Trichogramma* (*Trichogramma pretiosum* Riley) parasitise a high percentage (c.a. 50 to 80%) of *H. armigera* eggs early season in local Bollgard II® crops. *Trichogramma* contribute to the management of possible resistance in *H. armigera* populations as they consume the developing embryo in parasitised host eggs minimising larval hatch and exposure to *Bt* proteins. Natural enemies are critical to the local cotton IPM system, and their pest control efficacy governs the need for judicious insecticide selection and application.

Insecticides that target sucking insects with minimal disruption to natural enemies were examined. Preliminary trials suggest mirids require control at a threshold of 0.5 per metre in local cotton to maintain yield. To the detriment of experimental consistency, trials incorporated other sucking insects (redbanded shield bugs (RBSB, *Piezodorus grossi* (Staddon)) and green vegetable bugs (GVB, *Nezara viridula* (L.)), in threshold calculations despite their relative damage potentials not being quantified. Further, the seasonal relative abundance of different mirid species (green, *C. dilutus*, and brown, *C. pacificus*) and their

pest status in local cotton has not been clarified. Consequently, large scale trials attempting to clarify control thresholds for this suite of sucking insects collectively were largely unsuccessful. The pest status and damage potential of RBSBs and another sucking pest, *Austroasca alfalfae* (Evans) (leafhoppers) were examined in late season cotton in 2005 only.

Local populations of the noctuid pests *H. armigera*, *Helicoverpa punctigera* (Wallengren), *Pectinophora gossypiella* (Saunders) and *Spodoptera litura* (Fabricius) were monitored temporally and spatially for the life of the project with pheromone traps. Local *H. armigera* populations persist year round with population peaks in both the wet and dry seasons. *Helicoverpa punctigera* migrate into crops early dry season with the ability to persist season long. *Pectinophora gossypiella* populations develop during the wet season only so are avoided by cropping through cool and dry winter. *Spodoptera litura* are prevalent in the wet season and persist into cotton crops through the dry so, although less damaging to cotton, their pest status and possible control measures require examination. To date, *H. armigera* resistance to *Bt* proteins has not caused problems in Katherine, although resistance to conventional insecticides is of concern and has been detected, often to products not utilised during local cotton production. Continued monitoring of *H. armigera*, *H. punctigera* and *S. litura* populations would be required should cotton production trials proceed.

All cotton IPM trials conducted in the NT during this project were compromised by continual staff turnover, inadequate cotton production expertise and farming infrastructure, and a lack of priority at the management level. It is recommended future trials be scrutinised more closely by external stakeholders should cotton production proceed. This project has laid the foundation for environmentally sound pest management in future local transgenic cotton production systems despite difficult circumstances. Methods to further refine the local cotton IPM system are suggested.

1.0 General Introduction

The viability of environmentally sustainable commercial cotton production based on Bollgard II® cultivars was investigated from 2000 to 2005 at Katherine Research Station (KRS, S14°28' E132°18') in the Northern Territory, Australia. Bollgard II® produces specific *Bt* proteins that target the principal insect pest *Helicoverpa,* reducing reliance on traditional insecticidal control. Prolonged efficacy of transgenic cotton's inherent control potential is integral to future industry sustainability. Entomological research outlined herein focused on management of potential resistance to transgenic cultivars by the insect pest, *H. armigera*, and associated status elevation of pests previously suppressed under obsolete insecticidal control regimes.

Insect control systems research conducted during Katherine cotton trials relied heavily on IPM principles. IPM incorporates the control potential of natural enemies (Pyke & Brown 1996, Johnson *et al.* 2000) and compensatory abilities of cotton plants (Sadras & Fitt 1997, Wilson *et al.* 2003) to minimise the need for insecticidal control of pests. Companion crops are utilised in IPM systems to trap insect pests and provide natural enemy refuges. Insecticides specific to target pest groups are utilised only when absolutely necessary, and chemical constituents are rotated to minimise potential resistance development. Emerging pests must be examined from the species level to eliminate confounded species complex issues (Walter 2003) and ensure accurate assessment of attributable plant damage and effectiveness of possible control measures. The application of an IPM system is essentially built on research that separately investigates its constituent parts.

1.1 General methodology

Till 2003, all research was conducted at KRS (Figure 1.1, next page) managed by the Northern Territory Department of Primary Industry, Fisheries and Mines (2005 till present, Department of Primary Industries and Fisheries 2000 to 2001, Department of Business, Industry and Resource Development 2002 to 2005). Lateral irrigated trials were rotated between the A and B series of fields biannually (Figure 1.1 Steemsons's paddock & Figure 1.2, page 3) for the life of the project. Fields numbered 1 to 3 in each of the A and B series' measure 3.3 Ha in area and field 4 in each 1.8 Ha (Figure 1.2). Tape irrigated fields (T1 and T2, 2.2 & 2.5 Ha, respectively, Figure 1.2) were primarily utilised for agronomic research. The pivot irrigation area (27 Ha, Figure 1.1 Rideout Paddock & Figure 1.3, page 3) was constructed specifically for cotton trials at KRS in 2001. From 2003, a pivot irrigator (30 Ha) was leased from Peanut Company Australia (PCA, Figure 1.4, page 4) to increase the area for companion crop trials.

Cotton crops were planted each year at the end of the wet season. All fields were managed minimum till with sabi or summer grass wet season cover crops, except for PCA which utilised millet. Following cover crop blow-out, weed management relied on emergence herbicide pre-planting, and chipping or occasionally directed spraying during the season. Fertiliser was applied in furrow at planting, then via fertigation for the remainder of the season. Insecticides were applied by ground rig, except when rank cotton plant growth forced aerial application.

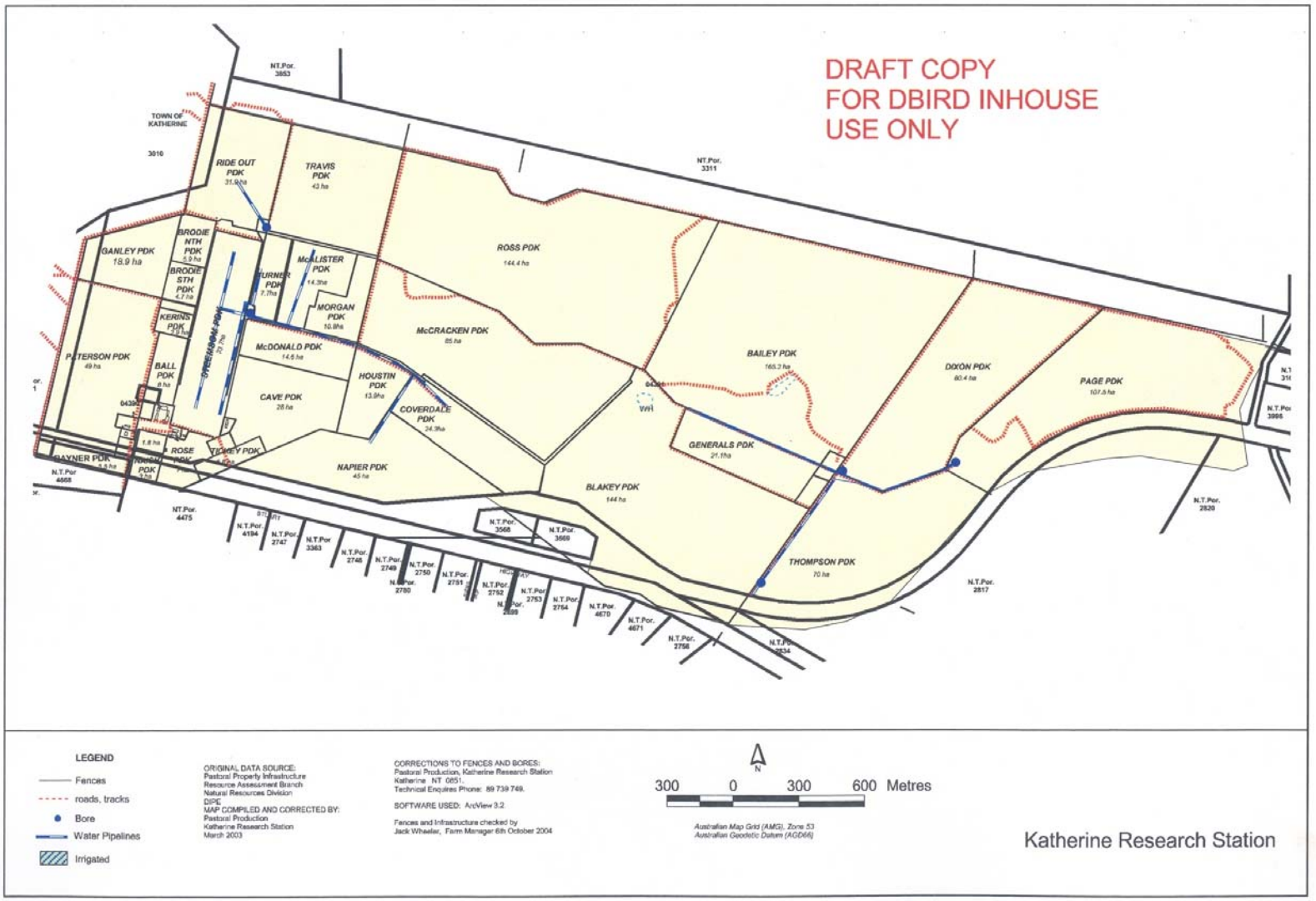

Figure 1.1. Map of Katherine Research Station. A and B series are located in Steemson's Paddock and the KRS pivot in Rideout Paddock.

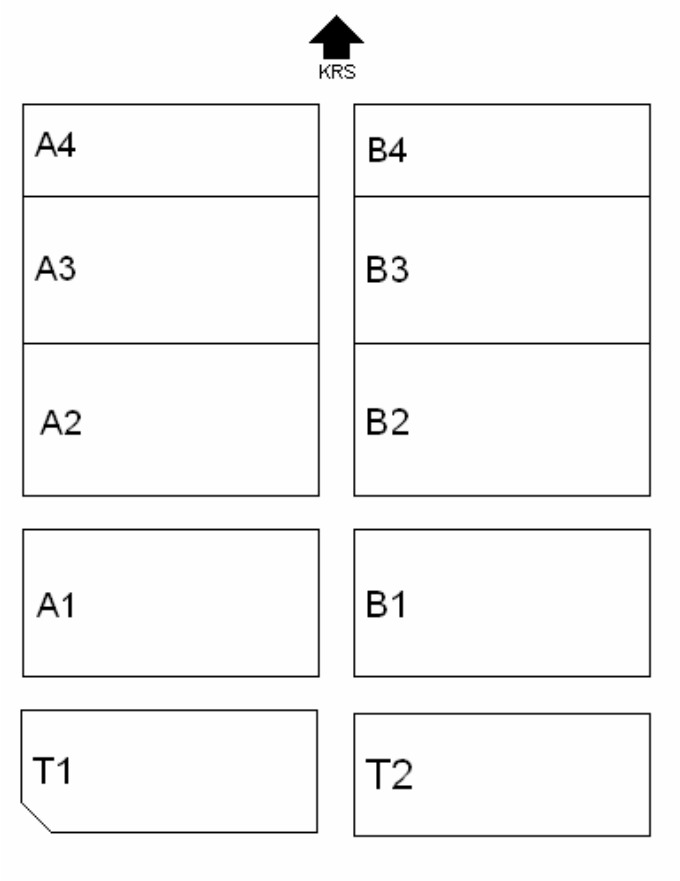

Figure 1.2. Layout of the A and B series lateral and Tape irrigation fields at Katherine Research Station. Figure is a representation only and is not to scale.

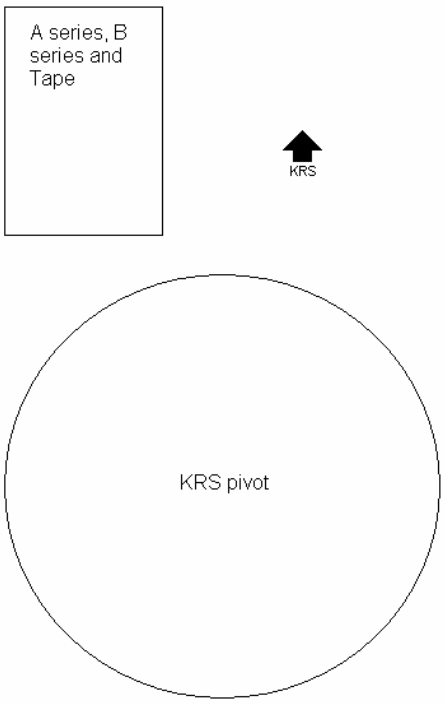

Figure 1.3 Layout of the KRS pivot in respect to the A and B series lateral and Tape irrigation fields at Katherine Research Station. Figure is a representation only and is not to scale.

Figure 1.4. Map of the Katherine region showing the relative locations of Katherine Research Station (KRS) and Peanut Company Australia (PCA). Figure is not to scale.

1.1.1 Companion crops

Companion crops, such as pigeon pea (*Cajanus cajan* (L.)), are used to bolster natural enemies for IPM systems in established Australian cotton growing regions. It was envisaged companion crops could fulfil a similarly effective role in Katherine cotton IPM. Various cultivars, including conventional cotton, were tested for their ability to harbour insect pests and natural enemies during the dry growing season and arbitrarily ranked according to relative densities of each. Lablab provided a suitable habitat for both cotton pest insects and natural enemies and so was selected for large scale companion crop evaluation. Lablab sustains relatively large numbers of *H. armigera* unexposed to *Bt* so is of benefit to resistance management within the local moth population, but does not influence pest insect and natural enemy densities nor cotton lint yield and fibre quality as a companion crop.

IPM systems may benefit from companion crops that provide suitable habitat for natural enemies but not insect pests. Through habitat manipulation of companion crops, such as mechanical disturbance and slashing, natural enemies could be encouraged to enter neighbouring cotton crops *ad hoc*, without directly increasing immigration of cotton insect pests. An IPM system incorporating such specialised companion crops would still require refuges for resistance management of susceptible insect pests. Sweet potato does not harbour major insect pests of cotton in Katherine, but is attractive to natural enemies and was trialled on a small scale as a companion. Although *Helicoverpa* and natural enemy, especially the model predator *Trichogramma*, densities were insufficient to draw robust conclusions regarding its influence on relative abundance of each, sweet potato at least reduced leafhopper densities in neighbouring cotton, so may be of more benefit than other companions examined.

1.1.2 Insecticides

Sucking insects presently require insecticidal control on Bollgard II® cotton in Katherine if densities breach designated thresholds. Fipronil, often in conjunction with salt, and imidacloprid were used to effectively suppress sucking insects and, although they hinder natural enemy efficacy in specific situations, do so to a lesser extent than more traditional, broad spectrum insecticides. *Spodoptera litura* develop unabated on *Bt* cotton so occasionally require insecticidal control, but only at unusually large densities (c.a. 5+ per metre). Indoxacarb effectively controls *S. litura* in Katherine cotton, however, its impact on certain natural enemies, such as coccinellids, can flare secondary pests, such as aphids, so its use must be carefully considered. Shield bugs, such as GVB and RBSB, and leafhoppers can become problematic late season in Katherine cotton and control measures targeting these pests require further examination. Earwigs required chemical control at planting in a few fields in one season only.

1.1.3 Natural enemies

Although companion crops rarely influenced relative insect densities in neighbouring cotton, natural enemies were effectively maintained in cotton fields through judicious use of target selective insecticides. Aphid and whitefly rarely required chemical control when natural enemies were not disrupted by insecticide application. This is encouraging when you consider *Bemisia tabaci* Biotype B, which is potentially resistant to conventional insecticides, has been isolated from Katherine cotton. Spiders were almost ubiquitous in Katherine cotton trials, however, their impact on insect pests is inferred to date and requires clarification. *Trichogramma,* although prevalent early season in Katherine cotton, are not as effective for *Helicoverpa* suppression as they are in the neighbouring Ord River Irrigation Area (ORIA). Besides these select beneficial species, little research into natural enemies has been undertaken in Katherine, other than cataloguing of species present.

1.1.4 Sucking insect pests

At present, thresholds of 0.5 sucking pests per metre are employed during Katherine cotton production. The seasonal pest status and damage potential of individual sucking pests require clarification at the species level before sucking pest thresholds can be further refined. Evidence suggests cotton plants can tolerate RBSB at relatively high densities without yield loss or fibre damage, and leafhoppers have the potential to seriously reduce photosynthetic ability through cotton leaf burn, late season in Katherine.

1.1.5 *Helicoverpa* and other lepidopteran pests

Helicoverpa armigera, H. punctigera and *S. litura* were the major noctuid pests of winter grown Katherine cotton. Resistance to both conventional and *Bt* insecticides was examined for *H. armigera* populations sampled in Katherine during this project.

Objectives

The primary aim of this project was to benchmark the ecology of key pest and beneficial insects that are likely to impact on a future cotton industry in the Katherine area, before assessing preliminary integrated pest management systems. More specifically, the objectives of the project were to:

Monitor the seasonal abundance of lepidopteran pests (*H. armigera*, *H. punctigera*, *S. litura* and *P. gossypiella*) weekly using pheromone traps at seven sights.

Conduct resistance testing and develop resistance management strategies for *H. armigera*.

Assess the role of companion crops in cotton IPM

Determine the refuge requirements for Bollgard II® cotton

Develop early and late season thresholds for the control of sucking insects attacking Bollgard II® cotton

Make a preliminary assessment of trap crops suitable for use in the Northern Territory

Rear and identify beneficial insect species (primarily parasitoids) and rank their status in the NT and link data to biodiversity studies.

These objectives evolved through the life of the project as follows:

2001

1. Monitor key lepidopteran pests (4) weekly using pheromone traps at 7 sites
2. Assess resistance levels in *H. armigera* to conventional insecticides monthly during the season
3. Determine the base-line susceptibilities of *H. armigera* & *H. punctigera* to *Bt*
4. Collaborate with CSIRO in a strontium mark-recapture study of regional *Helicoverpa* population dynamics
5. Monitor *Trichogramma* activity and identify local species
6. Rear & identify beneficial insect species and rank their status in the NT – link to biodiversity studies

2002

1. Monitor key lepidopteran pests (4) weekly using pheromone traps at 7 sites
2. Continue resistance testing and develop a resistance management strategy for *H. armigera* and *Aphis gossypii*
3. Monitor for *Bt* resistance
4. Assess the suitability of various companion crops for use in the NT.
5. Assess the impact of sucking insects on cotton grown in the NT
6. If not present, introduce *Trichogramma pretiosum* from Kununurra
7. Rear & identify beneficial insect species and rank their status in the NT

2003

1. Monitor key lepidopteran pests (4) weekly using pheromone traps at 7 sites
2. Continue resistance testing and develop a resistance management strategy for *H. armigera* and *Aphis gossypii*
3. Monitor for *Bt* resistance
4. Develop companion cropping protocols for sucking insects and *Helicoverpa* in the NT and monitor their impact in field scale trials
5. Assess the impact of sucking insects on cotton grown in the NT including the continued development of thresholds
6. Monitor the impact of *T. pretiosum.*
7. Rear & identify beneficial insect species and rank their status in the NT

2004

1. Monitor key lepidopteran pests (4) weekly using pheromone traps at 7 sites
2. Continue resistance testing and develop a resistance management strategy for *H. armigera* and *Aphis gossypii*
3. Monitor for *Bt* resistance
4. Develop companion cropping protocols for sucking insects and *Helicoverpa* in the NT and monitor their impact in field scale trials
5. Assess the impact of sucking insects on cotton grown in the NT including the continued development of thresholds
6. Monitor the impact of *T. pretiosum.*
7. Rear & identify beneficial insect species and rank their status in the NT

2005

1. Monitor key lepidopteran pests (4) weekly using pheromone traps at 7 sites
2. Re-initiate resistance testing and develop a resistance management strategy for *H. armigera*
3. Re-initiate monitoring for *Bt* resistance
4. Re-assess companion cropping protocols for sucking insects and *Helicoverpa* in the NT and monitor their impact in field scale trials
5. Assess the impact of sucking insects at the species level on cotton grown in the NT including the continued development of thresholds
6. Monitor and model the impact of *T. pretiosum* on *H. armigera.*

2.0 Companion Crops

Introduction

The future of a cotton industry in the Northern Territory based on transgenic cultivars depends on the successful application of IPM principles. Cotton IPM research in Katherine aimed to reduce reliance on insecticides through natural enemy impact on pests thereby minimising possible detrimental effects on the environment attributed to excessive chemical use, and promote sustainability through prolonged transgenic efficacy via dilution of potentially resistant genes in target pest species (see Fitt *et al.* 1994, Fitt 2000). The utilisation of companion crops forms an integral part of cotton IPM systems (Fitt 2000) and is investigated in this section.

Companion crops act as reservoirs for natural enemies in the form of unsprayed refuges (Baggen & Gurr 1998, Gurr *et al.* 1998a, 1998b, Baggen *et al.* 1999, Gurr & Wratten 1999, Mensah 1999, Anand *et al.* 2001, Schellhorn 2001, Mensah 2002a, Lawrence *et al.* 2003) strategically grown attractants for pests (trap or suicide crops) (Kennedy & Margolies 1985, Hokkanen 1991, Craig & Luttrell 1997, Grundy *et al.* 2004, Tillman & Mullinix 2004, Duraimurugan & Regapathy 2005) and a source of emergent pests unexposed to transgenic plant tissue (Fitt *et al.* 1994, Fitt 2000, Ravi *et al.* 2005, Sisterson *et al.* 2005). The latter provides dilution of resistant genes through subsequent mating should target pests survive and emerge from neighbouring transgenic crops. Grown as strips within or surrounding cotton fields, examination of companion crop efficacy requires continual monitoring for pest insect and natural enemy densities for comparison between companion crop types and to results derived from insect monitoring in associated cotton. Companion crops are usually managed by periodic slashing to avoid rank growth and ensure continual attractiveness to insects. Although visual and beat sheet sampling are considered more robust for estimation of insect densities in companion crops, vacuum sampling was utilised in Katherine cotton trials due to time and personnel restrictions.

Successful preliminary small scale companion crop trials testing cultivar efficacy suggested lablab as the primary option in Katherine, followed by pigeon pea and kenaf (*Hibiscus cannabinus* L.) (Appendix 1, page 121). Subsequent large scale trials examined lablab efficacy at the production level, however, analyses did not indicate an advantage with its use in terms of relative insect densities or reduced insecticide applications and were confounded by insufficient replication and treatments for comparison and variation in chemical use between fields. Alternate trap crops were utilised early and late season in different fields to the detriment of treatment conformity within experimental blocks. Applied experimental design required further refinement in an attempt to reduce analytical anomalies in conclusions derived from previous experiments.

In 2005, within field experimental replication was doubled and pigeon pea was utilised as a comparison to lablab and no companion. This provided a relatively robust analysis of companion crop efficacy. Insect control decisions incorporated monitoring estimates from all treatments and were applied across entire experimental fields when possible to maintain conformity for subsequent statistical analyses.

A further small scale comparison of sweet potato to lablab and no companion was conducted. This trial tested the hypothesis that attracting host species for the natural enemy egg parasitoid *Trichogramma* that did not attack cotton would improve *Trichogramma* biological control

efficacy while not directly encouraging increased pest densities in neighbouring cotton fields (examined further in **Natural Enemies**).

There is minimal scientific evidence in support of the use of lablab as a companion crop to transgenic cotton production in the Katherine region. Possible reasons for this, and expansion of conventional cotton refuges, are discussed.

Methodology

2000-2002

For preliminary studies, see Appendix 1.

2003-2004

Relatively large scale trials designed to compare yield potential of Sicot® 289B cotton grown with and without a lablab companion crop were conducted under pivot irrigation at KRS and PCA in 2003 and 2004. Experimental design is outlined in Figure 2.1, below. Companion strips comprised 5% of total treatment plot area (5% of 15 Ha). Three one metre visual samples to estimate insect densities (as per cottonLOGIC® (CSIRO plant industries, ACRI, Narrabri, NSW, Australia) sampling guidelines) were taken from each treatment plot (half pivot, Figure 2.1) twice per week during the 2003 and 2004 cotton growing season. Unfortunately, cotton lint samples to estimate yield difference between treatments were not taken in 2003 or 2004.

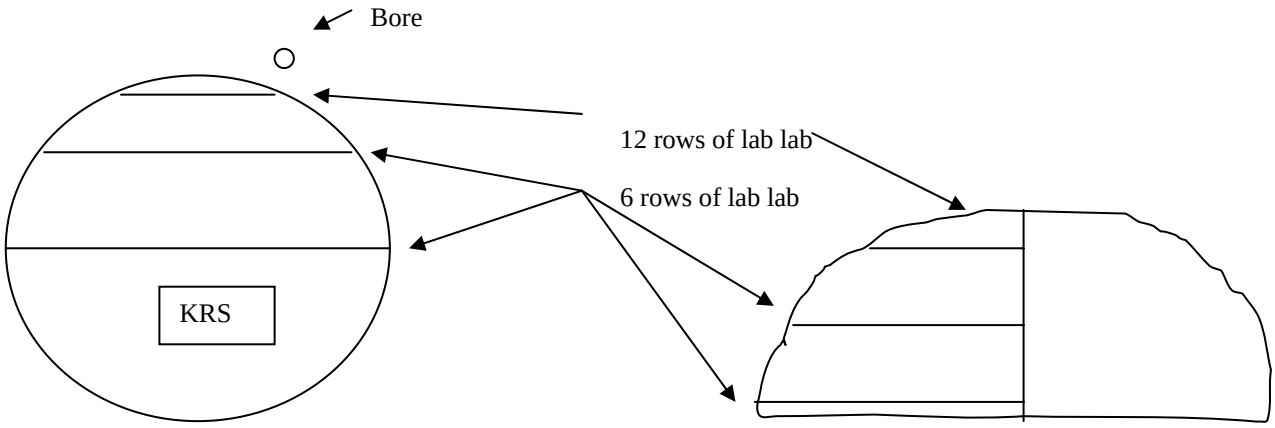

Figure 2.1. Experimental design for trials assessing the validity of lablab companion crops for transgenic cotton production in the Northern Territory during 2003 and 2004 (ex research pre-schedule for 2003, Dr Andrew Ward). PCA (half) pivot is on the right.

Insect relative abundance in all fields during the growing season was estimated using cottonLOGIC®. As the number of samples per unit area for each treatment on each sampling occasion was relatively low, data extracted for relative insect density analyses in 2006 were grouped into sucking pests, predators, *Helicoverpa* eggs and *Helicoverpa* larvae. A linear mixed effects model (REML) was utilised to discern companion crop treatment effects on relative insect group densities for each year.

2005

In 2005, experimental design was improved to minimise possible ambiguities in statistical analyses, incorporating four replications and three treatments (lablab, pigeon pea and no companion crop, Figure 2.2, next page). Companion crops comprised 5% of total plot area (5% of 5 Ha). The geographical isolation of replications one and two (PCA, Figure 2.2) from three and four (KRS, Figure 2.2) was not ideal, but logistically unavoidable. Cotton variety Sicot® 289B was utilised for conformity with 2003 and 2004 trials.

It was intended companion crops be slashed *ad hoc* and in half strips to avoid rank growth and ensure constant attractiveness to insects, respectively. Fallow strips were to be controlled by regular slashing. Companion strip management received minimum priority due to limited personnel and management issues. Companion crops were generally neglected well beyond slashing requirements, often encroaching on and invading neighbouring cotton trials. This provided a poor reflection of envisaged commercial production systems, yet companion crop management was equally poor across all treatments so statistical analyses were not strictly compromised. Estimated relative pest insect and natural enemy densities utilising data generated from insect monitoring were Log_{10} (counts) or arcsine square root (proportions) transformed prior to comparison between companion crop treatments with repeated measure ANOVA.

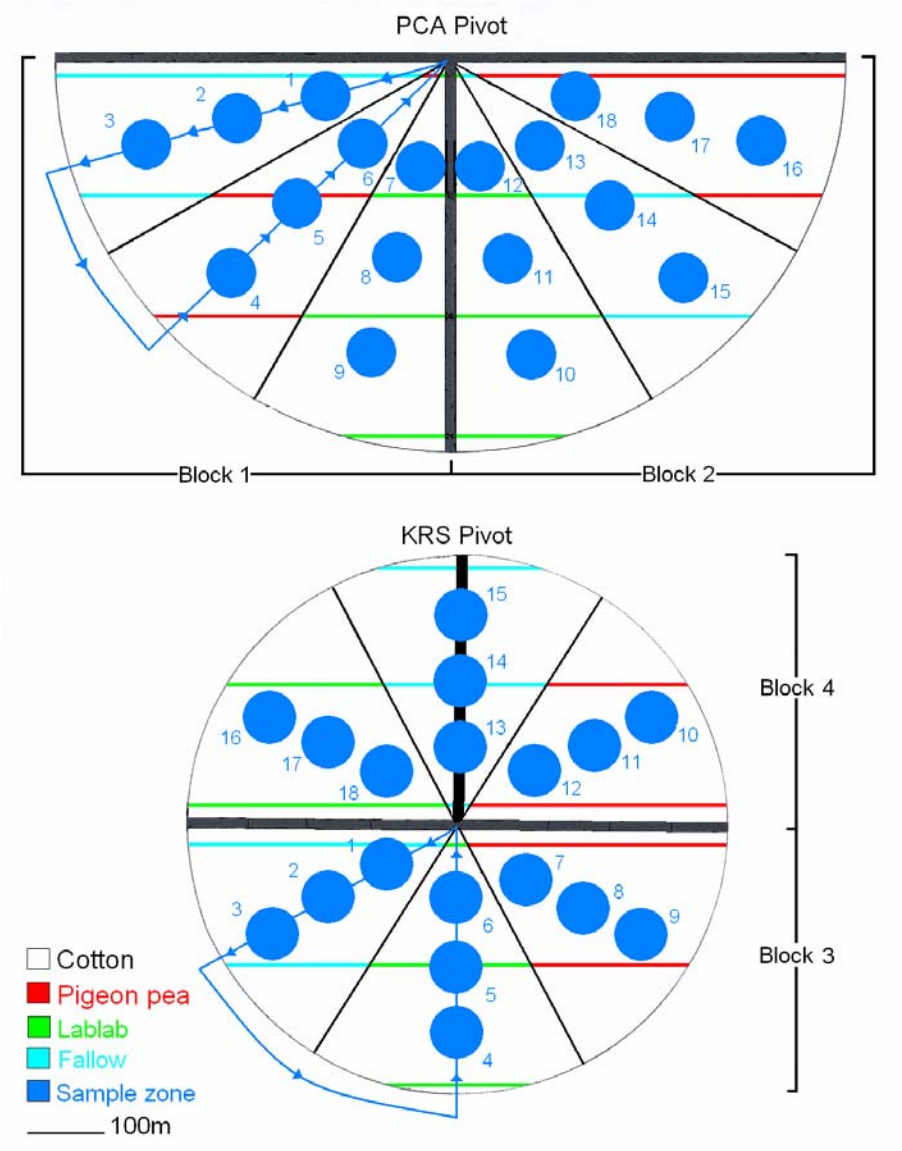

Figure 2.2. Insect monitoring system for Katherine Research Station (KRS) and Peanut Company of Australia (PCA) pivot irrigation cotton companion crop trials during the 2005 dry season. Treatments (see key) and block and sample site designations are given.

To examine sweet potato as a possible novel companion crop for cotton production in the Northern Territory, Field B1 (Figure 1.2) of Sicot® 289B was divided into four blocks of three replicates (sweet potato, lablab and no companion, Figure 2.3, next page). Beyond usual insect monitoring requirements, B1 was subject to intensive insect monitoring (four half metre samples per each experimental plot) twice per week to compare relative insect densities between companion crop treatments. Estimated relative pest insect and natural enemy densities were Log_{10} (counts) or arcsine square root (proportions) transformed prior to comparison between companion crop treatments with repeated measure ANOVA.

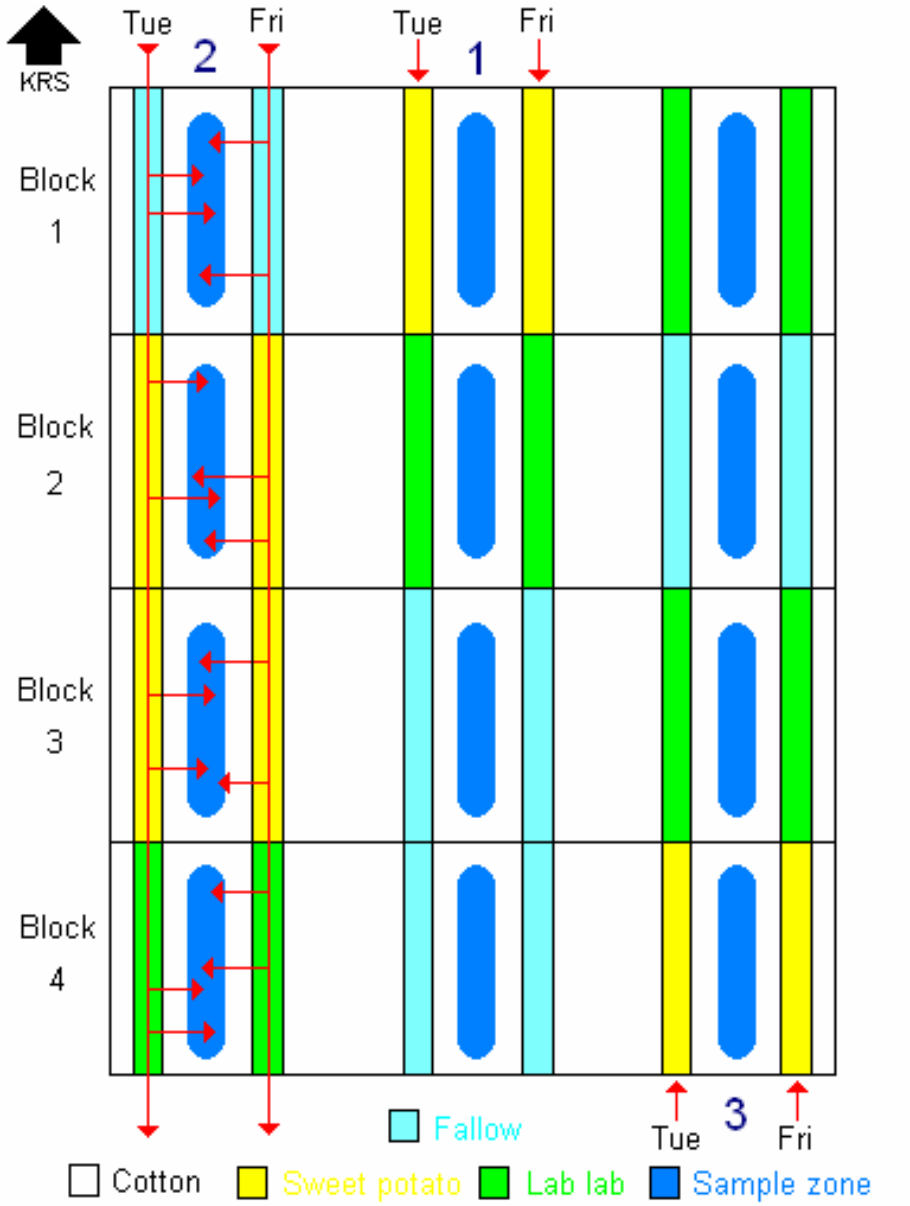

Figure 2.3. Insect monitoring system for field B1 Katherine Research Station lateral irrigation cotton companion crop trials during the 2005 dry season. Treatments (see key) and block and sample site designations are given. Numbers indicate respective monitors.

Companion crop strips in each treatment plot in both trials were vacuum sampled twice per week during the growing season to estimate pest insect and natural enemy relative densities. A randomly selected ten (pivots) or five (B1) metre section from one companion strip in each plot was vacuum sampled on every monitoring occasion (Figure 2.4, next page). The vacuum was run at full throttle while sampling in a sweeping motion across all plant surfaces within reach of the monitor traversing each sampled section in a straight line. The vacuum remained in idle while a paper tag designating the sample location was inserted and the sock safely removed and sealed with a rubber band to ensure insects did not escape during the process. On return to the laboratory, all socks were placed in large plastic containers with ethyl acetate soaked paper for 30 minutes to kill captured arthropods. For each sample, dead arthropods were gently brushed from collateral plant material into a collection petri dish. The contents lining each sock were then brushed into the same petri dish, sealed with masking tape, labelled with sample location and date, and frozen for post-season examination and analysis.

Extremely fine mesh socks capable of trapping minute *Trichogramma* were utilised. Due to a lack of technical support and time constraints, analysis of this experiment was not completed.

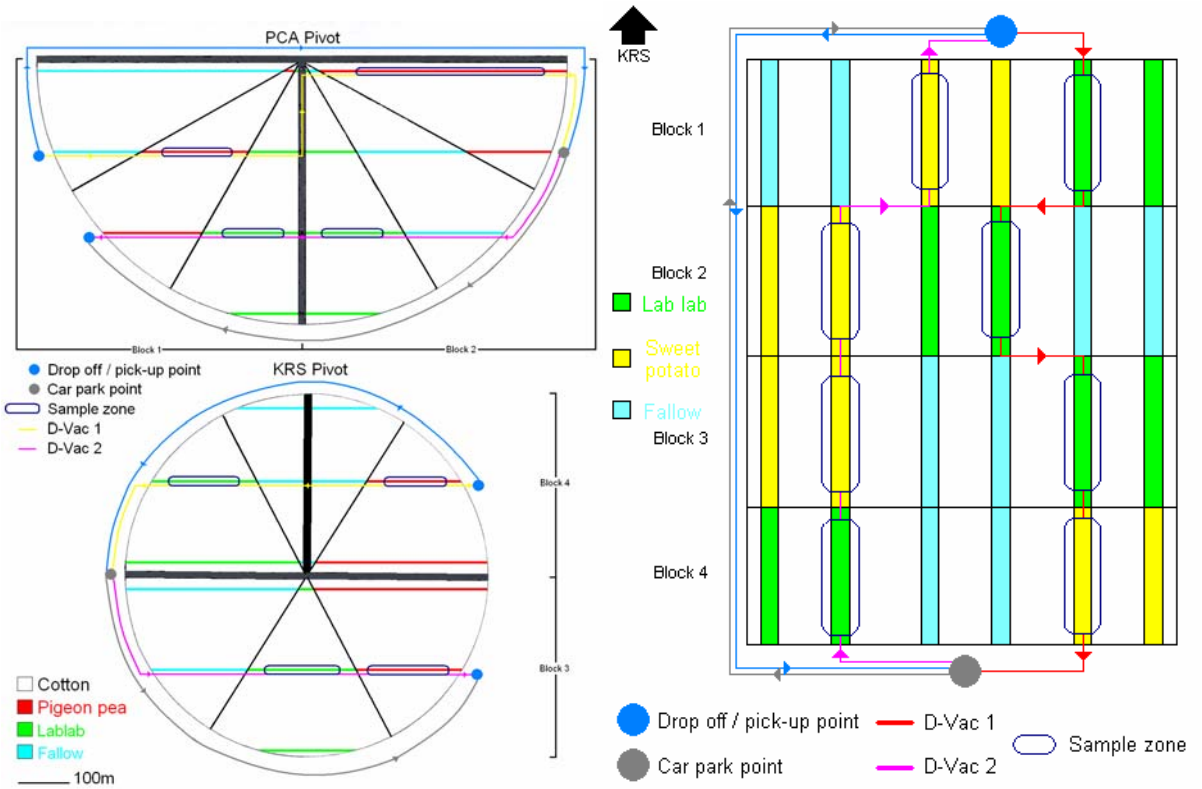

Figure 2.4. Vacuum sampling system for Katherine Research Station (KRS) and Peanut Company of Australia (PCA) pivot and KRS B1 lateral irrigation cotton companion crop trials during the 2005 dry season.

Comparative cotton yield and fibre quality between companion crop treatments was assessed in 2005. Both mechanical and hand picked yield estimates were generated in the pivot trials using two samples from each collection site (Figure 2.5, next page). Two randomly selected 15 metre sections of one cotton row (neither guess nor compaction row = sample row) at each sampling location were machine harvested. Sample sections were often difficult to designate due to significant gaps between cotton plants and excessive within and between row weediness. Two randomly selected five metre sections of another sample row within each sampling location were hand harvested. Four 5 metre hand picked samples (from sample rows) only were taken per plot in B1. Lint from each machine and hand harvested sample was weighed to estimate yield. Fibre quality was assessed for cotton grown in each treatment plot by ACRI using random samples from hand harvested lint only. Yield estimates and fibre quality variables were compared between treatments in both experiments by ANOVA with Tukey test.

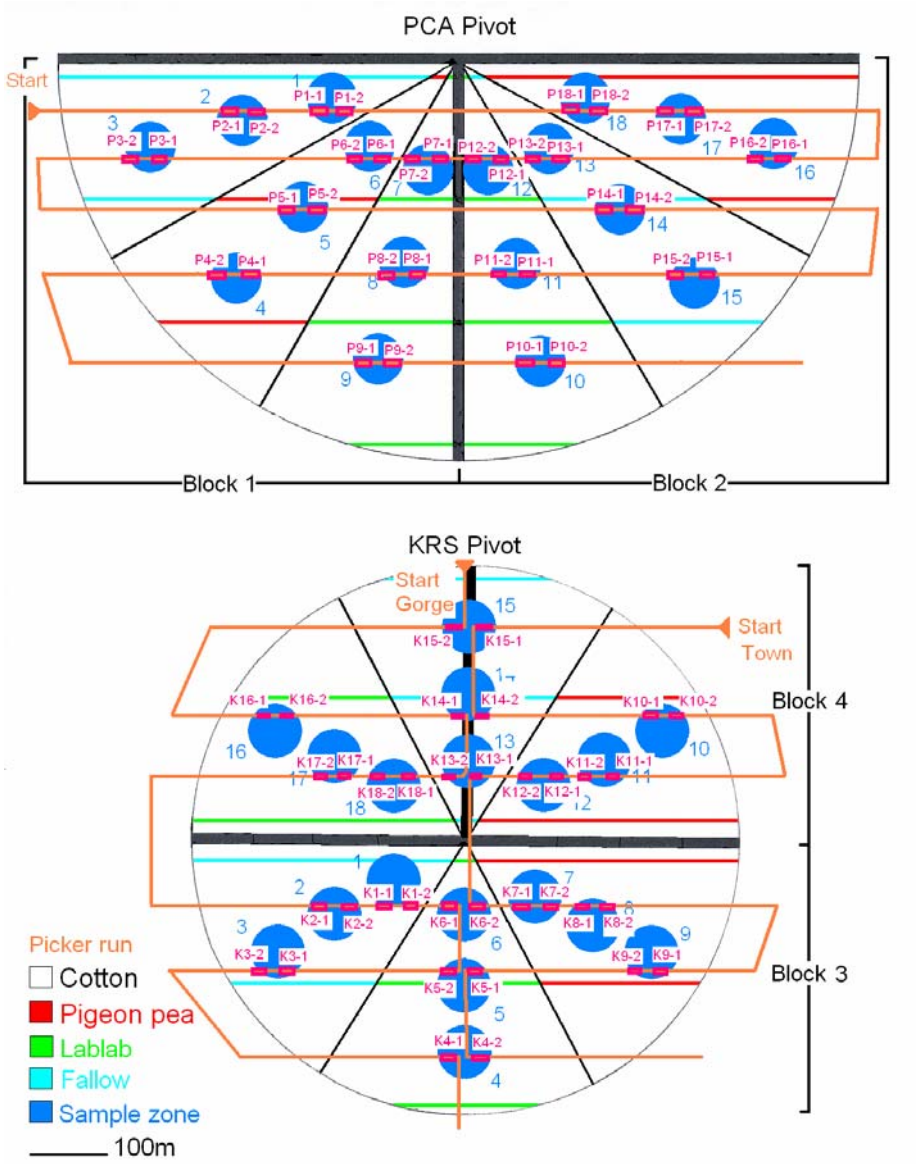

Figure 2.5. Cotton harvesting system for Katherine Research Station (KRS) and Peanut Company of Australia (PCA) pivot irrigation cotton companion crop trials during the 2005 dry season. Hand picked samples were collected in a similar fashion from different rows within each collection site. Treatments (see key) and block and collection site designations are given.

Results

2.1 Insect dynamics

2001-02

Preliminary studies conducted in 2001 and 2002 recommended large scale examination of lablab as a possible companion crop for transgenic cotton production in Katherine (see Appendix 1, 3.4, page 129)

2003-04

There was no significant difference between the densities of sucking pests, predators, *Helicoverpa* eggs or *Helicoverpa* larvae in cotton grown with or without lablab in 2003 and 2004 (Appendices 2a, 2b, page 142). It is unclear if this result is a reflection of comparative insect densities between treatments or due to insufficient sampling, replication or comparative treatments in the experimental design.

2005

Large scale trial

Results from repeated measure analyses of relative insect densities between the pivot irrigation companion crop treatments grown in 2005 are presented in Table 2.1 (next page). Fifty-seven percent of insect groups demonstrated a significant difference in relative abundance over time between pivots, suggesting geographic separation may have influenced localised insect densities (Table 2.1). There was no significant difference over time in the densities of medium *Helicoverpa* larvae, hoverfly larvae, lacewing eggs, mirid adults or nymphs, parasitised *Helicoverpa* eggs or spiders between cotton pivots (Table 2.1).

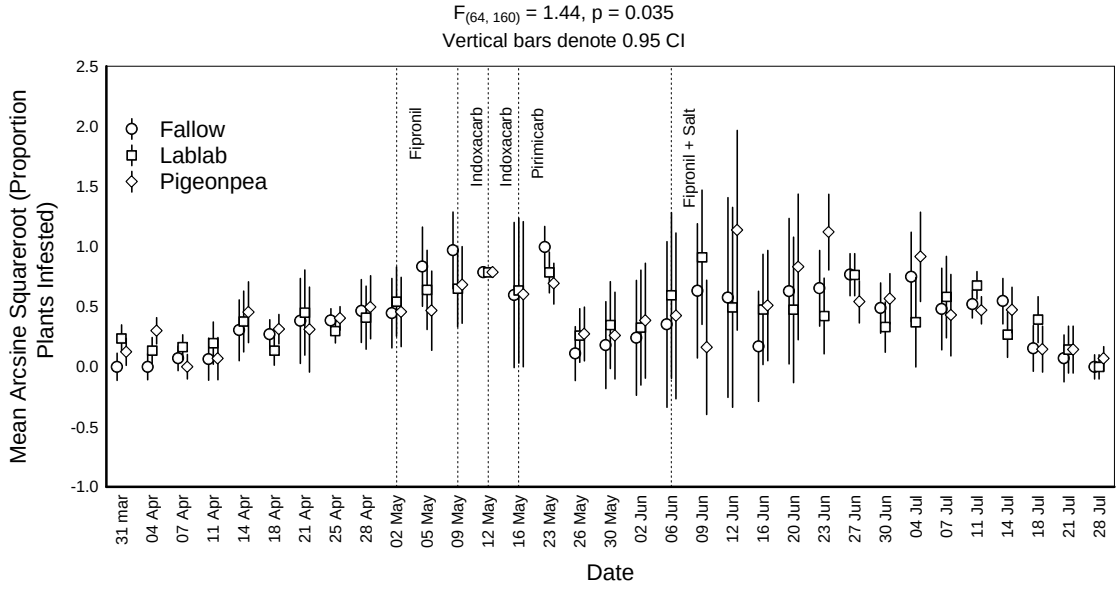

Figure 2.6. The relative density of aphids in cotton over time compared between companion crop treatments (see legend) grown under pivot irrigation at KRS and PCA in 2005. The nearest sample date preceding and active constituents of insecticide applications are given.

Aphids

Aphid densities demonstrated a significant difference between treatments over time (Table 2.1 & Figure 2.6, above). Higher aphid densities were present in cotton grown with fallow and pigeon pea companion strips, early and late season, respectively (Figure 2.6). Serious aphid infestation was limited to the KRS pivot (Table 2.1 & Figure 2.7, page 18).

Table 2.1. F values, degrees of freedom (df) and probabilities (P, significant values are shaded) generated from repeated measure analyses of relative insect numbers between companion crop treatments grown under pivot irrigation at KRS and PCA in 2005. Results are separated into analyses that utilised treatment only, pivot only and both treatment and pivot as factors. The relative status of insect groups are given.

		Treatment			Pivot			Treatment X Pivot		
Group	Status	*F* value	df	*P*	*F* value	df	*P*	*F* value	df	*P*
Aphids	Pest	1.44	64, 160	0.035	10.40	32, 160	<0.00001	1.00	64, 160	0.49
Green vegetable bugs	Pest	1.20	32, 80	0.26	2.10	16, 80	0.02	1.01	32, 80	0.46
Helicoverpa eggs	Pest	0.94	64, 160	0.61	2.02	32, 160	0.0025	0.72	64, 160	0.93
Very small larvae	Pest	0.96	40, 100	0.55	3.59	20, 100	0.00001	1.56	40, 100	0.04
Small larvae	Pest	1.40	24, 60	0.15	2.25	12, 60	0.02	1.31	24, 60	0.20
Medium larvae	Pest	3.26	6, 15	0.03	1.05	3, 15	0.40	1.05	6, 15	0.43
Total larvae	Pest	1.36	52, 130	0.08	3.94	26, 130	<0.00001	1.72	52, 130	0.0075
Hoverfly larvae	Pest	1.42	14, 35	0.19	0.71	7, 35	0.66	1.35	14, 35	0.23
Lacewing eggs	Predator	0.92	24, 60	0.57	0.92	12, 60	0.54	0.71	24, 60	0.82
Ladybeetle adults	Predator	1.08	62, 155	0.35	3.15	31, 155	<0.00001	1.30	62, 155	0.10
Ladybeetle larvae	Predator	0.67	40, 100	0.93	2.66	20, 100	0.00073	0.87	40, 100	0.69
Leafhoppers	Pest	1.35	56, 140	0.08	1.77	28, 140	0.017	1.20	56, 140	0.19
Mirid adults	Pest	0.89	38, 95	0.64	1.12	19, 95	0.35	0.95	38, 95	0.57
Mirid nymphs	Pest	0.87	50, 125	0.71	1.02	25, 125	0.45	1.09	50, 125	0.34
Mirid total	Pest	0.92	62, 155	0.64	1.57	31, 155	0.04	0.82	62, 155	0.81
Parasitised eggs	Predator	1.60	16, 40	0.11	1.35	8, 40	0.25	2.52	16, 40	0.0091
Predatory bugs	Predator	0.82	62, 155	0.81	1.62	31, 155	0.03	1.03	62, 155	0.44
Redbanded shield bugs	Pest	1.21	52, 130	0.20	1.62	26, 130	0.04	1.29	52, 130	0.13
Spiders	Predator	1.18	66, 165	0.20	0.73	33, 165	0.86	0.93	66, 165	0.63
Spodoptera larvae	Pest	1.52	48, 120	0.036	3.05	24, 120	0.00003	1.57	48, 120	0.003
Thrips	Pest	1.24	64, 160	0.14	0.81	32, 160	0.75	1.06	64, 160	0.38
Whiteflies	Pest	1.01	66, 165	0.47	3.38	33, 165	<0.00001	0.87	66, 165	0.73

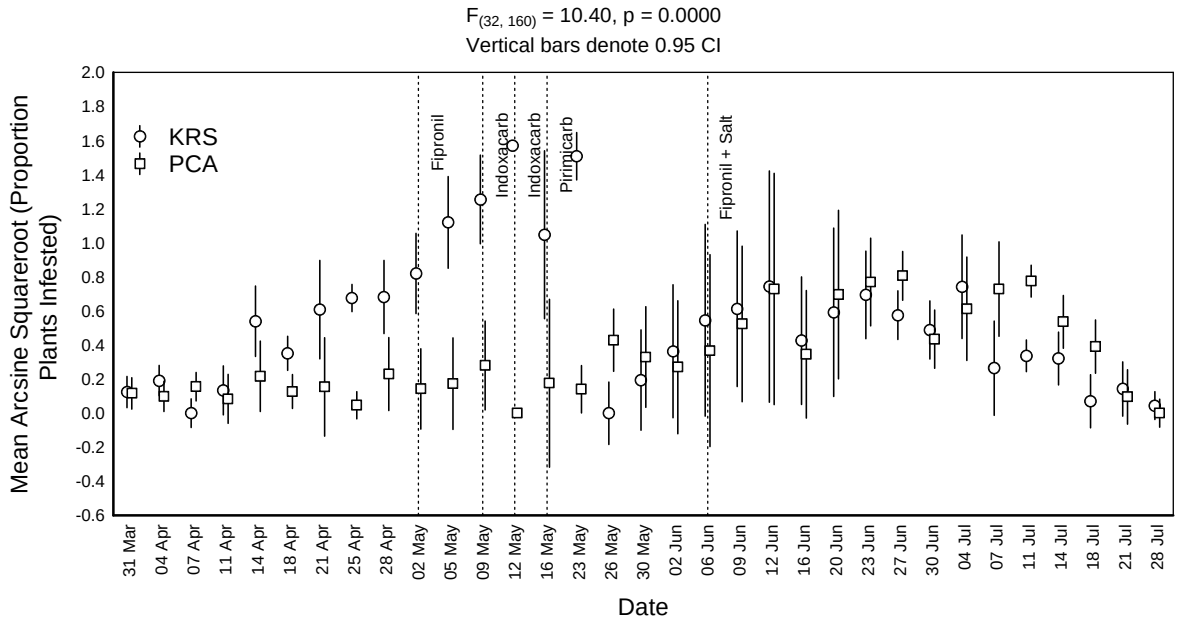

Figure 2.7. The relative density of aphids in cotton over time compared between pivot irrigation fields grown at KRS and PCA in 2005. The nearest sample date preceding and active constituents of insecticide applications are given.

Helicoverpa larvae

Significantly higher densities of very small *Helicoverpa* larvae infested the pivot at KRS (Table 2.1) early season (Figure 2.8, below) compared to the PCA pivot. PCA had a significantly higher density of very small *Helicoverpa* larvae than KRS on 12 May and 12 June only (Figure 2.8). The interaction between treatment and pivot was significant over time for very small *Helicoverpa* larvae (Table 2.1), however, trends are not obvious (Figure 2.9, next page), suggesting the difference between pivots is a strong influence (Figure 2.10, next page). The total densities of *Helicoverpa* larvae demonstrated similar though more significant results to very small *Helicoverpa* larvae (Table 2.1, Appendix 3, page 145).

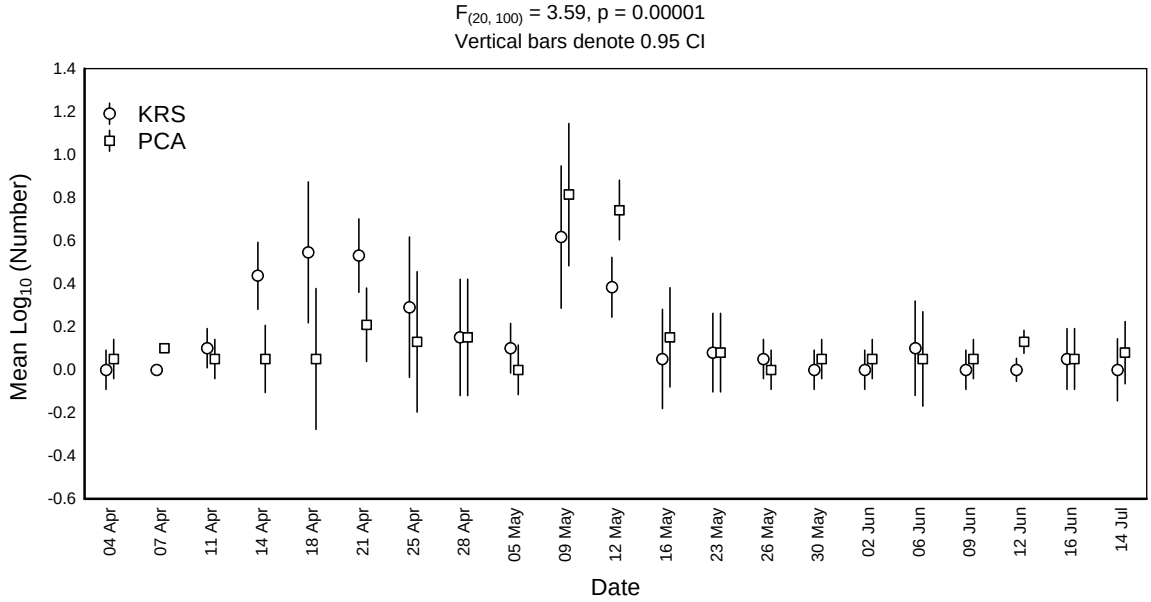

Figure 2.8. The relative density of very small *Helicoverpa* larvae in cotton over time compared between pivot irrigation fields grown at KRS and PCA in 2005.

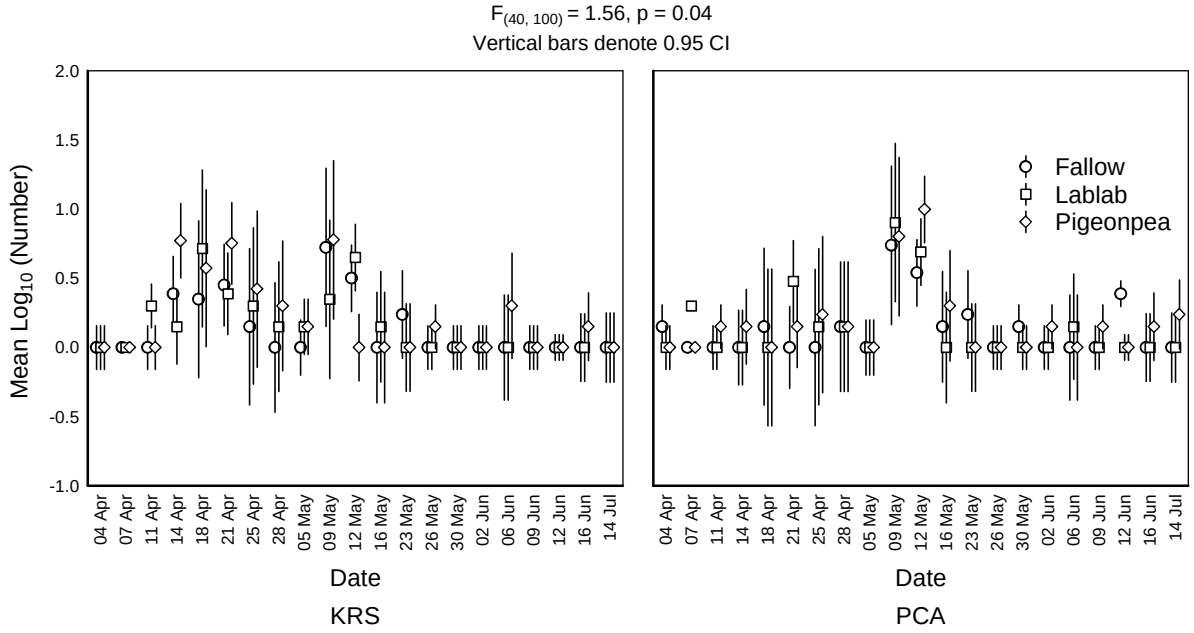

Figure 2.9. The relative density of very small *Helicoverpa* larvae in cotton over time compared between pivot irrigation fields and treatments (see legend) grown at KRS and PCA in 2005. Graphs are separated into pivots.

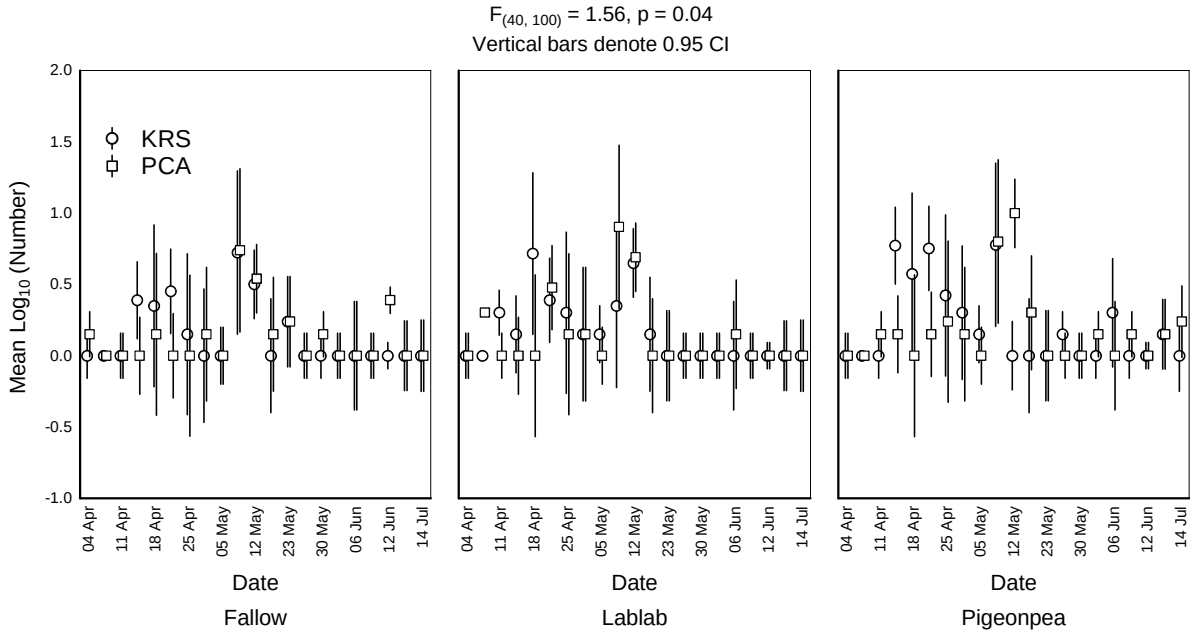

Figure 2.10. The relative density of very small *Helicoverpa* larvae in cotton over time compared between pivot irrigation fields and treatments grown at KRS and PCA in 2005. Graphs are separated into treatments.

Parasitised *Helicoverpa* eggs

Helicoverpa egg parasitism was relatively rare in pivot irrigated companion crop trials and confined to the first half of the season (pre-July). There was a significant interaction over time between treatment and pivot for parasitised *Helicoverpa* eggs (Table 2.1). Significantly higher densities of parasitised *Helicoverpa* eggs were recorded at PCA than KRS in cotton grown with lablab and pigeon pea companion strips on 9 May and 23 May, respectively (Figures 2.11 & 2.12, next page). At KRS, cotton grown with no companion strip (fallow) recorded a higher density of parasitised *Helicoverpa* eggs than the other two treatments on 23 May only (Figure 2.12). *Trichogramma* egg parasites are examined further in **Natural Enemies**.

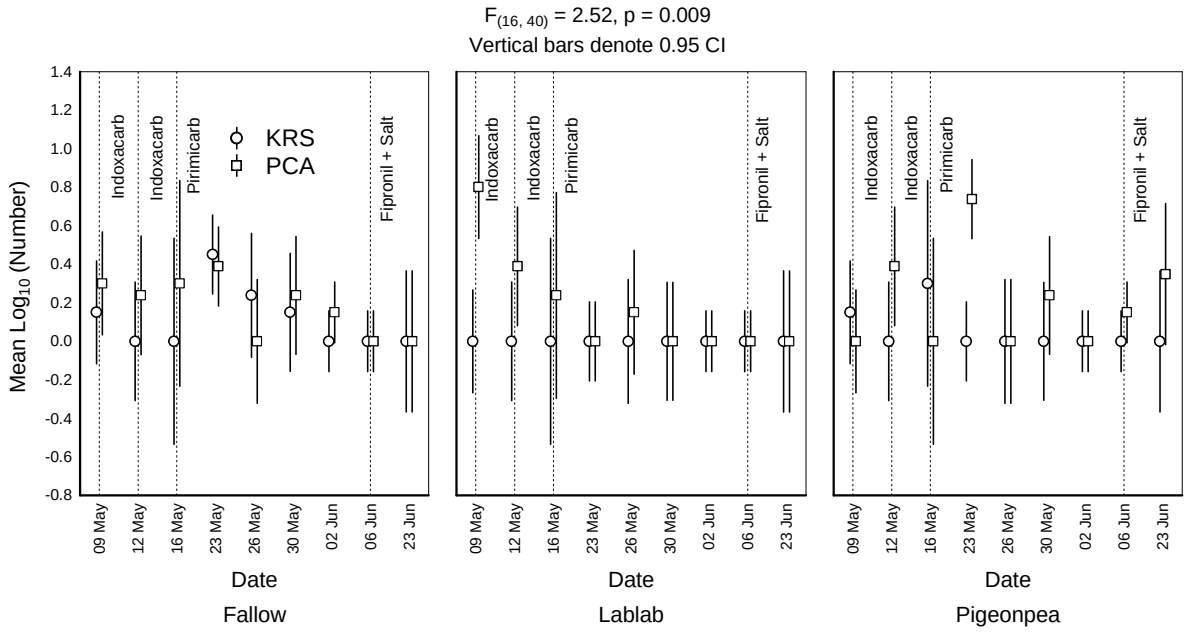

Figure 2.11. The relative density of parasitised *Helicoverpa* eggs in cotton over time compared between pivot irrigation fields and treatments grown at KRS and PCA in 2005. Graphs are separated into treatments. The nearest sample date preceding and active constituents of insecticide applications are given.

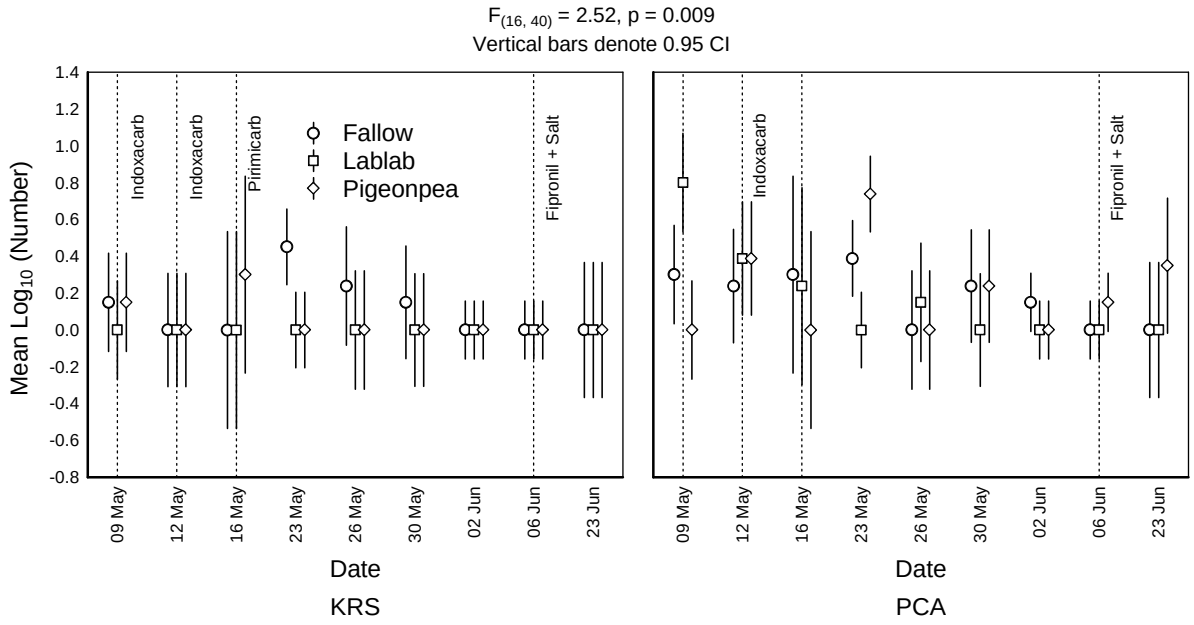

Figure 2.12. The relative density of parasitised *Helicoverpa* eggs in cotton over time compared between pivot irrigation fields and treatments (see legend) grown at KRS and PCA in 2005. Graphs are separated into pivots. The nearest sample date preceding and active constituents of insecticide applications are given.

Spodoptera litura larvae

The significant difference in *S. litura* larvae between treatments over time (Table 2.1) is a byproduct of significantly different densities recorded on isolated occasions, rather than specific trends (Figures 2.13 & 2.14, both next page). The KRS pivot recorded significantly greater *S. litura* larval densities than PCA during 2005 (Table 2.1 & Figure 2.15, page 22).

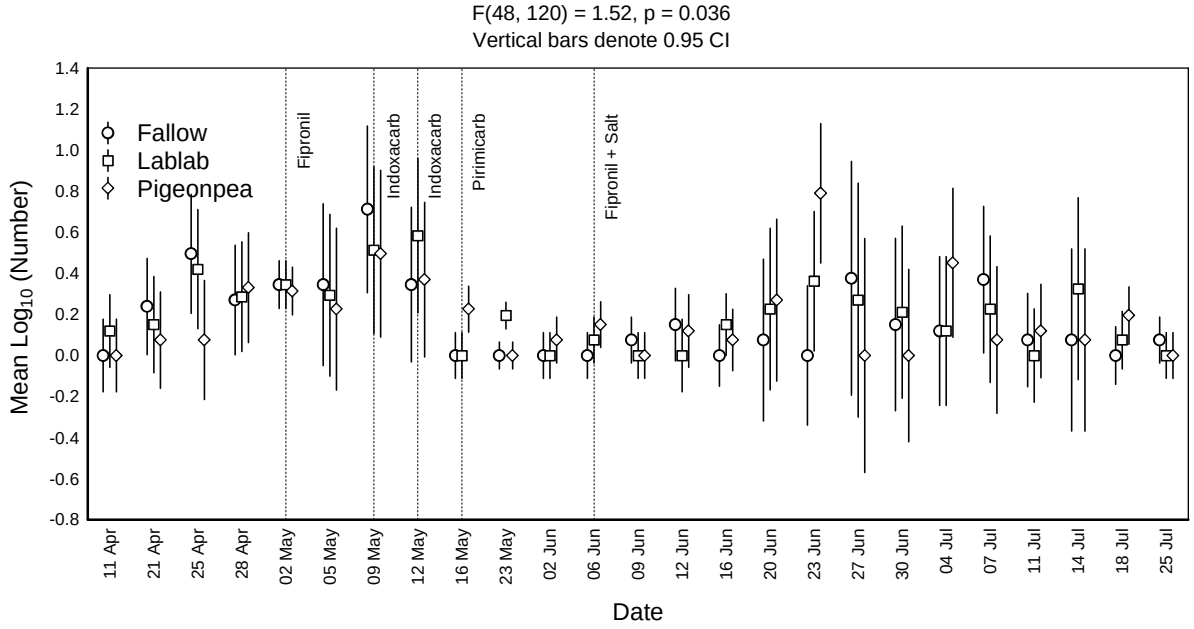

Figure 2.13. The relative density of *Spodoptera litura* larvae in cotton over time compared between companion crop treatments (see legend) grown at KRS and PCA in 2005. The nearest sample date preceding and active constituents of insecticide applications are given.

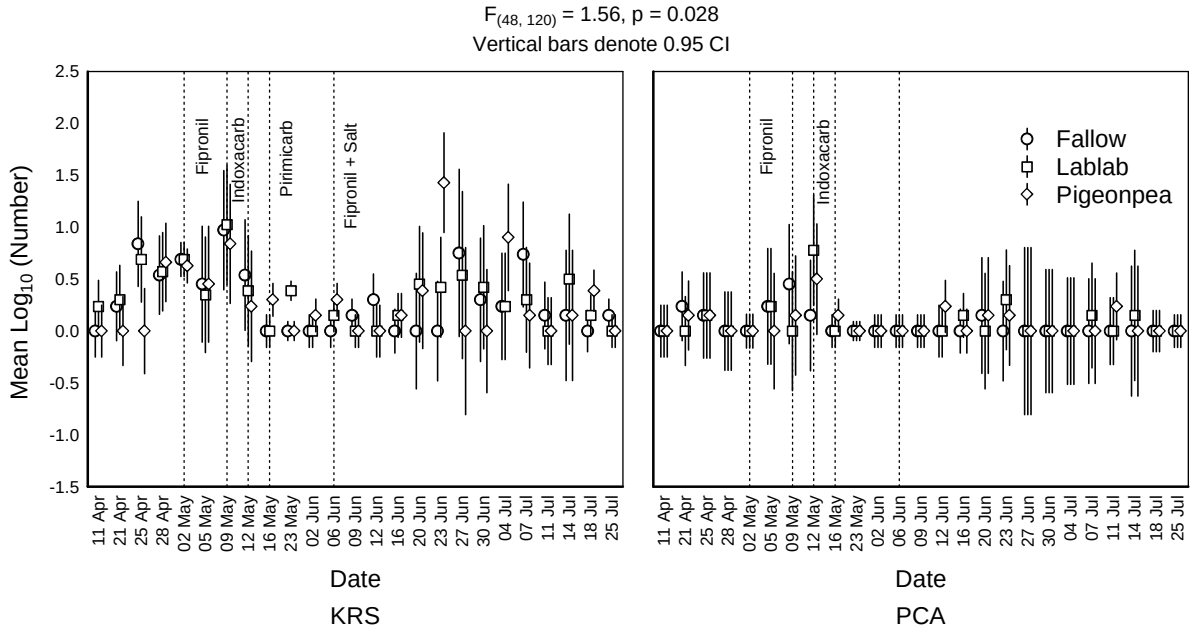

Figure 2.14. The relative density of *Spodoptera litura* larvae in cotton over time compared between pivot irrigation fields and treatments (see legend) grown at KRS and PCA in 2005. Graphs are separated into pivots. The nearest sample date preceding and active constituents of insecticide applications are given.

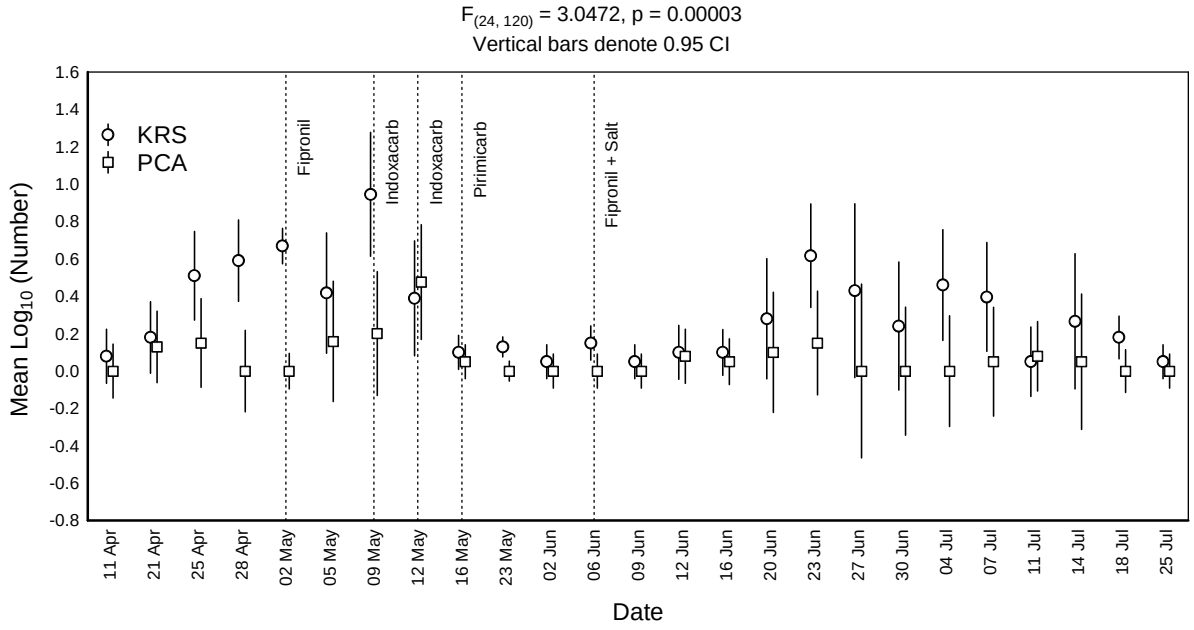

Figure 2.15. The relative density of Spodoptera litura larvae in cotton over time compared between pivot irrigation fields grown at KRS and PCA in 2005. The nearest sample date preceding and active constituents of insecticide applications are given.

There was a significant interaction between treatment and pivot for *Spodoptera litura* larvae (Table 2.1 & Figure 2.14), however, the difference between pivots (Table 2.1 & Figure 2.15) had a stronger influence than that between treatments (Figures 2.14 & 2.16, below).

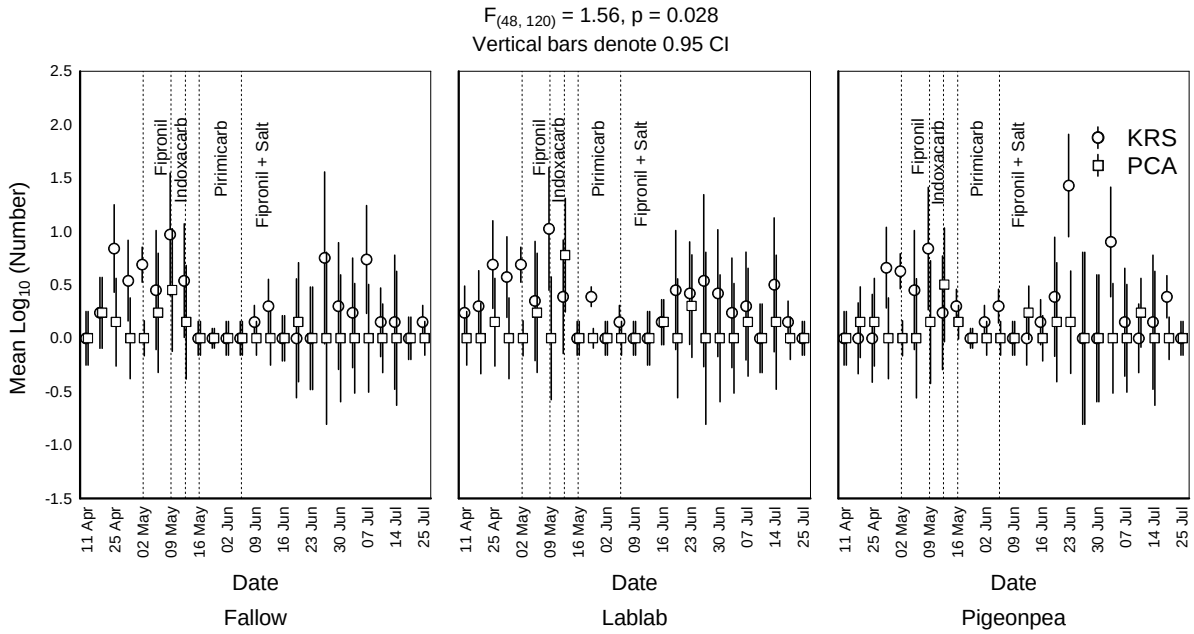

Figure 2.16. The relative density of Spodoptera litura larvae in cotton over time compared between pivot irrigation fields and treatments grown at KRS and PCA in 2005. Graphs are separated into treatments. The nearest sample date preceding and active constituents of insecticide applications are given.

Small scale trial

There was no significant difference over time in relative densities of insect groups in cotton companion crop treatments in field B1 small scale trials during 2005 (Appendix 4, page 146).

2.2 Yields

Pivots

There was no significant difference in hand picked yield estimates between treatments in pivot irrigated companion crop trials in 2005 ($F_{2, 46}$ = 1.55, P = 0.22). Cotton grown with pigeon pea companion yielded significantly less (6.00 ± 0.29 (mean ± se) bales/Ha) than cotton grown with both lablab (6.89 ± 0.28 bales/Ha) and no companion (fallow) (7.0 ± 0.16 bales/Ha) treatments when harvested mechanically from the same sites and fields ($F_{2, 46}$ = 7.16, P = 0.0019, Figure 2.17, below).

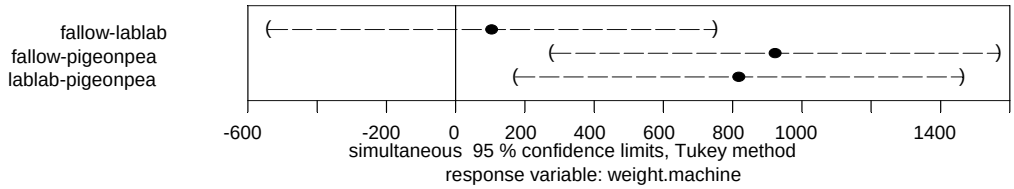

Figure 2.17. Tukey comparison of simultaneous 95% confidence limits for treatment effects on yield estimates machine harvested from pivot irrigated companion crop trials in Katherine in 2005.

B1

There was no significant difference in hand picked yield estimates between treatments in B1 in 2005 ($F_{2, 30}$ = 1.50, P = 0.24).

2.3 Fibre quality

Pivots

Fibre uniformity of cotton hand picked from pigeon pea (76.6 ± 0.25) was significantly different to that from lablab (77.4 ± 0.22) treatments (Table 2.2, below & Figure 2.18, next page).

Table 2.2. *F* values and probabilities (*P*) generated following analyses of fibre quality data in all companion crop trials in Katherine during 2005. sfi = short fibre index. Significant probabilities are highlighted.

Field	Variable	*F* value	*P*
B1	Length	0.43	0.65
	Uniformity	0.11	0.9
	sfi	0.4	0.68
	Strength	0.18	0.83
	Elongation	0.43	0.65
	Micronaire	0.44	0.65
Pivots	Length	2.61	0.08
	Uniformity	4.48	0.015
	sfi	5.73	0.0052
	Strength	1.43	0.25
	Elongation	0.6	0.55
	Micronaire	1.2	0.31

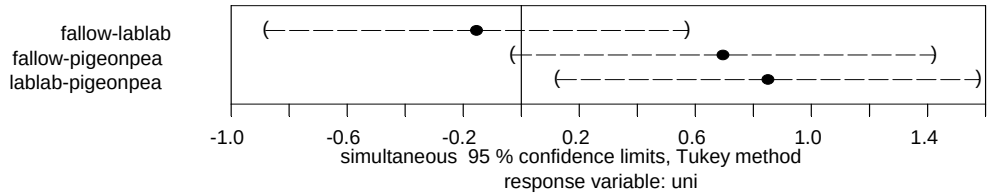

Figure 2.18. Tukey comparison of simultaneous 95% confidence limits for treatment effects on fibre uniformity of cotton hand harvested from pivot irrigated companion crop trials in Katherine in 2005.

Short fibre index of cotton hand picked from pigeon pea (14.7 ± 0.33) was significantly greater than that from both lablab (13.7 ± 0.23) and no companion (13.6 ± 0.24) treatments (Table 2.2 & Figure 2.19, below).

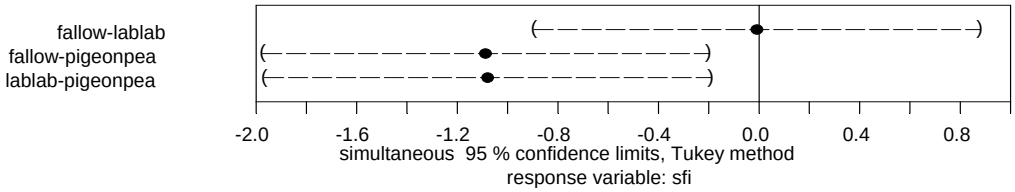

Figure 2.19. Tukey comparison of simultaneous 95% confidence limits for treatment effects on short fibre index of cotton hand harvested from pivot irrigated companion crop trials in Katherine in 2005.

B1

There was no significant difference in fibre quality variables between treatments in B1 companion crop trials in 2005 (Table 2.2).

Discussion

When considering relative densities of pest insects and natural enemies and cotton yield potential over the three years of large scale companion crop trials, there is limited evidence to suggest lablab companion crops provide sufficient benefit to warrant application in future transgenic cotton production in the Northern Territory. Where relative insect densities were significantly different between companion crop treatments over time (e.g. Aphids and *S. litura* larvae) it is difficult to discern trends, significant results most likely the product of differing treatment effects on specific sampling occasions (e.g. 23 May and 23 June for *S. litura* larvae, Figure 2.13) or other influences. In the vast majority of cases, insect densities were not significantly different between companion crop treatments over time for all three years.

Relatively few *Helicoverpa* larvae developed beyond very small category, presumably due to the control efficacy of Bollgard II®. The analysis of all *Helicoverpa* larval categories combined (Appendix 3) therefore closely reflected that of very small larvae only (Figures 2.8, 2.9 & 2.10) as the latter category constituted the vast majority of data utilised. For this reason, *Helicoverpa* larvae medium size and larger recorded in Bollgard II® cotton are often anecdotally blamed on influx from neighbouring companion crops. Medium *Helicoverpa* larvae were encountered on only four sampling occasions (14 & 18 April and 23 & 30 June) and the count was only greater than one (= 4) on 18 April in cotton grown with pigeon pea.

The significant difference in medium *Helicoverpa* larvae between companion crop treatments during 2005 (Table 2.1) is a reflection of a relatively poor data set so not considered of further interest. It is interesting to note, no *Helicoverpa* larvae beyond medium size were collected in 2005 cotton crops, suggesting evidence of individuals resistant to *Bt* proteins expressed in Bollgard II® being present in Katherine to date is minimal.

That the majority of insect groups sampled demonstrated a significant difference in density over time between pivots is not surprising given their geographical separation (c.a. 10 km, Figure 1.4). The KRS and PCA pivots are surrounded by different habitat mosaics likely to support different insect assemblages (see ***Helicoverpa* and other lepidioteran pests**). For example, the KRS pivot is located within a mixed farming system incorporating sesame, forage sorghum, rhodes and sabi grass hay production, mixed horticultural crops including mangoes, cucurbits and citrus, and pastoral production. The PCA pivot is bordered by peanuts and fallow hay fields dominated by cavalcade in the wet season, a neighbouring property produces irrigated forage crops such as maize and sorghum, and cattle feedlots separate the pivot from a wet swamp area. As relative attractiveness of surrounding habitats to pest and beneficial insects directly influences densities in neighbouring cotton (Grundy *et al.* 2004, Tillman & Mullinix 2004, Duraimurugan & Regapathy 2005), it is likely the different habitats surrounding the two pivots strongly influenced their relative insect assemblages, confounding experimental consistency and ecological conclusions considerably.

Insect groups that did not display differing densities over time between treatments or pivots included spiders and mirid nymphs and adults (Table 2.1). Spiders are largely regarded as among the most important predators of insect pests in cotton crops (Bishop 1980, Bishop 1981, Bishop & Blood 1981, Whitehouse *et al.* 2006). That spiders seem temporally and spatially ubiquitous in Katherine cotton regardless of companion crop treatments reflects their relative importance as predators in other regions, and encourages their continued utilisation in local cotton IPM systems (see **Natural Enemies**). Spiders that inhabited Katherine cotton trials included many families (17, see **Natural Enemies**), let alone species. Their broad categorisation as spiders only in this study, although necessary for logistical reasons, could well be reflected in the ubiquitous nature of analysis results. Research investigating temporal and spatial distribution of specific spider groups, preferably down to genus or species where possible, within cotton crops would improve our understanding of their role as natural enemies.

Transgenic cotton's inherent control properties effectively nullify *Helicoverpa* damage potential without the need for insecticide applications, so their elevated pest status renders mirids, previously controlled by insecticides targeting *Helicoverpa*, important from a pest control perspective. Like spiders, mirid densities were not significantly different between treatments or pivots over time in companion crop trials during 2005 (Table 2.1). Companion crops and the natural enemies they harbour (Appendix 1) do not influence mirid densities in neighbouring cotton crops in Katherine. Consequently, mirids contributed to the majority of required insecticide applications in all years of the project (see **Insecticides**). Companion crops have been successfully used to decrease mirid densities on neighbouring cotton (Stride 1969, Godfrey & Leigh 1994, Mensah & Khan 1997, Mensah 2002b, Carriere *et al.* 2006), encouraging continued investigation of their potential in Katherine should research proceed and facilities and techniques improve. Novel natural enemies of mirid cotton pests were recently discovered in eastern growing regions. Ongoing studies hope to utilise beneficial insects for biological rather than chemical suppression of mirid cotton pests and may be applicable in Northern Territory cotton production systems in the future.

Aphids are almost ubiquitous in Katherine cotton, displaying higher densities in crops with no companion early season and with a pigeon pea companion late season, in 2005 at least (Figure 2.6). Aphids are generally maintained below control thresholds by natural enemies in Katherine cotton IPM trials. Aphid control thresholds, however, were breached in the KRS pivot following an application of indoxacarb on 9 May (Figure 2.6) to control *S. litura* larvae (the reasons behind and implications of this incident are discussed further in **Insecticides**). *Spodoptera litura* larvae were similarly distributed season long in all companion crop treatments, but densities were significantly greater at KRS than PCA, suggesting the habitat surrounding the KRS pivot, discussed earlier, may be more conducive to *S. litura* population development.

Cotton yield estimates between treatments in pivot irrigated companion crop trials were not significantly different, except for mechanically harvested cotton grown with pigeon pea, which yielded relatively poorly (Figure 2.17). However, this result is most likely an anomaly caused by excessive weed infestation. The pigeon pea and half the neighbouring lablab plot in block 3 did not receive emergence herbicide treatment prior to planting due to human error, and consequently weeds, such as gooseberry and senna, predominated. Weed densities were such that mechanical harvesting was near impossible with continuous blockages causing stoppage and loss of cotton lint during clearing. Hand picking, however, was not affected by weeds, as reflected in the lack of significant difference in yield estimates between treatments for this harvest method. Large scale trial results from 2003 to 2005 suggest companion crops do not improve cotton yields in Katherine.

Fibre quality (uniformity and short fibre index only) of cotton grown with pigeon pea is significantly different to cotton grown with lablab or no companion in Katherine (Figures 2.18 & 2.19). It is unlikely poorer fibre quality is due to insect damage as treatment effects on relative insect densities were minimal. Cotton yield was similarly unaffected by treatment, except for one plot dominated by weeds. It is well documented that weeds impact on cotton growth, vigour and fibre quality in established growing regions (Sadras 1997, Simpson *et al.* 1998, Baily *et al.* 2003). Perhaps weeds impacted on cotton fibre production to such an extent in the Katherine pivot weed infested plot that fibre quality statistical analyses were compromised. A number of factors related to relatively poor farm management contributed to the possibility of spurious fibre quality results.

That there was no significant difference between treatments for all fibre quality variables analysed in the small scale trial was surprising. The area utilised (3.3 Ha) might not have been sufficient for ecological studies investigating insect assemblages, however, was considered so in the past (see Ward 2005). Area for novel research in Katherine was restricted under the circumstances. The possible use of sweet potato as a companion crop for cotton production in the tropics is discussed further in **Natural Enemies**, and **Sucking Pests**.

Trap crops attractive to ovipositing *Helicoverpa*, such as maize, sorghum and lablab, could be utilised at critical cotton growth stages to minimise exposure to *Bt* protein within the insect pest population (see Fitt 2000). Unfortunately, trap crop research in Katherine was limited to early season sorghum and late season chickpea planted in 2004 only and no relevant insect assemblage data was collected. The potential to effectively use trap crops for *Helicoverpa* control during cotton production in the Northern Territory has not been adequately investigated.

Early trials suggested lablab was capable of harbouring relatively high densities of pests and natural enemies associated with cotton production in the Northern Territory (Appendix 1). It is not unreasonable, therefore, to suggest lablab as a possible cotton companion crop in the region. Unfortunately, lablab's attractiveness to insects as a companion crop did not translate to improved pest control or yields in associated cotton. There may be several reasons for this.

Firstly, companion crops in Katherine trials were poorly managed, in 2005 at least. According to Hokkanen (1991), companion crops should be managed so they are continually attractive to pest insects and natural enemies to remain effective. Companion crops in Katherine were difficult to establish due to poor planting (the same planter and disks used for all seed sizes) and mismanagement of pre-emergence herbicide and were often neglected, invading neighbouring cotton, setting seed and dying back. It is difficult to evaluate the efficacy of companion crops under such conditions, however, as stated earlier, companion crop management was equally poor across all treatments so analyses, strictly speaking, were unlikely to be compromised. Trials were generally a poor reflection of intended production methods.

Secondly, the geographical separation of the lablab trial into two distinct halves (pivots) caused considerable variation in insect assemblages. It is difficult to say if variation in pest insect and natural enemy densities between pivots clouded treatment effects, however, it cannot be ruled out. Unfortunately, significant separation of experimental replicates was logistically unavoidable when conducting relatively large scale lablab companion crop trials in Katherine.

Thirdly, it is not unreasonable to assume that insects reared in one crop will possibly remain there for their entire lives provided conditions, such as climate, food availability and shelter, are suitable (Cunningham *et al.* 1998, 1999). Correctly managed companion crops encourage movement of insects into neighbouring fields via mechanical means, such as disturbance then slashing, and act as a suicide or trap crop for pest insects (Hokkanen 1991). The poor management of companion crops during Katherine cotton trials was unlikely to encourage natural biological control of pests in associated cotton, although there is little evidence to suggest it would be otherwise, even with improved management.

Considering weed fallow is as equally effective as companion crops when compared to lablab, pigeon pea and sweet potato (the latter on a small scale at least) and relatively easily managed, it seems transgenic cotton separated by weed follow is a logical production option in the Northern Territory. Unsprayed conventional cotton, as a proportion of each transgenic field, could provide a sink of *H. armigera* individuals unexposed to *Bt* to dilute resistance genes within the population should resistant individuals survive and emerge from neighbouring transgenic cotton. Further, unsprayed conventional cotton yields harvestable lint in Katherine, so could contribute to farm production while improving IPM. Recommendations require further investigation prior to commercial application, however, it is clear that lablab as a companion crop provides minimal, if any, discernible advantage to cotton production in the Northern Territory.

3.0 Insecticides

Introduction

Katherine cotton trials relied considerably on biological control with natural enemies. In-crop insect pest and natural enemy dynamics are influenced by environmental, such as climate and relative attractiveness of neighbouring vegetation, and cropping, such as weed control and soil management, factors. Cotton best management practices for the Northern Territory, such as minimum till and habitat manipulation via companion cropping, purportedly provide an environment suitable for natural enemy proliferation and effective biological control. Insecticide applications only become necessary in Katherine cotton if insect densities breach control thresholds, designed to minimise damage to cotton yield potential, and natural enemy impact is inadequate.

Effective insecticidal control of insect pests requires accurate and timely application. In order to minimise adverse impact on non-target insects in IPM systems, it is critical the active constituent be as target selective and of minimal concentration as possible. It is crucial, therefore, that IPM practitioners have a thorough, accurate and contemporary understanding of resident insect assemblage characteristics, such as an accurate catalogue of species present and their estimated densities, when insecticide application becomes imminent (Dillon & Fitt 1995, Willers *et al.* 2005). Till 2005, insect control decisions during Katherine cotton trials were based on recommendations generated by cottonLOGIC®.

CottonLOGIC® is a desk-top program designed to facilitate cotton farm management. It utilises models calibrated to eastern Australian climatic regions and a summer growing season to predict plant and insect phenologies and recommend management strategies (http://www.cotton.crc.org.au/CottonLogic/HTML/About.shtml). It is not regarded as a research tool, nor is it calibrated to far northern Australian tropical climes and a winter growing season. For the life of the cotton project in Katherine, yield loss and experimental anomalies were often anecdotally blamed on insect damage, claims relatively difficult to definitively refute when using cottonLOGIC® to manage data. For the above reasons, data from insect monitoring in the final year of the project was analysed in spreadsheets rather than cottonLOGIC®, enabling accurate statistical analyses of insect density estimates rather than un-calibrated model inference (see Method, next page). Unfortunately, time and logistical constraints did not allow an experimental comparison between fields managed with cottonLOGIC® and by the spreadsheet method, but it is generally accepted that insects were effectively managed during Katherine cotton trials in 2005, contributing to record experimental yields.

Seedlings were damaged by earwigs in lateral irrigated Katherine cotton trials following relatively early planting in 2005 to such an extent that re-planting was required. Earwigs had never caused serious seedling damage during the prior ten years of winter cotton production trials in Katherine, and are considered only an incidental pest in established cotton growing regions (Pyke & Brown 1996). Chlorpyrifos application in-furrow to control earwigs during and following re-planting was delayed, due to a lack of evidence supporting earwigs as the cause for poor seedling emergence, to ensure minimal soil biology disruption. Subsequent poor seedling emergence and robust evidence supporting earwig damage to seedlings necessitated a second re-plant with chlorpyrifos in-furrow. Analyses proved chlorpyrifos significantly reduced seedling loss which was attributed to earwigs.

Methodology

All insecticide spray decisions during 2001-2004 growing seasons were based on cottonLOGIC® recommendations. In 2005, insect density estimates (per metre) and standard error between samples were calculated for each field on each sampling occasion using spreadsheets. Mean insect pest densities were checked against control thresholds and standard errors indicated consistency between samples for each field. Control thresholds for each insect pest, other than sucking insects, discussed later, were taken from entoPAK® and chemical label recommendations. The control action of predatory insects, as indicated by their density relative to target pests, was considered prior to any insecticide application and active constituents that target mainly pests and not predators were utilised where possible. Insecticides were only applied as a last resort when pest insect thresholds were breached, target pests were relatively evenly distributed across the infested field, and control by predatory insects was inadequate. Insecticide efficacy was examined using a paired *t* test of target insect densities prior to and following application. Insecticide impact on predatory insect densities was similarly examined.

Following initial planting in 2005, seedling emergence appeared patchy and earwigs unusually prevalent in soil mulch of lateral irrigation fields (B series, Figure 1.2) at KRS. Ten randomly located 10 metre row counts of seedlings and gaps between seedlings greater than 0.5m were taken in each of the B series, tape area and pivot (Figures 1.2 & 1.3) by the agronomy team. Both variables were tested for field effect using ANOVA with Tukey test.

The desire to use furrow applied insecticide to control earwigs during a re-plant required statistical support. The density of earwigs and their influence on seedling emergence following first planting were simultaneously estimated as follows. Four randomly located sites in each of fields B1-4, the Tape area and four quarters of the pivot (due to the pivot's relatively large area) were chosen. Two soil samples, one with seedling canopy and one without, five to seven cm deep and wide and 17 cm (one shovel width) long were taken at each site and placed in sealed, plastic containers and labelled. The sampling process was undertaken as briskly as possibly to alleviate earwig escape. All containers were frozen overnight to kill arthropods, contents were washed with tap water through progressively smaller sieves and captured earwigs counted and preserved in 70% ethanol. A generalised linear model for field effects on raw earwig counts did not fit the Poisson distribution, so data were log (x + 1) transformed and field and canopy effects were tested with a factorial ANOVA.

Fields B1-4 were re-planted on 24 March due to excessive seedling gapiness not attributed to relative earwig densities, but rather mechanical planting inconsistencies. Seedling emergence was again tested, as above but limited to five samples per field, in fields B1 (emergence considered acceptable) and B3 (emergence considered inadequate). Pitfall traps were used as an alternative to mechanical soil sampling to test for relative earwig densities between fields. Each trap consisted of a standard plastic 70ml sterilised specimen jar, containing 15ml of water and detergent mix, buried so the rim was level with the soil surface. In each of B1-3, nine pitfall traps were placed in the soil as per Figure 3.1, next page, at a randomly selected site. Field effects on earwig captures were tested with ANOVA and Tukey test.

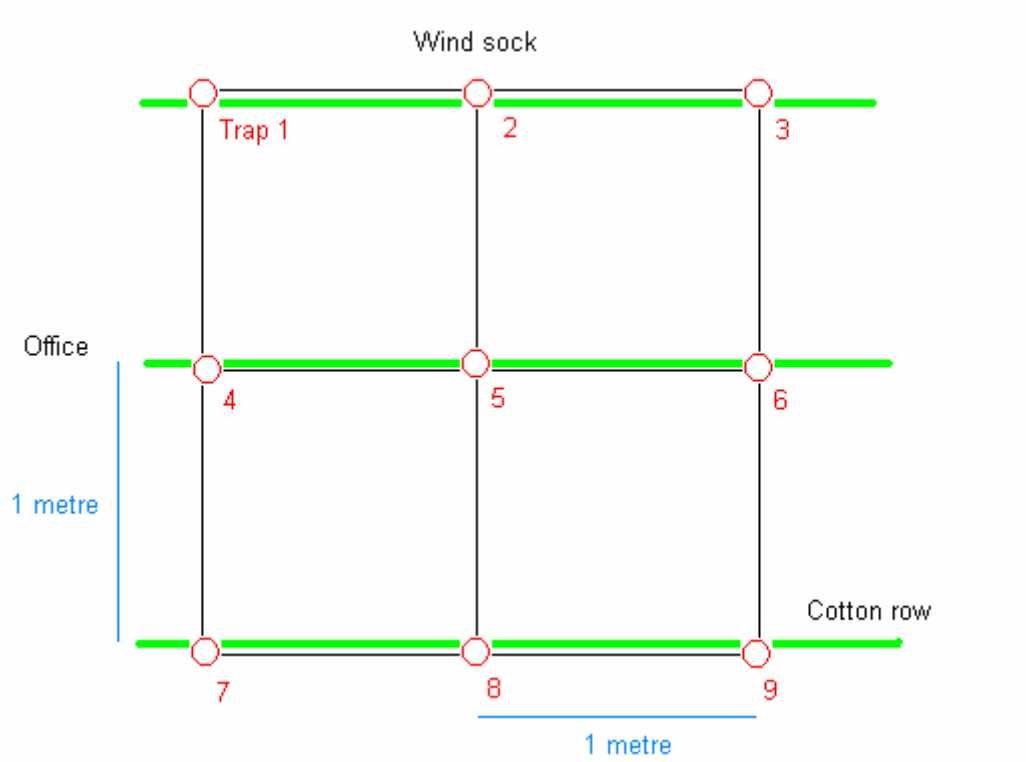

Figure 3.1. Stylised representation of the layout and orientation of pitfall traps targeting earwigs at sites in field B1, B2 and B3 following the re-plant of cotton, on 24 March, and seedling emergence.

Results

3.1 Insecticides utilised

2001-02

Insecticide regimes employed in 2001 and 2002 are presented in Appendix 1, Tables 13 & 14, pages 140 & 141, respectively. The number of required insecticide sprays and reasons for their application are summarised in Appendix 1, 6.0 Insect Management, page 138.

2003-04

Insecticide application records for 2003 are presented in Appendix 5, page 147. In 2003, cotton grown with no lablab was sprayed twice at KRS pivot and once at PCA, and cotton grown with lablab companion was sprayed once at both locations, with Regent® (=fipronil), presumably for sucking insects. In the lateral irrigation area, field A1 was sprayed once with fipronil and the sucking pest trial in fields A2 and A3 once with Ovasyn® (=amitraz) (excluding scheduled sucking pest trial insecticide applications - see **Sucking Pests**). Salt was mixed with fipronil on several occasions in some or all fields where applied.

The insecticide regime employed by the project agronomist in 2004 is summarised in Table 3.1, below. Two fields (KRS lablab and B2) required no sprays and T1, the 'best-bet' agronomy block, received four (Table 3.1). This table does not include sucking pest trial sprays.

Table 3.1. Insecticide regime employed for all trials at KRS and PCA in 2004.

Field	Date	Chemical	Rate
PCA Lablab half pivot	01-Sep	fipronil	125ml
PCA Bollgard half pivot	28-May	fipronil	125ml
KRS Lablab half pivot	NO SPRAYS		
PCA Bollgard half pivot	02-Aug	fipronil	125ml
T1	21-May	imidacloprid	250ml
	31-May	emamectin	700ml
	06-Aug	imidacloprid	250ml
	23-Aug	bifenthrin	800ml
T2	04-Jun	indoxacarb	500ml
	10-Jun	fipronil	125ml
B1	21-Sep	bifenthrin	600ml
B2	NO SPRAYS		

2005

The 2005 insecticide regime is presented in Table 3.2, page 34.

3.2 Impact on target pests

Mirids

Pivots

There was no significant difference in the density of mirids between treatments over time in both pivots during 2005 (Table 2.1 & Figure 3.1, next page). There was a significant reduction in the density of mirids in both pivots immediately following the fipronil application on 2 May (t = 3.32, df = 11, P = 0.0069, Figure 3.1). Neither the indoxacarb at PCA on 12 May, nor fipronil plus salt at KRS on 7 June, insecticide applications reduced mirid densities significantly (t = 0.89, df = 5, P = 0.41 and t = 1.94, df = 5, P = 0.11, respectively, Figure 3.1). Mirid control efficacy was considered good following all targeted applications in pivots during 2005 (Table 3.2).

B1

Likewise, there was no significant difference in the density of mirids between treatments over time in B1 during 2005 (Appendix 4 & Figure 3.2, below). The reduction in mirid density in B1 following fipronil application was not significant on 29 April (t = 1.86, df = 11, P = 0.09, Figure 3.2) but significant on 17 May (t = 2.25, df = 11, P = 0.046, Figure 3.2).

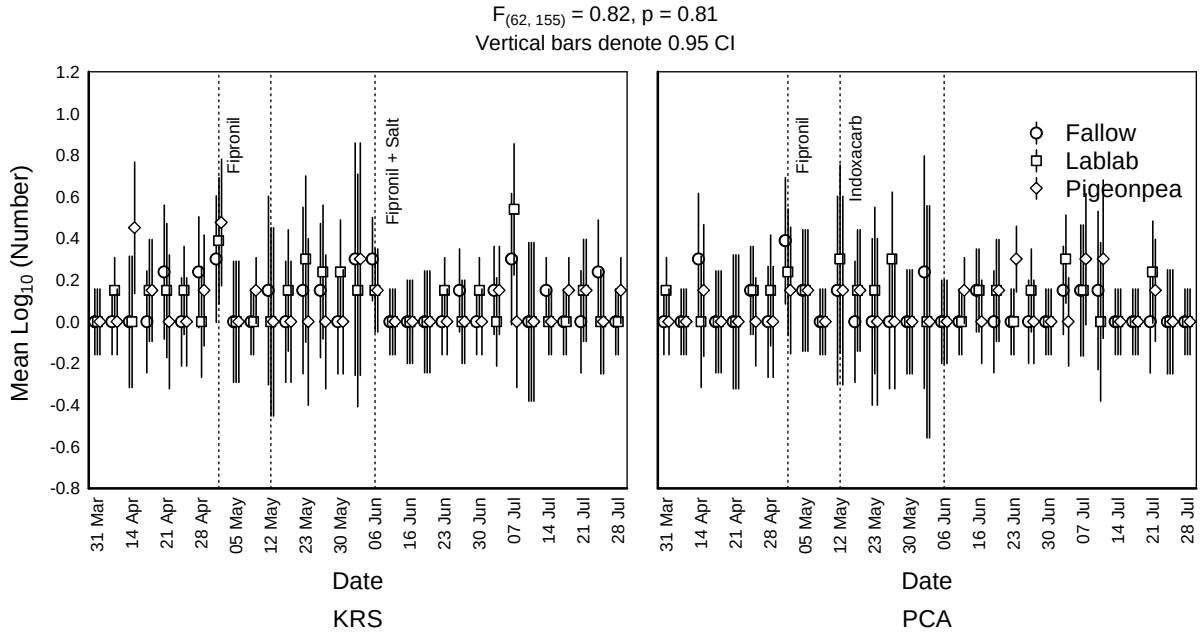

Figure 3.1. The relative density of total mirids in cotton over time compared between pivot irrigation fields and treatments (see legend) grown at KRS and PCA in 2005. Graphs are separated into pivots. The nearest sample date preceding and active constituents of insecticide applications are given.

The fipronil and salt application on 24 June significantly reduced mirid densities in B1 (t = 4.69, df = 11, F = 0.00066, Figure 3.2). Mirid control was considered good to excellent following all targeted insecticide applications in B1 during 2005 (Table 3.2).

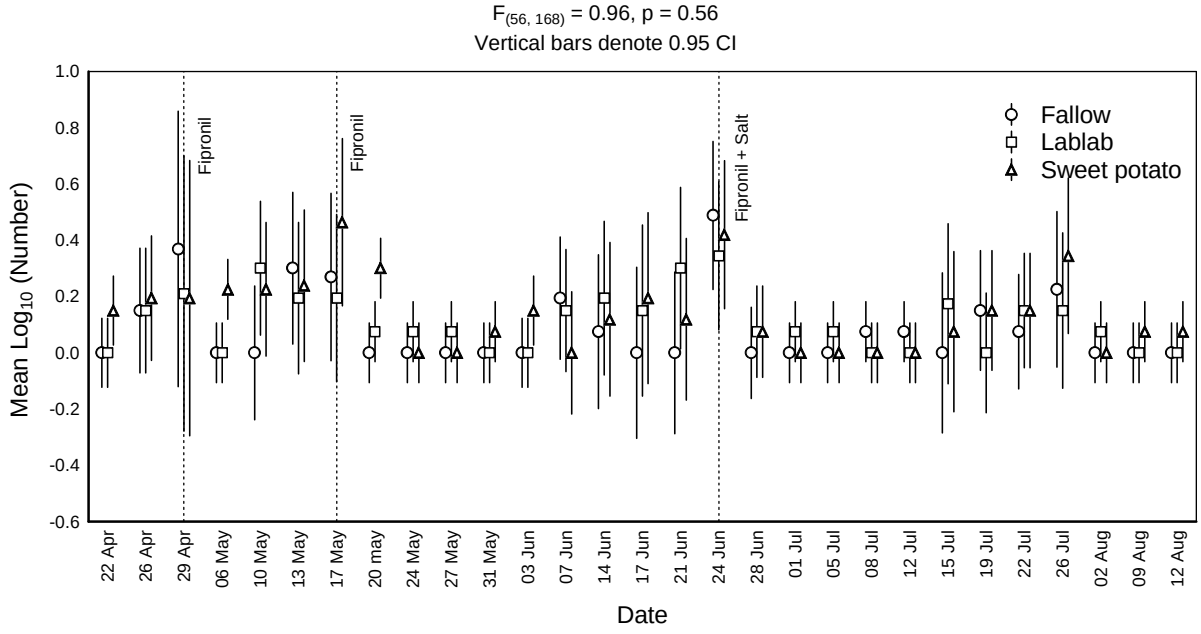

Figure 3.2. The relative density of mirids in cotton over time compared between companion crop treatments (see legend) grown under lateral irrigation at KRS in field B1 during 2005. The nearest sample date preceding and active constituents of insecticide applications are given.

Table 3.2. Insecticide regime employed for all trials at KRS and PCA in 2005. RBSB = redbanded shield bug, spods = *Spodoptera litura* larvae, heli = *Helicoverpa*.

Date	Insecticide	Primary target	Secondary targets	Rate	Rig	Nozzles	Carrier
02-May-05	Fipronil	Mirids	Small spods in KRS pivot	60ml/Ha	Ground	Flat fan	75L/Ha
03-May-05	Fipronil	Mirids		60ml/Ha	Ground	Flat fan	75L/Ha
09-May-05	Indoxacarb	Spods	V small heli larvae	650ml/Ha	Ground	Flat fan	100L/Ha
13-May-05	Indoxacarb	Mirids	Spods, vs heli larvae	650ml/Ha	Ground	Flat fan	100L/Ha
18-May-05	Fipronil	Mirids		60ml/Ha	Ground	Flat fan	75L/Ha
21-May-05	Pirimor	Aphids	NA	500g/Ha	Ground	Fans & droppers with hollow cones	100L/Ha
22-May-05	Pirimor	Aphids	NA	500g/Ha	Ground	Fans & droppers with hollow cones	100L/Ha
07-Jun-05	Fipronil and Salt	Mirids & RBSB		40ml/Ha 10g/L	Ground	Fans & droppers with hollow cones	100L/Ha
25-Jun-05	Fipronil and Salt	Mirids & RBSB		40ml/Ha 10g/L	Ground	Fans & droppers with hollow cones	100L/Ha
29-Jul-05	Dimethoate and Salt	GVB	RBSB, mirids, jassids	340ml/Ha 10g/L	Ground	Fans & droppers and hollow cones	100L/Ha

Date	PCA pivot	KRS Pivot	T1	T2	B1	B2-4	Efficacy/Comments
02-May-05		Bollgard					Good, small spods minor control if any
03-May-05							Good
09-May-05							Good, but flared aphids
13-May-05							Good
18-May-05				Bollgard			Good
21-May-05		P2					Good
22-May-05		P1					Good
07-Jun-05							Excellent on mirids, minimal control on RBSB. **DROPPERS FACING DOWN!**
25-Jun-05							Excellent on mirids, RBSB kill ok, some persistent - others sick/slow
29-Jul-05							No mirid nymphs (1 adult), GVB & RBSB persist but reduced, jassids gone

Redbanded shield bug (RBSB)

Pivots

The reduction in RBSB density in the KRS pivot following an application of fipronil plus salt on 7 June was not significant (t = 0.70, df = 5, P = 0.52, Figure 3.3, below). This application targeted both RBSB and mirids and was considered ineffective and excellent on each, respectively (Table 3.2). The density of RBSB was significantly different between the KRS and PCA pivots over time (Table 2.1 & Figure 3.3).

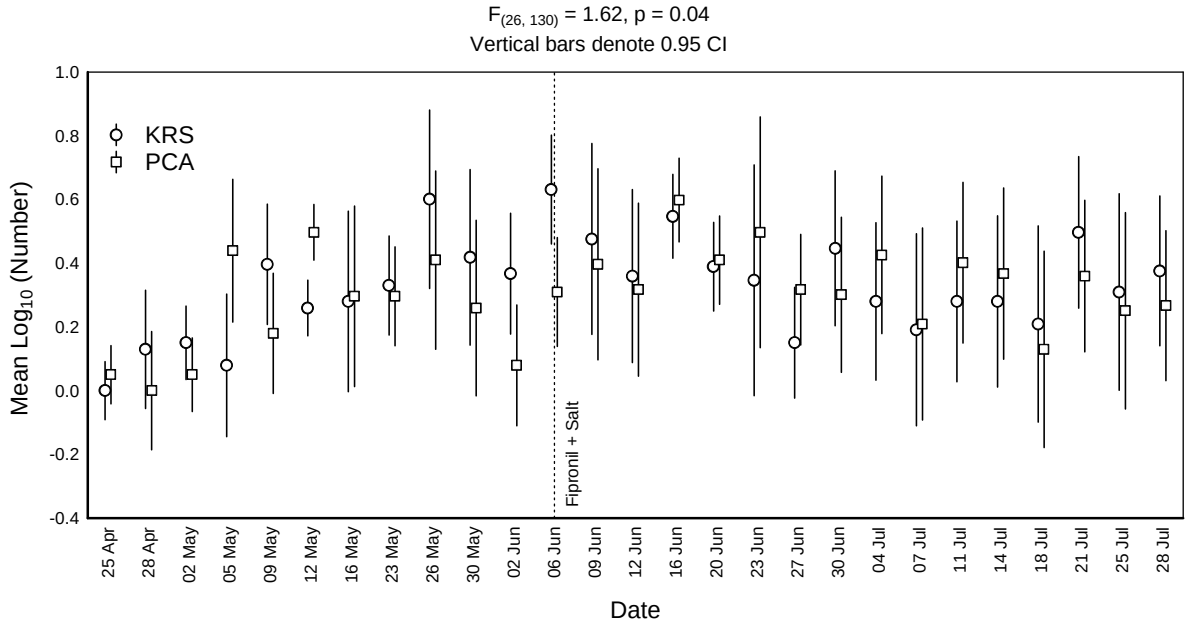

Figure 3.3. The relative density of redbanded shield bugs in cotton over time compared between KRS and PCA pivots during 2005. The nearest sample date preceding and active constituents of an insecticide application is given.

B1

There was no significant difference in the density of RBSB between treatments over time in B1 (Appendix 4 & Figure 3.4, next page). An application of fipronil and salt on 24 June significantly reduced the density of RBSB in B1 (t = 2.54, df = 11, P = 0.028, Figure 3.4). The reduction in RBSB density following an application of dimethoate plus salt on 29 July was not significant (t = 0.68, df = 11, P = 0.51, Figure 3.4). Results from the two insecticide applications were described as ‘okay’ and ‘reduced’ (density), respectively (Table 3.2).

Green vegetable bug (GVB)

Dimethoate plus salt was applied to B1 on 29 July to control a suite of sucking pests. There was no significant reduction in the density of the principal target, GVB, following application (t = 0.29, df = 11, P = 0.78, Table 3.2 & Figure 3.5, next page). The relative density of GVB was not significantly different between treatments over time in B1 (Appendix 4 & Figure 3.5).

Spodoptera litura larvae (Spods)

An application of indoxacarb significantly reduced the density of Spods at KRS on 9 May (t = 6.64, df = 5, P = 0.0012, Figure 2.10), but the reduction was not significant following a similar application at PCA on 13 May (t = 2.12, df = 5, P = 0.087, Figure 2.10). Control

efficacy was described as good following both applications (Table 3.2), but aphid densities increased at KRS soon after (see **Impact on predators**, next page). The relative density of Spods between treatments and pivots over time was significant (see **Companion Crops**, Figures 2.8 – 2.11).

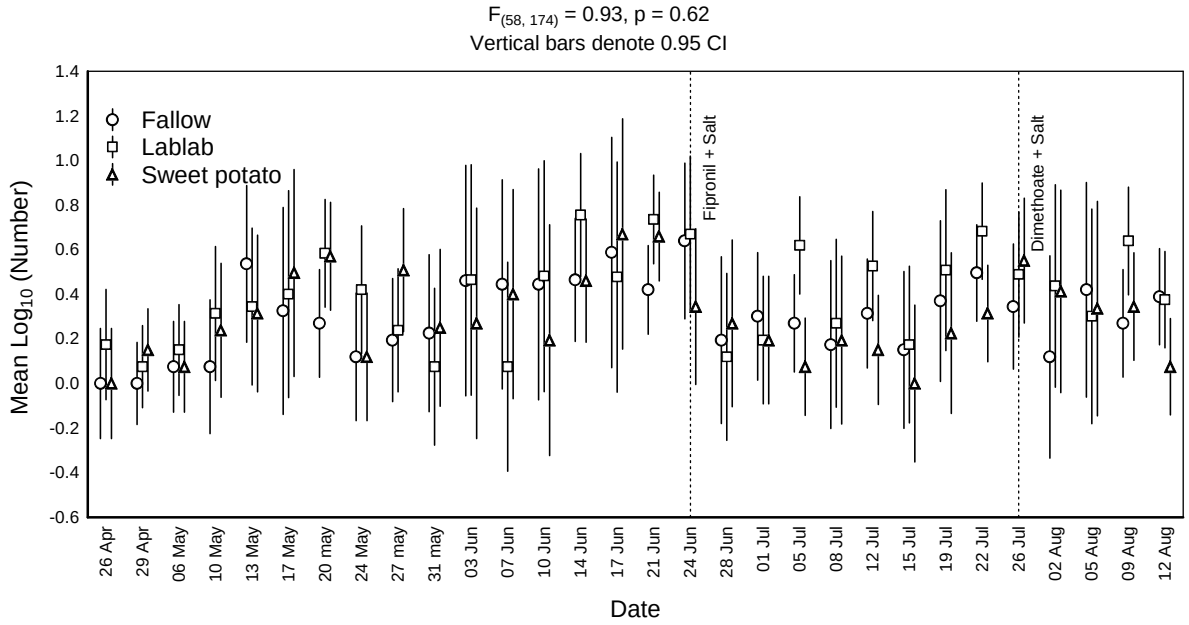

Figure 3.4. The relative density of redbanded shield bugs in cotton over time compared between companion crop treatments (see legend) grown under lateral irrigation at KRS in field B1 during 2005. The nearest sample date preceding and active constituents of insecticide applications are given.

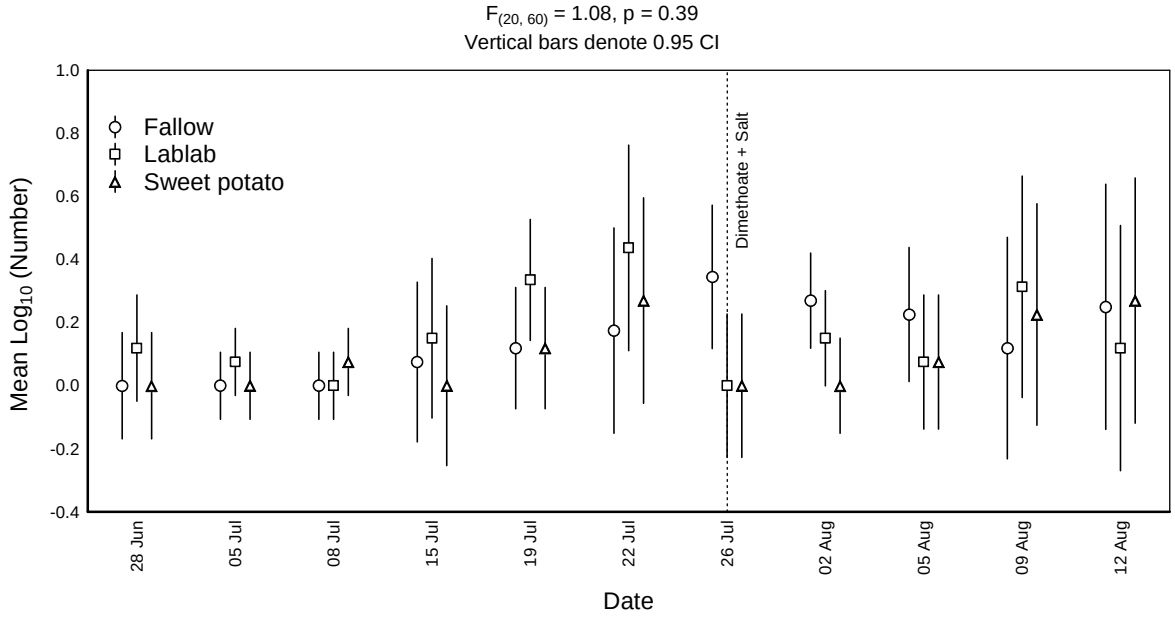

Figure 3.5. The relative density of green vegetable bugs in cotton over time compared between companion crop treatments (see legend) grown under lateral irrigation at KRS in field B1 during 2005. The nearest sample date preceding and active constituents of insecticide applications are given.

Leafhoppers

Although leafhoppers were secondary targets for the dimethoate plus salt application in B1 on 29 July, the reduction in their density was not significant (t = 1.00, df = 5, P = 0.36) despite apparent control (Table 3.2).

3.3 Impact on predators

Coccinellids (Ladybeetles)

Pivots

Ladybeetle larval densities were significantly different between pivots over time (Table 2.1 & Figure 3.6, below). There was no significant reduction in larval ladybeetle densities following all insecticide applications in the KRS and PCA pivots (Table 3.3, below, & Figure 3.6). Adult ladybeetle densities were significantly reduced by an application of fipronil in both pivots on 2 May, indoxacarb in the PCA pivot on 13 May and fipronil plus salt in the KRS pivot on 7 June (Table 3.3 & Figure 3.7, next page). Although the reduction in ladybeetle densities was not significant following an indoxacarb application in the KRS pivot on 9 May, it is important to note aphid densities breached control thresholds immediately afterwards (see Figure 2.6). A pirimicarb application to control aphids in the KRS pivot on 21-22 May did not significantly affect adult ladybeetle densities (Table 3.3 & Figure 3.7).

Table 3.3. Results from paired *t* tests comparing adult and larval ladybeetle densities prior to and following insecticide application in pivot irrigated cotton trials in Katherine during 2005. *t* = *t* value, df = degrees of freedom and *P* = probability. NA represents insufficient data (densities) for accurate *t* tests.

		Adults			Larvae		
Field	Insecticide	*t*	df	*P*	*t*	df	*P*
Both	fipronil	2.7	11	0.02	-1.59	11	0.14
KRS	indoxacarb	-0.49	5	0.64	1.27	5	0.26
PCA	indoxacarb	4.00	5	0.01	NA	NA	NA
KRS	pirimicarb	-0.021	5	0.84	NA	NA	NA
KRS	fipronil plus salt	2.91	5	0.03	NA	NA	NA

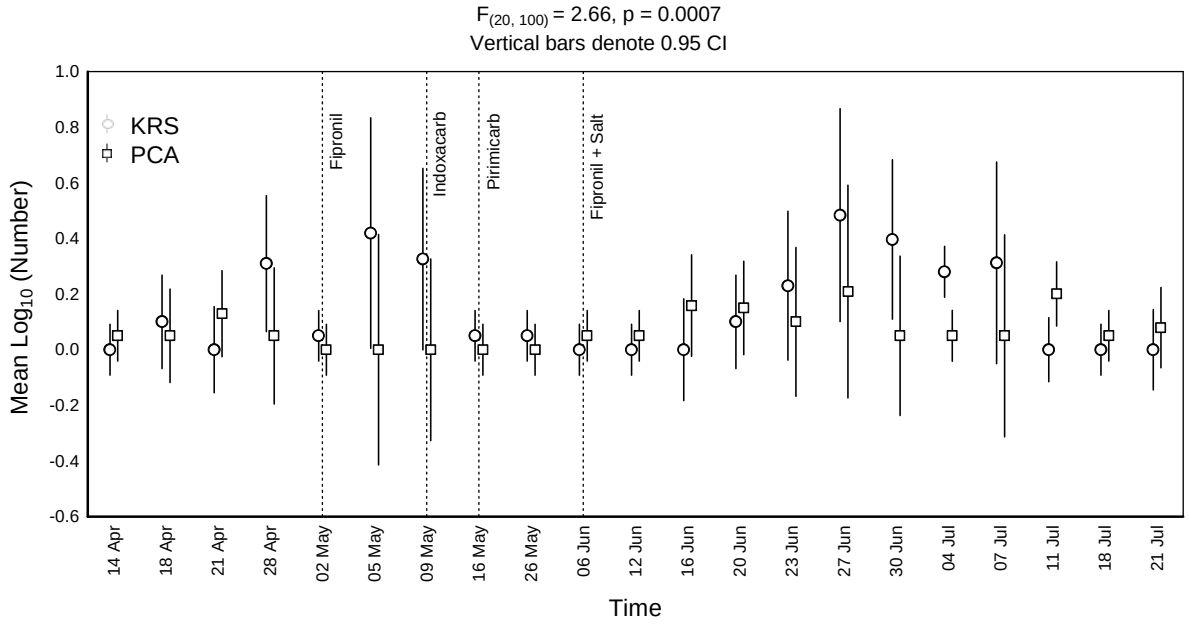

Figure 3.6. The relative density of ladybeetle larvae in cotton over time compared between KRS and PCA pivots during 2005. The nearest sample date preceding and active constituents of insecticide applications are given.

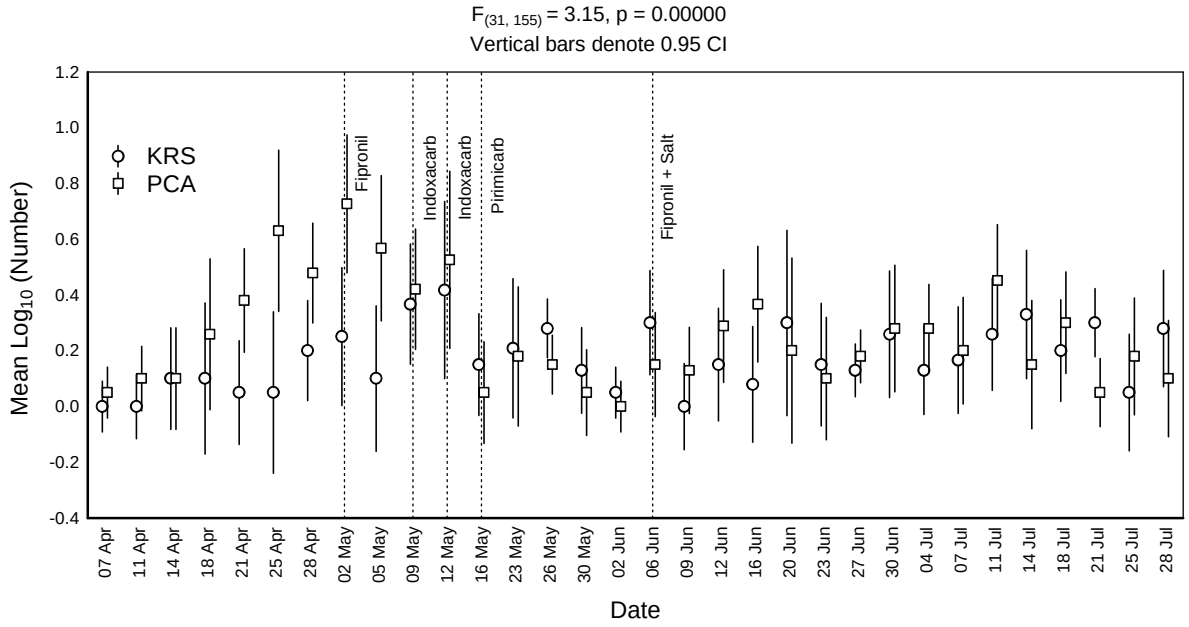

Figure 3.7. The relative density of ladybeetle adults in cotton over time compared between KRS and PCA pivots during 2005. The nearest sample date preceding and active constituents of insecticide applications are given.

B1

Neither the first two fipronil (2 May and 18 May) nor the fipronil plus salt (25 June) insecticide applications reduced ladybeetle larval densities significantly in B1 (t =1.00, df = 11, P = 0.34; t = -1.00, df = 11, P = 0.34; t = 1.76, df = 11, P = 0.11, respectively, Figure 3.8, below).

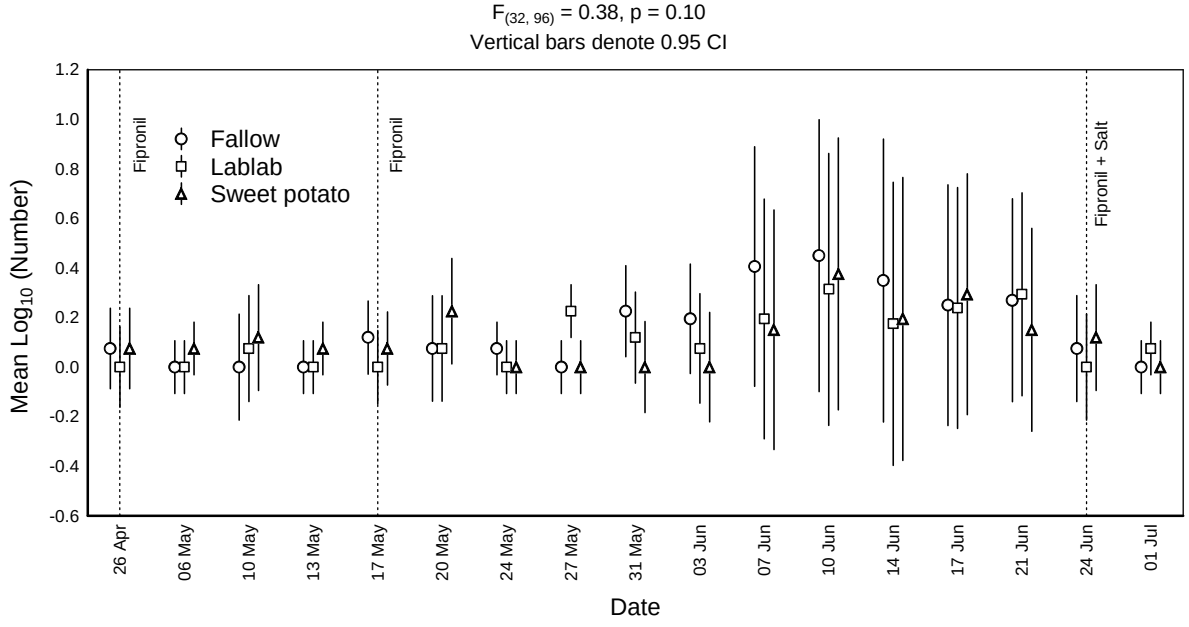

Figure 3.8. The relative density of ladybeetle larvae in cotton over time compared between companion crop treatments (see legend) grown under lateral irrigation at KRS in field B1 during 2005. The nearest sample date preceding and active constituents of insecticide applications are given.

Likewise, the first two fipronil applications did not change adult ladybeetle densities significantly (2 May: t = -1.91, df = 11, P = 0.08, 18 May: t = 0.96, df = 11, P = 0.36, Figure 3.9, next page). Both the fipronil plus salt (25 June) and the dimethoate plus salt (29 July)

insecticide applications reduced adult ladybeetle densities significantly (t = 3.60, df = 11, P = 0.0042 and t = 2.46, df = 11, P = 0.032, respectively, Figure 3.9).

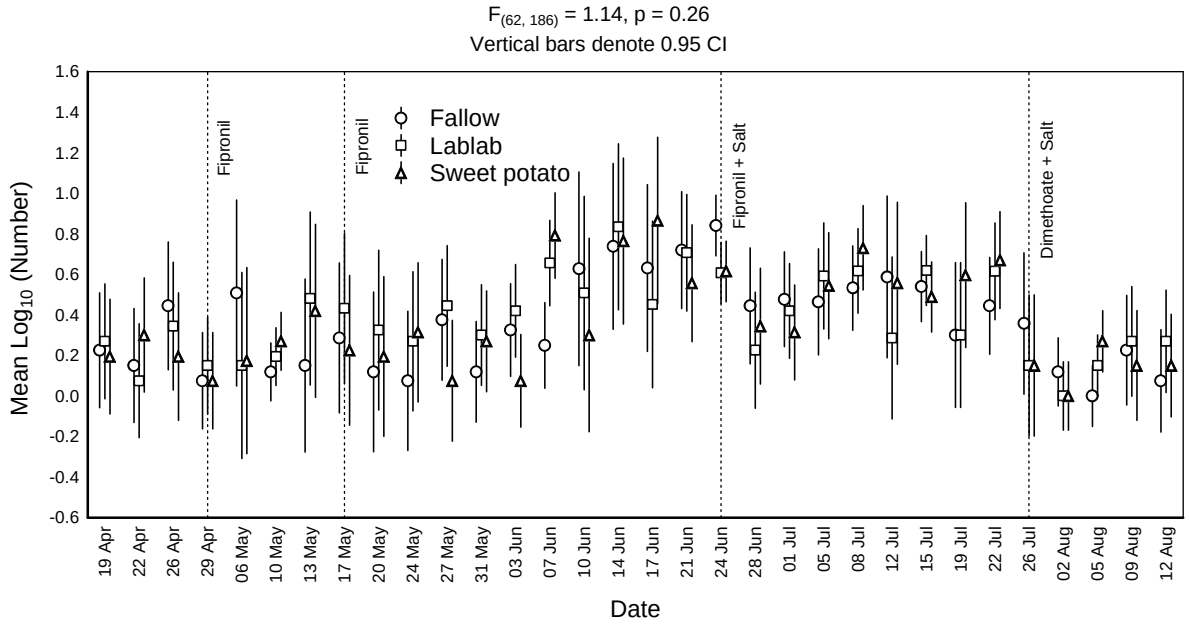

Figure 3.9. The relative density of ladybeetle adults in cotton over time compared between companion crop treatments (see legend) grown under lateral irrigation at KRS in field B1 during 2005. The nearest sample date preceding and active constituents of insecticide applications are given.

Predatory bugs

Pivots

Predatory bug densities were significantly different between pivots over time (Table 2.1 & Figure 3.10, below). No insecticide applications significantly affected predatory bug densities in both pivots during 2005 (Appendix 6, page 148, & Figure 3.10).

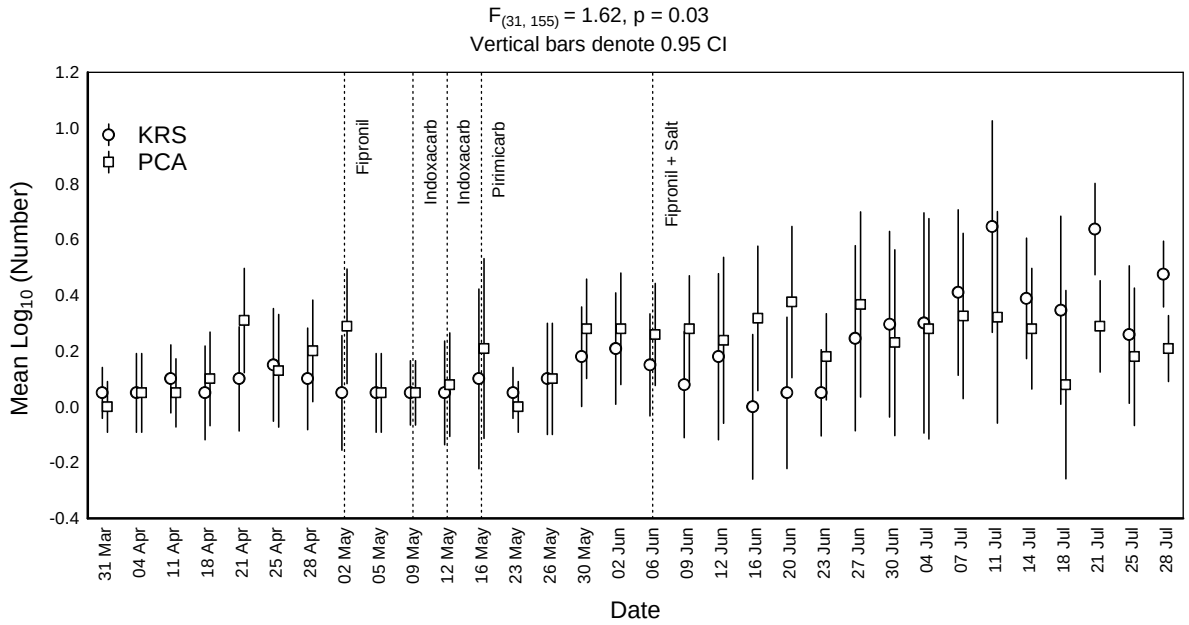

Figure 3.10. The relative density of predatory bugs in cotton over time compared between KRS and PCA pivots during 2005. The nearest sample date preceding and active constituents of insecticide applications are given.

B1

Insecticide applications did not significantly affect predatory bug densities in B1 during 2005 (Appendix 7, page 148 & Figure 3.11, below).

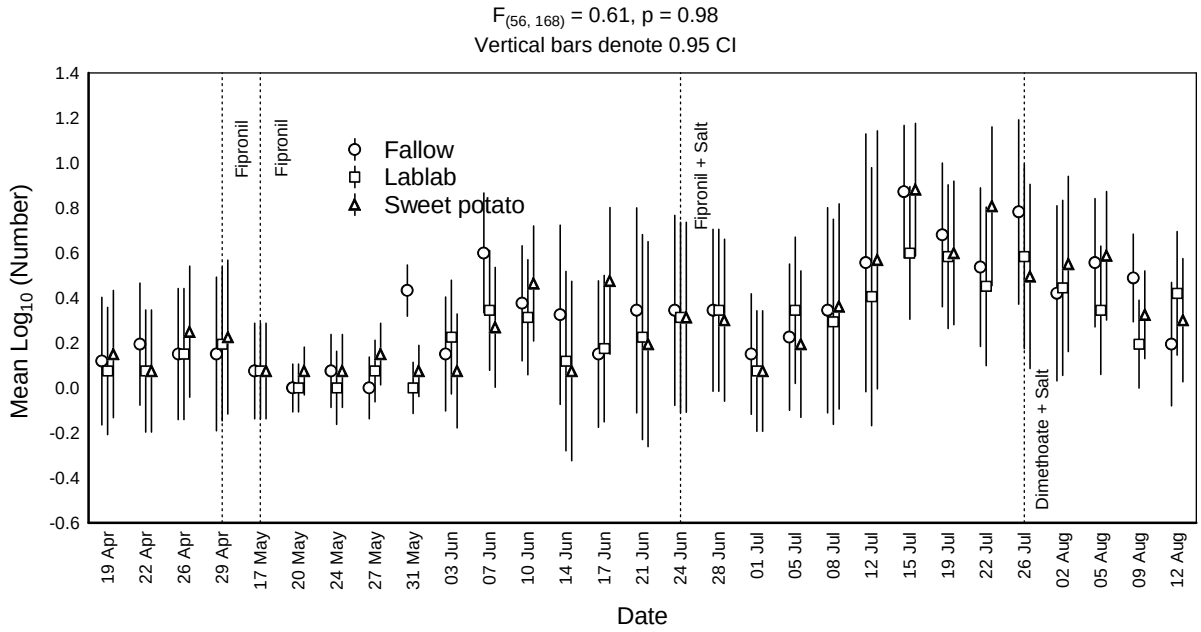

Figure 3.11. The relative density of predatory bugs in cotton over time compared between companion crop treatments (see legend) grown under lateral irrigation at KRS in field B1 during 2005. The nearest sample date preceding and active constituents of insecticide applications are given.

Spiders

Pivots

No insecticide applications significantly affected spider densities in both pivots during 2005 (Appendices 8 & 9, page 148).

B1

Spider densities increased significantly following fipronil (2 May: t = -2084, df = 11, P = 0.016) and fipronil plus salt (25 June: t = 2.24, df = 11, P = 0.047) insecticide applications in B1 (Figure 3.12, next page). The second fipronil alone (18 May: t = -0.51, df = 11, P = 0.62) and dimethoate plus salt (29 July: t = 1.46, df = 11, P = 0.17) insecticide applications in B1 did not change spider densities significantly (Figure 3.12).

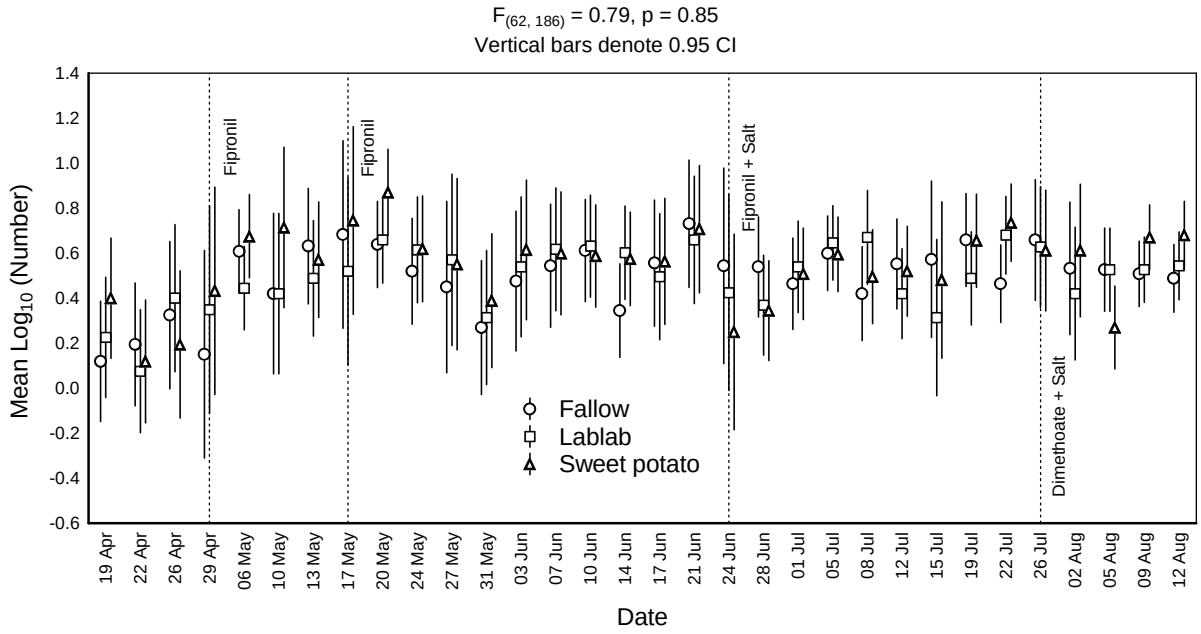

Figure 3.12. The relative density of spiders in cotton over time compared between companion crop treatments (see legend) grown under lateral irrigation at KRS in field B1 during 2005. The nearest sample date preceding and active constituents of insecticide applications are given.

3.4 Earwigs

Initial seedling emergence

The B series had significantly more seedling gaps (>0.5m) per sample than the pivot at KRS following first cotton planting in 2005, however, gap number in the Tape, which separates the pivot and B series, was not significantly different to either ($F_{2, 27}$ = 4.80, P = 0.016, Figure 3.13, below). Seedling density compared between all fields was not significantly different ($F_{2, 27}$ = 3.16, P = 0.058).

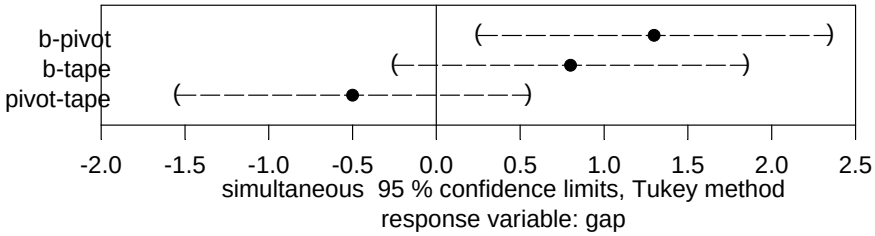

Figure 3.13. Tukey comparison of simultaneous 95% confidence limits for field (y axis) effects on the number of gaps in seedling emergence following initial cotton planting in Katherine in 2005.

Soil samples

There was no significant interaction between field (B series, Tape, KRS pivot P1 and KRS pivot P2) and the presence of cotton seedling canopy for soil collected earwigs ($F_{8, 54}$ = 0.59, P = 0.78).

Second planting (B1 – B4)

There was no significant difference in the number of seedling gaps (>0.5m) per sample between five counts only in each of B1 and B3 following the second planting in 2005 ($F_{1, 8}$ = 0.60, P = 0.46), however, seedling density was significantly different between the two fields ($F_{1, 8}$ = 9.27, P = 0.016).

Pitfall traps

B3 pitfall traps collected significantly more earwigs than both B2 and B4 traps ($F_{2, 24}$ = 14.46, P = 0.000076, Figure 3.14, below).

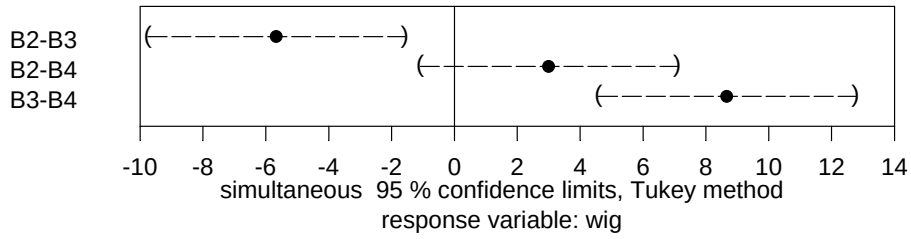

Figure 3.14. Tukey comparison of simultaneous 95% confidence limits for field (y axis) effects on the number of earwigs caught in pitfall traps following second cotton planting in Katherine in 2005.

Chlorpyrifos test

In B3, seedling emergence was significantly denser ($F_{1, 11}$ = 66.68, P = 5.37E-6) and gaps significantly fewer ($F_{1, 11}$ = 78.57, P = 2.43E-6) in plots planted with chlorpyrifos in the furrow when compared to controls with no insecticide following the third planting in 2005.

Discussion

Pest insect and natural enemy densities were effectively monitored using spreadsheets rather than cottonLOGIC® in 2005, removing yield anomalies previously attributed to insect damage from trial results, however, definitive evidence cottonLOGIC® was at fault is lacking. Further, data management via spreadsheets permitted ease of statistical analyses presented in this and other report sections. CottonLOGIC® is not regarded as a research tool, so it is surprising it was utilised for insect management during the majority of IPM trials in Katherine. There is definitely a need for a desk-top cotton crop management tool if commercial cotton production becomes a reality in the Northern Territory, but it must be developed or modified specifically for northern, winter growing applications.

The adoption of Bollgard II® significantly reduced insecticide reliance in all trials (see Appendix 1 - Tables 13 & 14, Appendix 5, Tables 3.1 & 3.2). From 2003 to 2005, the majority of insecticide applications targeted sucking insects, most notably mirids, previously controlled under obsolete chemical based *Helicoverpa* control regimes. It is unlikely companion crops reduced the impact of mirid damage on cotton yields in Katherine as mirid densities were not significantly different between treatments in companion crop trials (see **Companion Crops**). Chemical control using fipronil with (Khan 2003) or without salt effectively reduces mirid densities and associated cotton crop damage in Katherine. Fipronil does, however, impact on natural enemy efficacy (discussed later). Possibilities for biological control of these important sucking pests in Katherine cotton crops requires attention.

Redbanded shield bugs are an emerging sucking pest of Katherine cotton crops, becoming increasingly prevalent from season to season. The impact RBSB late season feeding has on potential cotton yield is examined further in **Sucking Pests**. In 2005, the use of fipronil plus salt to control RBSB was satisfactory at best, and dimethoate plus salt was ineffective. Due to the consistent and emerging nature of their pest status, an understanding of effective control measures for RBSB in Katherine cotton crops is required (see **Sucking Pests**).

Although green vegetable bugs were significant pests during Katherine cotton trials in 2001 and 2002, they required chemical control only periodically in following years. Insecticidal control of GVB, if required early season, could disrupt natural enemy populations instigating heavier reliance on chemical control of insect pests for the remainder of the season, as experienced in 2001 and 2002. In 2005, GVB were present late season and were targeted within a suite of sucking pests using dimethoate plus salt, which was largely ineffective. Although GVB attack appears periodic at best, there is no doubt it has the potential to disrupt IPM systems in Katherine cotton, and effective control measures should be elucidated further.

Leafhoppers have the potential to infest Katherine cotton crops at high densities late season. The impact of leafhopper infestation on Katherine cotton yields is investigated further in **Sucking Pests**. In 2005, dimethoate plus salt was applied in field B1 in an unsuccessful attempt to suppress leafhopper densities. That dimethoate plus salt failed to suppress RBSB, GVB and leafhoppers suggests the utilisation of salt to decrease the required rate of dimethoate may not be an effective control measure for sucking pests in Katherine cotton crops.

Disruption of natural enemies with insecticides can cause aphid flare in cotton (Wilson *et al.* 1999). In 2005, whitefly and aphid densities were effectively managed by natural enemies, such as coccinellids, syrphids and spiders, except for the one occasion natural enemy populations were severely disrupted by insecticides at critical pest density, as follows. Leaf damage attributed to *S. litura* larvae early season is generally tolerable in Katherine cotton crops. Damage to plant reproductives increased in the KRS pivot in 2005 when *S. litura* larvae reached relatively high densities (5+ per metre) necessitating their control to reduce possible impact on cotton yield. Indoxacarb effectively suppresses *S. litura* larvae in Katherine cotton crops, at least at high densities (Figure 2.15), yet ongoing efficacy of natural enemies may be disrupted and should be considered prior to its application. As seen in 2005, the reduction in coccinellid densities in the KRS pivot following indoxacarb application was not significant, yet previously suppressed aphid densities flared beyond control thresholds. The resultant application of selective pirimicarb decimated aphid densities, yet could have been avoided if insecticides were not employed initially, or applied in conjunction with pirimicarb. The potential for spods to reduce yield in winter grown cotton crops in Katherine is evident yet unquantified, hence the decision to employ chemical control at unusually high densities. Other insecticides, such as fipronil with or without salt, have the potential to significantly reduce adult coccinellid densities and subsequently their impact on pest insect densities (Table 3.2). Thorough consideration of insecticide impact on natural enemies is critical prior to their application.

Predatory bugs and spiders were not affected by insecticide applications in Katherine cotton crops during 2005. In fact, the build-up of spider densities increased unabated following early season application of fipronil and fipronil plus salt in field B1. The impact spider feeding has

on pest densities in Katherine cotton crops is discussed further in **Natural Enemies**. The relative abundance and importance of predatory bugs as natural enemies is considered low.

Earwigs demonstrated their potential to devastate seedling emergence of Katherine cotton crops in 2005, despite never requiring control in previous trial years. This may in part be due to the relatively dry wet season experienced prior to the 2005 growing season. Firstly, that the soils were rarely saturated and never flooded may have provided earwigs with ideal habitat for proliferation, although densities were not equally high across all fields. Secondly, a lack of soil moisture is not conducive to bacterial breakdown of the mulch layer, perhaps providing additional vegetative sustenance for earwig population expansion. Thirdly, an extended break in late wet season rains permitted unusually early planting and, although not documented, it is possible earwigs are present at higher densities during the warmer months, dissipating as the dry approaches. That earwigs were only a problem in one cotton growing area suggests their presence was an anomaly highlighting the need to check mulch for unusually high earwig densities prior to planting. Unfortunately, earwigs are nocturnal and habitual and their densities are best estimated over an extended period (7 days) with specially designed shelter traps, yet this was not possible in 2005 as re-planting decisions required immediate action due to impending rain. Earwigs were effectively suppressed by the application of chlorpyrifos in-furrow during re-planting in 2005, although disruption of soil biology with insecticides should be avoided where possible (Acosta-Martinez *et al.* 2004, Park & Lees 2004).

The combination of Bollgard II® control efficacy in an IPM system minimised reliance on insecticidal control of pests in Katherine cotton during 2005, with fields requiring an average of 2.8 sprays mainly for sucking pests. Implications are discussed further in **General Discussion and Recommendations**.

4.0 Natural Enemies

Introduction

IPM strategies incorporate available control options into a pest management system designed to limit reliance on insecticides and associated detrimental environmental and social impacts and production costs. Sustained natural enemy impact on pest insects is regarded as a crucial aspect of cotton IPM systems (Pyke & Brown 1996, Lawrence *et al.* 2000), and their pest control potential has been successfully utilised in established Australian cotton growing regions where IPM has been universally adopted (Fitt 1994, Fitt 2000). Research in Katherine investigated management options, designed to maximise natural enemy impact on pest insects, ultimately intended for incorporation into IPM strategies for local transgenic cotton production.

Natural enemies rarely eradicate pests without augmentation as biopesticides, but rather maintain pest insect densities below designated chemical control thresholds negating the need for insecticide application. In order to maximise their control efficacy, the cropping environment must be suitable for natural enemy proliferation, and this is achieved using several approaches. Natural enemies are often inoculatively introduced in classical biological control following colonisation by invasive pest insects, however, successful pest suppression is rarely realised via this method primarily due to insufficient prior understanding of the complex ecological and environmental interactions involved (Walter 2003). Periodic or seasonal augmentation of natural enemy populations is often achieved via mass release methods, although these can be costly, or even impossible, in relatively remote regions in comparison to insecticidal control options. Alternatively, it is possible to augment and conserve endemic natural enemy populations via habitat manipulation, such as companion cropping, where food, shelter and hosts are supplied for natural enemy proliferation and movement into associated production crops (Baggen & Gurr 1998, Gurr *et al.* 1998b, Baggen *et al.* 1999, Gurr & Wratten 1999).

Companion cropping was investigated as a management option to maximise natural enemy impact in Katherine cotton IPM trials (see **Companion Crops**). Endemic and adventitiously introduced natural enemies residing in local cotton production systems were catalogued and their relative abundance in various possible companion crops assessed. Among the many possible natural enemies worthy of investigation, spiders and the egg parasitoid *Trichogramma* and its impact on pest species were specifically examined.

The role of spiders as natural enemies in Australian cotton IPM systems is well documented (Bishop 1980, 1981, Bishop & Blood 1981, Whitehouse *et al*. 2006). Spiders tend to dominate natural enemy fauna in Australian cotton, yet despite their impact on pest species being difficult to quantify, there is little doubt the large number of spider families and various predatory strategies they employ are crucial for biological pest suppression in Australian cotton IPM systems (Whitehouse *et al*. 2006). Further, their ubiquitous nature and robust ability to tolerate insecticide applications relative to other natural enemies (Mansfield *et al.* 2006) renders them crucial in IPM systems once insecticides are employed.

Trichogramma egg parasitoids are considered the principal natural enemy of target insect pests in neighbouring Ord River Irrigation Area (ORIA, tropical Western Australia) transgenic cotton trials due to their prolific nature in the region (Strickland & Lacey 1996, Davies 2005). Egg parasitoids prevent larval hatch of their insect host effectively minimising the number of emergent pest larvae ingesting transgenic plant tissue thereby reducing their

potential for possible resistance development. It is for this reason *Trichogramma* are considered ideal biological control agents (Scholz 2000), especially for insect resistance management strategies targeting *Helicoverpa* in transgenic cotton. Further, the biology of *Trichogramma* provides researchers with a unique model to assess predator (egg parasitoid) / prey interaction in crops, a notoriously difficult task to achieve, through relatively simple collection of host eggs. Populations of *Trichogramma* in Katherine cotton crops have received minimal attention to date.

Despite achieving relatively high abundance periodically in the ORIA (ca. 99% egg parasitism) effective control of *Helicoverpa* is only achieved when successive populations of *Trichogramma* rarely overlap (Davies 2005). This is primarily due to *Helicoverpa's* relatively short egg stage (about three days) in comparison to the ten days required for *Trichogramma* to develop within host. Habitat manipulation can provide refuge for *Trichogramma* populations prior to *Helicoverpa* egg lay in cotton crops effectively avoiding the lag period that normally occurs during *Trichogramma* establishment (Virk *et al.* 2004). By providing ample hosts that do not attack cotton plants, a substantial population of *Trichogramma* can theoretically establish in specific companion refuges with minimal harm, through transfer of pests, to neighbouring cotton crops.

The suggested hypothesis was tested using sweet potato as a companion crop for comparison to lablab and no companion. Insects harboured by sweet potato foliage are poorly documented as pest control research focuses on harvested tubers that mature below ground. The principal foliage pest of sweet potato in Australia is the sphyngid, *Agrius convolvuli* (L.) or the agrius moth, that lays large eggs attractive to ovipositing *Trichogramma*. Importantly, the agrius moth does not attack cotton as it is specific to Convolvulaceae and is considered ubiquitous in Australia and common in Katherine. Large numbers of *Trichogramma* develop per *Agrius* egg (ca. 18) relative to *Helicoverpa* egg (ca. 2.5) resulting in dense populations at high host densities (Davies 2005). By disrupting established *Trichogramma* populations in companion sweet potato *ad hoc*, it is envisaged they could potentially move into neighbouring cotton crops following *Helicoverpa* egg lay to provide biological control.

The relative attractiveness of each host egg to *Trichogramma* may adversely affect outcomes if *Agrius* eggs are strongly preferred to *Helicoverpa*, and sweet potato must not encourage the establishment of pests in cotton crops to be successful as a companion crop (Davies 2005). In terms of management, sweet potato strips will persist unaided once established with rank growth controlled in a similar fashion to the accepted lablab.

Methodology

4.1 Natural enemy catalogue

Natural enemies encountered *in situ* and collected from Katherine cotton crops were catalogued during the entire cotton IPM project. Unfortunately, emphasis on natural enemy research was generally marginalised by budget and personnel constraints, other than collaboration with CSIRO scientists at ACRI regarding latitudinal gradients in spider diversity across Australian cotton regions. Emphasis on *Trichogramma* research eventuated primarily due to the appointment of a project leader experienced in this field of research.

4.2 Spiders

Spiders were collected periodically throughout 2002 Katherine cotton IPM trials in both cotton and associated companion crops via beat sheet and pitfall trap. Samples were sent to ACRI for identification down to spider family. The relative diversity of spiders in cotton across Australian growing regions, including Katherine, was then examined (see Whitehouse *et al.* 2006). The relative abundance of each spider family in both cotton and associated companion crops in Katherine was examined when relevant data were returned by ACRI.

4.3 *Trichogramma*

Cotton in both companion crop trials was examined and *Helicoverpa* eggs removed twice per week to examine relative parasitism by *Trichogramma* between companion crop treatments. A random metre long sample site in each treatment plot was selected and each cotton plant that fell within that metre examined for host eggs on each sampling occasion. Encountered *Helicoverpa* eggs were removed (up to 20 eggs only per site due to time restrictions) using specially designed hole punches (following Hoffman *et al.* 1970), gently placed in gelatin capsules and stored in labelled cloth bags for return to the laboratory in a chilled esky. The number of host eggs present in each sample metre was recorded. Gelatin capsules from each site were then stored in specimen jars labelled with collection date and location, examined daily, and emergence dates recorded. Emergent *Trichogramma* were preserved in ethanol, following freezing for ten minutes to induce torpor, for later identification. Emergent *Helicoverpa* larvae were reared on diet in the insectary at 30 ± 5°C, 12:12 L:D and 65 ± 10% RH for identification as adults.

The arcsine square root relative proportion *Helicoverpa* larval hatch and *Trichogramma* emergence from collected eggs over time were examined for pivot companion crop trials only using repeated measure ANOVA, as B1 did not maintain sufficient *Helicoverpa* egg densities for accurate parasitism studies. Percent parasitism was calculated and compared graphically for each companion crop treatment and the impact of insecticides on relative parasitism rates prior to and following application examined with a paired *t* test.

Results

4.1 Natural enemy catalogue

Natural enemies observed in the field and collected in Katherine cotton trials are presented in Table 4.1, below.

Table 4.1. Natural enemies observed *in situ* and collected from Katherine cotton trials during 2004 and 2005. Compiled by Natasha Galvin.

Common name	Scientific name	Family	Group
Assassin Bug	NA	Reduviidae	Predatory bug
Bigeyed Bug	*Geocoris lubra*	Lygaeidae	Predatory bug
Braconid	*Chelonus* spp.	Braconidae	Parasitoid
Brown Smudge Bug	*Deraeocoris signatus*	Miridae	Predatory bug
Crab spider	NA	Thomisidae	Spider
Damsel Bug	*Nabis kinbergii*	Nabidae	Predatory bug
Glossy Shield Bug	*Cermatulus nasalis*	Pentatomidae	Predatory bug
Green Lacewing	*Chrysopa spp.*	Chrysopidae	Lacewing
Hoverfly	NA	Syrphidae	Hoverfly
Minute Two-spotted ladybird	*Diomus notescens*	Coccinellidae	Predatory beetle
Mite eating Ladybird	*Stethorus spp.*	Coccinellidae	Predatory beetle
Orb spider	NA	Araneidae	Spider
Phorid	NA	Phoridae	Parasitoid
Pirate Bug	*Orius spp.*	Anthocoridae	Predatory bug
Predatory Shield Bug	*Ochelia schellenbergii*	Pentatomidae	Predatory bug
Red and Blue Beetle	*Dicranolaius bellulus*	Melyridae	Predatory beetle
Six Spotted Thrips	*Scolothrips sexmaculatus*	Thripidae	Predatory thrips
Small Carabid Beetle	NA	Carabidae	Predatory beetle
Striped Ladybird	*Micraspis frenata*	Coccinellidae	Predatory beetle
Tachinid	NA	Tachinidae	Parasitoid
Transverse Ladybird	*Coccinella transversalis*	Coccinellidae	Predatory beetle
Trichogramma	*Trichogramma* spp.	Trichogrammatidae	Parasitoid

In 2001 and 2002, sufficient *Helicoverpa* larvae for parasitism studies were only collected late season (July-September, Fig 6, Appendix 1, page 129). Larval parasitism did not reach more than 30% in either year (Fig 6). *Helicoverpa* larvae were collected and reared to test for parasitism *ad hoc* in 2003 and 2004, with no results recorded. Parasitism studies focused on *Trichogramma* in 2005.

4.2 Spiders

In 2002, the Katherine cotton IPM team participated in a collaborative project assessing the latitudinal gradient in spider diversity in Australian cotton. The relative abundance of spider families in Katherine cotton and companion refuge (assumed to be lablab) during 2002 is summarised in Table 4.2, next page. Further analyses were not attempted due to inconsistent data sets. Oxyopids were the dominant spider family caught in Katherine cotton in 2002 (57, 3.17, 22.35%; total, mean per trap, proportion; Table 4.2). Cotton and its refuge were both dominated by oxyopids (23, 2.56, 19.33% and 34, 3.78, 25%, respectively, Table 4.2) as were spiders caught by beat sheets (54, 6.00, 22.35%, Table 4.2). The majority of spiders collected in pitfall traps were lycosids (31, 3.44, 33.33%, Table 4.2). More spiders overall were caught in the refuge crop (53.33%, Table 4.2) and by beat sheets (65.53%, Table 4.2).

Table 4.2. Number of each spider family captured in Katherine cotton trials during 2002. The total, mean (per trap) and proportion (Prop) of each spider family and total and proportion caught in and by each crop and collection method (Beat = beat sheet) are given. Maximums from each crop and collection method are shaded. Ar = Araneidae, Cl = Clubionidae, Co = Corinnidae, Cy = Cycloctenidae, Di = Dictynidae, Gn = Gnaphosidae, Li = Linyphiidae, Ly = Lycosidae, Mi = Mimetidae, Ne = Nesticidae, Oo = Oonopidae, Ph = Philodromidae, Sa = Salticidae, Sc = Scytodidae, Te = Theridiidae and To = Thomisidae.

			Ar	Cl	Co	Cy	Di	Gn	Li	Ly	Mi	Ne	Oo	Ox	Ph	Sa	Sc	Te	To	Total	Prop
Crop	Cotton	Total	6	7	2	8	3	9	8	20	1	1	1	23	4	15	0	9	2	119	46.67
		Mean	0.67	0.78	0.22	0.89	0.33	1.00	0.89	2.22	0.11	0.11	0.11	2.56	0.44	1.67	0.00	1.00	0.22		
		Prop	5.04	5.88	1.68	6.72	2.52	7.56	6.72	16.81	0.84	0.84	0.84	19.33	3.36	12.61	0.00	7.56	1.68		
	Refuge	Total	1	10	1	17	3	12	1	20	1	1	0	34	0	16	1	14	4	136	53.33
		Mean	0.11	1.11	0.11	1.89	0.33	1.33	0.11	2.22	0.11	0.11	0.00	3.78	0.00	1.78	0.11	1.56	0.44		
		Prop	0.74	7.35	0.74	12.50	2.21	8.82	0.74	14.71	0.74	0.74	0.00	25.00	0.00	11.76	0.74	10.29	2.94		
Method	Beat	Total	6	12	3	22	4	8	2	9	1	1	0	54	4	13	0	18	5	162	63.53
		Mean	0.67	1.33	0.33	2.44	0.44	0.89	0.22	1.00	0.11	0.11	0.00	6.00	0.44	1.44	0.00	2.00	0.56		
		Prop	3.70	7.41	1.85	13.58	2.47	4.94	1.23	5.56	0.62	0.62	0.00	33.33	2.47	8.02	0.00	11.11	3.09		
	Pitfall	Total	1	5	0	3	2	13	7	31	1	1	1	3	0	18	1	5	1	93	36.47
		Mean	0.11	0.56	0.00	0.33	0.22	1.44	0.78	3.44	0.11	0.11	0.11	0.33	0.00	2.00	0.11	0.56	0.11		
		Prop	1.08	5.38	0.00	3.23	2.15	13.98	7.53	33.33	1.08	1.08	1.08	3.23	0.00	19.35	1.08	5.38	1.08		
		Total	7	17	3	25	6	21	9	40	2	2	1	57	4	31	1	23	6	255	
		Mean	0.39	0.94	0.17	1.39	0.33	1.17	0.50	2.22	0.11	0.11	0.06	3.17	0.22	1.72	0.06	1.28	0.33		
		Prop	2.75	6.67	1.18	9.80	2.35	8.24	3.53	15.69	0.78	0.78	0.39	22.35	1.57	12.16	0.39	9.02	2.35		

4.3 *Trichogramma*

Helicoverpa egg parasitism studies in 2001 and 2002 revealed *Trichogramma* effectively parasitise pest eggs in Katherine cotton early season, but parasitism wanes into cooler months and is not present late season (Fig 5, Appendix 1, page 129). In 2002, a subsample of Katherine *Trichogramma* specimens were identified via molecular means as *Trichogramma pretiosum*, suggesting the species has been introduced adventitiously, since its inoculation in the nearby ORIA in 1974, and superseded local species in Katherine cotton crops.

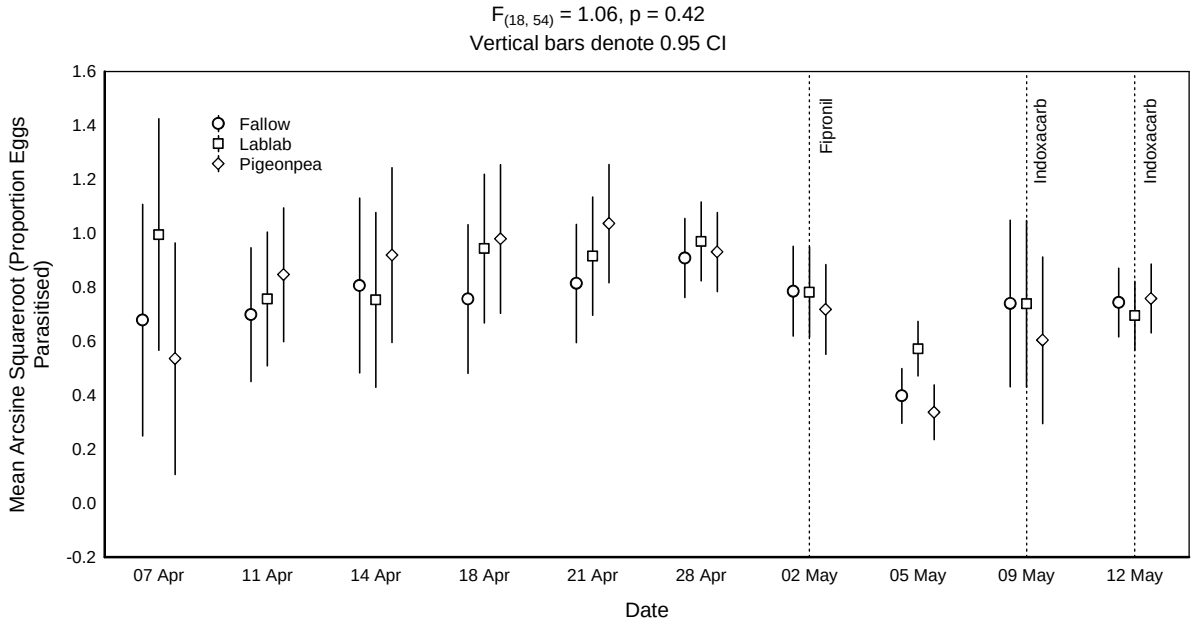

Figure 4.1. The relative proportion *Helicoverpa* eggs parasitised in cotton over time compared between companion crop treatments (see legend) grown under pivot irrigation at KRS and PCA in 2005. The nearest sample date preceding and active constituents of insecticide applications are given.

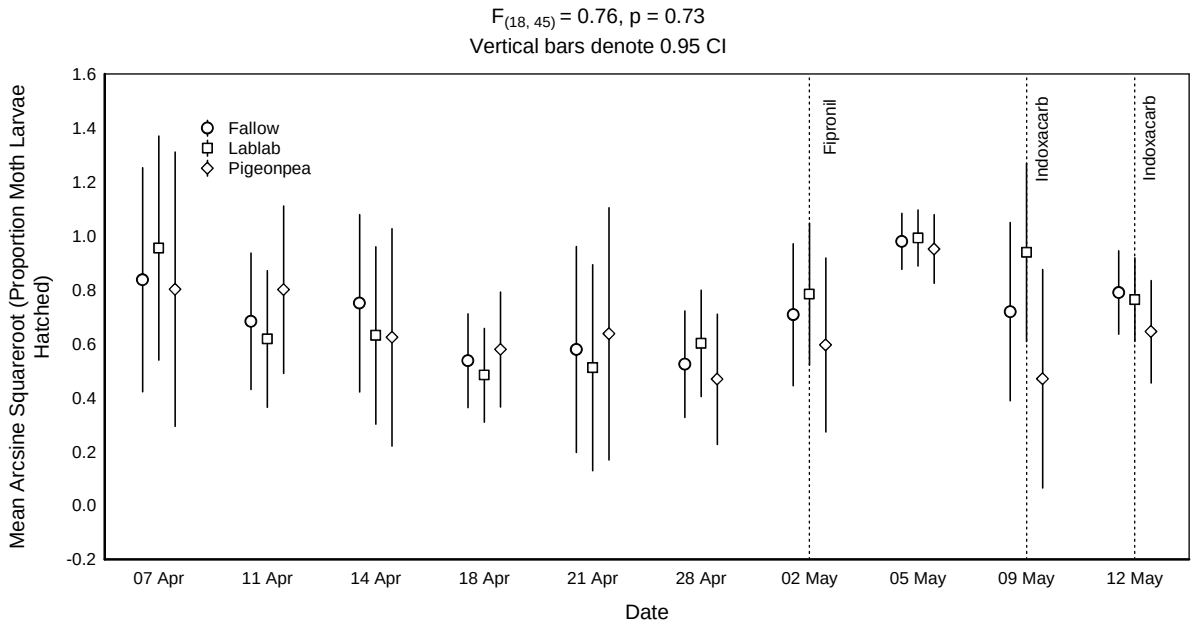

Figure 4.2. The relative proportion larval hatch from *Helicoverpa* eggs in cotton over time compared between companion crop treatments (see legend) grown under pivot irrigation at KRS and PCA in 2005. The nearest sample date preceding and active constituents of insecticide applications are given.

In both pivots during 2005, the proportion *Helicoverpa* eggs parasitised or successfully hatched as larvae was not significantly different between companion crop treatments ($F_{18, 54}$ = 1.06, P = 0.42; Figure 4.1 and $F_{18, 45}$ = 0.76, P = 0.73; Figure 4.2, respectively, both previous page).

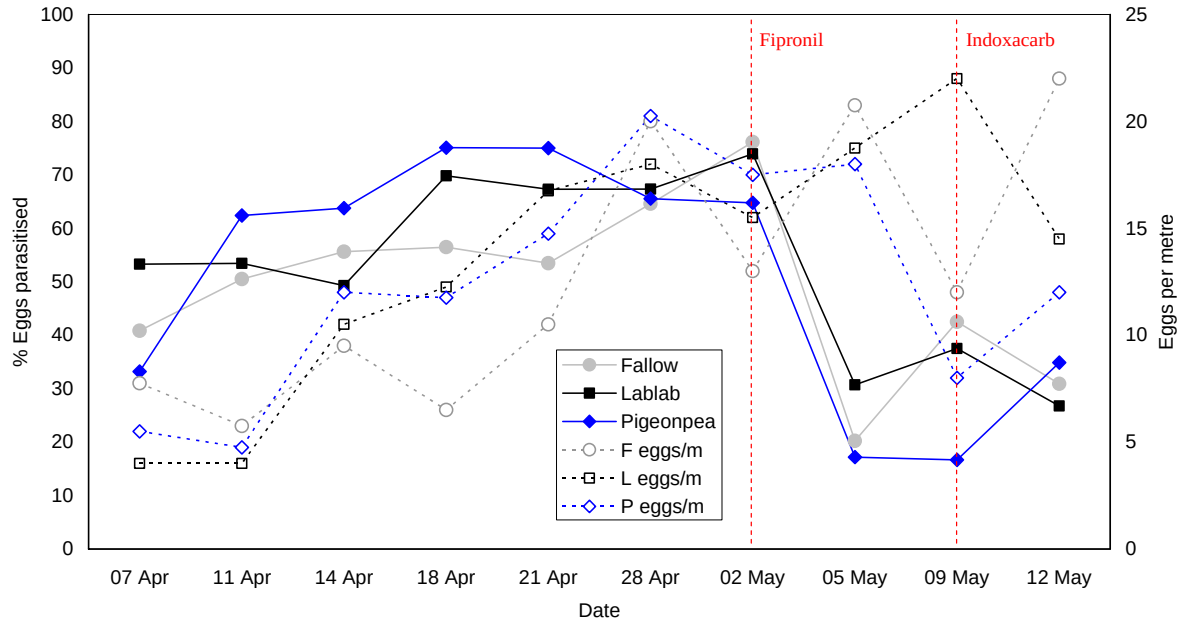

Figure 4.3. The percentage *Helicoverpa* eggs parasitised and egg density on each sampling occasion during intensive *Trichogramma* studies in cotton compared between companion crop treatments (see legend, F = Fallow, L = Lablab and P = Pigeon pea) grown under pivot irrigation at KRS and PCA in 2005. The nearest sample date preceding and active constituents of insecticide applications are given.

The percentage *Helicoverpa* eggs parasitised in all treatments increased from between 33.21 and 53.28% on 7 April to 64.71 and 76.15% on 2 May (Figure 4.3, above). The following fipronil application to control mirids across both pivots significantly reduced the proportion *Helicoverpa* eggs parasitised by 5 May (t = 6.61, df = 11, P = 0.000038, Figure 4.3). Indoxacarb was applied in the KRS pivot only on 9 May (Figure 4.3) so a t test for its influence on egg parasitism across both pivots was not possible. Following the fipronil and indoxacarb applications, parasitism levels remained relatively low and *Helicoverpa* egg densities declined to, and remained below, densities sufficient for accurate percentage parasitism calculations during the remainder of the season.

Discussion

Of the natural enemies catalogued during this project, spiders, coccinellids, syrphids and *Trichogramma* are most notable due to their relatively high abundance. *Helicoverpa* rarely develop past very small larval stage on Bollgard II® cotton in Katherine, so the importance of larval parasitise to control these pests is reduced, however, may increase in years when *Helicoverpa* densities drastically increase or should *Bt* resistance emerge in the future. *Spodoptera litura*, however, readily develop on and occasionally damage Bollgard II® in Katherine, so larval parasite species, their biology and the rate at which they attack this pest are worthy of investigation. Anthocorids are relatively minute predators credited with significant impact on pest insect densities in established crops other than cotton (Drukker *et al.* 2000, Shipp & Wang 2003, Rondon *et al.* 2004). Although pirate bugs have been recorded in Katherine cotton crops (Table 4.1), the impact of other anthocorids and small carabid

beetles on pest insects have been largely overlooked to date primarily due to their minute size. Assassin, big-eyed, brown smudge, damsel and predatory shield bugs, whose presence is recorded periodically, all contribute to pest insect density suppression, however, their individual impact and preferred prey species have not been investigated and this warrants rectification. Lacewings, red and blue beetles and six-spotted thrips seem relatively rare in Katherine cotton crops.

The impact of spiders on insect pests is often overlooked in biological control systems. Spiders do not fit the classical profile of biological control agents as they are generalist predators with relatively long generation time that are often territorial so relatively sedentary (Whitehouse *et al.* 2006). The seventeen spider families recorded from Katherine cotton in 2002 represent many spider predatory strategies, namely web spinning, snaring, jumping, stalking and ambush. The most common families, Oxyopidae (lynx or stalking spiders, 22.35%), Lycosidae (ground running or wolf spiders, 15.59%) and Salticidae (stalking spiders, 12.16%) are all hunting spiders. Oxyopids are known to be effective mirid hunters (Breene *et al.* 1989). Lycosids tend to hunt across the soil surface, hence the relatively large number caught in pitfall traps, and are known to hunt pest Lepidoptera and mirids in cotton (Hayes & Lockley 1990). Salticids are good predators of leafhoppers (Brown *et al.* 2003) and mirids (Breene *et al.* 1989). Resident spider fauna and their impact on pest insect densities, although relatively robust, require conservation season long in Katherine cotton IPM systems via judicious use of target selective insecticides.

Coccinellids and syrphids effectively suppress aphid and whitefly densities in Katherine cotton crops when not disrupted by insecticide use. If insecticide application is required to control insect pests, especially early season, careful consideration of their impact on these predators and the relative abundance of aphid and whitefly should be given. More specifically, target selective insecticides that do not harm these predators should be used at low rates whenever possible to avoid predatory disruption and possible flare in aphid or whitefly densities (Wilson *et al.* 1999). It is critical, therefore, to avoid broad spectrum insecticides where possible. This strategy is especially relevant when you consider that Biotype-B whitefly, potentially resistant to conventional insecticides and devastating at high densities in cotton, have previously, though rarely, been isolated in Katherine cotton crops.

Trichogramma are effective egg parasites of relatively dense *Helicoverpa* populations early season in Katherine cotton crops. By minimising *Helicoverpa* larval hatch and subsequent ingestion of cotton tissue, *Trichogramma* reduce the possibility of resistance development to *Bt* proteins in local Bollgard II® cotton. Unlike in the ORIA, where *Trichogramma* are important predators season long (Davies 2005), they are only prevalent early season in Katherine cotton, and do not persist past the onset of insecticide applications or into cooler winter months. *Trichogramma*, like all insects, are constrained by climatic conditions (Andrewartha & Birch 1954) and appear especially susceptible to insecticide applications in Katherine. Unsprayed companion crops provide refuge for *Trichogramma* populations and promote their effectiveness as predators in cotton (Virk *et al.* 2004), however, evidence suggests the presence of companion crops does not bolster the relatively low *Trichogramma* densities experienced during Katherine cotton trials. It is unfortunate that the sweet potato trial did not attract sufficient *Helicoverpa* nor *Trichogramma* to test if provision of hosts that do not attack cotton for *Trichogramma* proliferation would improve parasitism in neighbouring cotton crops. Sweet potato companion crops do not proliferate pests detrimental to cotton at least, so further investigation of their potential to increase *Trichogramma* densities and testing of this hypothesis is warranted.

This study is preliminary in nature and, in most cases, only catalogues predatory species in winter grown Katherine cotton. Scope for investigation of individual natural enemy impact on pest insects and further examination of spider, coccinellid, syrphid and *Trichogramma* biological control potential is warranted should cotton production progress in Katherine. Of critical importance is evidence suggesting insecticides severely disrupt natural enemy performance. Hence the conundrum regarding initiation of insecticidal control. It is crucial possible influence on all resident natural enemy and pest insect densities is carefully considered when insecticide application becomes imminent, and that insecticides are only used when absolutely necessary, at lowest possible rates and preferably later in the growing season to prolong natural enemy efficacy within the IPM system.

5.0 Sucking Pests

Introduction

Reduced broad-spectrum insecticide application attributed to the pest control action of transgenic cottons has elevated the status of previously controlled secondary pests, the majority of which are sucking insects impervious to *Bt* proteins produced by Bollgard II® (Fitt *et al.* 1994, Fitt 2000, Khan & Bauer 2002, Ward 2005). Relatively high density sucking pest populations can cause considerable damage to cotton plants (Khan & Bauer 2002), and thresholds to determine the necessity for chemical control in Katherine were examined. Selective insecticides that specifically target sucking pests prove useful when trying to maintain natural enemy numbers.

The major sucking pests in Katherine cotton crops are green mirids, brown mirids, GVB, RBSB, aphids and leafhoppers. Experiments designed to determine sucking pest thresholds for transgenic cotton crops in Katherine examined sucking insects as a suite of target pests. Graduated thresholds in replicated blocks were treated with selective insecticides when breached and cotton plant mapping and yield estimates were compared between thresholds and unsprayed and regularly sprayed control plots. Preliminary early season (pre-flowering) collective sucking pest thresholds were estimated at 0.5 per metre, although this threshold was not breached during trials (Appendix 1). Likewise, a control threshold of 0.5 sucking pests per metre was recommended late season (flowering to cutout) for Katherine cotton (Ward 2005). These results were not matched in large scale trials which proved inconclusive.

Rather than considering all sucking pests collectively, the pest status of, and plant damage attributed to, some sucking pests at the species level was examined. Comparative thresholds for species, once determined, can subsequently be incorporated into refined large scale trials with appropriate damage potential attributed to each. Mirid damage to transgenic cotton plants has been extensively examined in established Australian growing regions (Khan *et al.* 2004) and considered in Katherine. The recommended threshold of 0.5 per metre, which incorporates the ability for plants to compensate for mirid damage, is regarded as acceptable in Katherine. However, the species and relative pest status of green and brown mirids has been questioned (Malipatil & Cassis 1997) and requires clarification in Katherine. Several fields harboured dense populations of an unknown mirid (possibly *Campylomma austrina* Malipatil) for a short period mid-season, but voucher specimens were lost by departmental taxonomists prior to identification. Understanding the species and pest status and damage potential of emerging pests, such as RBSBs and leafhoppers, is necessary prior to development of an holistic approach to sucking pest control.

Methodology

5.1 Preliminary sucking pest trial

For preliminary sucking pest trial method see Appendix 1 & Ward (2005).

5.2 Large scale sucking pest trials

The suite of sucking pests examined in this trial included mirid adults, mirid nymphs, GVB and RBSB. Experimental plots were visually sampled twice per week and the density of each species summed to determine total sucking pests per metre in each treatment. Plots were sprayed with fipronil (62.5ml/Ha) when threshold treatments were breached or on a calendar basis (every nine days) in regularly treated controls. Early season trials were conducted from

first square to first flower reverting to standard control measure (0.5 sucking pests per metre) till cutout, and late season trials were subject to standard control measures till first flower, then experimental treatments till cutout. Three one metre row plant maps per plot were completed for each trial at cutout, and yields were estimated by hand (three randomly selected five metre rows per plot) and machine (20 metre row per plot in 2003 only) picking. In 2004, cotton fibre samples from both trials were examined for treatment effects. All plots were 20 rows wide and 30 metres long.

In 2003, the early season sucking pest trial in A2 comprised six (unsprayed - Uc, regularly sprayed - Reg, 0.5, 1.0, 2.0 and 3.0 sucking pests per metre) and the late season trial in A3 five (Uc, Reg, 0.5, 1.0 and 1.5 sucking pests per metre) treatments in four replicates (RCB).

In 2004, both the early (PCA) and late (KRS) trials utilised six treatments (Uc, Reg, 0.5, 1.0, 2.0 and 3.0 sucking pests per metre) in four replicates (RCB).

All analyses for sucking pest trials involved ANOVA with Tukey test for treatment effects when samples numbers were consistent between plots. For plant map data, proportions were arcsine square root transformed to normalise distribution prior to ANOVA and significant treatment differences were examined by plotting means with 95% CI's. If CI's did not overlap, means were considered significantly different.

5.3 RBSB

Four 289B® cotton seedlings were raised in each of 24 30cm diametre pots of topsoil, potting mix and vermiculite (15L: 2.5L: 5kg = 3 pots) with Osmocote® for potted plants as fertiliser and culled to single healthiest at four leaves for the KRS shadehouse small scale RBSB trial. During ongoing growth, cotton plants were supported by cane poles due to rank height associated with light constraints in overcast wet season conditions under shadecloth, and periodic mite infestations were controlled *ad hoc* with neem extract. Six organza lined cages (100 by 40 by 40cm) only were available for the experiment, so six plants (three treatment and three controls) were selected at random from the healthiest for each of early (first flower) and late (15 node) exposure to RBSBs. Three newly moulted adult RBSB's from a culture maintained on washed green beans at 33 ± 5 ºC, 12:12 L:D and 65 ± 10% RH in the KRS insectary were assigned to each of three randomly selected plants placed in cages. The remaining three plants were caged with no RBSBs. For each of the two exposure periods, all experimental cotton plants were caged on a table outside the shadehouse, to avoid its possible infestation, for ten days. The number of surviving RBSB per cage was counted daily, and plants were searched for removal of RBSB and their eggs and returned to the shadehouse following exposure. When bolls began to split, boll count by position and node per plant was analysed for treatment effects with ANCOVA and bolls removed for weighing. Boll weight by node was analysed for positions one and two separately for treatment effects with ANCOVA.

The field scale RBSB trial area in the KRS pivot was chosen according to location (outside of regular insect sample zones) and relative consistency of cotton plant vigour with 10 metre buffer zones to regular pest control areas. Exposure cages (single bed mosquito nets) covering one metre of cotton plants were erected every second row in radially segregated replicated blocks to ensure all treatments fell beneath the same pivot sprinkler (see Figure 5.1, next page). Due to inconsistent plant stand, thinning to standardise plant number was not possible, so the number of plants in each metre cage was recorded. Treatments included 3, 6 and 12

RBSBs and controls with no RBSB per cage. Adult RBSBs were collected by mechanical vacuum from pigeon pea companion crops on the day the experiment began as sufficient numbers could not be maintained in the insectary. Robust RBSB were selected for the experiment, with ten control individuals maintained on green beans at 33 ± 5 ºC, 8:16 L:D and 65 ± 10% RH in a cage in the KRS insectary for experiment duration. Exposure lasted ten days at late cotton plant flowering as this is when RBSB were prevalent. All cages were sprayed out with bioallethrin and bioresmthrin (Mortein® ultra low allergenic fly spray) prior to and at completion of insect exposure. RBSB were inserted following the irrigation after initial spray out to limit the impact of residual Mortein® on their survival. The number of live RBSB per cage was recorded prior to spraying out at completion of exposure. Cotton plants in each cage were plant mapped at time of netting and prior to harvest. Cages were not removed and small insects that penetrated cage mesh sprayed out *ad hoc* with Mortein® till harvest to avoid possible further insect damage. At harvest, each boll, its position and plant and cage number were recorded prior to lint weighing. Lint was then sent to ACRI for fibre testing.

Figure 5.1. Exposure cage orientation for the RBSB cotton plant damage assessment trial in the KRS pivot in 2005. Replicated blocks are separated by star pickets and the slashed area on the right (for tractor turn around) indicates the edge of the buffer zone.

Boll number, lint weight, fibre quality variables and the number of new bolls per plant following exposure were tested for treatment effects with ANOVA and Tukey test. Plant map variables were compared pre- and post-RBSB exposure with paired dependent variable (delta variable) ANOVA using no bug controls as constants for all bolls, bolls above position 10 only and bolls in third and fourth position only. The latter two analyses were designed to assess treatment effects on young bolls at exposure. A Dunnett analysis of treatment means for plant map variables was also performed.

5.4 Leafhoppers

Leafhoppers infested B1 and T1 (Figure 1.2) late season in 2005 so analyses were confined to these fields.

The impact of graduated leafhopper damage on cotton yield was examined in B1. Ten one metre row sections in areas each of light, medium and heavy leafhopper feeding damage (hopper burn) were marked with flagging tape prior to defoliation. At harvest, each flagged sample had all cotton lint hand picked, weighed and a subsample sent to ACRI for fibre testing. Lint weight and fibre quality variables were analysed for treatment effects with ANOVA.

The rate of hopper burn in B1 cotton was examined for companion crop treatment effects as follows. In each treatment plot, the leaves on nodes 1, 4, 7, 10 and 13 counting down from the growing tip of ten randomly chosen cotton plants were assessed for proportion damage symptoms (surface reddening) evident. The presence or absence of *alternaria* infection was also recorded for each leaf. Proportion damage was arcsine square root transformed to normalise the distribution before further analysis. Treatment effects were examined by GLM (Poisson) with a logit link and Tukey test, treatment and node effects by GLM (Poisson) and *alternaria* effects with ANOVA as infection was recorded as a categorical variable.

Symptoms of alternaria infection over time were recorded photographically for 5 randomly selected cotton leaves in B1.

Cotton varietal response to heavy leafhopper infestation was evident in the CSIRO variety trial in T1. Photographic evidence of this phenomenon was recorded.

Results

5.1 Preliminary sucking pest trial

2002

Small scale trials conducted before 2003 examined the impact of graduated treatment thresholds for a suite of sucking pests on cotton yield in Katherine. A combined treatment threshold of 0.5 per metre was recommended to minimise sucking pest damage in late season cotton (Ward 2005, Appendix 13, page 151). Trials aimed at verifying preliminary results at larger scales were relatively unsuccessful.

5.2 Large scale sucking pest trials

2003

Thresholds were breached and fipronil applied on only two occasions (16 May and 20 May) in two different treatments (0.5 and 1.0 thresholds, respectively) during the 2003 early season sucking pest trial (Figure 5.2, below).

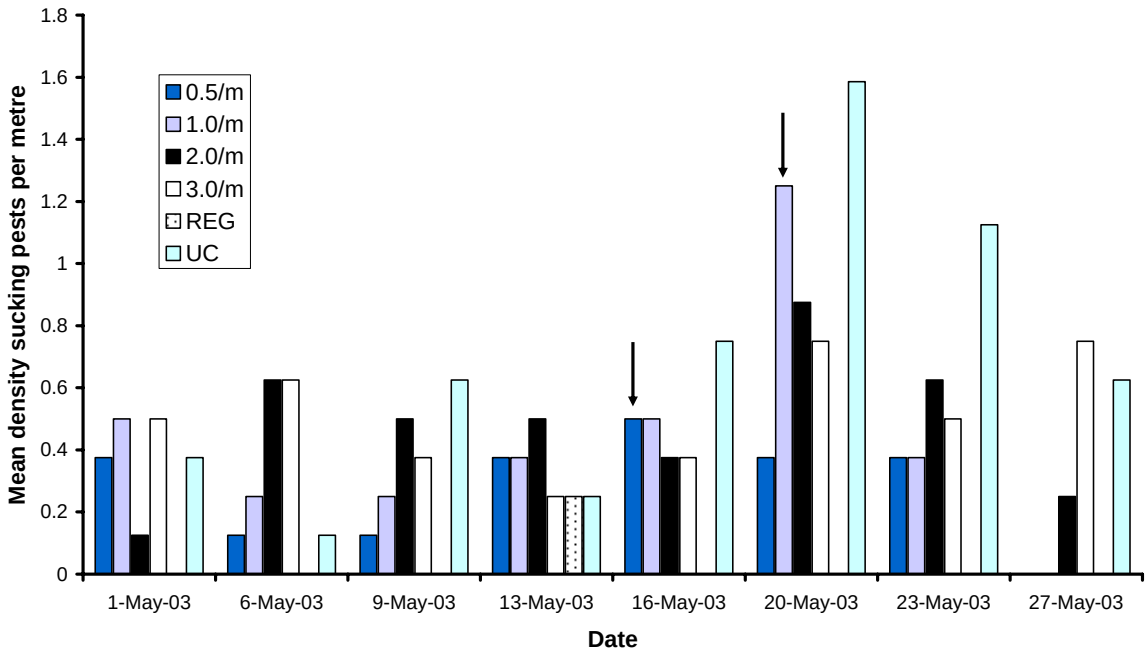

Figure 5.2. The mean density of sucking pests in each treatment (see legend) during the 2003 early season sucking pest trial at PCA. Arrows indicate threshold exceeded and fipronil insecticide applied. REG = regularly controlled and UC = uncontrolled.

Likewise, thresholds were breached and fipronil applied on only two occasions (17 June and 14 August) in two different treatments (1.5 and 0.5 thresholds, respectively) during the 2003 late season sucking pest trial (Figure 5.3, next page).

There was no significant difference in hand picked and machine harvested yield estimates between treatments in both the early and late season sucking pest trials in 2003 (Appendix 10, page 149). Likewise, all plant mapping variables were not significantly different between treatments in both 2003 sucking pest trials (Appendix 11, page 149).

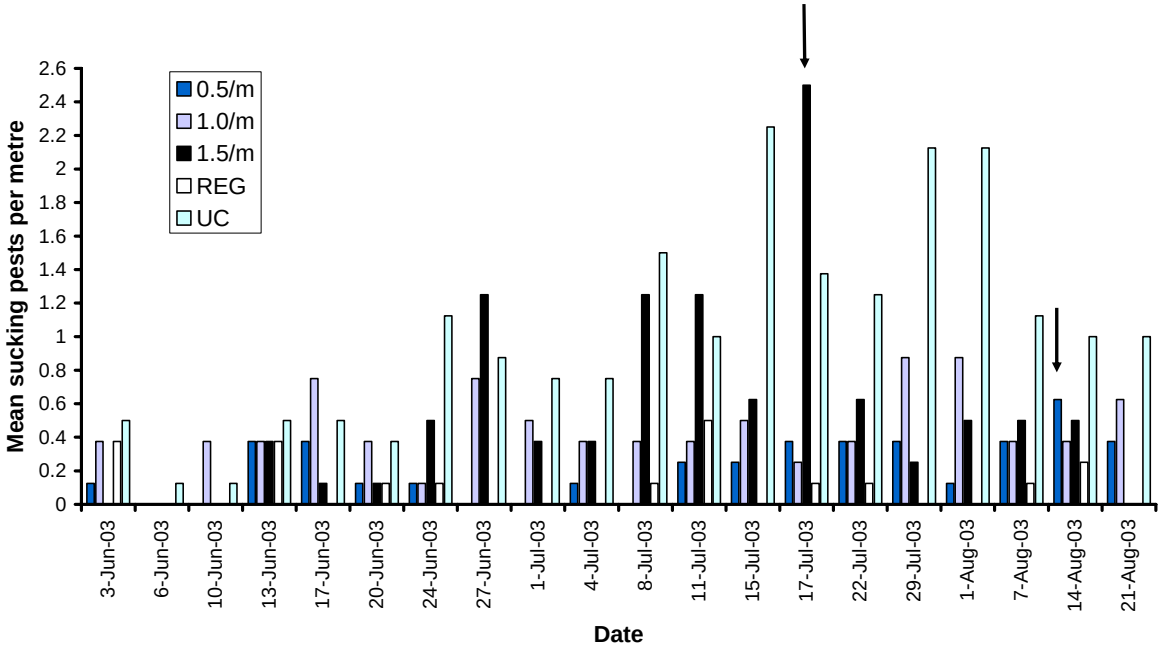

Figure 5.3. The mean density of sucking pests in each treatment (see legend) during the 2003 late season sucking pest trial at KRS. Arrows indicate threshold exceeded and fipronil insecticide applied. REG = regularly controlled and UC = uncontrolled.

2004

Early season

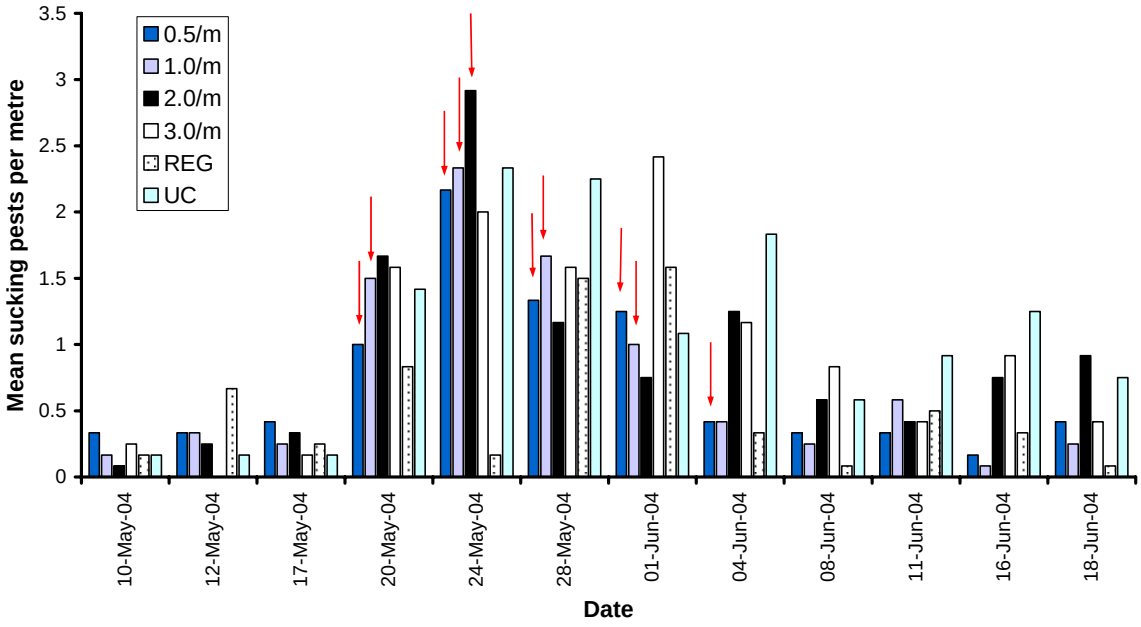

Figure 5.4. The mean density of sucking pests in each treatment (see legend) during the 2004 early season sucking pest trial at PCA. Arrows indicate threshold exceeded and fipronil insecticide applied. REG = regularly controlled and UC = uncontrolled.

During the 2004 early season sucking pest trial, thresholds were exceeded and fipronil insecticide applied following consecutive sampling occasions five times from 20 May to 4 June in 0.5, four times from 20 May to 1 June in 1.0 and once only on 24 May in 2.0 threshold treatments (Figure 5.4, previous page). The 3.0 threshold was never breached (Figure 5.4).

There was no significant difference in the number of bolls, bolls by position, proportion bolls per plant, plant stand nor handpicked yield estimates between treatments in the 2004 early season sucking pest trial ($F_{5, 87} = 1.27$, $P = 0.28$; $F_{5, 1770} = 0.96$, $P = 0.44$; $F_{5, 87} = 1.27$, $P = 0.29$; $F_{5, 87} = 0.74$, $P = 0.60$; $F_{5, 15} = 1.87$, $P = 0.16$, respectively). Fibre quality analyses from the same trial revealed no significant difference between treatments (Table 5.1, below).

Table 5.1. Results from analyses of fibre quality comparisons between treatments from early and late season sucking pests trials conducted under pivot irrigation at PCA and KRS, respectively, in 2004. sfi = short fibre index, *P* = probability and there are 5 and 15 degrees of freedom for all analyses. Significant probabilities are shaded.

Trial	Variable	*F* value	*P*
Early season	Length	1.27	0.33
	Uniformity	1.79	0.18
	sfi	1.37	0.29
	Strength	0.60	0.70
	Elongation	0.77	0.59
	Micronaire	0.4	0.84
Late season	Length	1.02	0.43
	Uniformity	0.62	0.69
	sfi	1.07	0.42
	Strength	4.23	0.013
	Elongation	1.44	0.27
	Micronaire	2.12	0.12

Analyses of early season sucking pest trial plant map data revealed a significant difference in the height, number of nodes and fruiting branches only between treatments (Table 5.2, next page). Figure 5.5, page 63, suggests field variability may have played a larger role than sucking insect control thresholds in determining plant vigour, as plants in regularly controlled plots (Reg) were significantly shorter than those in uncontrolled (Uc), 0.5 (Half) and 3.0 (Three) threshold plots, and shorter than, but not significantly different to, plants in 1.0 (One) and 2.0 (Two) threshold plots . The number of nodes and fruiting branches in regularly controlled plots was significantly lower than all other treatments, except 2.0 threshold (Figures 5.6 & 5.7, pages 63 & 64, respectively).

Table 5.2. Results from analyses of plant map data comparisons between treatments from early and late season sucking pests trials conducted under pivot irrigation at PCA and KRS, respectively, in 2004. *P* = probability and df = degrees of freedom. Significant probabilities are shaded.

Trial	df	Variable	*F* value	*P*
Early season	5, 575	Height	9.22	1.84E-08
		Nodes	3.16	0.0079
		Fruiting branches	3.08	0.0094
		First fruit branch	0.38	0.86
		Vegetative branches	0.36	0.87
		Vegetative bolls	0.33	0.9
		Total bolls	1.12	0.35
		Fruit position 1	1.08	0.37
		Fruit position 2	1.87	0.1
		Fruit position 3	1.22	0.3
		Fruit position 4	1.22	0.35
		Proportion vegetative bolls	1.28	0.27
		Retention position 1	1.7	0.13
		Retention position 2	1.71	0.13
		Retention position 3	0.92	0.47
		Retention position 4	0.92	0.47
Late season	5, 554	Height	12.69	1.08E-11
		Nodes	12.94	6.33E-12
		Fruiting branches	12.15	3.39E-11
		First fruit branch	1.28	0.27
		Vegetative branches	1.43	0.21
		Vegetative bolls	1.98	0.08
		Total bolls	12.72	1.03E-11
		Fruit position 1	4.65	0.00036
		Fruit position 2	9.59	8.61E-09
		Fruit position 3	7.18	1.60E-06
		Fruit position 4	0.63	0.68
		Proportion vegetative bolls	2.2	0.052
		Retention position 1	7.38	1.04E-06
		Retention position 2	13.09	4.65E-12
		Retention position 3	9.53	9.87E-09
		Retention position 4	0.99	0.42

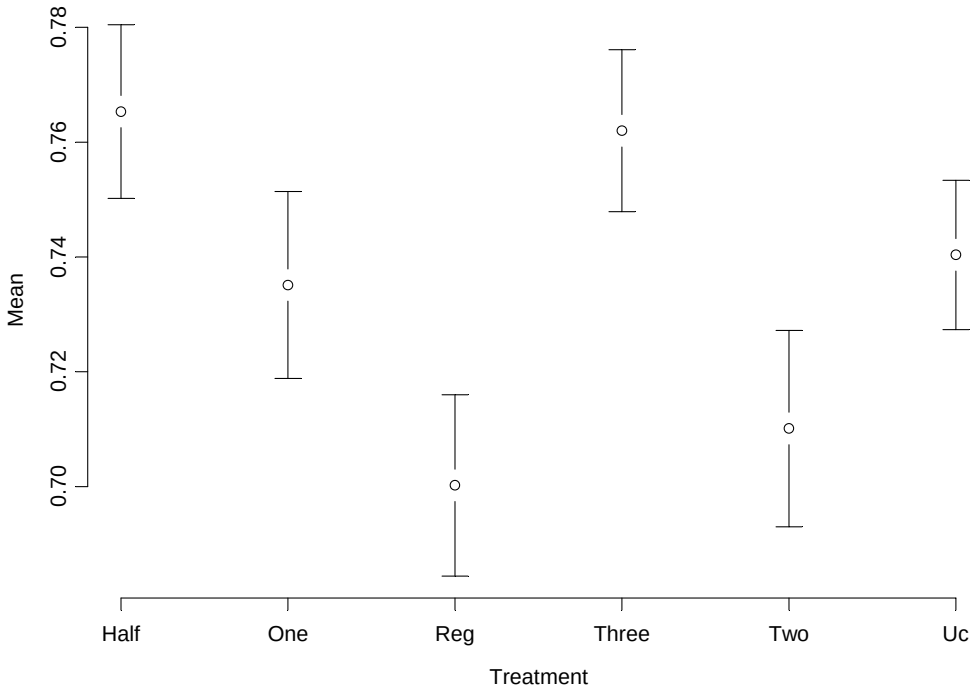

Figure 5.5. Mean cotton plant heights for each treatment in the early season sucking pest trial conducted at PCA in 2004. Error bars denote 95% CI.

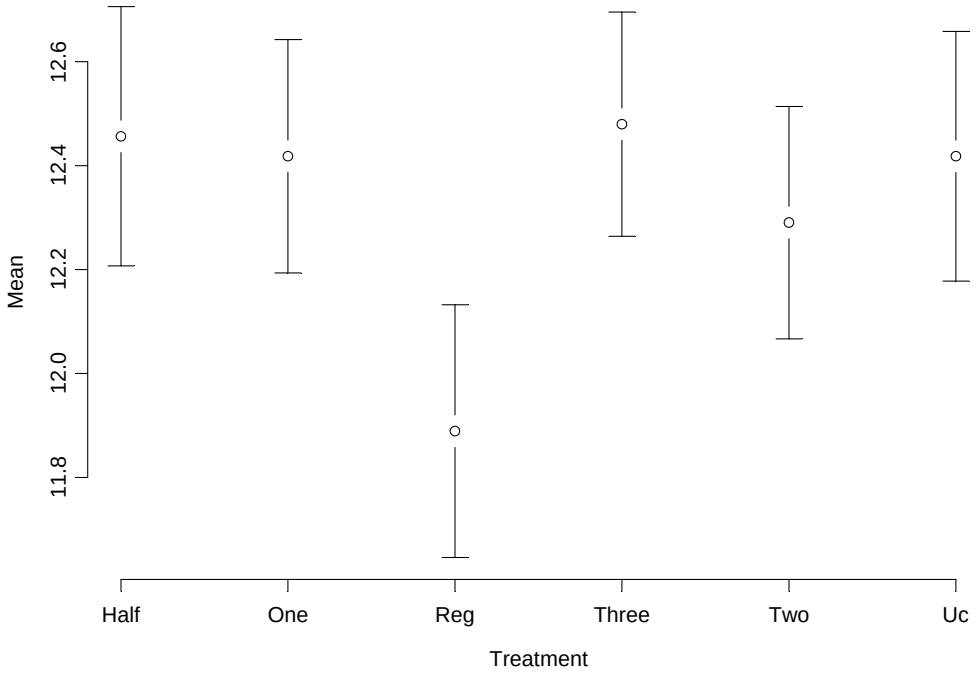

Figure 5.6. Mean number of cotton plant nodes for each treatment in the early season sucking pest trial conducted at PCA in 2004. Error bars denote 95% CI.

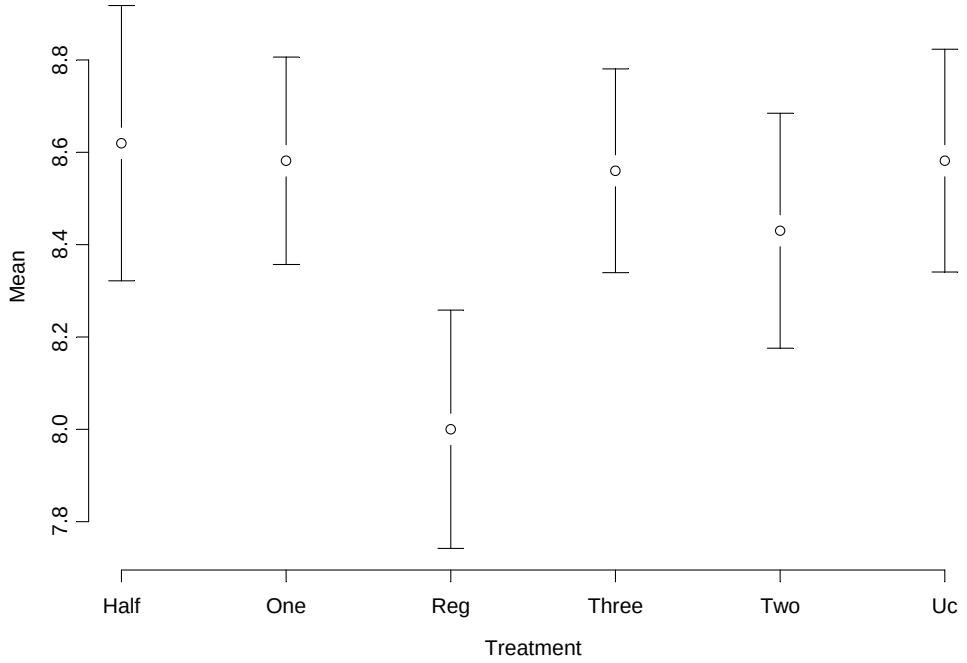

Figure 5.7. Mean number of cotton plant fruiting branches for each treatment in the early season sucking pest trial conducted at PCA in 2004. Error bars denote 95% CI.

Late season

During the 2004 late season sucking pest trial, the 0.5 threshold was breached and fipronil insecticide applied on 1, 4, 18 and 25 June and 30 July (Figure 5.8, next page). Fipronil was applied to 1.0 threshold plots when breached on 1 and 29 June (Figure 5.8). The 2.0 and 3.0 thresholds were never breached (Figure 5.8).

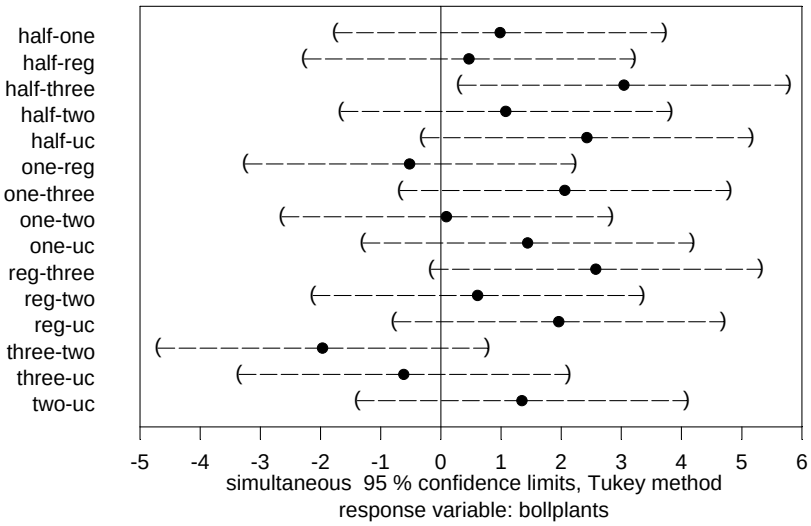

Figure 5.9. Tukey comparison of simultaneous 95% confidence limits for treatment effects on proportion bolls per plant from the pivot irrigated late season sucking pest trial at KRS in 2004.

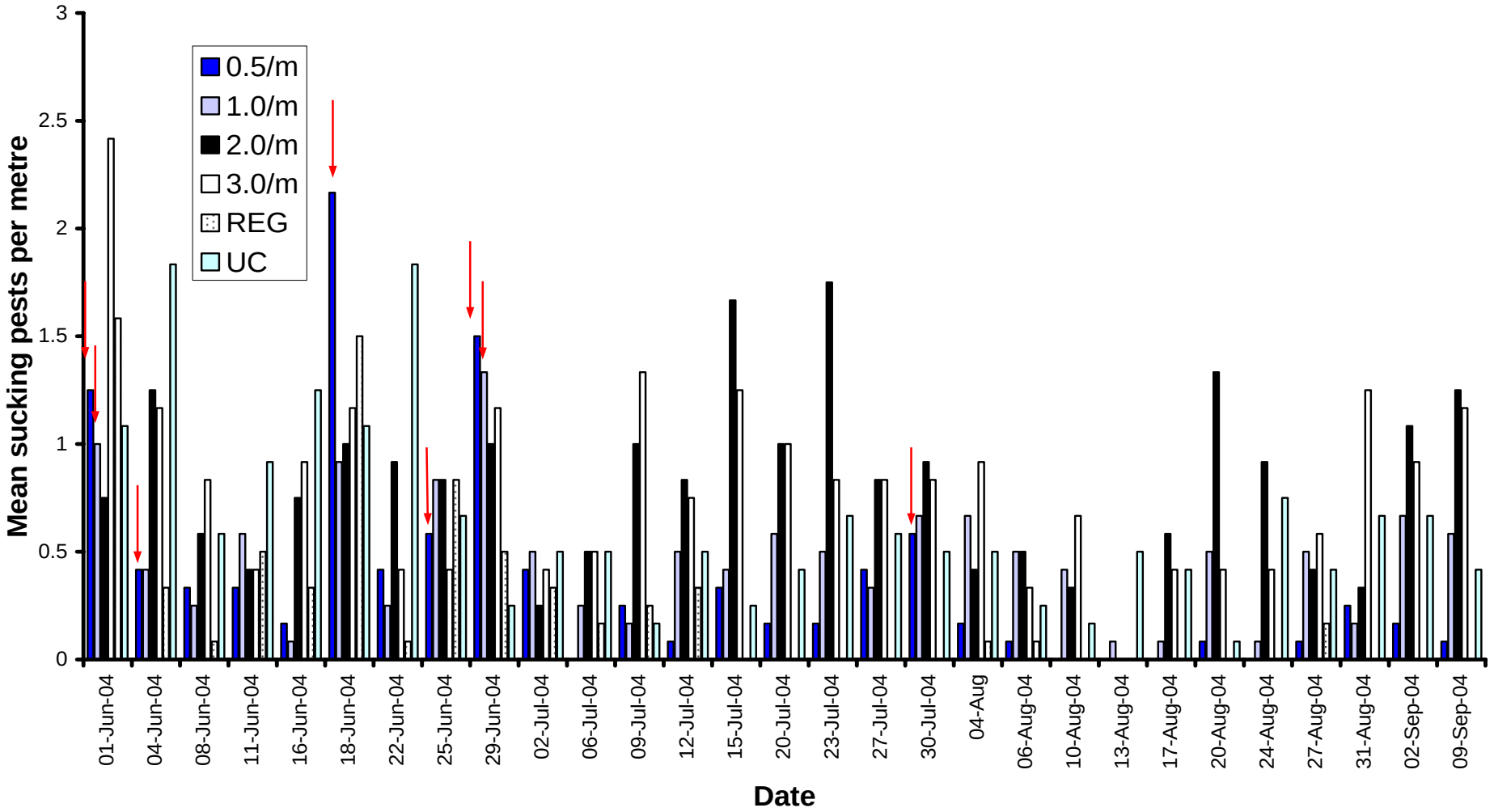

Figure 5.8. The mean density of sucking pests in each treatment (see legend) during the 2004 late season sucking pest trial at KRS. Arrows indicate threshold exceeded and fipronil insecticide applied. REG = regularly controlled and UC = uncontrolled.

There was no significant difference in the number of bolls, plant stand or hand picked yield estimates between treatments in the 2004 late season sucking pest trial ($F_{5, 87}$ = 1.79, P = 0.12; $F_{5, 87}$ = 1.27, P = 0.28; and $F_{5, 15}$ = 1.21, P = 0.35, respectively). The proportion bolls per plant was significantly different between the 0.5 and 3.0 threshold treatments only ($F_{5, 87}$ = 3.10, P = 0.01, Figure 5.9, page 64) and the number of bolls by position in 3.0 threshold was significantly different to all other treatments ($F_{5, 1707}$ = 11.41, P = 7.46E-11, Figure 5.10, below).

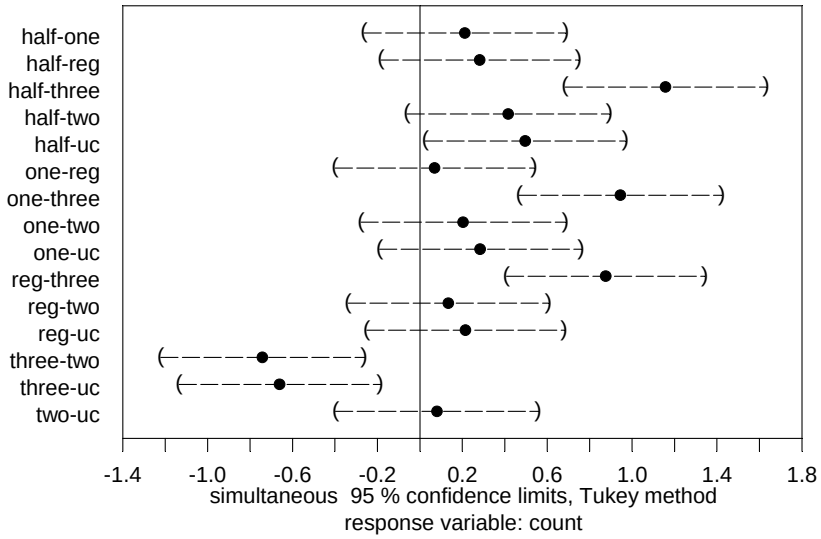

Figure 5.10. Tukey comparison of simultaneous 95% confidence limits for treatment effects on boll number by position from the pivot irrigated late season sucking pest trial at KRS in 2004.

The fibre strength of cotton harvested from uncontrolled was significantly lower than that from 1.0 threshold and regularly controlled treatment plots (Table 5.1 & Figure 5.11, below). All other fibre quality variables were not significantly different between treatments in the late season sucking pest trial (Table 5.1).

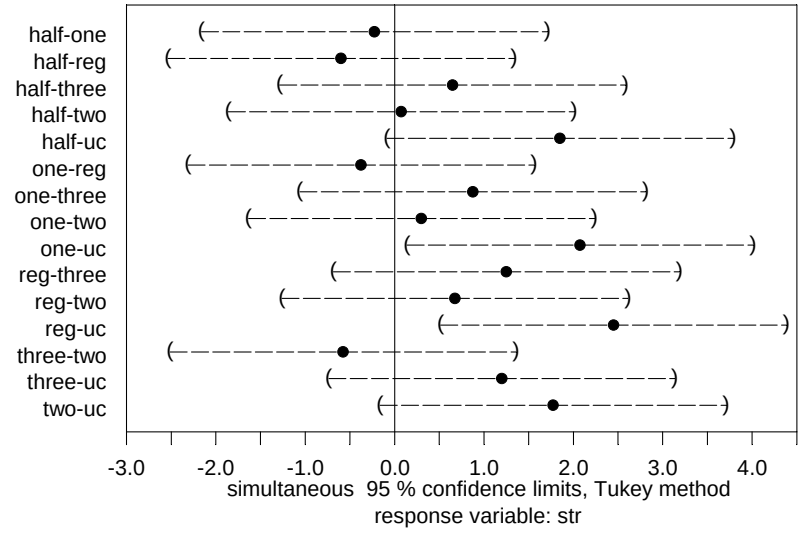

Figure 5.11. Tukey comparison of simultaneous 95% confidence limits for treatment effects on cotton fibre strength from the pivot irrigated late season sucking pest trial at KRS in 2004.

Sixty-two point five percent of plant map variables were significantly different between treatments in the late season sucking pest trial (Table 5.2). The first fruiting branch, the number of vegetative branches and bolls, proportion vegetative bolls and number and retention of fruit in position four were not significantly different (Table 5.2). Plant heights in the uncontrolled and regularly controlled plots were significantly shorter than those in all other treatments (Table 5.2 & Figure 5.12, below).

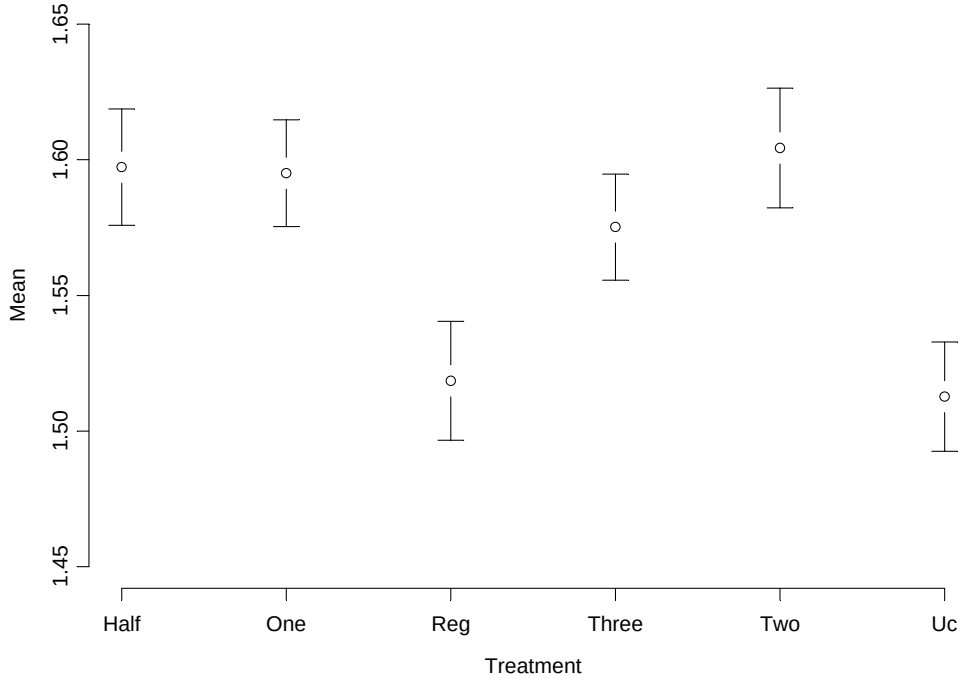

Figure 5.12. Mean cotton plant height for each treatment in the late season sucking pest trial conducted at KRS in 2004. Error bars denote 95% CI.

Plants in the 0.5 threshold, regularly and uncontrolled plots had significantly less nodes and fruiting branches than those in the 1.0, 2.0 and 3.0 threshold plots (Table 5.2 & Figures 5.13 & 5.14, below and next page, respectively).

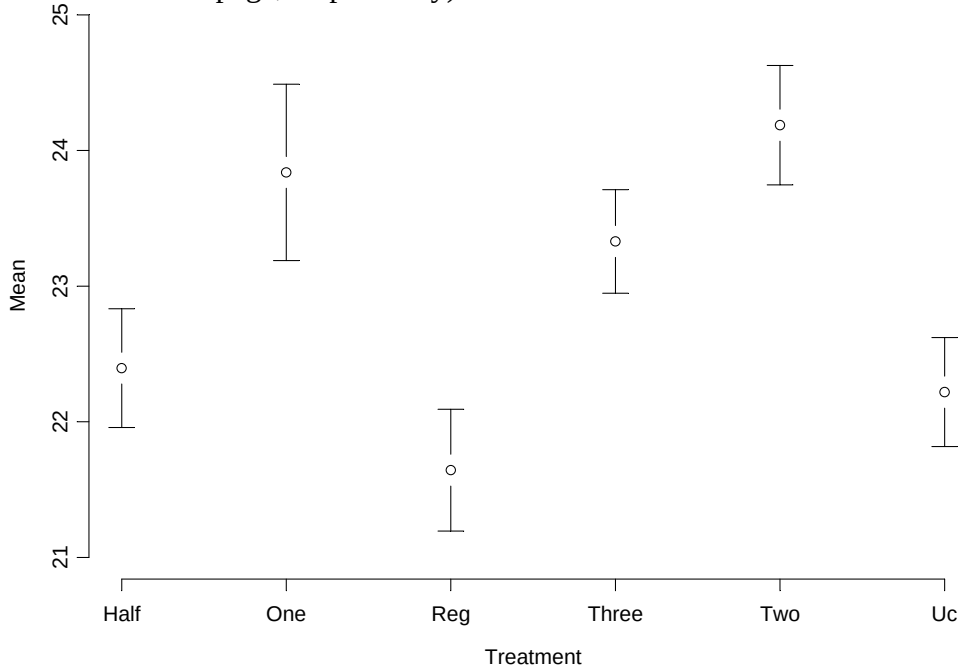

Figure 5.13. Mean number of cotton plant nodes for each treatment in the late season sucking pest trial conducted at KRS in 2004. Error bars denote 95% CI.

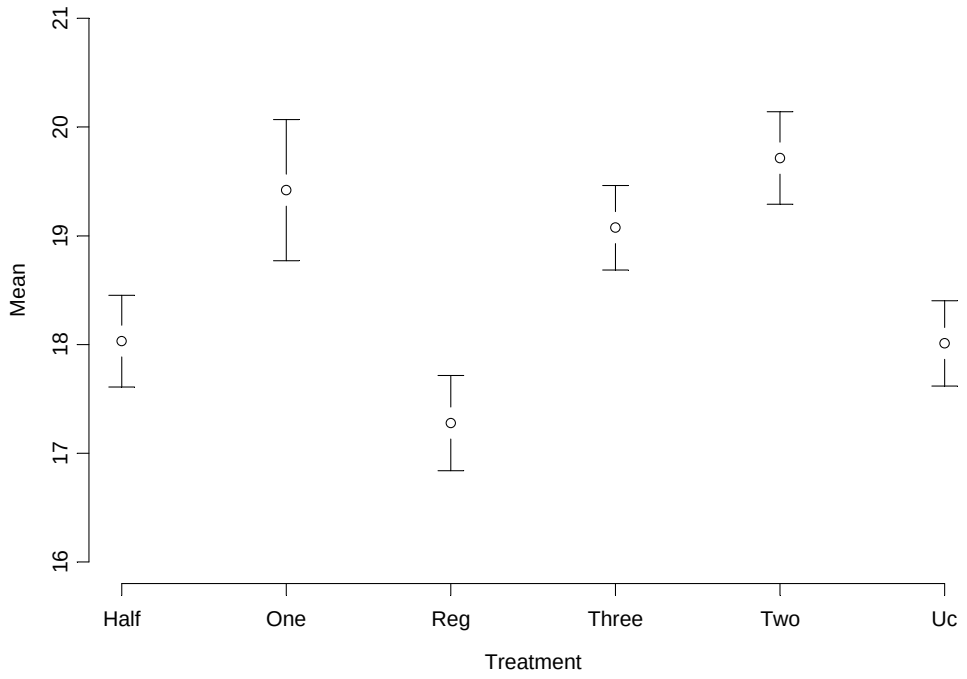

Figure 5.14. Mean number of cotton plant fruiting branches for each treatment in the late season sucking pest trial conducted at KRS in 2004. Error bars denote 95% CI.

There were significantly less bolls in 3.0 threshold plots than all other treatments (Table 5.2 and Figure 5.15, below).

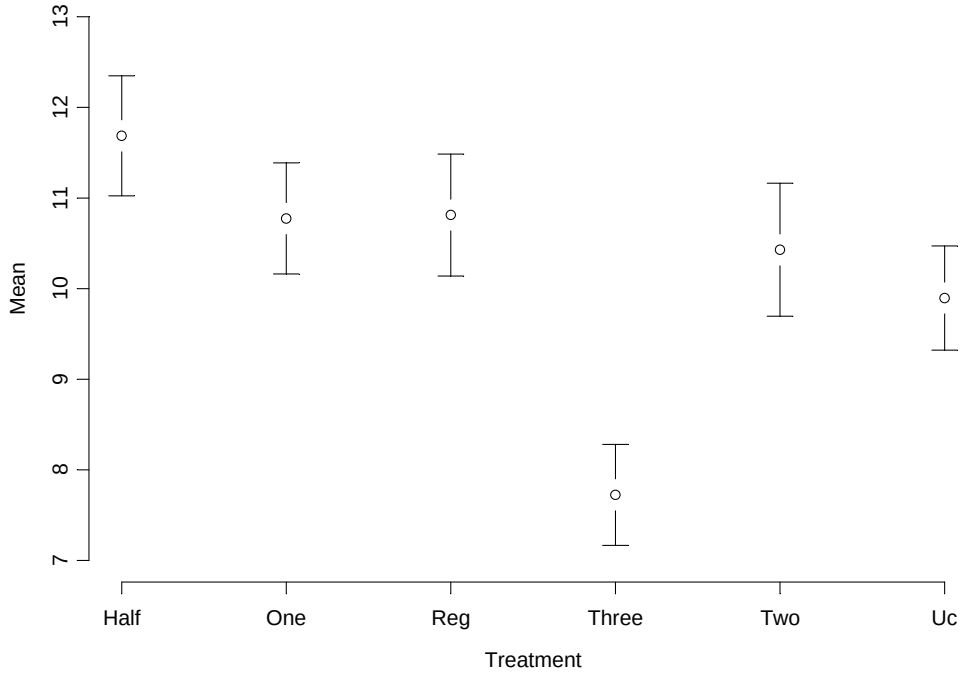

Figure 5.15. Mean number of cotton bolls for each treatment in the late season sucking pest trial conducted at KRS in 2004. Error bars denote 95% CI.

The number of position one fruit was significantly lower in the 3.0 threshold than all treatments excluding uncontrolled (Table 5.2 & Figure 5.16, below).

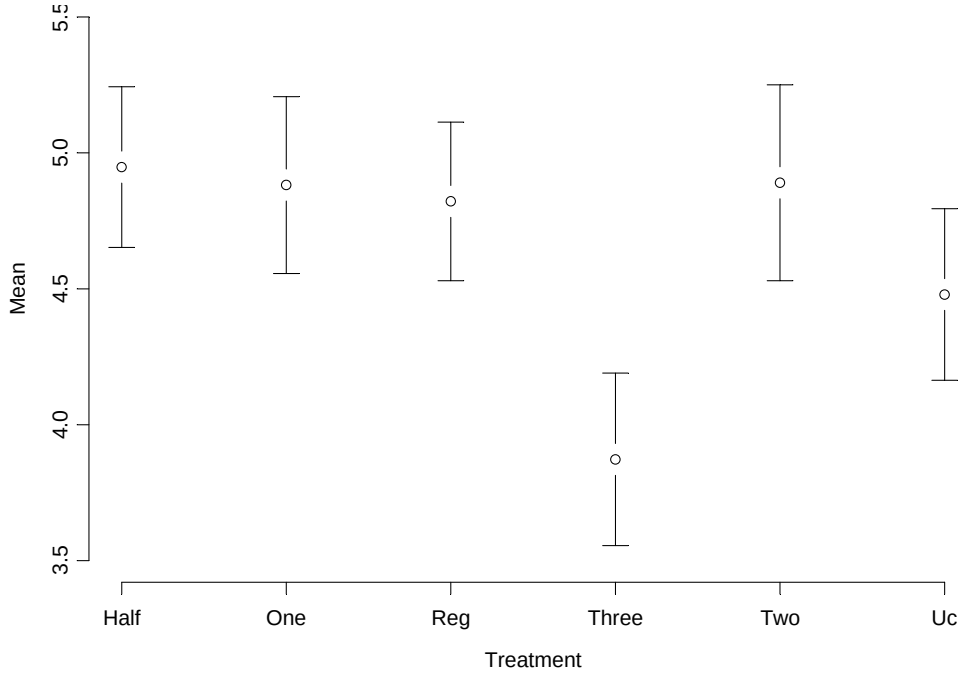

Figure 5.16. Mean number of position one fruit for each treatment in the late season sucking pest trial conducted at KRS in 2004. Error bars denote 95% CI.

Significantly less position two fruit were present in the 3.0 threshold than all other treatments (Table 5.2 & Figure 5.17, below). The 0.5 threshold had significantly more position two fruit than all other treatments, excluding 1.0 threshold (Table 5.2 & Figure 5.17).

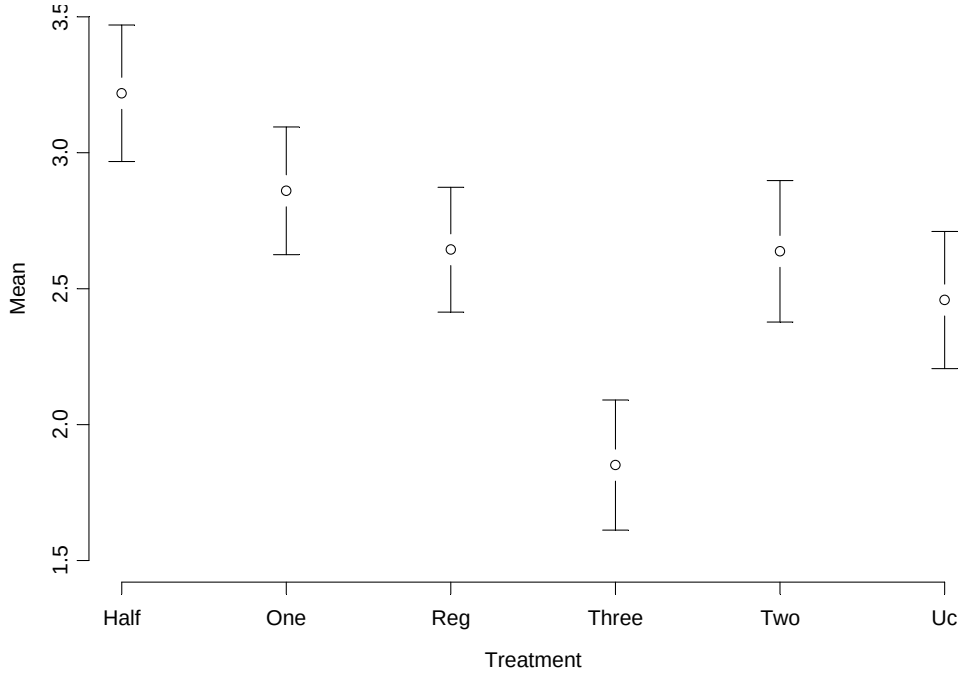

Figure 5.17. Mean number of position two fruit for each treatment in the late season sucking pest trial conducted at KRS in 2004. Error bars denote 95% CI.

The 3.0 threshold had significantly less position three fruit than all other treatments, excluding 2.0 threshold (Table 5.2 & Figure 5.18, below).

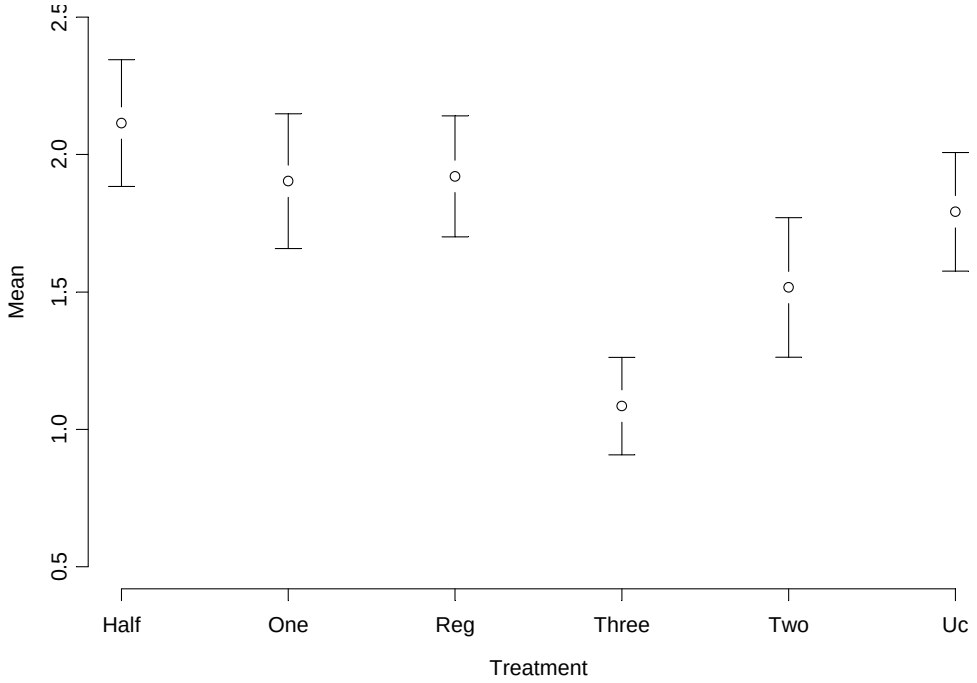

Figure 5.18. Mean number of position three fruit for each treatment in the late season sucking pest trial conducted at KRS in 2004. Error bars denote 95% CI.

Position one fruit retention was significantly less in 3.0 threshold than all other treatments (Table 5.2 & Figure 5.19, below).

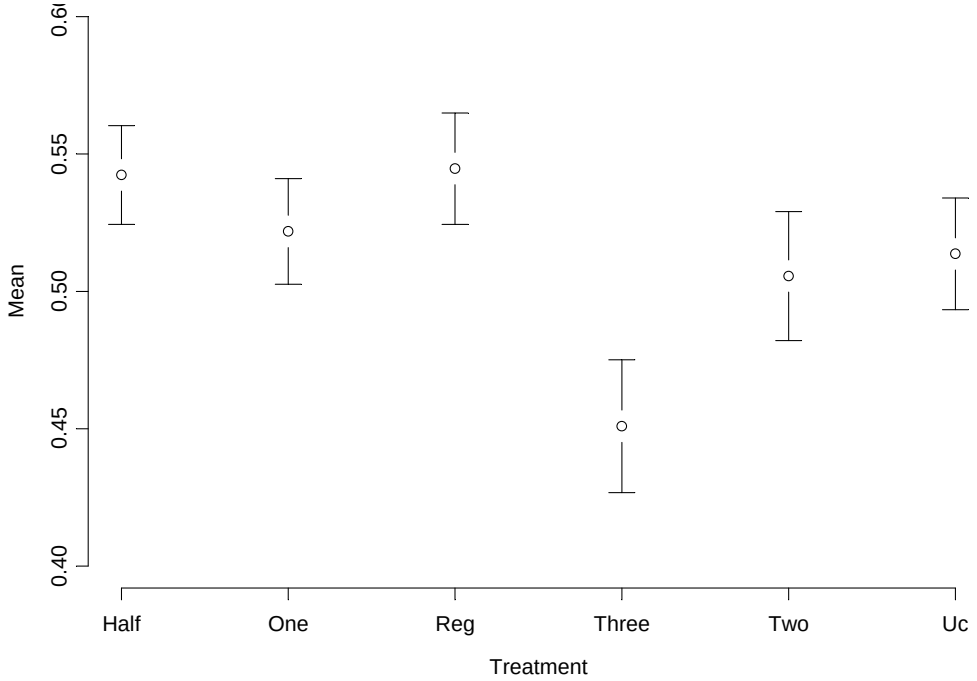

Figure 5.19. Mean retention of position one fruit for each treatment in the late season sucking pest trial conducted at KRS in 2004. Error bars denote 95% CI.

The 3.0 threshold had significantly less position two fruit retention than all other treatments (Table 5.2 & Figure 5.20, below). Except for 1.0 threshold and regularly controlled, 0.5 threshold had significantly more position two fruit retention than other treatments (Table 5.2 & Figure 5.20).

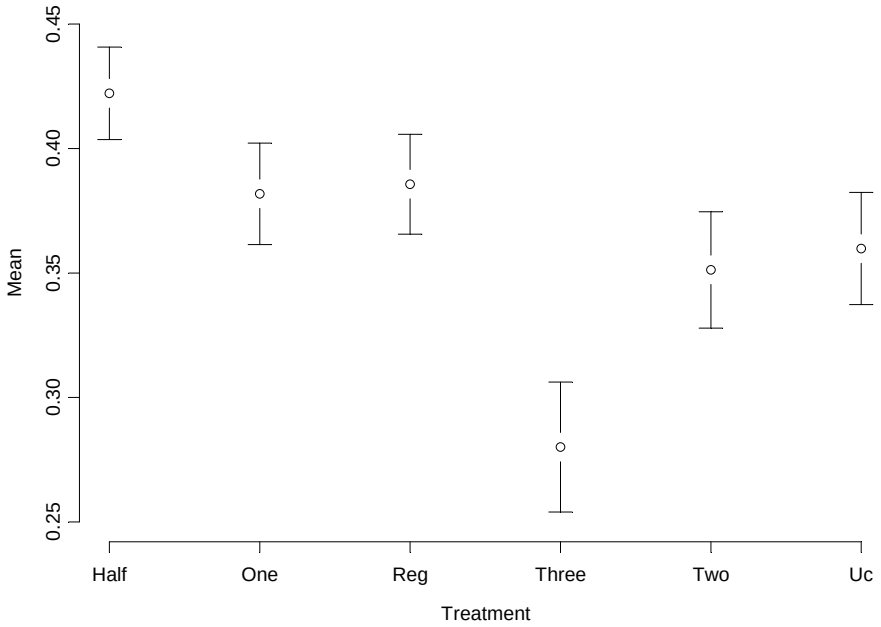

Figure 5.20. Mean retention of position two fruit for each treatment in the late season sucking pest trial conducted at KRS in 2004. Error bars denote 95% CI.

There was significantly more position three fruit retention in 0.5 than 2.0 and 3.0 but not 1.0 threshold and regularly and uncontrolled treatments (Table 5.2 & Figure 5.21, below). The 3.0 threshold had significantly less position three fruit retention than all other treatments, excluding 2.0 (Table 5.2 & Figure 5.21).

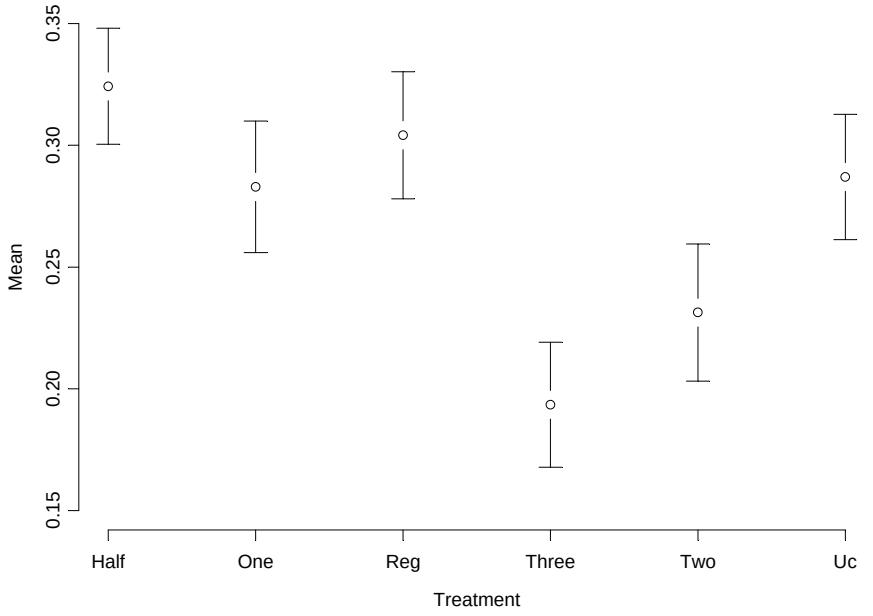

Figure 5.21. Mean retention of position three fruit for each treatment in the late season sucking pest trial conducted at KRS in 2004. Error bars denote 95% CI.

5.3 RBSB

Shadehouse cage trial

Potted cotton plants maintained for this trial displayed relatively long internode length and required structural stem support (stake tethers) to remain upright, probably due to continued wet season cloud cover and shadecloth reducing light availability. Mite infestation periodically required chemical control. On 24 November, Pegasus® (diafenthiuron) at 3ml in 50ml water carrier was applied by hand spray bottle to four heavily mite infested plants, which were later excluded from the trial due to chemical related foliage burn. All plants were subject to milder chemical mite suppression with Neemtech® (Neem extract, 30ml in 1 litre water carrier applied by hand spray bottle) on 21 December (following removal of experimental plants from cages in the late exposure trial).

During the early exposure trial, 1, 2 and 1 RBSBs died in pot 4 on the 7th, 8th and 9th day of exposure, respectively. All other RBSBs survived the early trial. During the late exposure trial, 1 RBSB died on the 9th day of exposure. All other RBSBs survived the late trial.

There was no significant difference in the number of bolls between treatments in the RBSB shadehouse trial ($F_{3, 44}$ = 0.35, P = 0.79). Boll weight by node was not significantly different between treatments for both position one ($F_{3, 69}$ = 0.39, P = 0.77) and two ($F_{3, 25}$ = 1.20, P = 0.33) and neither analysis displayed an interaction of slope with treatment ($F_{3, 69}$ = 0.32, P = 0.81 and $F_{3, 25}$ = 1.59, P = 0.22, respectively).

Field cage trial

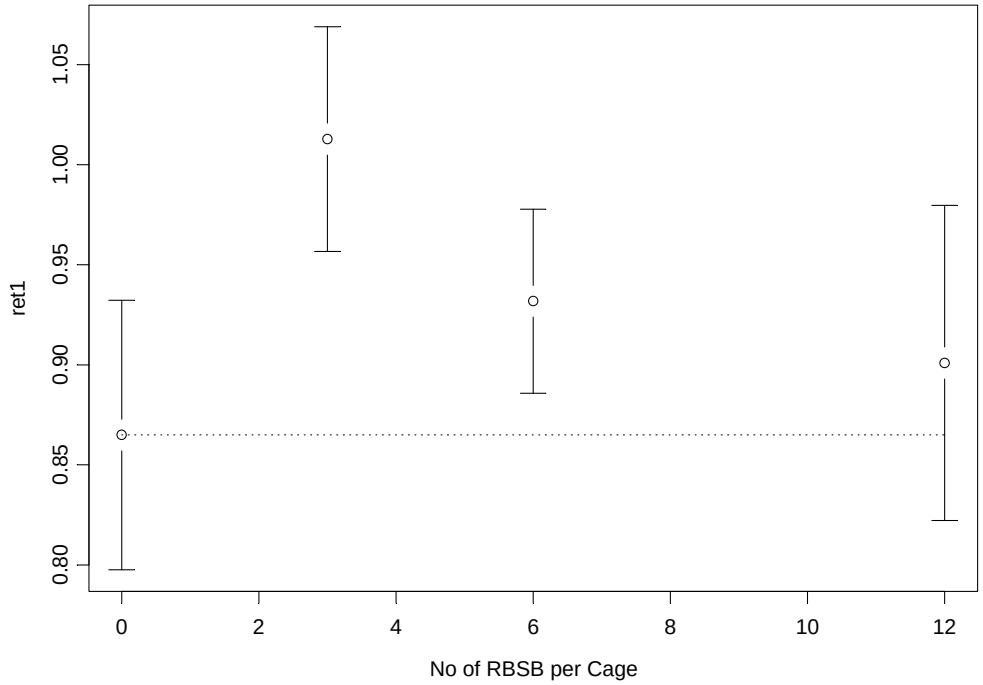

Figure 5.22. Net variation in position one fruit retention for each treatment in the redbanded shield bug field cage trial conducted at KRS in 2005. Error bars denote 95% CI.

There was no significant difference between treatments in boll weight from all nodes and fruit positions ($F_{3, 1703}$ = 2.50, P = 0.058), when restricted to positions three and four only ($F_{3, 244}$ = 1.57, P = 0.20) and when restricted to nodes 10 and above only ($F_{3, 758}$= 1.83, P = 0.14). From plant mapping data variables pre- and post-exposure, there was significantly more position one fruit retention on cotton plants in cages with three RBSBs than control cages with no

RBSBs only ($F_{3, 12}$ = 3.91, P = 0.036; Appendix 12, page 150; Figure 5.22, previous page). A Dunnett analysis using treatment means from the same data produced no significant difference between treatments.

5.4 Leafhoppers

Yield estimates

There was no significant difference in boll weight between cotton plants subject to heavy, medium and light leafhopper damage in B1 late season 2005 ($F_{2, 27}$ = 3.06, P = 0.063, Figure 5.23, below). There were significantly more bolls per metre ($F_{2, 27}$ = 7.56, P = 0.0025) producing significantly more yield per metre ($F_{2, 27}$ = 8.40, P = 0.0015) in the heavy rather than medium and light damaged cotton plants (Figures 5.24 & 5.25, both below).

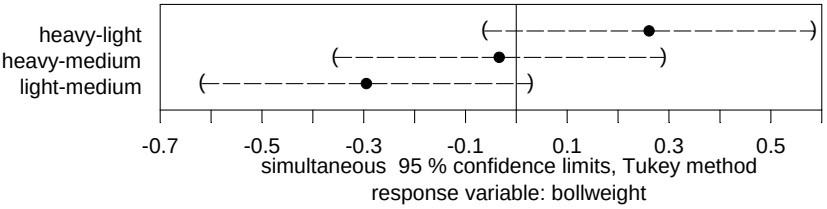

Figure 5.23. Tukey comparison of simultaneous 95% confidence limits for treatment effects on boll weight from the lateral irrigated late season leafhopper damage trial in B1 at KRS in 2005.

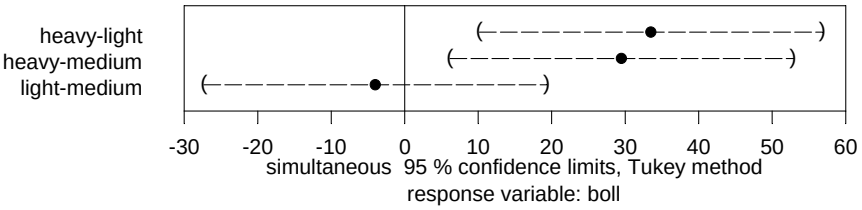

Figure 5.24. Tukey comparison of simultaneous 95% confidence limits for treatment effects on boll density from the lateral irrigated late season leafhopper damage trial in B1 at KRS in 2005.

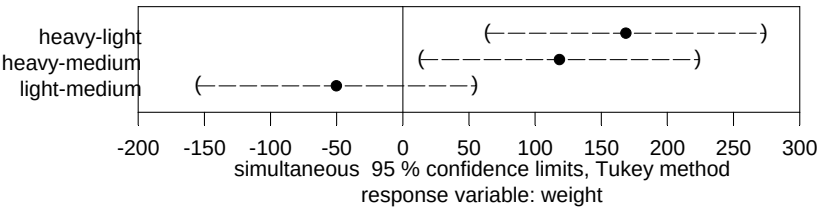

Figure 5.25. Tukey comparison of simultaneous 95% confidence limits for treatment effects on yield estimates from the lateral irrigated late season leafhopper damage trial in B1 at KRS in 2005.

Fibre quality

Fibre elongation was significantly less in cotton plants exposed to heavy rather than light leafhopper damage in B1 late season 2005 (Table 5.3 & Figure 5.26, next page). There was no significant difference in all other fibre quality characteristics between treatments (Table 5.3).

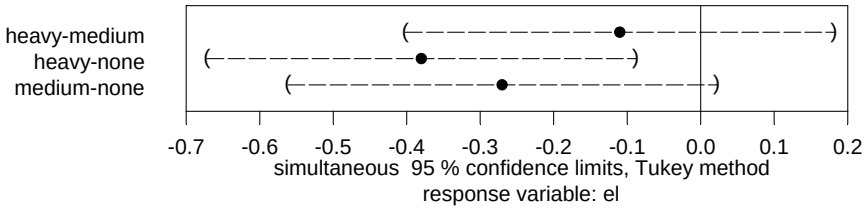

Figure 5.26. Tukey comparison of simultaneous 95% confidence limits for treatment effects (in this case, none = light infestation) on fibre elongation from the lateral irrigated late season leafhopper damage trial in B1 at KRS in 2005.

Table 5.3. *F* values and probabilities (*P*) generated from quality variable analyses examining fibre samples from cotton plants displaying symptoms of heavy, medium and light leafhopper infestation in B1 at KRS late season in 2005.

Variable	*F* value	*P*
Length	1.32	0.28
Uniformity	1.49	0.24
Sfi	1.77	0.19
Strength	0.63	0.54
Elongation	5.56	0.0098
Micronaire	1.28	0.29

Leaf damage

Significantly less plants were damaged by leafhoppers in cotton grown with sweet potato than lablab or no (fallow) companion strips in B1 at KRS in 2005 ($F_{2, 37}$ = 5.50, P = 0.0041, Figure 5.27, below).

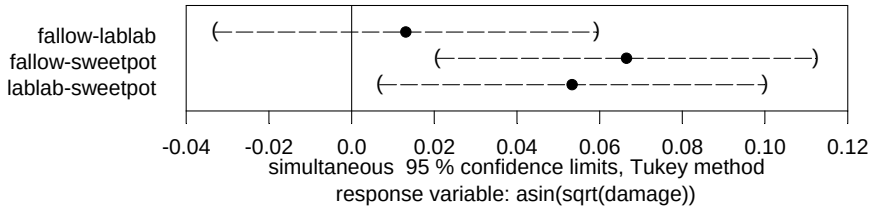

Figure 5.27. Tukey comparison of simultaneous 95% confidence limits for treatment effects on leafhopper damage from the lateral irrigated late season leafhopper damage trial in B1 at KRS in 2005.

Evidence of cotton plant varietal response to leafhopper damage in the CSIRO variety trial was recorded photographically (Appendix 14, page 157).

The proportion leaf damaged by node (counted down from plant tip) did not differ between treatments ($F_{8, 1472}$ = 1.83, P = 0.068). Damage was generally restricted to leaves between nodes 1 and 13, which had similar proportion damage, and significantly different between all other nodes (Figure 5.28, next page).

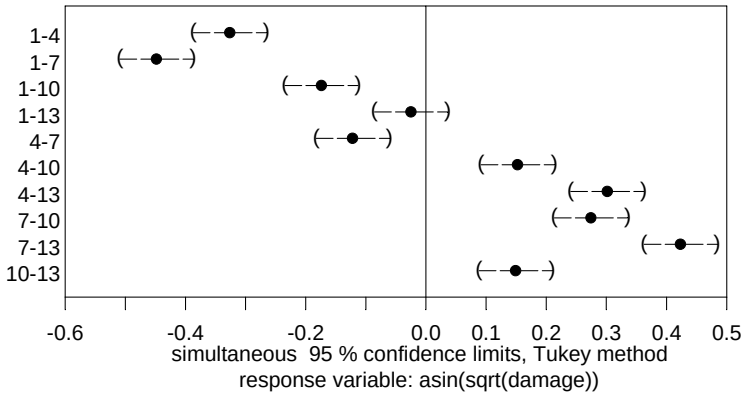

Figure 5.28. Tukey comparison of simultaneous 95% confidence limits of node (y axis) effects on leafhopper damage from the lateral irrigated late season leafhopper damage trial in B1 at KRS in 2005.

For late season 2005 leafhopper damage in B1, there was a significant interaction between treatment, node and the presence of *alternaria* infection ($F_{8,\ 1130} = 2.58$, $P = 0.0085$). Proportion leaf damaged was greater on larger leaves (nodes 7 to 13 from plant tip) with *alternaria* infection than leafhopper damage alone (Figure 5.29, below). The trend of proportion leaf damaged with *alternaria* infection from node 7 to 13 differed between all treatments (Figure 5.29).

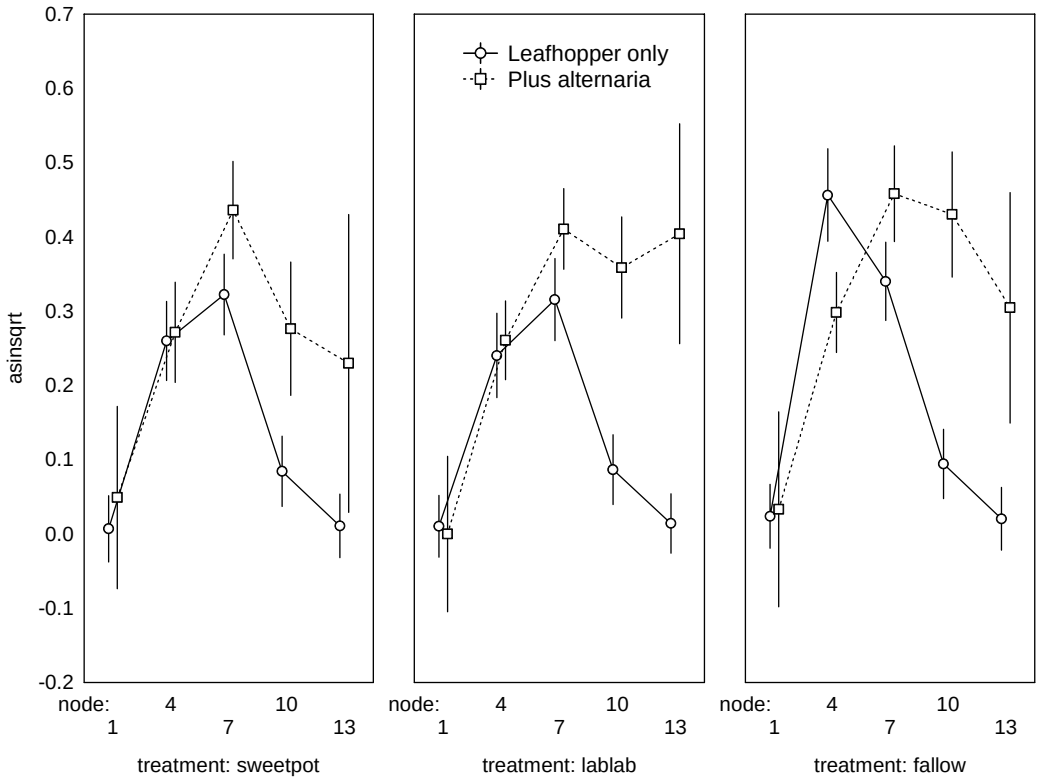

Figure 5.29. Proportion leaf damage from nodes one to thirteen (counted down from tip) attributed to leafhopper feeding and *alternaria* infection (see legend) on cotton plants grown with three companion crop treatments in B1 at KRS in 2005.

Progressive stages of leaf damage caused by *alternaria* infection in B1 at KRS during 2005 were recorded photographically (Appendix 15, page 160).

Discussion

Both the early and late season 2003 sucking pest trials attracted relatively low densities of sucking insects, with control thresholds breached only twice at differing thresholds on each occasion in each trial. It is interesting to note that in both trials, lower control thresholds were not breached when higher were, suggesting sucking pests were perhaps periodically colonising as hot-spots rather than infiltrating in high enough densities to colonise across the entire trial. That treatments were only marginally varied during both trials is reflected in the lack of significant difference between treatments for all variables examined. Despite regularly controlled plots registering resident sucking pests on only one sampling occasion in the early trial, and relatively rarely in the late trial, cotton lint yield was not significantly different between all treatments for both. This suggests cotton plants may tolerate relatively low levels of sucking pest damage without loss of yield in Katherine.

In 2004, both the early and late season sucking pest trials were more regularly infested with target insects and higher thresholds were only breached in conjunction with lower ones (Figures 5.4 & 5.8), suggesting sucking insects were more evenly distributed across trials. Despite regular threshold breaches (Figures 5.4 & 5.8), again yield estimates were not significantly different between all treatments in both trials. When you consider that consecutive breaches of thresholds up to 1.0 sucking pests per metre were recorded and fipronil applied from 20 May to 4 June in the early season trial (Figure 5.4), and yield estimates from these treatments were no different to others including both regularly and uncontrolled plots, it is not unreasonable to suggest that experimental outcomes may be confounded.

Different sucking insect pests cause different damage at seasonably variable rates. In established Australian cotton growing regions, RBSBs are purported to cause one third the damage of GVBs to cotton plants at the same densities (Khan & Bauer 2002). Summing densities between these pests to determine control thresholds will cause irregularities in experimental consistency when considering yield estimates if, as you would expect with insects, their relative abundances have varied across the season. Further, the pest status of *C. pacificus*, the dominant mirid in Katherine cotton crops, has been questioned (Malipatil & Cassis 1997). It is critical the pest status and damage potential of each sucking pest species be investigated and duly attributed if they are to be controlled successfully as a suite of pests. However, this may not be the only reason results seem confounded.

Where variables were significantly different between treatments in the 2003 and 2004 sucking pest trials, expected trends with graduated thresholds were generally not evident. For example, in the 2004 early season trial, regularly controlled plants, purportedly devoid of sucking insects for the entire trial, were significantly shorter, and had significantly fewer nodes and fruiting branches than those in uncontrolled plots (Figures 5.5, 5.6 & 5.7). An explanation may be that regular fipronil application stunted cotton growth, but this is unlikely given fipronil is commonly used to control sucking pests in Katherine and established growing regions with no discernible harm to cotton plants. Similar results for the same variables were evident in the 2004 late season trial, although regularly controlled plots fared worse than graduated threshold treatments, except twice for the 0.5 threshold, and were not significantly different to uncontrolled plots (Figures 5.12, 5.13 & 5.14). This suggests regular application of fipronil did not influence these results. Given thresholds were never breached nor fipronil applied in the 3.0 threshold plots in the same trial, it is not unreasonable to assume variables from these plots should be similar to those in uncontrolled plots. Yet the number of position three fruit and retention of fruit in positions one, two and three were

significantly lower in 3.0 threshold plots than in uncontrolled plots (Figures 5.18, 5.19, 5.20 & 5.21).

The closest resemblance to an expected trend is evident in Figures 5.15, 5.16 & 5.17, where the total number of bolls and position one and two fruit in the 2004 late season trial are significantly lower in 3.0 threshold than all other treatments, except uncontrolled plots for position one fruit only. Although not statistically significant, uncontrolled plots also returned the closest means to 3.0 threshold for the other two variables. It is difficult to draw definitive conclusions from these results, yet there is weak evidence to support that, post first flower, cotton plants may tolerate a threshold up to 2.0 sucking pests per metre with minimal impact on total boll number and position one and two fruit in Katherine. I am not confident these results are genuine enough to warrant a recommendation without further experimental evidence from more refined experiments, as explained previously.

It is evident sucking pest thresholds require further clarification. The significant difference in variables discussed above appear spurious and not worthy of reliable conclusions. Soil inconsistency is historically blamed for significant cross field variation in cotton yield in Katherine, although this has not been examined experimentally. Areas among suitable soils where soil drainage is excessive or minimal seem to produce a patchwork mosaic of healthy and stunted cotton plants. Across trial variability in cotton vigour attributed to agronomics perhaps contributed to the spurious results achieved during the sucking pest trials in 2003 and 2004. It would have been preferable to investigate the pest status and damage potential of cotton sucking pests individually without plant variability confounding results prior to examining sucking insects as a suite of pests, but unfortunately in Katherine, the latter problem seems unavoidable.

RBSBs in Australia were separated as *P. grossi* from the cosmopolitan species *P. hybneri* due to variations in their reproductive structures by Staddon (1997). Unfortunately this is rarely recognised (as done by Loch 2000), and attributing behavioural characteristics from a closely related and well studied (Higuchi 1992, 1993, 1994, 1995, van den Berg *et al.* 1995, Higuchi & Suzuki 1996, Hirose *et al.* 1996, Icuma & Hirose 1996, Coombs & Khan 1997, Leal *et al.* 1998) but distinct pest species to a pest insect under investigation (as done by Coombs & Khan 1997, Ward 2005) is fraught with assumptions of similarity so potentially flawed (see Walter 2003). Further, previous Australian studies examining the damage potential of RBSBs to cotton yield focused on boll damage only. Khan & Bauer (2002) caged RBSBs on individual bolls in styrofoam cups and assessed damage after ten days, concluding RBSBs cause one third the damage of GVBs to bolls, so require control at three times (3.0 per metre) the threshold of GVBs (1.0 per metre). The design of this experiment was weighted, as pest RBSBs had no choice but to feed continuously from the boll on which they were caged, and not also beneath young leaves and shoots as witnessed in the field in Katherine. RBSB were released onto entire plants in relatively large cages during Katherine trials in an attempt to closely mimic normal field conditions.

Evidence suggests RBSB's cause minimal if any damage to cotton plants in controlled caged conditions in Katherine. The wet season RBSB shadehouse trial was preliminary in nature and aimed to refine RBSB rearing techniques while determining if RBSB feeding reduced the yield potential of cotton. Although there was no significant difference between plant boll number nor weight with or without RBSB exposure at both early and late plant growth stages, extrapolating results to field conditions in Katherine is not possible due to weak data (limited cages) and seasonal variation in plant growth and possibly RBSB activity.

RBSBs proved difficult to rear, so commencement of field cage trials was dependent on sufficient RBSBs to conduct the experiment being collectable from field populations. In 2005 as in previous years, RBSBs were not prevalent till late season (approaching cutout) and even then tended to congregate in flowering pigeon pea rather than neighbouring cotton. From experimental results, RBSBs at densities up to 12 per metre do not seem to damage cotton plants approaching cutout in Katherine to the extent that growth, as measured by plant mapping, and yield are not compromised. It is difficult to explain why the number of first position fruit on plants in unexposed control cages was significantly lower than the number in cages exposed to three RBSB per metre. As GVB require late season control in established cotton growing regions at 1.0 per metre, then, according to Khan & Bauer (2002), RBSB should require control at densities above 3.0 per metre, yet damage to late season cotton plants seems tolerable at RBSB densities up to 12.0 per metre in Katherine. Thresholds for this experiment were definitive and not inferred like those from scouting estimates, so caution is needed before suggesting that such high densities can be tolerated under normal field conditions. Yet, if densities of up to 12.0 RBSBs per metre are indeed tolerable late season without yield loss in Katherine, then results support earlier conjecture that assigning sucking insects equal damage potential during sucking pest trials may be flawed. The damage potential of RBSB, both early and late season, and the potential for biological control with local parasitoids in Katherine cotton requires further investigation.

Leafhoppers in Katherine cotton have historically been incorrectly categorised as jassids (Jassidae). Despite departmental taxonomists wrongly identifying leafhopper samples from Katherine cotton (as *Empoasca* sp., further, it is critical insects are identified to species to avoid complications when designing IPM systems (Walter 2003)), samples were concurrently sent to recognised expert taxonomists and identified correctly as *Austroasca alfalfae*. Heavy leafhopper infestation, like RBSB, appears to be a late season phenomenon in Katherine cotton, at least in 2004 and 2005. Despite heavy leafhopper infestation causing severe hopper burn or leaf reddening, yield potential does not seem to be reduced when compared to plants with relatively light and medium hopper damage. In fact, yield was greater when hopper damage was most severe (Figure 5.25), however, severely hopper damaged plants tended to carry a higher density of bolls than plants with less damage (Figure 5.24), suggesting they may have been more attractive to hopper infestation due to their healthy vigour. Hopper damage appears to stunt fibre elongation (Figure 5.26). Loss of leaf photosynthetic ability is a recognised cause for stunted elongation in cotton fibre growth (Bauer *et al.* 2000, Pettigrew *et al.* 2001), so perhaps plant damage symptoms attributed to leafhopper feeding at high densities, such as severe leaf reddening, were responsible.

Leafhoppers were less likely to damage cotton plants grown with sweet potato compared to lablab and no companion crop within field (Figure 5.27), which encourages further examination of sweet potato companion crop potential. Within plant damage symptoms varied between nodes (Figure 5.28) and concentrated on younger leaves (nodes 4 to 7 down from tip, Figure 5.29), although symptoms would not be apparent on relatively younger leaves (nodes 1 to 3) so their attractiveness to hopper feeding requires clarification. That symptoms were less obvious on nodes 10 to 13 (Figure 5.29) could reflect either their relative unattractiveness to or ability to tolerate hopper feeding. Most alarming is the damage caused by leafhoppers in conjunction with *alternaria* infection (Figure 5.29). *Alternaria* infection causes leaf necrosis (Appendix 15). Leaves not infected with *alternaria* remain attractive to leafhoppers, so if hopper infestation occurs in *alternaria* infected fields, as occurred in B1 in 2005, cotton yield and fibre quality potential suffers considerably as no healthy leaves are available to continue

photosynthesis through to harvest. Hopper damage could possibly be minimised through companion cropping with sweet potato, or tolerated with possible hopper resistant varieties (Appendix 14). Leafhopper control options require further consideration.

6.0 *Helicoverpa* and other lepidopteran pests

Introduction

Helicoverpa moths are a major economic pest in agroecosystems Australia wide (Wardaugh & Room 1980, Fitt 1989, Fitt *et al.* 1995, Zalucki *et al.* 1986), and are the principal insect pests attacking Katherine cotton. Their larvae feed on cotton plant tissue causing serious damage, most importantly to squares and immature and maturing bolls. The introduction of transgenic cottons that produce *Bt* proteins specifically targeting this group of moths has effectively reduced *Helicoverpa* damage potential and minimised reliance on insecticidal applications for their control (Fitt *et al.* 1994, Fitt 2000). The sustainability of transgenic cultivar production is dependent on the continued efficacy of *Bt* proteins to control the potentially resistant pest species, *Helicoverpa armigera.*

Helicoverpa armigera populations have previously demonstrated the inherent ability to develop resistance to insecticides over time by artificial selection through overzealous application (Forrester 1994). Should *H. armigera* individuals successfully develop on transgenic cotton, it is imperative resistant genes are diluted within the population. Alternate host plants, in the form of un-sprayed companion crops (discussed previously) designed to harbour natural enemies, provide a continuous source of non-resistant moths for this purpose (Fitt *et al.* 1994, Fitt 2000). However, *Helicoverpa* populations require constant monitoring to ensure resistant genes are not proliferating. Other moth pests, namely *Helicoverpa punctigera, Pectinophora gossypiella* and *Spodoptera litura*, have caused serious damage to cotton crops in tropical northern Australia in the past, especially in the ORIA (Michael & Woods 1980). Winter cropping effectively avoids periods when moth pests proliferate, and is the primary reason a winter growing strategy has been adopted for current northern cotton production trials (Yeates 2001, Strickland and Lacey 1996, Strickland and Annells 1998).

It is imperative the local seasonal phenology of these major lepidopteran cotton pests is examined and incorporated into recommended IPM and resistance management strategies. Pheromone traps were utilised to monitor the seasonal phenology of *H. armigera*, *H. punctigera*, *P. gossypiella* and *S. litura* during the Katherine cotton IPM project. Despite only targeting male moths and variable lure attractiveness nullifying comparison of relative abundance between species (Gregg & Wilson 1991, Titmarsh *et al.* 1991), pheromone trapping proved an effective method for monitoring fluctuations in moth populations both temporally and spatially.

Methodology

6.1 Pheromone traps

Temporal and spatial variation in *Helicoverpa* and other pest moth populations within the Katherine region were monitored via strategically positioned commercially available pheromone traps containing synthesised chemical lures as attractants and dichlorvos infused pest strip sections (1 cm^2) or chalk blocks (1 cm^3) to kill attracted moths. Once per week for the entire project, traps targeting primarily *H. armigera* and *H. punctigera* and occasionally *S. litura* and *P. gossypiella* males at seven sites (see Figure 6.1, next page) were checked and captured moths removed and counted. Sites were chosen according to variation in habitat (Table 6.1, next page). All four moths were targeted at Campbell, KRS, PCA and Shaw, but only the two *Helicoverpa* species at Stuart Highway, Ballongilly and Power pheromone trap sites. Pheromone lures and insecticide strips were replaced as prescribed in the directions for

use. From 2001 to 2004, pheromone traps were wired to fencelines one metre above the ground at each site. In 2005, traps were mounted with 4mm gauge fencing wire (see figure 6.2, next page) on curtain rods 1.5 to 1.7 metres above the ground (see Figure 6.3, next page), depending on give in soil structure accepting the post, and ten metres apart to promote uniformity of capture potential between sites. Posts were sprayed with concentrated salt solution during the wet season in 2005 to deter predatory frogs.

Figure 6.1. The locations (red dots) of pheromone traps targeting *Helicoverpa armigera*, *H. punctigera*, *Spodoptera litura* and *Pectinophora gossypiella* in the Katherine region during the cotton IPM project (2001 to 2005).

Table 6.1. Habitat description for each of the seven sites used for pheromone trapping of *Helicoverpa armigera*, *H. punctigera*, *Spodoptera litura* and *Pectinophora gossypiella* in the Katherine region during the cotton IPM project (2001 to 2005).

Site	GPS	Description	Habitat
Ballongilly	S14°34.076'E132°02.180'	Farm gate	Bushland & mangoes
Campbell	S14°35.591'E132°00.237'	Farm gate	Bushland & mixed farming
KRS	S14°27.978'E132°18.596'	Fenceline	Mixed farming
PCA	S14°29.531'E132°12.753'	Swamp	Swampland & peanuts nearby
Power	S14°33.521'E132°29.652'	Fenceline	Cucurbits
Stuart Highway	S14°29.981'E132°25.120'	Fenceline	Bushland
Shaw	S14°32.005'E132°27.626'	Fenceline	Mangoes & cucurbits

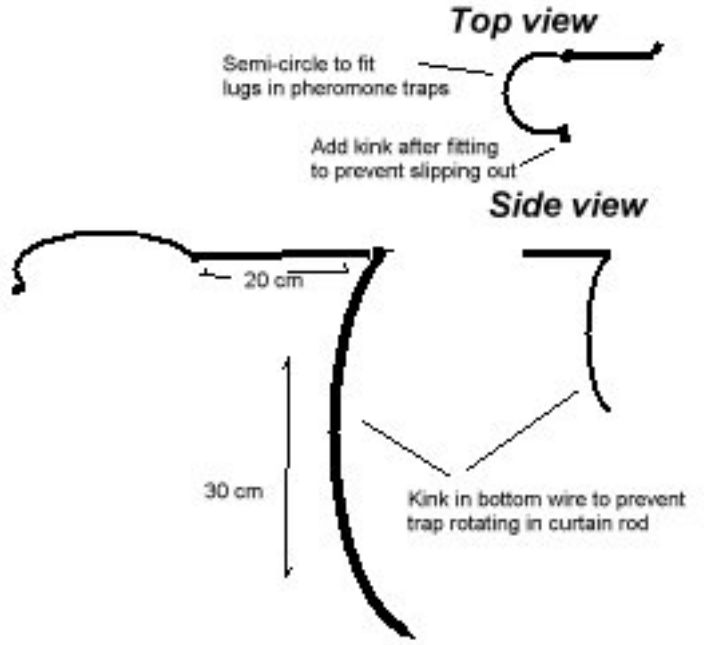

Figure 6.2. Structure of fencing wire hanger for pheromone traps utilised in 2005. Diagram courtesy Peter Gregg.

Figure 6.3. Example of a pheromone trap supported by a wire hanger in a curtain rod mounted in the soil. Photo courtesy Peter Gregg.

Data from pheromone trap collections were subject to time series plots for individual species per site and across all sites for the duration of the project (2001 to 2005). Partial cross correlation analyses determined periodicity in count lag for each species at each site, and removal of partiality provided evidence of cycle. Both correlations were plotted with 95% CI. Spectral analyses of count frequencies were used to isolate evidence for data cycles for each species across all sites at 95% CI, and plotted as spectral periodograms.

6.2 Resistance testing

When *Helicoverpa* populations within the cropping region were of sufficient density to allow collection within a suitable time frame, 200 plus larvae were captured and reared to pupae in the Katherine insectary for shipment to ACRI to test for conventional insecticide and Bollgard II® *Bt* protein resistance. This task was attempted at least twice per year up till suspension of resistance testing for unknown reasons in 2003 and 2004, and pupae were not viable or *H. armigera* populations too small for successful resistance testing in 2005.

6.3 Emergence traps and pupae digging

In 2002, emergence traps were used to compare the potential for conventional cotton and Bollgard II® for adult, and pupae digging used to monitor conventional cotton, sorghum and peanuts for pupal, *H. armigera* production (see Appendix 1).

On six occasions from 9 June to 16 August 2004, ten shadecloth traps 1 metre long, 0.5 metres wide and 0.8 metres tall were placed for two weeks over one metre row at randomly selected sites in each of three Bollgard II® (KRS pivot, KRS T1 and PCA pivot), one peanut (PCA) and one sorghum (PCA) crop and checked twice weekly for possible resistant moth emergence. Emergence traps were not used in 2005.

Results

6.1 Pheromone traps

H. armigera

H. armigera were caught in pheromone traps relatively rarely at both Ballongilly and Stuart Highway (Figure 6.4, page 87), probably due to the predominantly dry conditions and lack of suitable host vegetation at these sites. Campbell pheromone trap catches reflect relatively large populations of *H armigera* being present during the dry growing season, with relatively low numbers caught during the wet season at the same site (Figure 6.4). The *H. armigera* pheromone trap at PCA caught relatively low moth numbers fairly consistently through 2001 to end 2005, with noticeable peaks in the wet season (Figure 6.4). Pheromone traps at KRS, Power and Shaw mixed farming sites attracted relatively large numbers of *H. armigera* year round, with relatively small peaks in the wet, and larger population booms toward the end of the wet and into early growing season when emerging crops provided conditions suitable for *H. armigera* proliferation (Figure 6.4).

The build-up of *H armigera* populations early growing season is demonstrated across all sites in Figure 6.8, page 90, although the tendency for *H. armigera* to persist year round is evident. The persistent nature of *H. armigera* populations is further supported in cross correlation plots of data from Campbell, KRS and Power sites, which demonstrate significant positive lags and evidence of cycle at one month (Figures 6.9, 6.10 & 6.11, page 91), although results from

Shaw were not significant. There is also evidence of a two month cycle at Campbell and Power (Figures 6.9 & 6.11) and a three month cycle at Power only (Figure 6.11). Although relatively low numbers of *H. armigera* were caught at Stuart Highway, they were always caught in the wet season with a strong 12 month lag and cycle in catches (Figure 6.12, page 91).

A spectral periodogram of all *H. armigera* data across all sites demonstrates significant population cycles at 12.64 (A), 6.32 (B), 3.41 (C) and 2.1 (D) months (Figure 6.24, page 95).

H. punctigera

Ballongilly, PCA, Stuart Highway and Shaw pheromone trap sites collected relatively low numbers of *H. punctigera* on rare occasions (Figure 6.5, page 88). Larger numbers of *H. punctigera* were caught at Power site (Figure 6.5) but evidence for population trend are difficult to discern. *Helicoverpa punctigera* were caught at Campbell in relatively large numbers early to mid cropping season in 2002, 2003 and 2004 (Figure 6.5). Similar numbers of *H. punctigera* were caught at KRS (Figure 6.5), however, population trends are not so evident. A time series plot of all *H. punctigera* data across all sites demonstrates populations tend to build during the growing season, but not to the same level every year (Figure 6.8).

Cross correlation plots of *H. punctigera* pheromone trap collection data from Campbell (Figure 6.13, page 92) and Stuart Highway (Figure 6.15, page 92) demonstrate strong and weak evidence, respectively, for a twelve month positive lag and cycle in population. This evidence was not supported by pheromone trap collection data from KRS, which demonstrated a one month positive and two month negative lag, with population cycles of one and five months (Figure 6.14, page 92).

A spectral periodogram of all *H. punctigera* data across all sites demonstrates significant population cycles at 6.20 (A) and 2.95 (B) months (Figure 6.25, page 95).

P. gossypiella

Two relatively large *P. gossypiella* population peaks were recorded at KRS pheromone trap site in the 2002 and 2003 wet season, although catches were relatively rare across remaining years (Figure 6.6, page 89). The majority of *P. gossypiella* caught at Campbell, PCA and Shaw sites were collected during the same periods (2002 and 2003 wet season), with additional evidence for relatively smaller population peaks post 2004 wet season, except for Campbell, where 2002 and post 2004 wet season peaks were similar in size (Figure 6.6). The relatively large numbers of *P. gossypiella* caught in 2002 and 2003 wet seasons are evident in the time series plot of all *P. gossypiella* data across all sites in Figure 6.8.

There is strong evidence for a three month positive lag and cycle in *P. gossypiella* populations at Campbell (Figure 6.16, page 92), PCA (Figure 6.18, page 93) and Shaw (Figure 6.19, page 93) pheromone trap sites. PCA *P. gossypiella* collection data also demonstrated a one month positive and six month negative lag and one month population cycle following cross correlation (Figure 6.18). A significant positive lag of one month and population cycles of 12 and 13 months were calculated from data collected at KRS site (Figure 6.17 page 93).

A spectral periodogram of all *P. gossypiella* data across all sites demonstrates significant population cycles at 14.00 (A), 6.50 (B), 3.05 (C), 2.49 (D) and 2.17 (E) months (Figure 6.26, page 96).

S. litura

Spodoptera litura were consistently collected in pheromone traps at Campbell and KRS sites across all years and seasons, with minimal evidence of population trend (Figure 6.7, page 89). Collection of *S. litura* from PCA and Shaw pheromone traps demonstrate distinctive wet season population peaks at these sites (Figure 6.7). The time series plot across all sites and years demonstrates relatively large populations of *S. litura* build up during the wet, and persist but gradually decline during the dry growing season (Figure 6.8).

Populations of *S. litura* tended to consistently persist across all sites, with significant one month positive lag and cycle in pheromone trap catches at all sites (Figures 6.20, 6.21, 6.22 & 6.23, pages 93 & 94). A significant negative lag of two months is evident at Campbell, KRS and PCA pheromone trap collection sites (Figures 6.20, 6.21 & 6.22, respectively).

A spectral periodogram of all *S. litura* data across all sites demonstrates significant population cycles at 11.20 (A), 6.22 (B), 4.30 (C), 2.80 (D) and 2.00 (E) months (Figure 6.27, page 96).

6.2 Resistance testing

Testing of Katherine collected *H. armigera* by ACRI displayed relatively low resistance to conventional insecticides, other than for Thiodicarb on 9 May 2001, Bifenthrin on 24 march and 21 July 2002, and Endosulfan on 24 March 2002 (Table 6.2, page 97). Resistance to insecticides not utilised in the Katherine cotton project (chlorphenapyr, profenofos and endosulfan) is evident (Table 6.2).

Katherine collected *H. armigera* tested for *Bt* resistance demonstrated baseline resistance was significantly higher than the susceptible strain at ACRI (Appendix 1, Table 3, page 128). Probit analysis showed the LD99.9 to be 477794 µl/ml of diet (Appendix 1, Table 3). Survival at the discriminating MVP dose of 3 µl/ml of diet produced similar survival for 2001 (11.6%) and 2002 (13.5%) Katherine collected *H. armigera* third instar larvae (Appendix 1, Table 4, page 128). No survival was recorded for Katherine collected *H. armigera* larvae exposed to Dipel® (*Bacillus thuringiensis* subsp. *kurstaki*, strain HD-1) and Xentari® (*Bacillus thuringiensis* subsp. *aizawai,* serotype H-7, strain ADTS-1857) (Appendix 1, Table 4).

6.3 Emergence traps and pupae digging

For 2002 results, see Appendix 1.

In 2004, only two moths were caught in emergence traps on the one occasion; one moth emerged from KRS pivot Bollgard II® and one from PCA sorghum on 3 August.

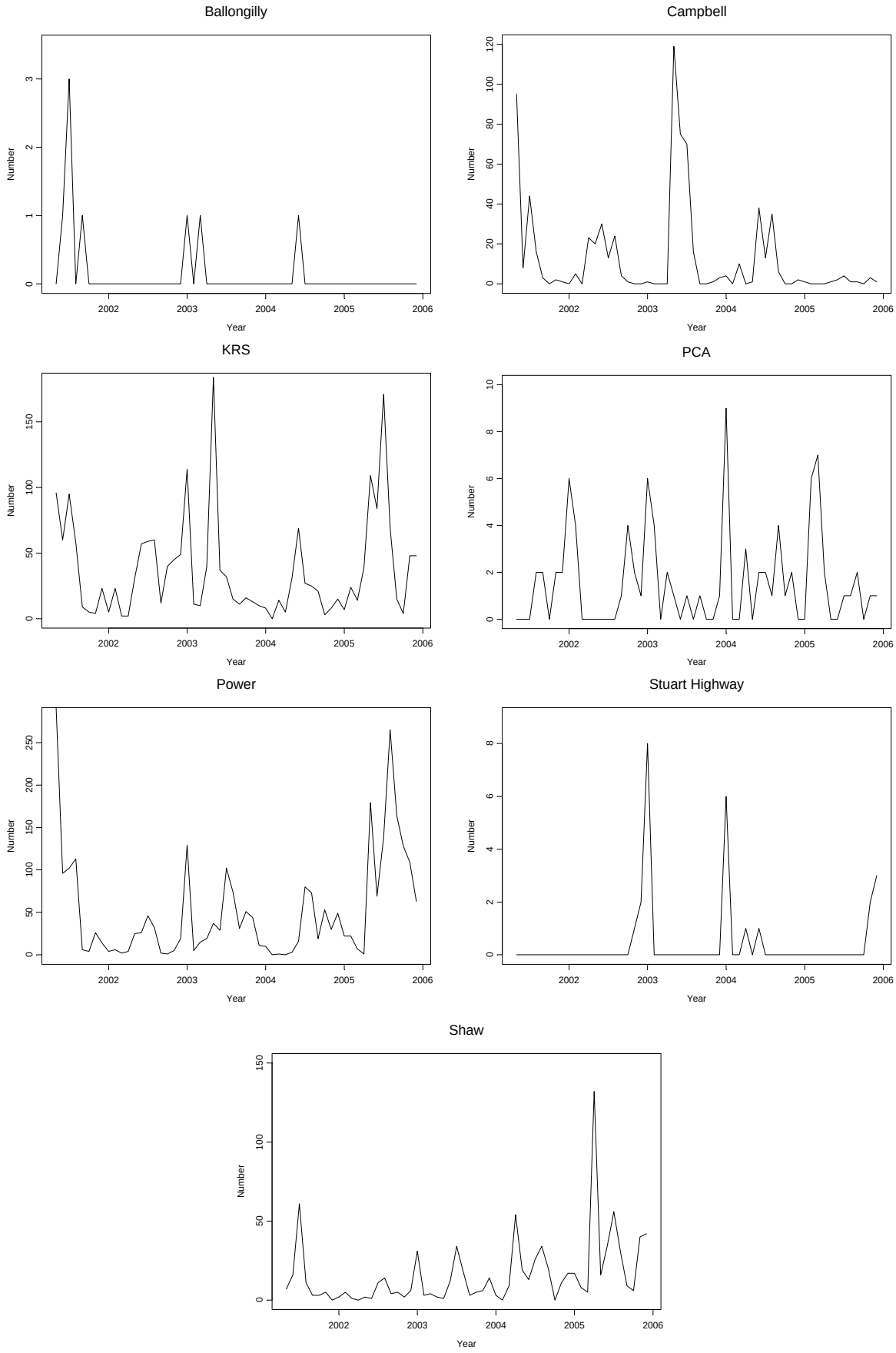

Figure 6.4. Time series plots of *Helicoverpa armigera* pheromone trap counts at all Katherine region sites (see graph titles) from 2001 to 2006.

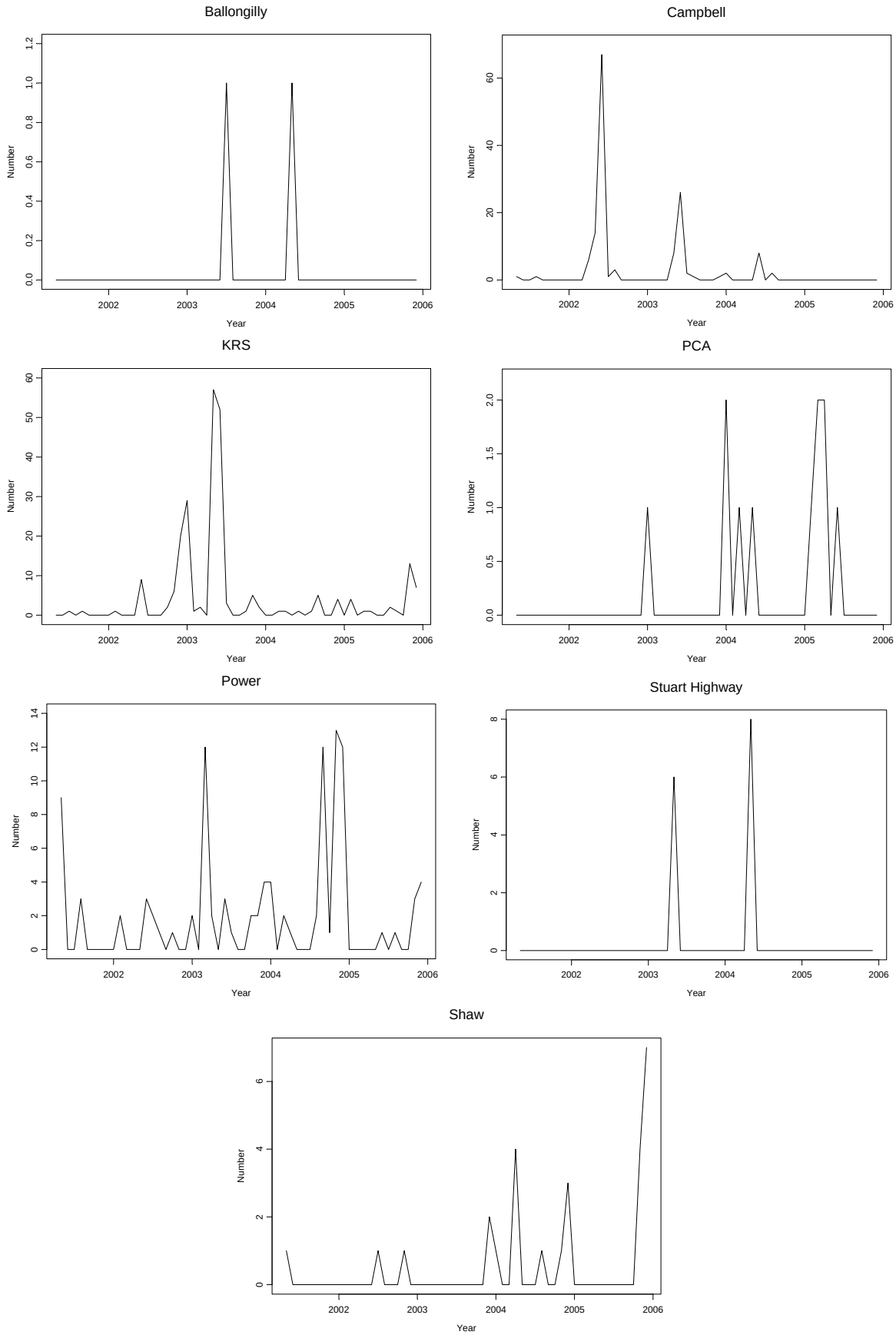

Figure 6.5. Time series plots of *Helicoverpa punctigera* pheromone trap counts at all Katherine region sites (see graph titles) from 2001 to 2006.

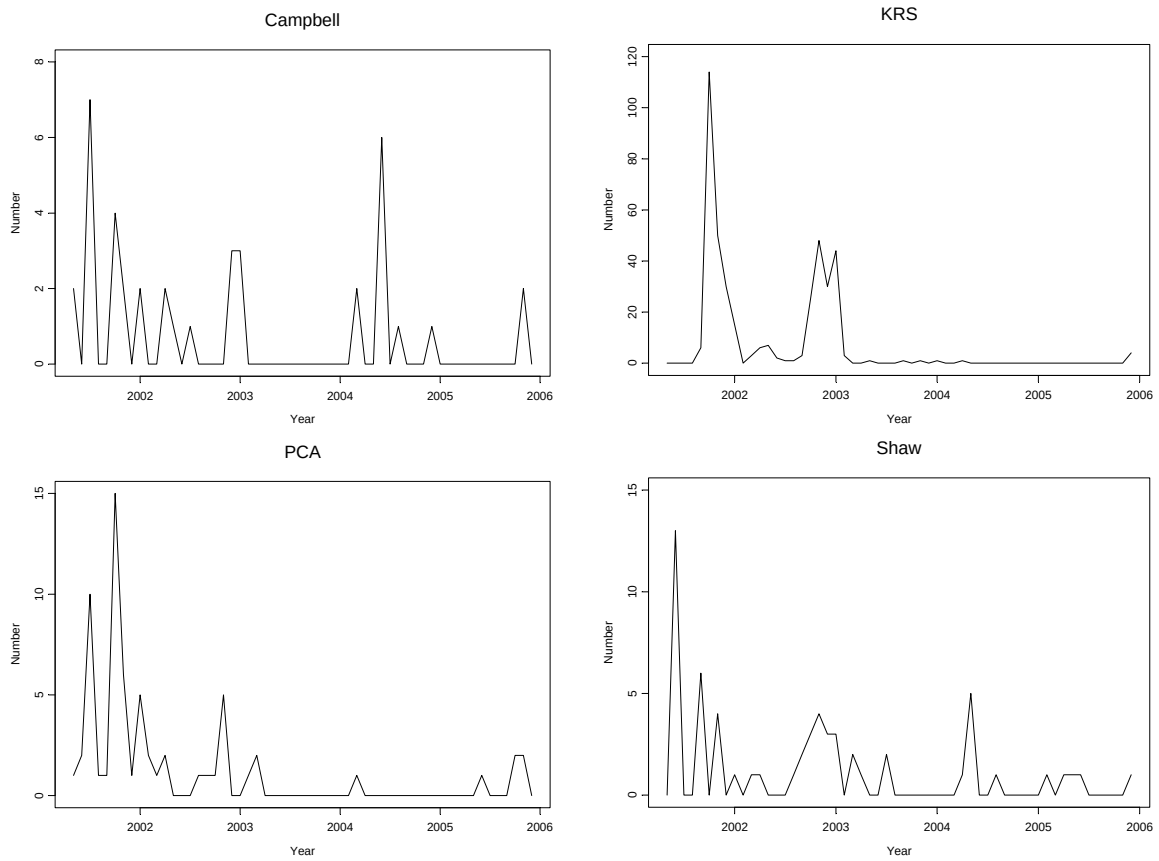

Figure 6.6. Time series plots of *Pectinophora gossypiella* pheromone trap counts at all Katherine region sites (see graph titles) from 2001 to 2006.

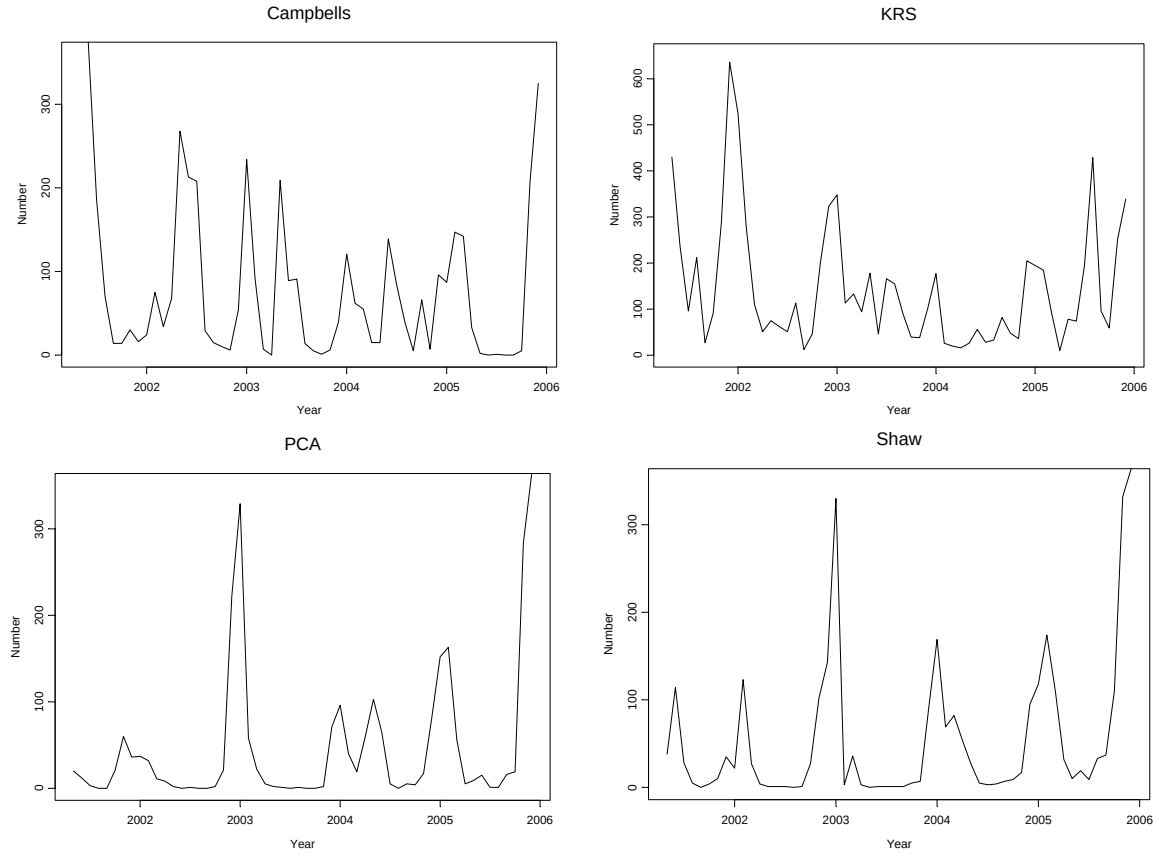

Figure 6.7. Time series plots of *Spodoptera litura* pheromone trap counts at all Katherine region sites (see graph titles) from 2001 to 2006.

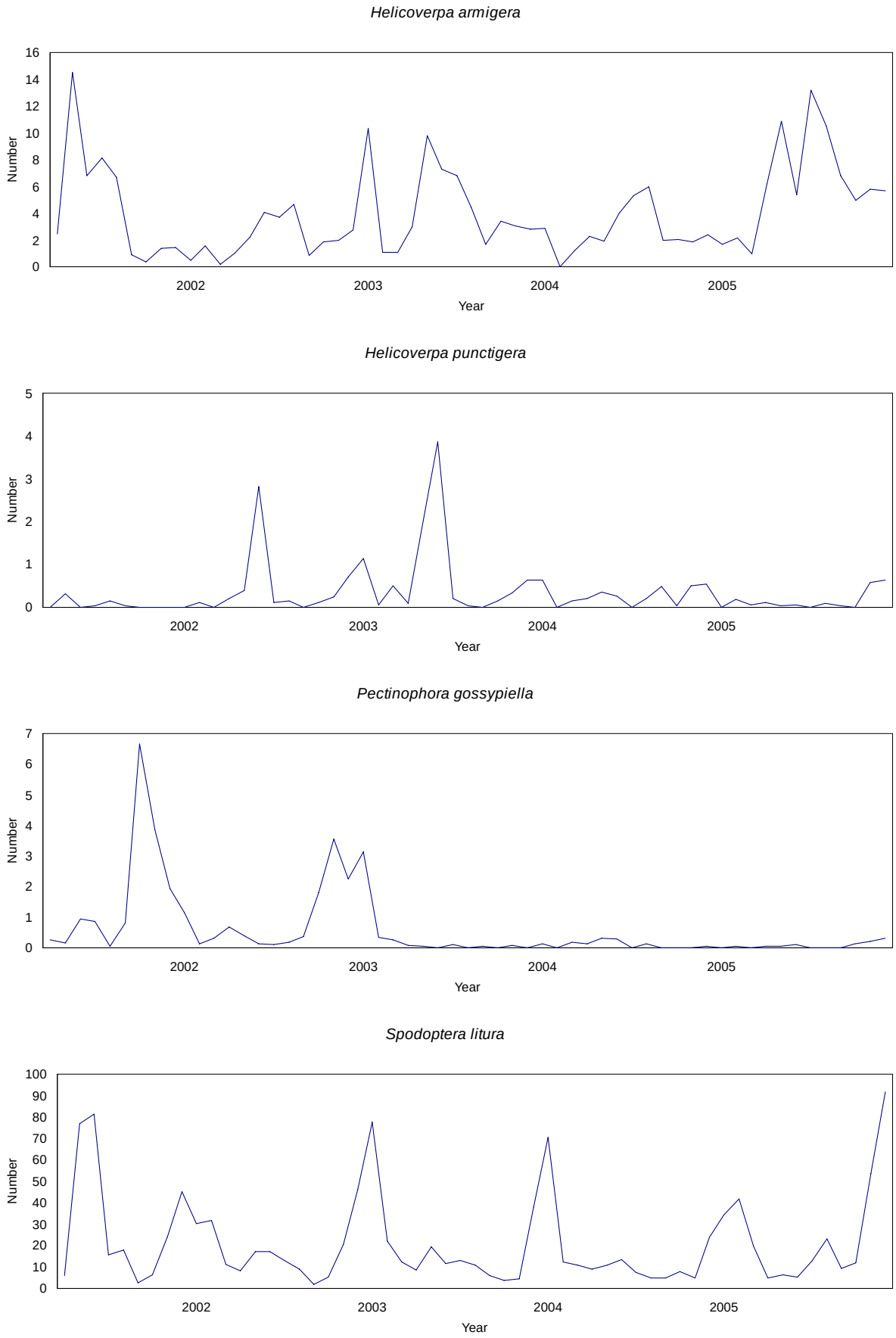

Figure 6.8. Monthly mean moth (see graph titles) pheromone trap counts across all Katherine region sites from 2001 to 2006.

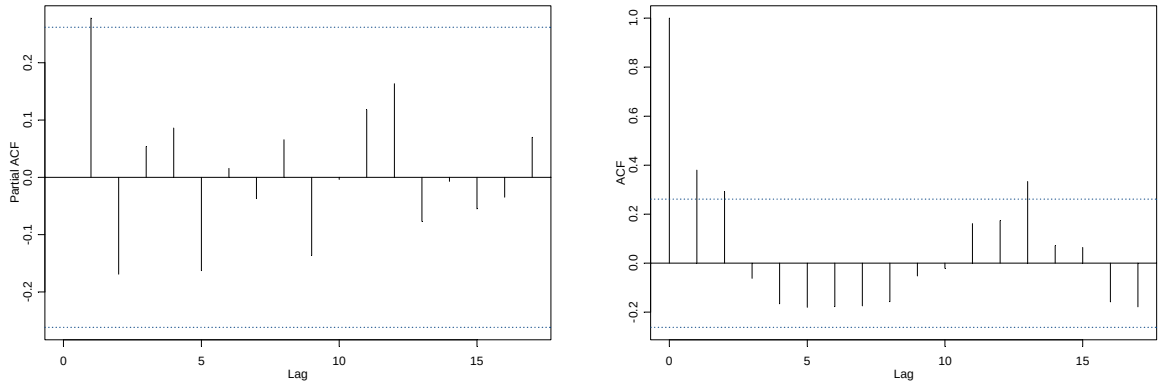

Figure 6.9. Evidence of lag (left) and cycle (right) in *Helicoverpa armigera* counts from a pheromone trap at Campbell site between 2001 and 2006. Lag (x axis) is in months. Dotted lines represent 95% CI.

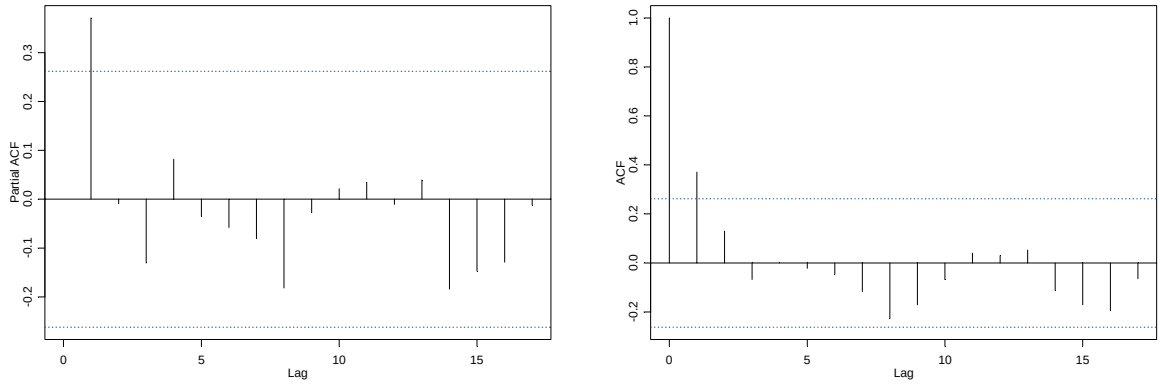

Figure 6.10. Evidence of lag (left) and cycle (right) in *Helicoverpa armigera* counts from a pheromone trap at KRS site between 2001 and 2006. Lag (x axis) is in months. Dotted lines represent 95% CI.

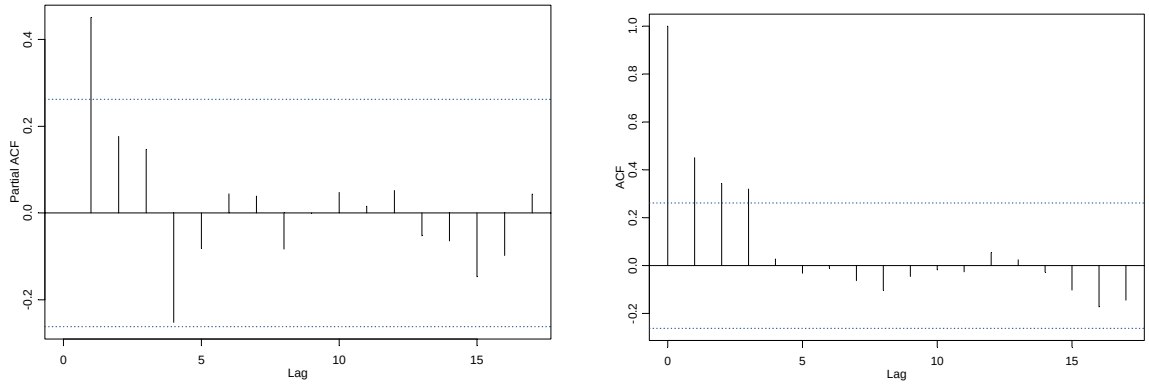

Figure 6.11. Evidence of lag (left) and cycle (right) in *Helicoverpa armigera* counts from a pheromone trap at Power site between 2001 and 2006. Lag (x axis) is in months. Dotted lines represent 95% CI.

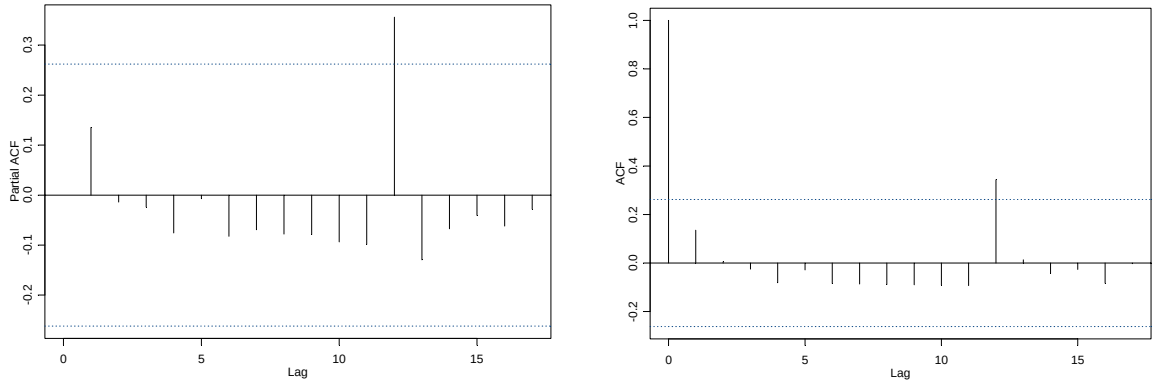

Figure 6.12. Evidence of lag (left) and cycle (right) in *Helicoverpa armigera* counts from a pheromone trap at Stuart Highway site between 2001 and 2006. Lag (x axis) is in months. Dotted lines represent 95% CI.

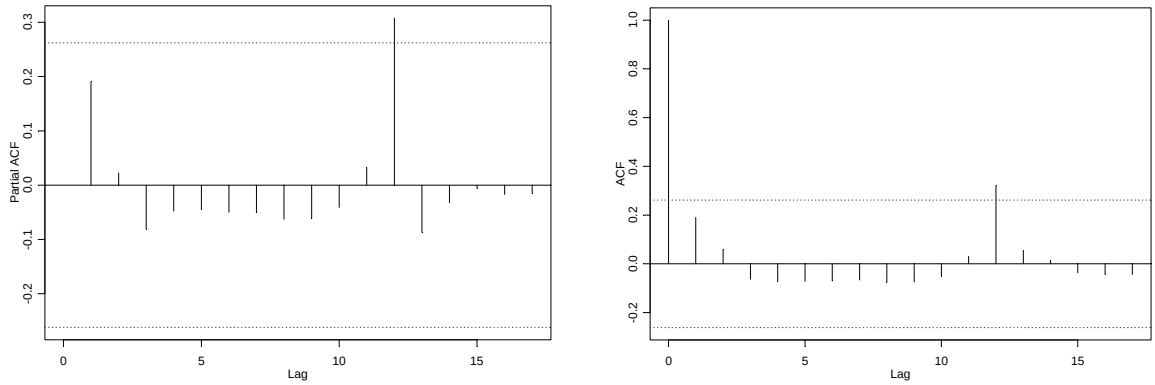

Figure 6.13. Evidence of lag (left) and cycle (right) in *Helicoverpa punctigera* counts from a pheromone trap at Campbell site between 2001 and 2006. Lag (x axis) is in months. Dotted lines represent 95% CI.

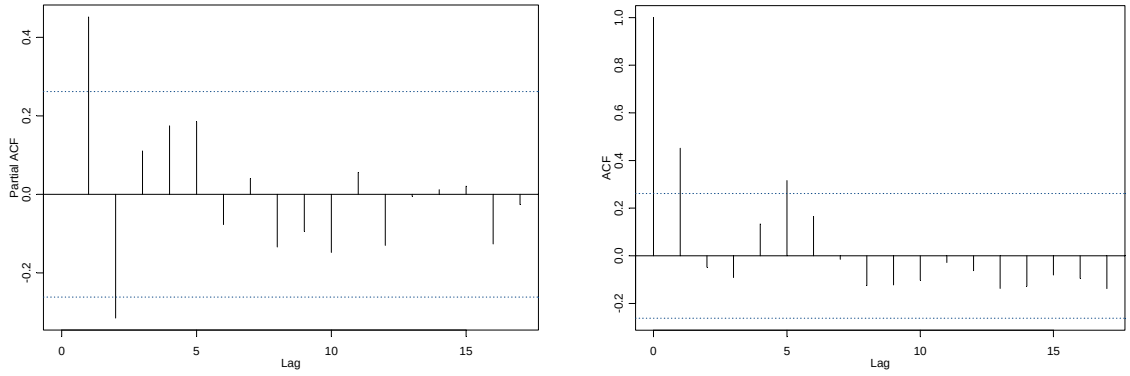

Figure 6.14. Evidence of lag (left) and cycle (right) in *Helicoverpa punctigera* counts from a pheromone trap at KRS site between 2001 and 2006. Lag (x axis) is in months. Dotted lines represent 95% CI.

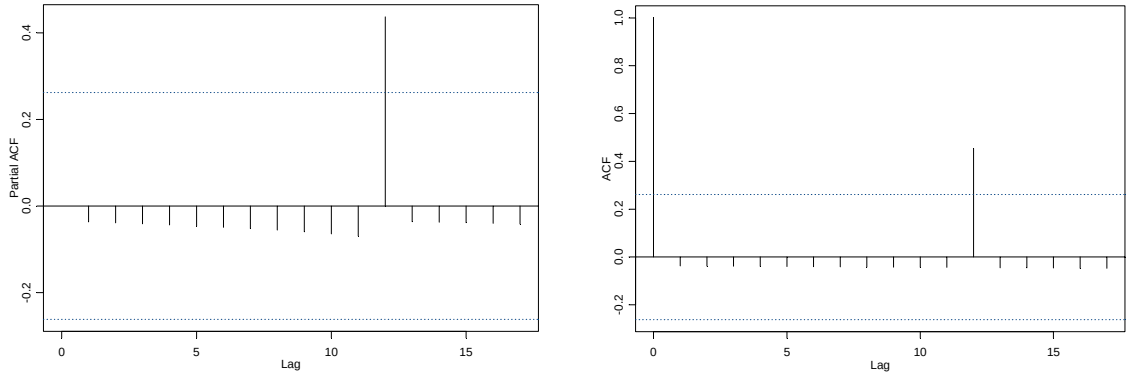

Figure 6.15. Evidence of lag (left) and cycle (right) in *Helicoverpa punctigera* counts from a pheromone trap at Stuart highway site between 2001 and 2006. Lag (x axis) is in months. Dotted lines represent 95% CI.

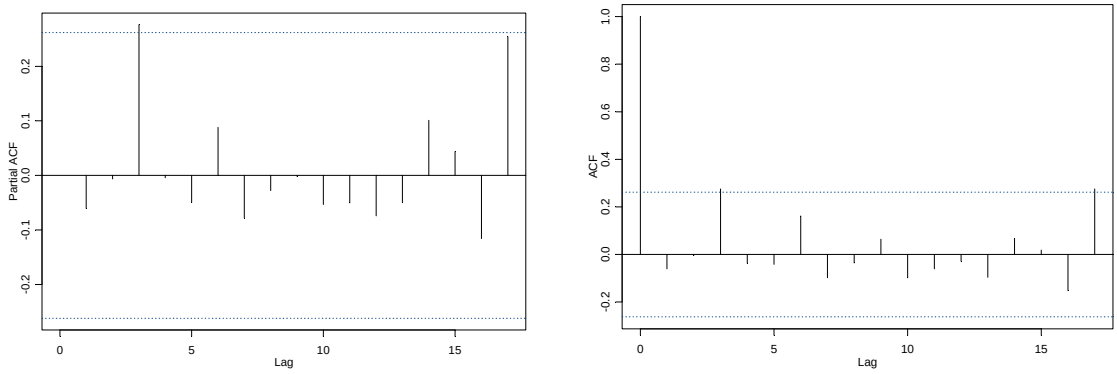

Figure 6.16. Evidence of lag (left) and cycle (right) in *Pectinophora gossypiella* counts from a pheromone trap at Campbell site between 2001 and 2006. Lag (x axis) is in months. Dotted lines represent 95% CI.

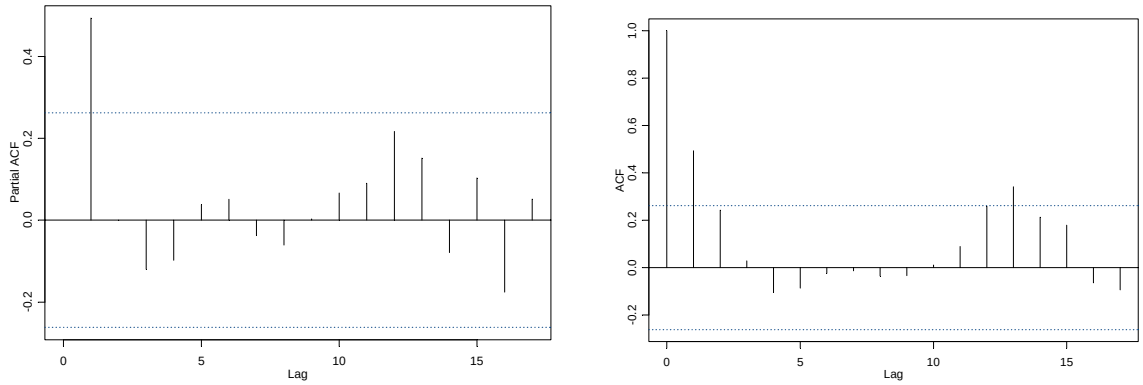

Figure 6.17. Evidence of lag (left) and cycle (right) in *Pectinophora gossypiella* counts from a pheromone trap at KRS site between 2001 and 2006. Lag (x axis) is in months. Dotted lines represent 95% CI.

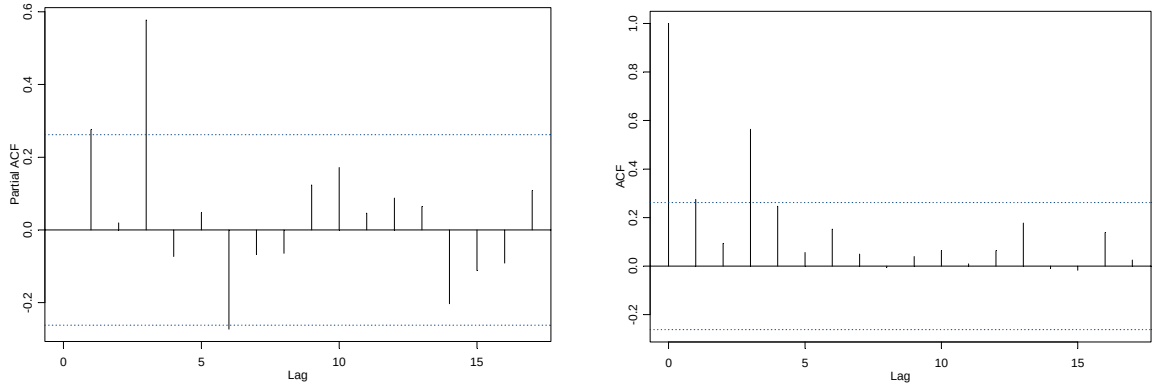

Figure 6.18. Evidence of lag (left) and cycle (right) in *Pectinophora gossypiella* counts from a pheromone trap at PCA site between 2001 and 2006. Lag (x axis) is in months. Dotted lines represent 95% CI.

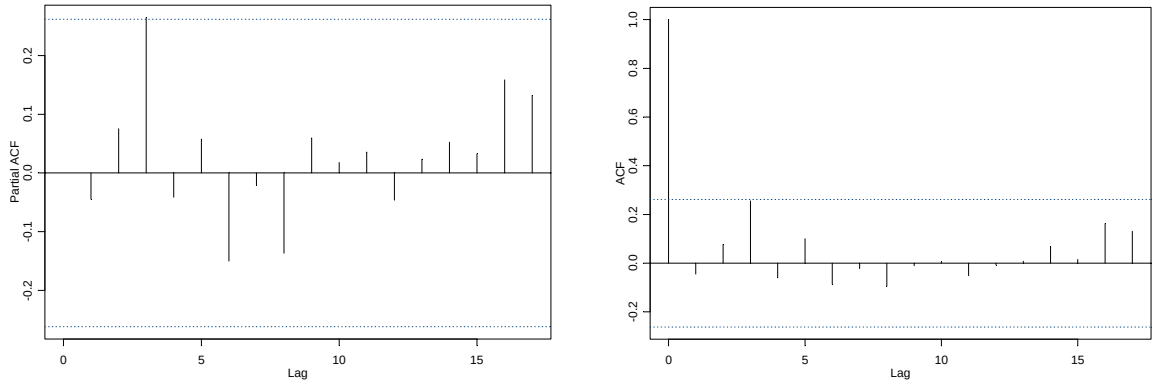

Figure 6.19. Evidence of lag (left) and cycle (right) in *Pectinophora gossypiella* counts from a pheromone trap at Shaw site between 2001 and 2006. Lag (x axis) is in months. Dotted lines represent 95% CI.

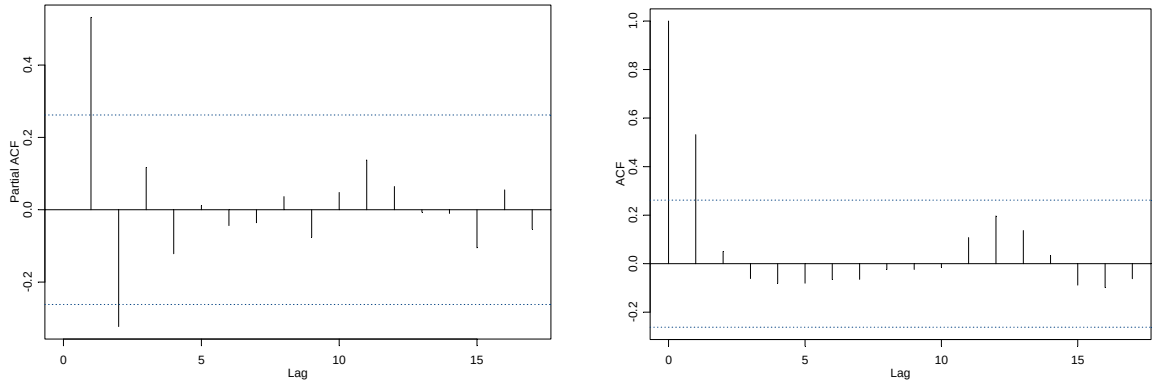

Figure 6.20. Evidence of lag (left) and cycle (right) in *Spodoptera litura* counts from a pheromone trap at Campbell site between 2001 and 2006. Lag (x axis) is in months. Dotted lines represent 95% CI.

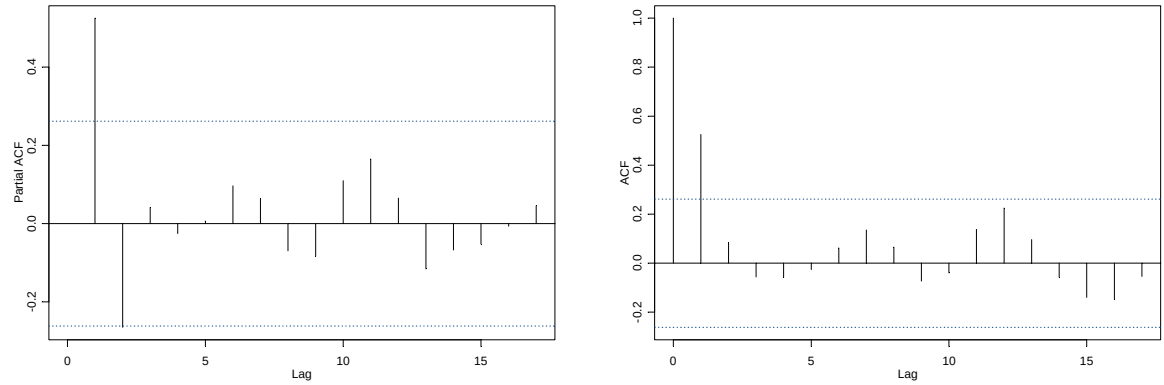

Figure 6.21. Evidence of lag (left) and cycle (right) in *Spodoptera litura* counts from a pheromone trap at KRS site between 2001 and 2006. Lag (x axis) is in months. Dotted lines represent 95% CI.

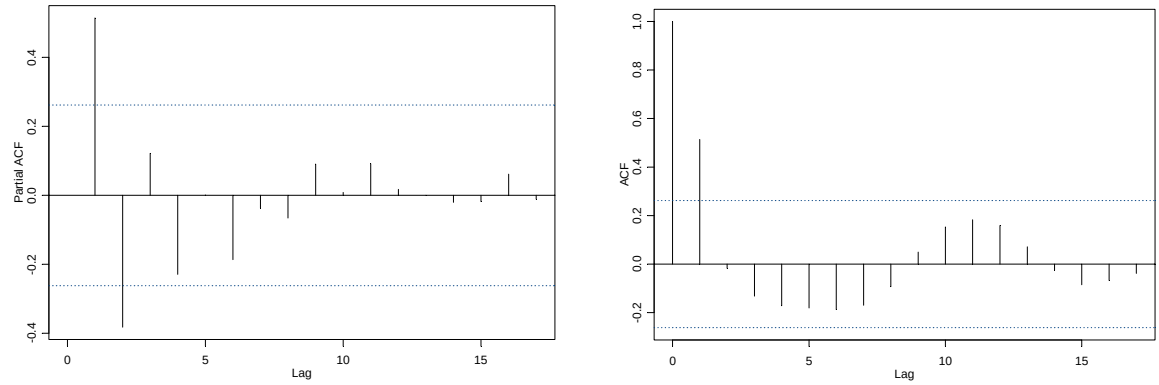

Figure 6.22. Evidence of lag (left) and cycle (right) in *Spodoptera litura* counts from a pheromone trap at PCA site between 2001 and 2006. Lag (x axis) is in months. Dotted lines represent 95% CI.

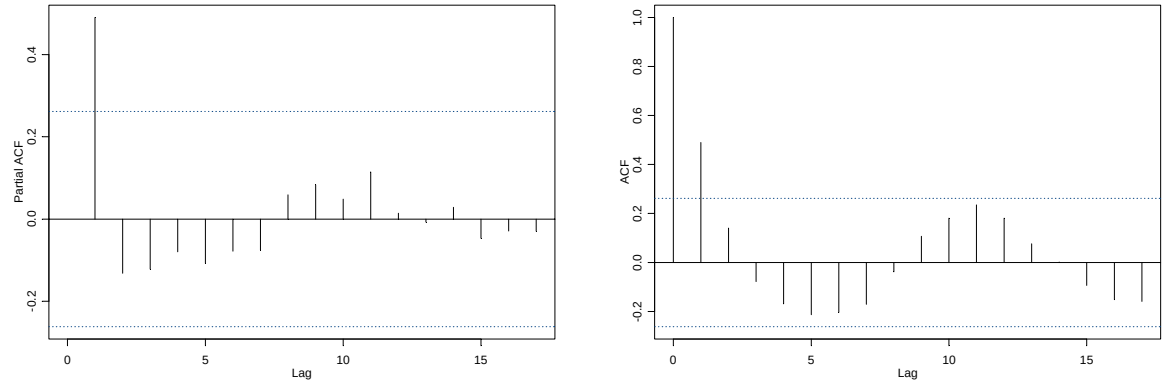

Figure 6.23. Evidence of lag (left) and cycle (right) in *Spodoptera litura* counts from a pheromone trap at Shaw site between 2001 and 2006. Lag (x axis) is in months. Dotted lines represent 95% CI.

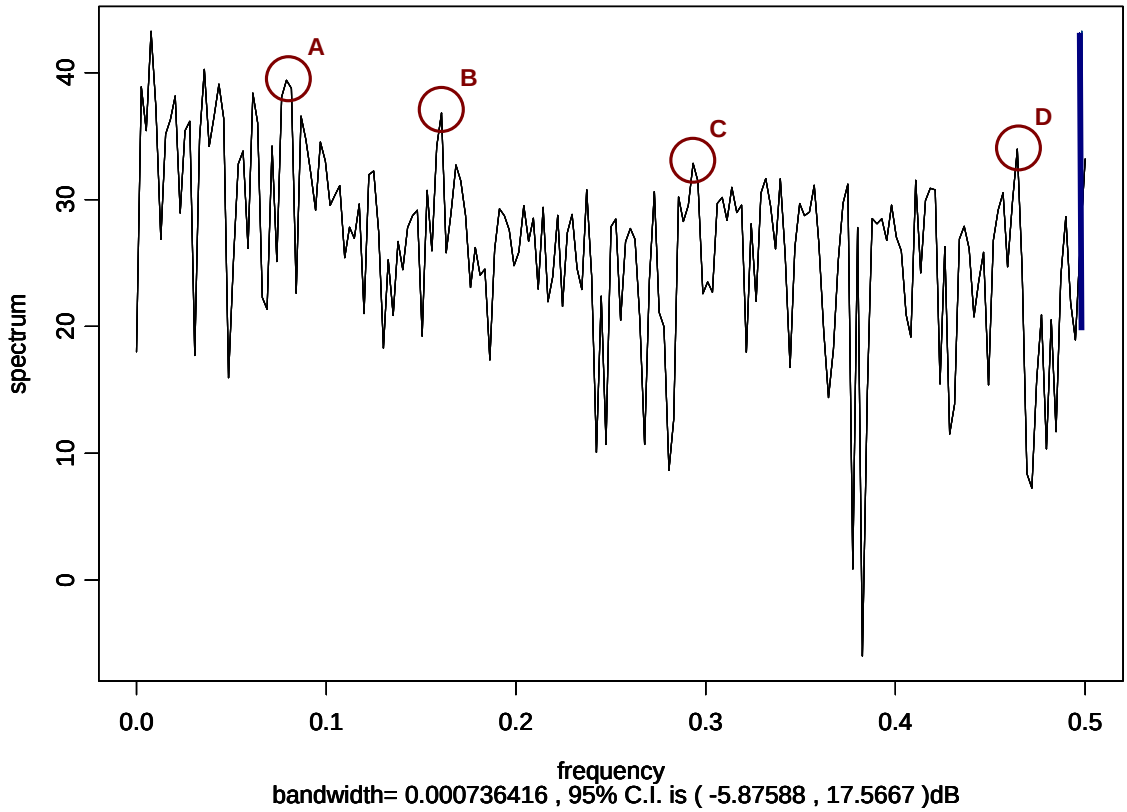

Figure 6.24. Spectral periodogram for *Helicoverpa armigera* counts from pheromone traps across all sites in the Katherine region from 2001 to 2006. Significant peaks are circled and the solid line (right) represents 95% CI.

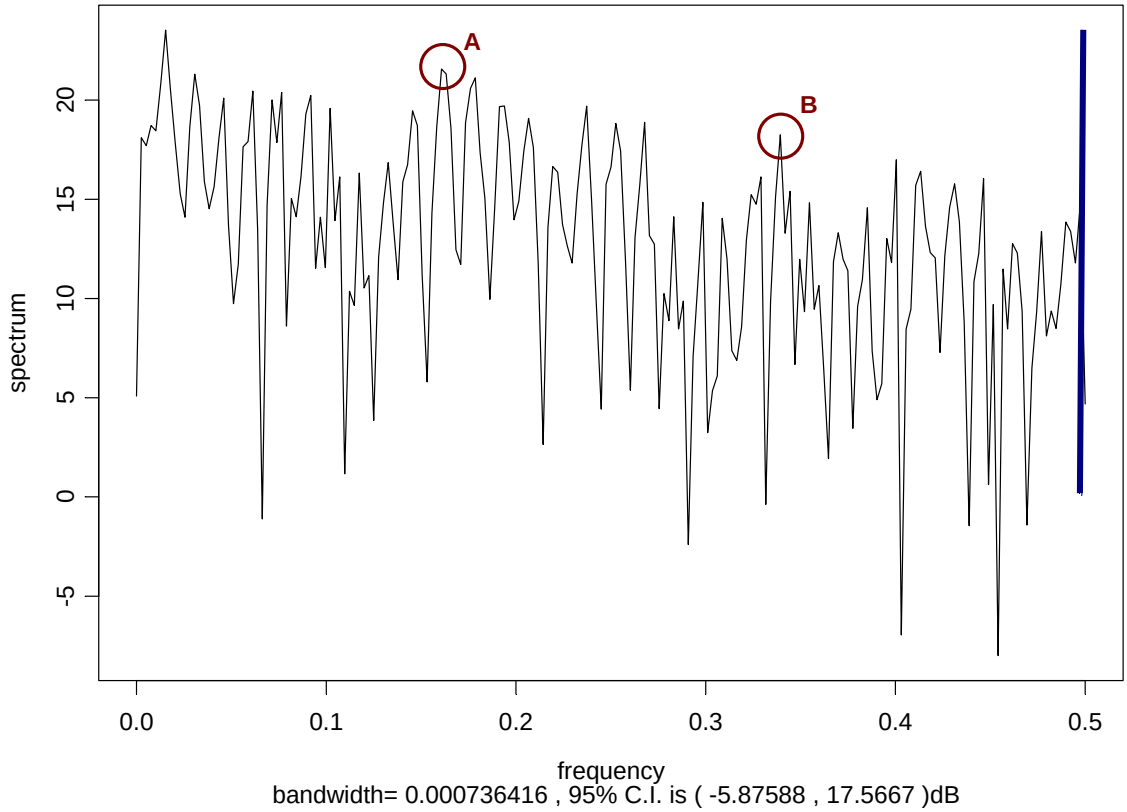

Figure 6.25. Spectral periodogram for *Helicoverpa punctigera* counts from pheromone traps across all sites in the Katherine region from 2001 to 2006. Significant peaks are circled and the solid line (right) represents 95% CI.

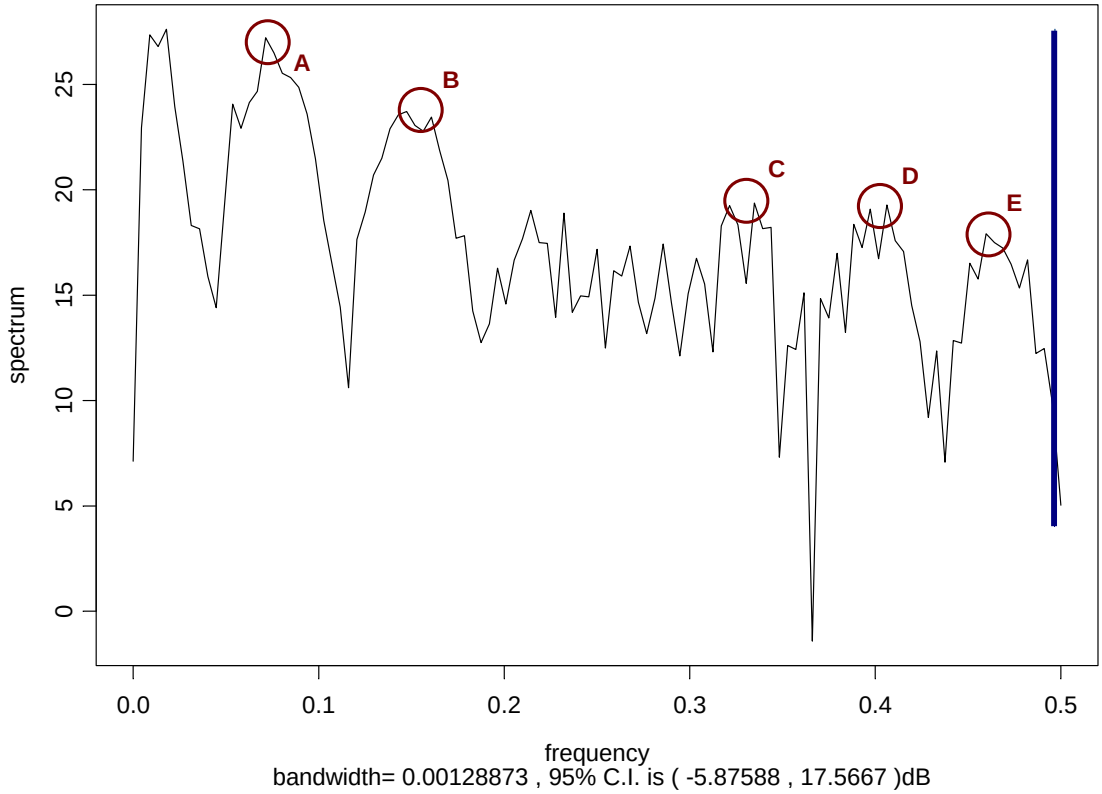

Figure 6.26. Spectral periodogram for *Pectinophora gossypiella* counts from pheromone traps across all sites in the Katherine region from 2001 to 2006. Significant peaks are circled and the solid line (right) represents 95% CI.

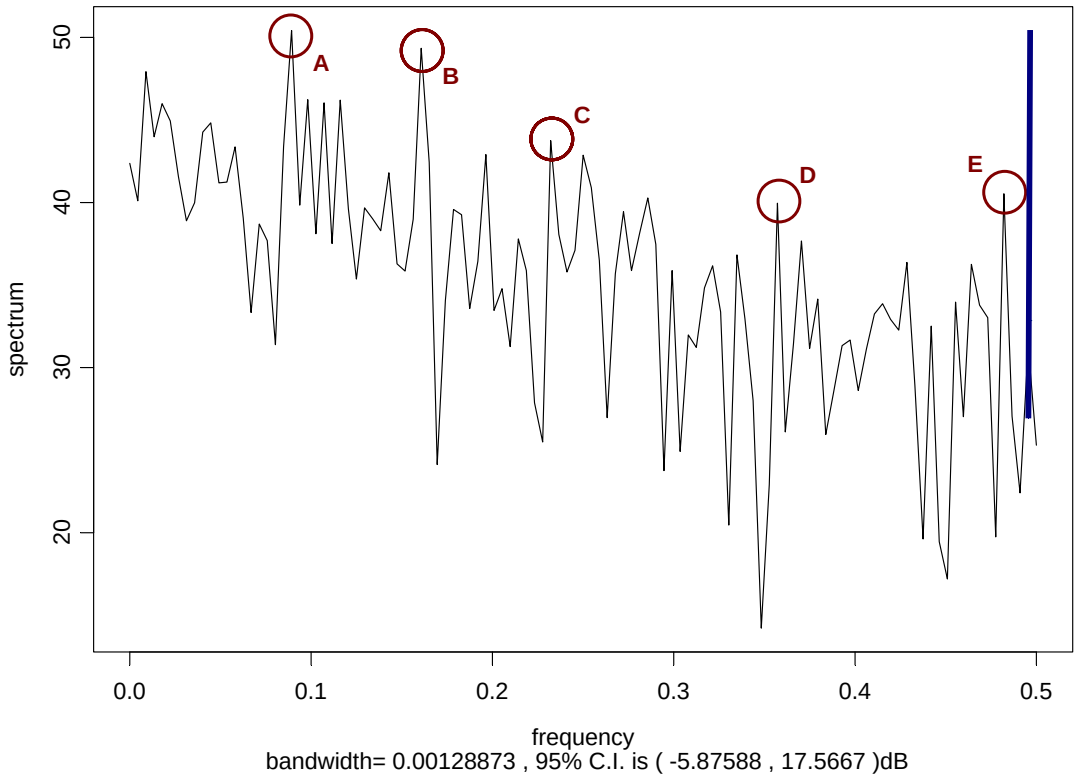

Figure 6.27. Spectral periodogram for *Spodoptera litura* counts from pheromone traps across all sites in the Katherine region from 2001 to 2006. Significant peaks are circled and the solid line (right) represents 95% CI.

Table 6.2. Results from testing of Katherine collected *Helicoverpa armigera* for resistance to conventional insecticides by ACRI. Values are given as percentages. *Helicoverpa armigera* were not collected in Katherine for resistance testing from 2003-04.

Date	Spinosad^	Bifenthrin	Chlorpyrifos^	Chlorfenapyr*	Profenofos*	Thiodicarb	Endosulfan*
09-May-01	**0**	**41**	**0**	**11**	**11**	**83**	NA
10-Jul-01	Pupae did not emerge						
18-Sep-01	**11**	**27**	NA	**33**	**31**	NA	NA
24-Mar-02	**0**	**60**	**0**	**4**	**3**	NA	**61**
21-Jul-02	**30**	**71**	NA	NA	**39**	NA	NA
21-Aug-02	Results not returned						
25-Sep-02	Pupae did not emerge						
14-Mar-05	Colony not viable at ACRI						
01-Jul-05	Insufficient *Helicoverpa* for remainder of season						

* Not utilised as an insecticidal treatment during the NT cotton project

^ Not utilised as an insecticidal treatment during 2002-04

Discussion

Helicoverpa armigera persist year round in Katherine, especially in areas where dry season mixed cropping provides a continuation of suitable habitat between wet seasons. For example, there is evidence that *H. armigera* populations continuously overlap in Katherine cropping regions (Figures 6.4, 6.8, 6.9, 6.10, 6.11 & 6.24). Dry season transgenic cotton crops are likely to experience influx of dense *H. armigera* populations continuously from seedling emergence in Katherine, as previously lush bushland bordering crops dries and becomes unsuitable habitat following cessation of the wet. Most significant is the twelve month cycle (Figure 6.24, A) between build up of dry season populations (Figure 6.8) on irrigated crops, interspersed with a six month cycle (Figure 6.24, B) incorporating wet season population peaks (Figure 6.8). Clearly irrigated dry season crops are very attractive to *H. armigera* populations that persist in lush bushland vegetation during the wet.

It is critical, therefore, that effective management strategies for potentially resistant *H. armigera* are universally implemented to maintain inherent Bollgard II® control efficacy. Recommended insect resistance management (IRM) strategies include companion cropping, discussed earlier, judicious use of *Bt* based insecticides, and continual monitoring of local *H. armigera* populations for possible emergence of resistance genes. *Bt* resistance is not a problem in Katherine *H. armigera* populations, but they have not been tested since 2002. Emergence trap results suggest Bollgard II® is possibly susceptible to resistant *H. armigera* development late season, if at all, so it is crucial refuge populations are maintained season long and beyond. Most disturbing is the prevalence of *H. armigera* resistance to conventional insecticides. A resistance management strategy, suggested in **General Discussion and Recommendations**, for conventional insecticides should be universally adopted to ensure effective and sustained area wide management of *H. armigera* in the Katherine region.

As opposed to persistent *H. armigera* populations in Katherine, *H. punctigera* tend to be more migratory (Wardaugh & Room 1980, Zalucki *et al.* 1986, Fitt 1989, Zalucki *et al.* 1994, Gregg *et al* 1989, Oertel *et al.* 1999) invading irrigated crops early season (Figures 6.5 & 6.8). Polarised twelve month *H. punctigera* population cycles are evident in Katherine with dry season population peaks in mixed cropping (Figure 6.13) and wet season population peaks in bushland (Figure 6.15) sites, although catch numbers were relatively low at the latter. Pheromone trap results for *H. punctigera* are further confounded by apparent year round population overlap at KRS (Figure 6.14) where population lag and cycle estimates conflict. Spectral analyses reflect 12 month cycles polarised between bushland and cropping sites, with strong evidence for a six month cycle in *H. punctigera* populations peaking in both dry and wet seasons (Figure 6.25, A). Less significant population cycles of 3 months (Figure 6.25, B) divide the two seasonal peaks. Clearly, *H. punctigera* have the potential to invade, persist in and cause damage to dry season irrigated cotton crops in Katherine.

Pectinophora gossypiella populations appear largely a wet season phenomenon in the Katherine region (Figures 6.8 & 6.26 (A)), although they do persist into early dry on occasion at some sites (Campbell, Shaw and PCA, Figures 6.6 & 6.26 (B)). Evidence suggests the population undergoes several generations during this period (Figures 6.16, 6.17, 6.18, 6.19 & 6.26). Utilising a winter cropping system for Katherine cotton production effectively avoids potentially damaging *P. gossypiella* population build up.

Wet season peaks are also evident for *S. litura* populations in Katherine (Figures 6.7 & 6.8). Although considered less damaging to cotton than the other pheromone trap targets, *S. litura* capably persist in dry season Katherine cotton crops occasionally breaching control

thresholds. This is evident in one month population lag and cycle estimates from analyses of all *S. litura* pheromone trap sites (Figures 6.20, 6.21, 6.22 & 6.23). From spectral analyses, wet season peaks and dry season cropping area build up are strongly reflected in peaks A and B, respectively in Figure 6.27. Year round population persistence produces less significant (C, D & E) cycle peaks (Figure 6.27). *Spodoptera litura* have a demonstrated potential to damage winter grown cotton crops in Katherine. The ability for cotton plants to tolerate or compensate for *S. litura* damage requires clarification, as does determination of control thresholds for this potentially important pest.

7.0 General Discussion and Recommendations

Results presented in this report have created a foundation from which future cotton IPM systems in the Northern Territory will evolve. This project has essentially been preliminary in nature and, while the insect management system outlined herein was fully vindicated when applied to relatively successful transgenic cotton production in the final year of the project, the majority of recommendations are for continued research aimed at more refined IPM. Ultimately, with application of novel technologies, biological control techniques and advanced research, reliance on conventional insecticide application in local cotton production will decrease further.

7.1 Insect pest control

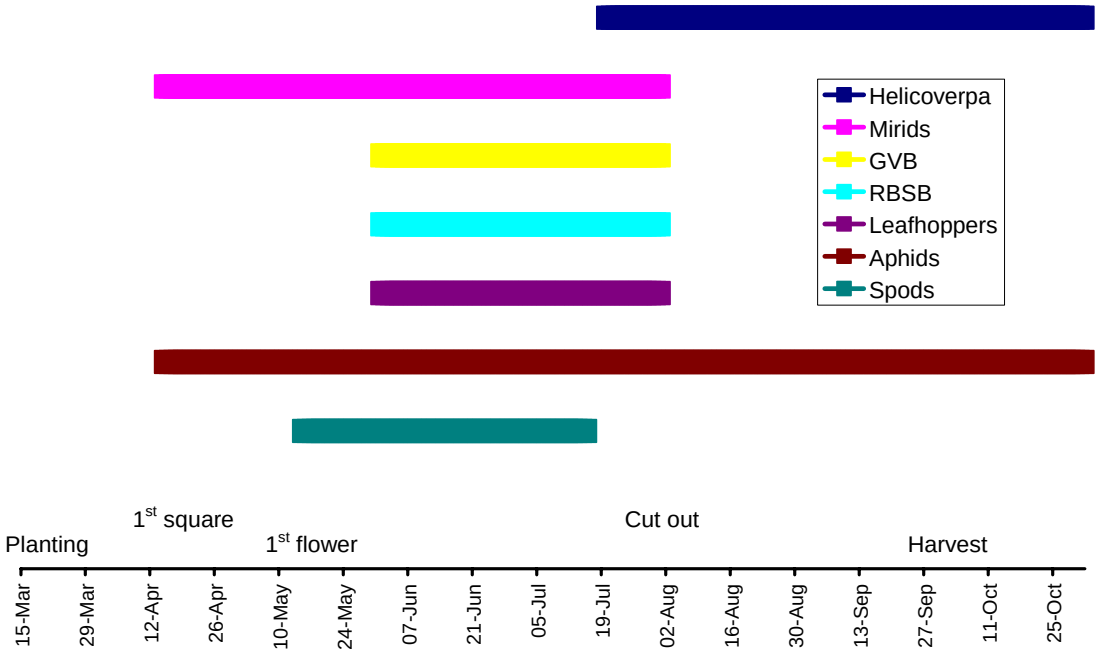

Figure 7.1. Major insect pests of Katherine cotton and the plant growth periods when monitoring and control, if necessary, is critical.

Helicoverpa

Bollgard II® effectively controls *Helicoverpa* larvae in Katherine. There is evidence *Helicoverpa* larval development on Bollgard II® is possible late season when *Bt* protein expression wanes, so careful monitoring of *Helicoverpa* phenology must continue beyond cutout. Relatively large *Helicoverpa* egg and larval densities may need control with appropriate insecticides, especially if signs of medium to large larval development are detected. Local *H. armigera* populations should be continuously monitored for resistance to conventional and *Bt* insecticides, and resistance management practices (discussed below) applied at all times.

Mirids

Mirids cause significant damage to fruiting cotton plants and must be monitored and controlled from first square to cutout (Figure 7.1). A control threshold of 0.5 mirid nymphs and adults per metre is currently recommended. Fipronil at the lowest rate mixed with salt (NaCl 10g/L (following Khan 2003)) effectively controls mirids early season with minimal

impact on natural enemies, other than *Trichogramma*. Imidacloprid can be rotated with fipronil to avoid possible resistance development, although the impact of imidacloprid on natural enemies has not been quantified in Katherine. Higher concentrations of fipronil may be required late season when the impact of natural enemies wanes and if mirids persist. Higher doses should only be used if absolutely necessary and the densities of other insect pests and the natural enemies controlling them should be taken into consideration.

GVB
Although GVB are intermittent pests in Katherine cotton, they have the potential to cause serious damage to developing and established cotton bolls (Figure 7.1) at high densities. GVB control is recommended at thresholds above 1.0 per metre. Unfortunately, mainly broad spectrum insecticides are currently effective against GVB, so natural enemy impact on other pests must be carefully monitored following application. Broad spectrum insecticides should be avoided if possible as they generally corrupt IPM systems, often beyond repair. GVB appear to be a late season phenomenon in Katherine cotton, when stability in the IPM system is not generally as critical.

RBSB
Like GVB, RBSB tend to infest Katherine cotton late season (Figure 7.1), but their pest status is questionable. There is evidence they cause significantly less damage than that attributed to GVB and mirids, although actual damage potential requires further clarification, especially early to mid season. At high densities, RBSB can be controlled with fipronil but monitor for efficacy, especially if low rates are used with salt.

Leafhoppers
Feeding damage to cotton leaves (hopper burn) caused by leafhoppers reduces leaf integrity and probably photosynthetic ability. Although tolerable at low densities, leafhoppers can cause serious damage late season if population growth is left unabated (Figure 7.1). Control thresholds and recommended insecticides for leafhoppers require clarification in Katherine cotton.

Aphids
Aphids persist season long but are maintained below control thresholds by natural enemies, such as coccinellids and syrphids, if not disrupted. Should insecticides that are disruptive to coccinellids and syrphids be required when aphid densities are approaching control thresholds, pirimicarb may be added as per product label suggestions to avoid aphid flare. Pirimicarb should be used no more than twice per season and never consecutively to avoid possible resistance development in aphid populations as per label suggestions.

Spods
Spods rarely require control in Katherine cotton. If spod densities increase to levels where fruit damage becomes apparent (5+ per metre) control with indoxacarb may be required. As always, monitor natural enemy activity prior to and following insecticide application. The damage potential and control thresholds for spods in Katherine cotton require clarification.

7.2 Resistance management

Research to date is not sufficient to construct a definitive resistance management strategy for *H. armigera* in Katherine. From the two years resistance testing was successful (2001 and 2002) it is clear local *H. armigera* populations already display resistance to conventional insecticides, including pyrethroids (Table 6.2). Resistance testing should continue and a

resistance management strategy be devised for late season *H. armigera* populations if Katherine cotton trials proceed.

Bollgard II® effectively suppresses *H. armigera* population development for the majority of the growing season in Katherine. As *H. armigera* have a demonstrable ability to develop resistance to *Bt* proteins (Bird & Akhurst 2005), it is crucial to the long term sustainability of an industry based on transgenic cotton that possible spread of resistance to *Bt* proteins in local *H. armigera* populations is managed (Fitt *et al.* 1994, Fitt 2000). Refuge crops are an accepted strategy to prolong Bollgard II® efficacy as they provide *H. armigera* individuals un-exposed to *Bt* which dilute the possible spread of resistance genes within the population. Questions remain in Katherine regarding which crops to use as refuges and at what percentage area should they be maintained. Optimal refuge crops will only be finalised after further research. It is possible to model refuge requirements to specific transgenic crops and regions (see Peck *et al.* 1999, Storer *et al.* 2003a, 2003b, Carriere *et al.* 2004, Sisterson *et al.* 2004, Sisterson *et al.* 2005), however, this is beyond the capabilities of the present study and should be examined in the future.

7.3 Research suggestions

It is envisaged research refining the IPM system would continue should cotton production trials proceed in Katherine. By refining the IPM system, reliance on insecticides could be further reduced. The aim is to promote sustainability and environmental acceptability of cotton production in the NT.

7.3.1 Companion crops

Although lablab displays vigorous growth and hardiness as a companion crop to winter grown cotton in Katherine, its benefit to IPM is questionable when you consider it does not seem to influence pest insect or natural enemy densities and lint yield and fibre quality in neighbouring cotton. Alternate cultivars, including some that do not attract cotton pests but natural enemies, should be considered in future trials. Trap crops to reduce pest densities in cotton also require further investigation in Katherine. Preliminary trials suggest lablab and pigeon pea are attractive to cotton pests, although they also attract high numbers of natural enemies. Early and late season trap cropping systems involving these and other cultivars should be investigated.

7.3.2 Sucking insects

Mirid control thresholds require clarification. Brown mirids (*C. pacificus*) are the dominant species in Katherine cotton, however, their pest status has been questioned (Malipatil & Cassis 1997). Quantifying their damage potential becomes complicated, especially if they are confused with less common green mirids (*C. dilutus*), which are known to devastate early season cotton crops at high densities. The relative abundance of mirid species and their pest status must be clarified across the growing season, so control thresholds can be further refined.

Likewise, the damage potential and pest status of GVB, RBSB and leafhoppers require clarification in Katherine cotton. Biological control options and possible chemical control thresholds should be examined.

7.3.3 Natural enemies

Natural enemy research has been largely neglected during Katherine cotton trials. A catalogue a natural enemies present is useful as a starting point for more relevant research investigating relative abundance and possible impact on pest species. The control capabilities of coccinellids and syrphids on aphids and whitefly require clarification and an index of relative densities developed to facilitate control decisions when necessary. It would be useful to continue *Trichogramma* research, especially in years when relatively high densities of *Helicoverpa* are experienced. The impact of natural enemies on sucking pests has garnered little attention to date, and should become a research focus should cotton trials proceed.

8.0 Communication of results

Project results were presented at annual CRC reviews and biannual Australian Cotton Conferences. An article for the Australian Cotton Grower was also produced. Results were presented at CRC Northern Program reviews, internal departmental reviews, grower and community meetings, and on ABC radio. During the final years of the project, communication was stifled by censorship and stakeholder apathy. Two manuscripts have been published or submitted in peer reviewed scientific journals, and several more will be gleaned from analyses in this report.

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10.0 Acknowledgements

Project participants acknowledge the financial support of the Cotton CRC and the Northern Territory Government. Thanks to all at ACRI Narrabri, especially Ms Lynda George, Ms Kym Orman and Mr Guy Roth, for never giving up hope. Dr Andrew Ward was responsible for developing this project and his research provided an excellent foundation from which the current IPM system evolved. Mr Geoff Strickland and Mr Stephen Yeates also contributed knowledge and support for the life of the project. The farm staff at KRS, headed by Mr Jack Wheeler, performed required tasks to the best of their abilities. Departmental staff at KRS and in Darwin helped things run smoothly when they could. Dr Ali-Nur Duale filled the position of cotton entomologist in Katherine for one year from 2003 to 2004, and Dr Colin Martin was project leader for project entirety. Dr Brian Thistleton, Ms Dianna Owens, Ms Joannah Avenell, Mr Mike Kahl, Mr Richard Renfree, Mr Clintunn Newbould, Ms Keera Schrimp, Ms Melinda Boyd, Mr Douglas Summers and Ms Megan Connolly provided excellent technical support. Ms Rowena Eastick, Mr Andrew Dougall and Mr John Moulden were invaluable sources of knowledge and inspiration during difficult times. Many thanks to all.

Appendix I: Project budgets.

Financial management at DPIFM was confounded by incorrect monetary allocations and subsequent corrections and frustratingly difficult to comprehend. It was not possible to accurately calculate actual budget expenditure for the 2005/06 financial year until May 2006, for example. Below are projected expenditures from project applications and reports, which are the best available indications of actual expenditure under the circumstances.

Item	2000/01 Original Estimate $	2001/02 Original Estimate $	2002/03 Original Estimate $
A STAFFING			
Salary (New Entomologist–P2)	29,263	58,527	60,989
Salary (New Technician-T1)	13,729	27,459	28,614
Oncosts (25% salary)	10,748	21,497	22,401
TOTAL STAFFING	$53,740	$107,483	$112,004
B TRAVEL			
Cotton conference fares	0	0	2,600
Travel allowance (interstate)	500	4,000	6,000
Intra-territory travel allowance	2,500	4,000	5,000
TOTAL TRAVEL	$3,000	$8,000	$13,600
C OPERATING			
Furniture relocation (A. Ward) visit	$6,000	0	0
Narrabri training, Darwin wk/shop, move to Darwin visit	$11,000	0	0
Interview expenses (fares, TA, A. Ward)	$1,623	0	0
Relocation and hiring New Technician	$8,000	0	0
Freight	0	1,500	1,500
Field consumables	4,000	4,000	4,000
Lab. Consumables	2,500	3,500	3,500
Software	0	500	500
Casual labour	1,500	3,000	3,000
4WD vehicle	5,000	12,000	12,000
TOTAL OPERATING	$39,623	$24,500	$24,500
TOTAL REQUESTED	$96,363	$139,983	$150,104

Item	2002/03		2003/04	
	Original Estimate $	Now Requested $	Original Estimate $	Now Requested $
A STAFFING	112004	115700	56851	58871
TOTAL STAFFING	112004	115700	56851	58871
B TOTAL TRAVEL	13600	13600	5500	5500
TOTAL TRAVEL	13600	13600	5500	5500
C OPERATING	24500	24500	24500	24500
TOTAL OPERATING	24500	24500	24500	24500
D CAPITAL	Nil	Nil	Nil	Nil
TOTAL CAPITAL	0	0	0	0
TOTAL REQUESTED (A+B+C+D)	150104	153800	86851	88871

Item	2005/06 $	2006/07 $	2007/08 $
Staffing [please itemise] P2 Research Scientist T2 Technician (till November 05) On Costs (25%)	 67,899 13,012 20,228		
Total Staffing	101,139		
Travel [please itemise] Interstate (ACRI twice) Intrastate (CRC and DPIFM cotton meetings in Darwin)	 2,500 1,100		
Total Travel	3,600		
Operating [please itemise] Vehicle (till November 05) Office and IT expenses Consumables (lab/field)	 3,800 7,500 1,000		
Total Operating	12,300		
Capital [please itemise]			
Total Capital			
Total Requested	117,039		

Appendix II: Abstracts from published papers

Evidence of a latitudinal gradient in spider diversity in Australian cotton.

Mary E. A. Whitehouse, Scott Hardwick, Brad C.G. Scholz, Amanda J. Annells, Andrew Ward, Paul R. Grundy, Steven Harden

1. If a latitudinal gradient in species diversity is largely governed by spatial heterogeneity, then the diversity of a community in a monoculture should be identical, irrespective of where it occurs.
2. Spiders are the dominant community in Australian cotton which is grown along a latitudinal gradient. We tested to see if spider diversity in cotton changed with latitude, and if the spider community in cotton in different parts of Australia was structurally identical.
3. We sampled seven sites extending over 20° of latitude. At each site we sampled 1-3 fields 3-5 times during the cotton growing season using pitfall traps and beat sheets, recording all the spiders collected to family.
4. We found that spider communities in cotton are diverse, making them suitable for a conservation biological control program.
5. We also found that spider diversity increased from high to low latitudes, and the communities were different, even though the spiders were in the same monocultural habitat.
6. Spider beat sheet communities around Australia were dominated by different families, and responded differently to seasonal changes, indicating that different pest groups would be targeted at different locations.
7. Synthesis and applications. These results show that diversity can increase from high to low latitudes, even if spatial heterogeneity is held constant, and that other factors external to the cotton crop are influencing spider species composition. Other models which may account for the latitudinal gradient observed in this study are discussed.

Development of a treatment threshold for sucking insects in determinate Bollgard II transgenic cotton grown in winter production areas

Andrew L. Ward

Little is known about the impact of sucking insects on cotton grown in tropical production areas. To examine this, an experiment was conducted at Katherine in northern Australia to determine the impact of mirids (*Creontiades dilutus* (Stål) and *Creontiades pacificus* (Stål), green vegetable bug (*Nezara viridula* (L.)) and red-banded shield bug (*Piezodorus hybneri* (Gmelin)) on the yield and fibre quality of the transgenic cotton variety DP50bx containing genes for the expression of Cry1A(c) and Cry2A(b) endotoxins of *Bacillus thuringiensis* Berliner variety *kurstaki*. The trial examined the impact of sucking insect populations in the range 0.5-2.0/m^2. Yields of unsprayed plots were approximately 30% of those obtained in plots sprayed regularly to exclude sucking insects. The increase in yield in the low pest density treatments was the result of improved fruit retention in the middle part of the plant. Fibre quality was similar in all treatments. However, damage ratings to individual bolls did differ between treatments. Bolls in the high insect density treatments received more damage than those in the low density treatments. As a result of this study, a tentative treatment

threshold of 0.5 sucking insects/m^2 is recommended in determinate Bollgard II cotton varieties grown in winter production areas.

Other Appendices

Appendix 1. Project progress report 2002. Dr Andrew Ward.

Research progress report
Insect dynamics of cotton ecosystem in Northern Territory
CRC Project 1.3.1
Dr Andrew Ward
Table of Contents

1. Executive summary

Observations made in the 2001 and 2002 seasons suggest that the major insect problems at Katherine are Helicoverpa armigera and a range of sucking insects including mirids, red banded shield bugs and green vegetable bugs. Spodoptera litura also have the potential to cause sporadic damage early in the season but seem to “disappear” as the season progresses. Aphids were also a problem in 2002 but in most fields were managed effectively by beneficial insect populations.

With the move to producing Bollgard II varieties, Helicoverpa are likely to become less of a problem. As a result, a major focus of the research program has become sucking insects. A broad aim of the research being conducted at Katherine Research Station is to develop a cotton production system with minimal reliance on insecticides. To this ends the Katherine program has focused on the development of techniques and tools that minimise the requirement for insecticide use, including the use of trap and companion crops to reduce pest pressure in cotton and the development of action thresholds. The results to date suggest that populations of sucking insects up to at least 1.5/m can be tolerated up to first flower. However, after first flower populations of 0.5 /m appear to result in significant yield loss.

Trials conducted in both 2001 and 2002 have examined a number of companion crops with the view of selecting a crop that is attractive to sucking insects, Helicoverpa and beneficials. Crops that have been examined include lab lab, niger, chickpea, kenaf, pigeon pea and sesame. At this stage it appears the best all round option is lab lab which was the most attractive crop to Helicoverpa and sucking insects and was also reasonably attractive to beneficial insects.

Beneficial insects have been observed to have a significant impact on the pest populations in Katherine. This has been particularly evident in aphids with hover fly larvae successfully controlling large outbreaks. Parasitism of Helicoverpa eggs as a result of Trichogramma pretiosum has been evident in the early part of the season but quickly falls away to zero by early June. Microplitis and tachinid parasitism is also evident late in the season. However, its contribution in terms of total pest management appears to be minimal.

A net work of pheromone traps has been operating for the last 2 years at 8 sites to examine the seasonal abundance of the major lepidopterous pests likely to impact on any future cotton industry in Katherine. The trap catches support the basis for a move to dry season production to avoid high populations of Spodoptera litura and Pectinophora gossypiella with the highest catches of both species occurring during the wet season. Helicoverpa armigera populations were the reverse with the highest populations being recorded during the dry season while the cotton was growing. Very few Helicoverpa punctigera were trapped at any of the 8 sites were monitoring took place. This was supported by lepton test results which demonstrated that most Helicoverpa were H. armigera.

Insecticide resistance testing has demonstrated that resistance levels are generally low. This is not surprising due to the limited area of crops in the Katherine area. The susceptibility of Helicoverpa at Katherine to Bt as indicated by probit analysis demonstrated that the Katherine strain sent for testing had higher tolerance to Bt than the Narrabri susceptible strain. Despite this the Katherine strain is more susceptible to Bt than the Helicoverpa in Kununurra.

2. Project milestones 2001 and 2002

2001

1. Monitor key lepidopteran pests (4) weekly using pheromone traps at 10 sites
2. Assess resistance levels in H. armigera to conventional insecticides monthly during the season
3. Determine the base-line susceptibilities of H. armigera & H. punctigera to BT
4. Collaborate with CSIRO in a strontium mark-recapture study of regional Helicoverpa population dynamics
5. Monitor Trichogramma activity and identify local species
6. Rear & identify beneficial insect species and rank their status in the NT – link to biodiversity studies

2002

1. Monitor key lepidopteran pests (4) weekly using pheromone traps at 8 sites
2. Continue resistance testing and develop a resistance management strategy for H. armigera and Aphis gossypii
3. Monitor for BT resistance
4. Assess the suitability of various trap crops for use in the NT.
5. Assess the impact of sucking insects on cotton grown in the NT
6. If not present, introduce Trichogramma pretiosum from Kununurra
7. Rear & identify beneficial insect species and rank their status in the NT

2003

1. Monitor key lepidopteran pests (4) weekly using pheromone traps at 10 sites
2. Continue resistance testing and develop a resistance management strategy for H. armigera and Aphis gossypii
3. Monitor for Bt resistance
4. Develop trap cropping protocols for sucking insects and Helicoverpa in the NT and monitor their impact in field scale trials
5. Assess the impact of sucking insects on cotton grown in the NT including the continued development of thresholds
6. Monitor the impact of T. pretiosum.
7. Rear & identify beneficial insect species and rank their status in the NT

3. Milestone related research

3.1 Milestone 1 (2001 and 2002)

Monitor key lepidopteran pests weekly using pheromone traps at 8 sites

Aim: To monitor the seasonal abundance of the four key lepidopterous pests Helicoverpa armigera, Helicoverpa punctigera, Spodoptera litura and Pectinophora gossypiella.

Methods: In April 2001 a network of pheromone traps were established around Katherine and at Douglas Daly. Sites were chosen according to their proximity to existing agricultural areas, proximity to bush refuges and proximity to areas that remain wet well into the dry season. A list of each site, the species monitored and its attributes are listed in Table 1. The traps were monitored weekly and the lures and killing agents changed monthly.

Table 1: Location of pheromone traps, attributes of each site and the species monitored

Site	Attributes	Species				
		H.a*	H. p	S. l	P.g	S. e
KRS	Cotton farming	√	√	√	√	
Aust. Asia	Wet refuge	√	√	√	√	
Ballongilly farm	Dry refuge	√	√			
Campbells	Peanut farming	√	√	√	√	√
Stuart Hwy	Dry refuge	√	√			
Shaws	Small crops	√	√	√	√	
Powers	Small crops	√	√			
DDRF	Peanut farming	√	√	√	√	√

(* H. a – Helicoverpa armigera, H. p – Helicoverpa punctigera, S.l - Spodoptera litura, P. g – Pectinophorra gossypiella, S. e – Spodoptera exigua.)

Results and discussion:

The populations of all species showed distinct seasonality across all sites. Helicoverpa armigera populations were highest in the dry season, which corresponded to the times when crops were being produced (Fig 1). These populations fell considerably in the wet season. This was probably the result of dispersal into the environment away from irrigated cropping locations after the commencement of the wet season. Despite this, the non-cropped sites caught few if any moths as did the wet refuge at Aust. Asia.

In general few if any H. punctigera were caught at any of the sites monitored (Fig 2). This was particularly evident in 2001. In 2002 a large immigration occurred at Campbells in which moths were caught over several weeks. The relative abundance of H. armigera and punctigera in the pheromone traps reflect the results obtained in the lepton tests where most eggs tested were H. armigera.

Pectinophora gossypiella populations were typically highest in the wet season and fell away sharply in the dry season (Fig 3). These results are reflected in the crop monitoring data, where very few P. gossypiella have been detected in crop.

The population dynamics of Spodoptera litura differed between different sites (Fig 4). At KRS their numbers peaked in the wet season but fell away sharply once cotton spraying started, whilst at Campbells where the peanuts remained unsprayed the Spodoptera numbers peaked during the dry. The continued presence of low catches of S. litura after their peak in December – February suggests that any attempt to push cotton planting forward into early March may result in high Spodoptera pressure in the early part of the season.

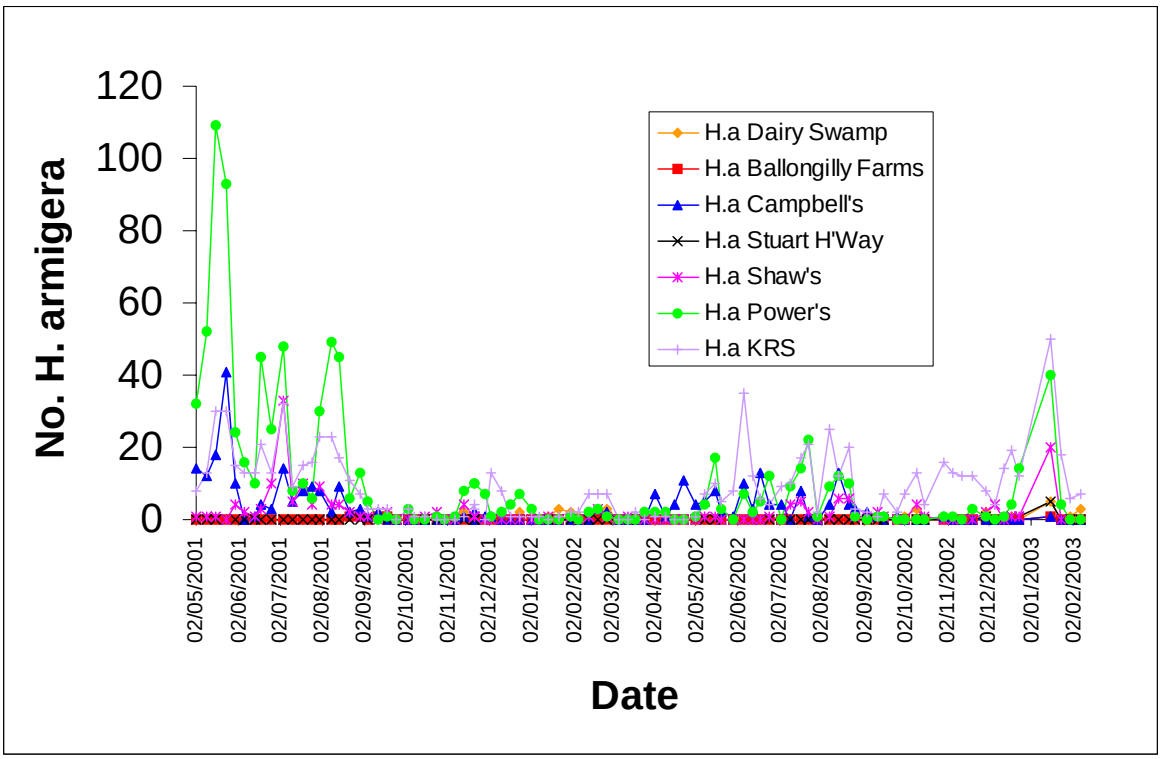


Fig 1: Pheromone trap catches of Helicoverpa armigera at sites where moths were caught.

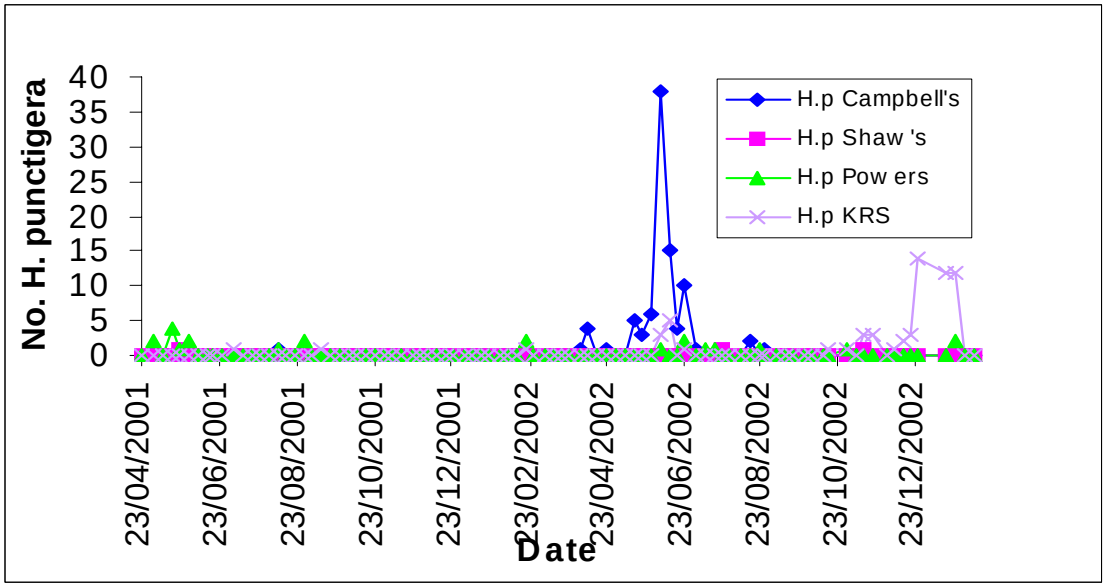


Fig 2: Pheromone trap catches of Helicoverpa punctigera at sites where moths were caught.

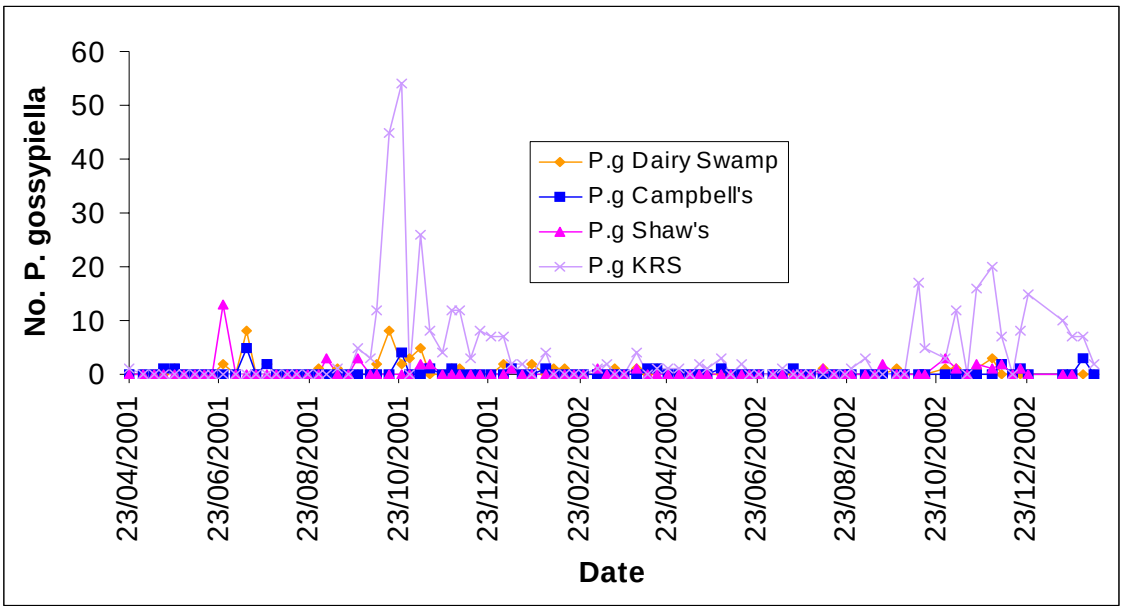


Fig 3: Pheromone trap catches of Pectinophora gossypiella at sites where moths were caught.

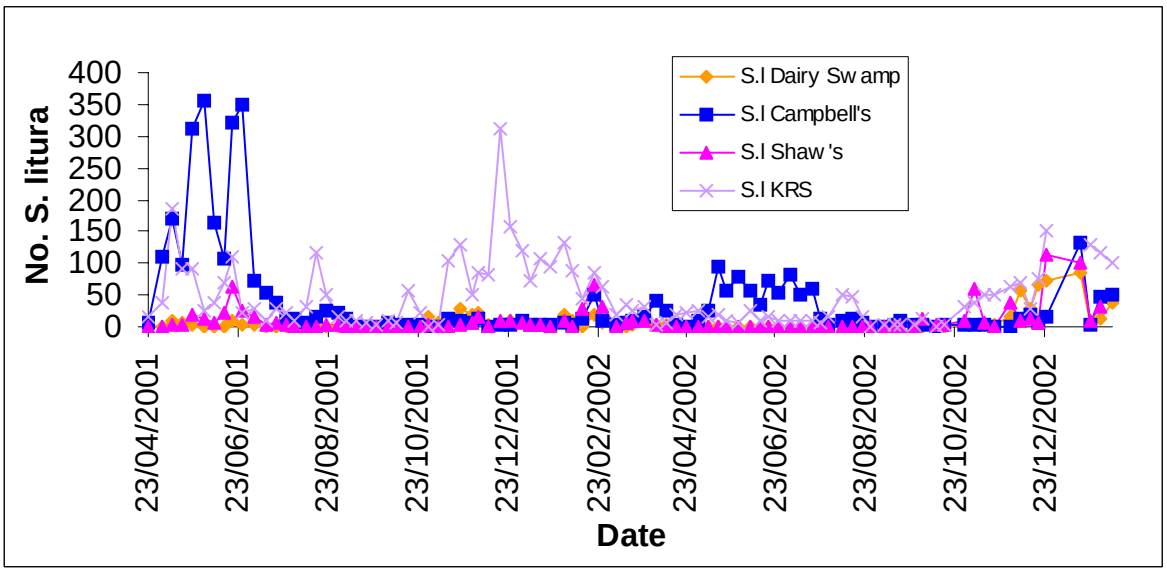


Fig 4: Pheromone trap catches of Spodoptera litura at sites where moths were caught.

3.2 Milestones 2 and 3 (2001 and 2002)

Resistance monitoring to BT and conventional chemistry

Aim: To monitor the susceptibility of Helicoverpa armigera to Bt and conventional insecticides.

Methods: Resistance monitoring for Bt and conventional insecticides was undertaken in both 2001 and 2002. In each case Helicoverpa armigera larvae were collected in the field and reared through to pupae which were couriered to Hoe Dang, Lisa Bird and Robin Gunning. A minimum of 50 pupae were sent each time. The standard methods used by each tester were employed in the testing.

Results and discussion

The KRS heliothis populations sent for resistance testing displayed heightened resistance to a range of products including chemicals that have never been used in the cotton program (Table 2). Chlorpyrifos was the only chemical tested that resistance was not detected to. However, in general the resistance levels were low. With the exception of bifenthrin there was no consistent trend to increasing resistance levels. These results have been supported by generally good insect control when spraying was undertaken providing conditions were optimal.

Table 2: Results of resistance tests conducted by Dr Robin Gunning on H. armigera larvae sourced from KRS.

Collection date	Spinosad	Bifenthrin	Chlorpyifos	Chlorfenapyr*	Profenofos*	Thiodicarb	Endosulphan*
2001							
9/5/01	0 %	41 %	0 %	11 %	11 %	83%	
10/7/01	Pupae did not emerge						
18/9/01	11 %	27 %		33 %	31 %		
2002							
24/3/02	0 %	60 %	0 %	4 %	3 %		61 %
21/7/02	30 %	71 %			39%		
21/8/02	Results not returned						
25/9/02	Pupae did not emerge						

* Profenophos, Chlorfenapyr and Endosulphan have never been used in the cotton project at KRS. Chlorpyifos and Spinosad were not used in 2002.

BT

The results of the Bt resistance monitoring indicated that the baseline resistance obtained was higher than the susceptible strain used in Narrabri by Hoe Dang. Probit analysis showed the LD99.9 to be 477794 ul/ml of diet which was significantly higher than the Narrabri susceptible strain (Table 3).

Survival at the discriminating MVP dose of 3ul/ml of diet resulted in similar survival in both 2001 and 2002 with between 11.6 and 13.5 % survival. This was less than the survival obtained in strains sent from Kununurra which was 12.1 %. No larvae survived when exposed dipel and Xentari (Table 4).

Table 3: Base line B.t susceptibility of Helicoverpa armigera at Katherine compared to the Narrabri susceptible strain as determined using probit analysis

LD	Katherine (ul/ml MVP)	Narrabri Susceptible strain (ul/ml MVP)
50	1.05	0.103
90	69.15	0.78
95	193.13	1.29
99	769.8	2.53
99.9	47794	18.8

Table 4: Summary of Bt Resistance testing data at Katherine Research Station

Collection date	Tester	Dipel (2mg/ml)	Xentari (2mg/ml)	MVP (3u/l/ ml)
2001				
10/7/01	H. Dang	0 %	0 %	11.6 %*
18/9/02	H. Dang	Pupae did not emerge		
2002				
25/1/02	H. Dang	Colony infested with stunt virus		
24/3/02	H. Dang	0%	0%	13.5%*
25/9/02	L. Bird			0%#

* tested on third instar larvae
# tested on 1st instar larvae

Milestone 4 2001

On the advice of Geoff Strickland (Program 1, Program leader) a decision was made not to undertake the strontium mark recapture program at this stage. In its place, preliminary studies were undertaken examining trap crops suitable for use in the Northern Territory as well as studies examining the impact of sucking insects (brown mirids, red banded shield bugs and green vegetable bugs). These studies will continue for the remainder of the project.

3.3 Milestones 5 and 6, 2001 and Milestones 6 and 7, 2002

Parasitism of Helicoverpa armigera eggs and larvae and lepton testing

Aim: To determine the incidence of parasitism of H. armigera and the species involved.

Methods: Egg and larval parasitism levels of Helicoverpa armigera were monitored periodically at KRS throughout 2001 and 2002. The periodic nature of the sampling was the result of difficulties in sourcing enough eggs and larvae to sample. When sampling for egg parasitism a minimum of 100 white eggs were collected from the field and reared in Microtitre trays covered with sticky tape. In the case of larvae a minimum of 100 second or third instar larvae were collected and reared on artificial diet (Toowoomba DPI recipe). The eggs or larvae were maintained at approximately 25 °C and the incidence of parasitism was recorded. The emerged parasitoids were identified.

When sufficient eggs were available Lepton tests were also conducted to determine the species composition of the Heliothis population.

Results

In both 2001 and 2002 egg parasitism contributed significantly to the mortality of H. armigera eggs in the early part of the season (Fig 5). However, as the season progressed the egg parasitism levels declined rapidly until virtually no parasitism was evident by June. The sole Trichogramma species identified was Trichogramma pretiosum (Andrew Davies 2002).

Larval parasitism levels were in general low in both years with parasitism levels of 10-20% on each sampling occasion (Fig 6). The predominate species identified were tachinids and Microplitus.

Results of the Lepton tests conducted in both 2001 and 2002 showed that most Heliothis eggs were H. armigera. These results were consistent with the pheromone trap catches which showed that most moths were H. armigera.

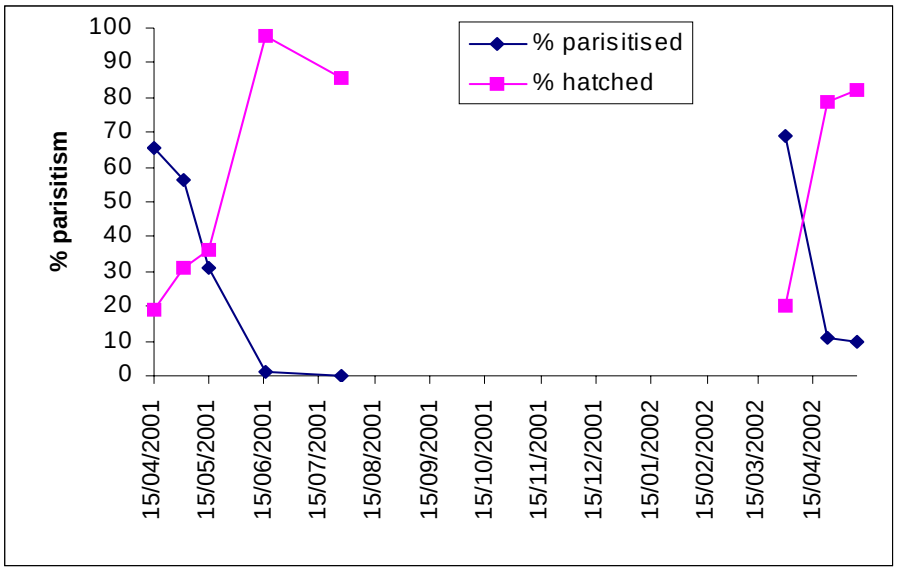


Fig 5: Egg parasitism levels at Katherine Research Station in 2001 and 2002

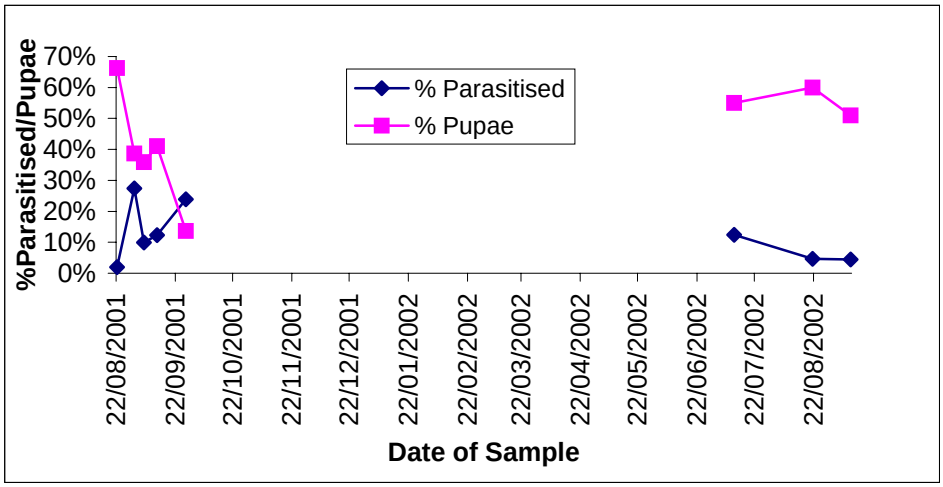


Fig 6: Percentage of larvae collected that were parasitised or reached pupation in the 2001 and 2002 cotton seasons.

3.4 Milestone 4 2002

Assess the suitability of various trap crops for use in the NT.

Aim: To identify trap crops suitable for use in Katherine as part of a cotton IPM program

Background

Trap cropping has become an important component of southern IPM programs. To investigate whether trap crops could be incorporated into the entomology package being developed in Katherine trials commenced in 2001 looking at six different crops. The most promising trap crops were Niger, lab lab and pigeon pea which were all found to be attractive to sucking insects, Helicoverpa and beneficial insects. In response to these results, further trials were conducted in 2002 to assess which crop has the best fit in our program.

Materials and Methods

Trials were conducted in both 2001 and 2002 to evaluate the most effective trap crops for use in the Northern Territory.

The 2001 trial was conducted under the lateral irrigator and was planted using the cone seeder. The trail examined 6 crops arranged in a randomized block. The six crops examined were Pigeon pea, chickpea, sesame, niger, lablab and kenaf. Each plot was 13 m long x 12 rows (m) wide.

The 2002 trial was planted using the trash culti drill with a row spacing of 50cm for all crop with the exception of cotton which was planted on a 1 m row spacing. Each plot was between 9 and 12 m wide and 49 m long. The perimeter of the trial was surrounded by a 6 m wall of Kenaf. Four crops were planted; cotton, pigeon pea, niger and lab lab with each be replicated 6 times. A Steward spray was accidentally applied to the trial on 17 July.

Sampling was conducted weekly in both trials using a devac sampler. Each sample consisted of 2, 10 m sections of row per plot. In each sample the number of pest and beneficial insects were recorded. Insects regarded as beneficial insects included ants, lady beetles, red and blue beetles, damsel bugs, brown smudge bugs, stilt bugs, big eye bugs and wasps.

Each half of each plot was slashed consecutively to delay seed set and promote flowering.

Results and discussion

In 2001 the most attractive crop to both sucking insects and Heliothis was Lab Lab (Table 5). This was followed by niger and pigeon pea which were of similar attractiveness to the pest insects.

Although Kenaf was the least attractive crop to pest insects it was the most attractive crop for beneficial insects followed by Niger and lab lab. Pigeon pea was the least attractive (Table 5). Sesame failed to establish and chickpea was destroyed by wallaby feeding meaning their attractiveness could not be assessed. Cotton was the least attractive crop.

Similar results were obtained in 2002 with lab lab being the most attractive crop to both pest and beneficial insects (Table 6). Pigeon pea and niger which were of similar attractiveness. Cotton was by far the least attractive of the crops examined to both pest and beneficial insects.

The number of insects in both years were relatively stable through out the season with the exception of the mirds and mirid nymphs in 2002 which increased in numbers considerably at the end of the season. This suggests that trap crops should be examined regularly so as out break populations are identified before they become a problem in crop.

The lack of attractiveness of cotton suggests that trap and companion crops have a role to play in reducing pest pressure in cotton crops and also as a source of beneficial insects. However, a significant challenge that needs to be overcome is how to get the beneficial insects out of the companion crops into the cotton.

Table 5: Ranking of trap crops tested at Katherine for their attractiveness to a range of pest and beneficial insects in 2001. Crops labeled 1 are the most attractive and 5 are the least attractive.

Crop	H. armigera larvae	GVB	RBSB	Brown Mirids	Total Pests	Beneficials	Total all Insects
Lab Lab	1	1	1	1	4	3	7
Pigeon Pea	4	2	2	2	10	5	15
Niger	2	5	3	1	11	2	13
Kenaf	5	3	5	3	16	1	17
Cotton	3	4	4	5	16	4	20

Table 6: Ranking of trap crops tested at Katherine for their attractiveness to a range of pest and beneficial insects in 2002. Crops labeled 1 are the most attractive and 4 are the least attractive.

Crop	H. armigera larvae	GVB	RBSB	Mirids	Total Pests	Beneficials	Total all Insects
Lab Lab	1	1	2	2	4	2	6
Pigeon Pea	3	3	1	1	8	3	11
Niger	2	2	4	3	11	1	12
Cotton	4	2	3	4	13	4	17

3.5 Milestone 5 2002

Pre Flowering Mirid Threshold trial

Aim: To determine the impact of mirids on the yield of cotton in Katherine when damage occurs in the pre-flowering period with the view of developing thresholds.

Background: In 2001 two bottle necks for insect management were identified. The first of these was the period between first square and first flower when brown mirids were observed to be a problem. In 2001 two sprays were required to control these insects. Despite this, past experience has indicated that there is some question about the pest status of brown mirids. To investigate this a trial was conducted examining both thresholds and control strategies for brown mirids.

Methods: The trial consisted of seven treatments replicated 3 times and was located in the center span of A1. The variety was Siokra V16I (Table 7). Each plot was 30 m long and 12 rows wide.
The seven treatments were as follows:

Unsprayed Control
5kg /ha of temik applied at planting
10 kg / ha of temik applied as a side dress at first square (26/4/02)
125 ml of regent applied at 9 day intervals commencing at first square (no mirids)
0.5 mirids / m
mirids / m
mirids / m

In the case of the 0.5, 1 and 2 mirids per m treatments, control was implemented using regent applied at 65 ml / ha. Population densities were calculated by averaging the sucking insect densities across the three replicates.

Plots were sampled twice a week commencing on 26/4/02 when the crop had reached first square. Sampling concluded on 7/6/02 when the crop reached first flower. On each sampling occasion four, 0.5 m sections of row were sampled per plot. During the trial one application of Tracer (150 ml / ha) was made to control Heliothis. At the trials conclusion a number of yield properties were recorded in 3 x five m sections of row per plot (Table 8).

Plant mapping was undertaken at cutout in 3 x 1 m sections of row per plot. Yield was determined by machine picking 3 x 15 m long transects of row per plot on 8 October 2002. Fibre quality was assessed at ACRI.

Results and discussion

During the course of the experiment sucking insect pressure was light with the unsprayed control reaching a peak in density of 1.4 sucking insects / m towards the end of the experiment (Fig 7). Of the threshold treatments, only the 0.5 /m plots required spraying (14 May and 24 May). The side dress Temik treatment appeared to suppress sucking insect populations for approximately 1 month after it was applied.

The low insect populations were reflected in high fruit retention in all treatments at the conclusion of the intensive monitoring period (Table 7). With the exception of the 5 kg Temik applied at planting treatment there were no differences between the remaining treatments.

At picking the highest yield was obtained in the Temik side dress treatment which yielded approximately 4 bales / ha (Fig 8). The remaining treatments including the insect exclusion treatment all yielded in the vicinity of 3.0 – 3.5 bales / ha. Fibre quality was very poor across the board and was similar in each treatment (Table 8).

Plant mapping demonstrated that fruit retention was high on the lower branches. However, after the fifth fruiting branch retention fell sharply for the remainder of the growing season (Fig. 9). The sharp reduction in fruit retention after the fifth fruiting branch was probably the result of the long cold season that commenced at about that time. In addition, the high incidence of Alternaria in the trial may have contributed to the low fruit set in the latter part of the season.

These results suggest that cotton can tolerate sucking insect populations of up to 0.5 / m (which was the density in most of the plots) without any adverse impact on yield up until first flower. However, the ability of the crop to handle higher populations is unclear due to the low pressure experienced.

Table 7: Crop phenology data for the pre flowering threshold trial early season sucking insect trial

Property	Date
Planting date	4/4/02
First square	26/4/02
First Flower	7/6/02
Start Sampling	26/4/02
Conclude sampling	7/6/02
Picking	8/10/02

Table 8: Yield properties / m of row measured on 11 and 12/6/02 at the conclusion of the intensive monitoring period

	#plants	#FB	#bolls	#flowers	#scars	# squares	bolls with scars	veg squares
Unsprayed	10.5	93.6	19.9	7.3	13.6	133.3	6.1	13.9
9 day Regent	11.0	93.4	19.9	8.1	11.5	127.9	6.9	11.9
5kg	15.1	93.7	7.7	4.6	8.5	127.6	8.5	9.0
Side dress	11.2	98.9	19.4	5.6	12.6	127.4	7.2	10.8
0.5/m	10.5	92.1	19.9	8.0	11.9	132.3	6.3	10.1
1/m	10.4	94.7	20.7	9.7	14.1	135.6	6.1	17.6
2/m	10.0	94.8	20.7	7.1	11.3	127.7	7.5	11.9

Table 9: Fibre quality measurements for each treatment.

Treatment	Turnout	Length	Uniformity	Short Fibre Index	Strength	Elongation	Micronaire
0.5/m	39.03	0.98	79.36	11.13	25.03	6.96	4.33
1.0/m	39.4	0.97	78.93	12.7	24.4	7.33	4.3
10kg SD	38.23	1	80.33	10.26	24.86	6.8	4.53
2.0/m	39.06	1	80.53	9.46	25.23	6.96	4.2
5kg planting	38.8	0.97	80.36	10.9	25.1	7.16	4.36
9day regent	40	0.99	79.7	10.9	24.9	6.7	4.46
control	38.7	0.98	80	10.9	25.13	6.7	4.2

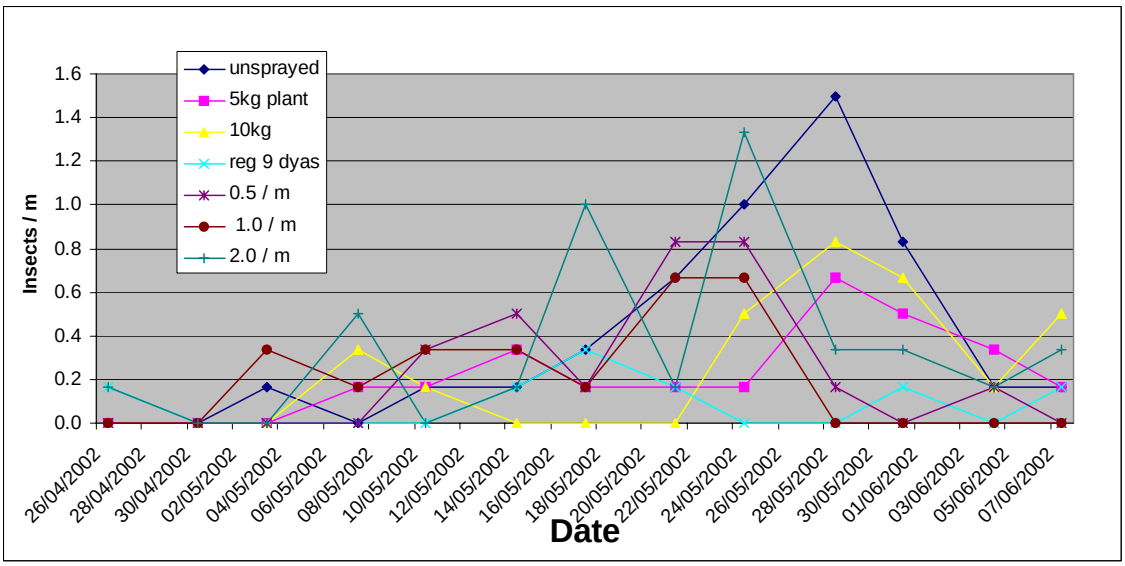


Fig 7: Sucking insect density in pre flowering threshold trial.

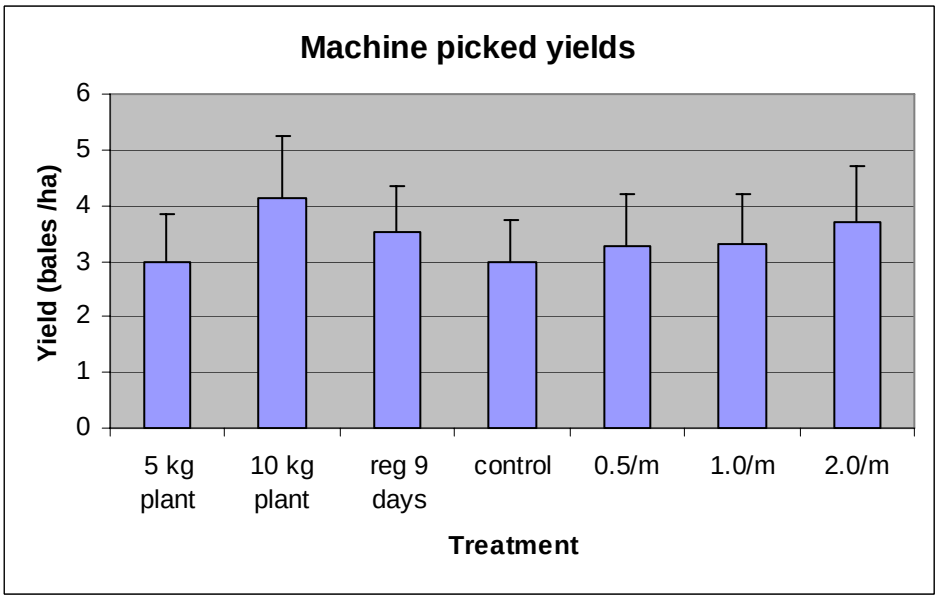


Fig 8: Machine picked yield taken from 3 x 15 m sections of row per plot. Vertical bars represent the standard error of the mean

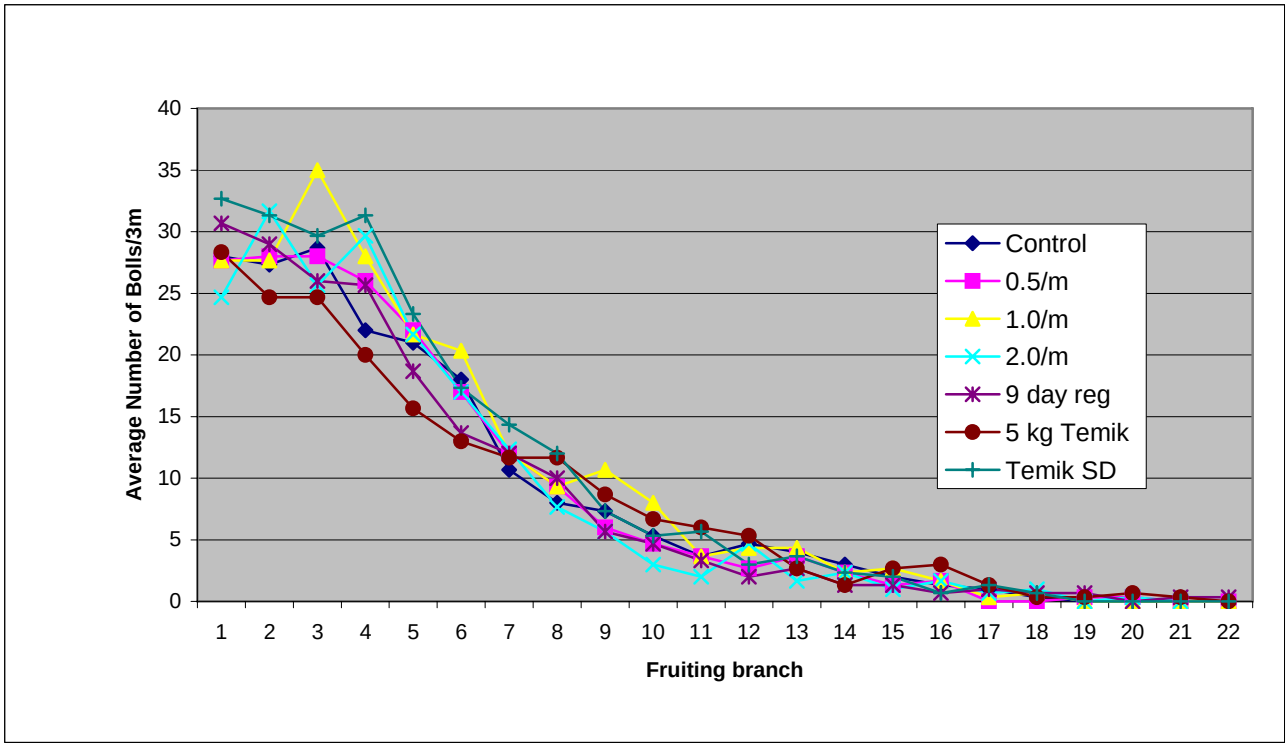


Fig 9: Distribution of bolls according to node in Mirid trial

3.6 Milestone 5 2002

Management of sucking insects in period between first flower and cutout.

Draft Manuscript to be submitted to Journal of Economic Entomology is included as Appendix 1

4. Other trials undertaken not related to project milestones

4.1 Refuge studies

Aim: To determine the production of pupae under Conventional and Bollgard 2 cotton varieties as well as under the potential rotation crops of peanuts and sorghum.

Methods: Two trials were undertaken in 2002. The first examined the relative production of pupae in Conventional and Bollgard 2 cotton at Katherine research station. The second trial examined the relative pupal production in conventional cotton, hybrid seed sorghum and peanuts . Significant phenological dates are outlined in Table 10.

In the trial at KRS populations were assessed using ten 1 m2 emergence traps centered over the row. The traps were examined every two days and were relocated once every 2 weeks. At Campbells sampling was undertaken using a small trowel. Ten metres of row were checked each time.

Results and discussion:
At KRS pupal production under Bollgard II was minimal with only 2 moths being detected in the emergence traps. Both moths emerged in late September (Fig. 10). By comparison 10 moths emerged from within the conventional cotton over the same trapping duration. Of interest was the lateness of the start of the production of pupae in the conventional cotton with the first moths not being detected until the end of August, more than 1 month after the start of the trapping. This was despite the crop carrying four medium and large grubs per m on 22 July suggesting that very few of the pupae resulting from this larval cohort survived.

At Campbells the pattern of emergence was similar in the Sorghum and unsprayed conventional cotton with approximately the same numbers of pupae being produced over the course of the sampling period (Fig. 11). By comparison more than twice the number of pupae were produced in the peanuts over the same period in a more

or less continuous manner. These results suggest that both peanuts and sorghum would make good refuge crops for transgenic cotton with both having the additional bonus of being suitable for use as rotation crops.

Other species reared from pupae collected:

1. Anomis flava
2. Leucania separata
3. Spodoptera litura

Table 10: Crop phenology data for the crops sampled in the refuge studies in 2002

		Campbell's			KRS	
	Conventional cotton	Peanuts	Sorghum		Conventional cotton	Bollgard 2
Variety	Siokra V16	Florunner	Pacific Pacer MR5		Sicala 40	DP50BX
Planting date	29/3/02		5/5/02		23/4/02	24/4/02
Peak flowering	July 2002		30/5/02			
Harvest	Oct 2002	Oct 2002	15/8/02		4/11/02	4/11/02
Start of pupae sampling	22/5/02	22/5/02	22/5/02		25/7/02	25/7/02
Conclusion of pupae sampling	11/9/02	11/9/02	11/9/02		16/10/02	16/10/02

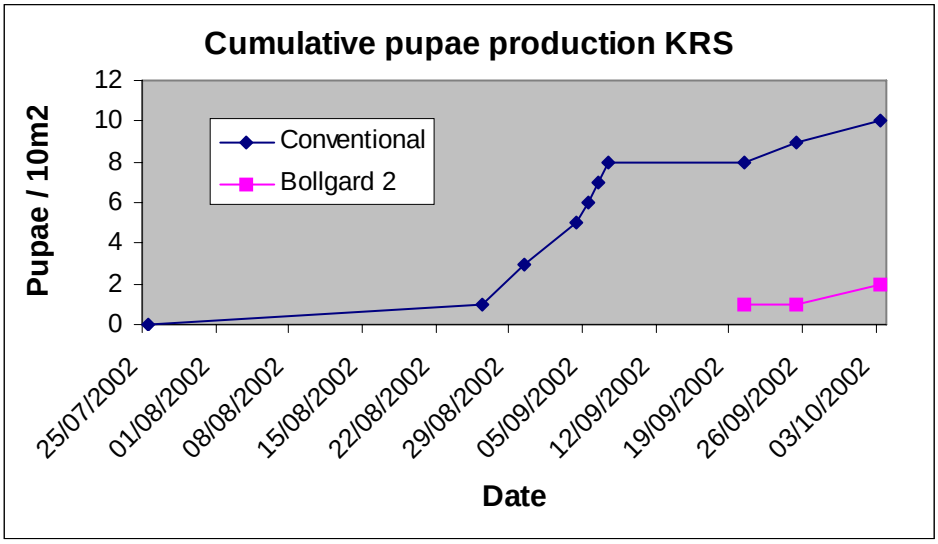


Fig. 10: Cumulative pupae production at KRS in Unsprayed conventional cotton and Bollgard II cotton. Sampling was undertaken using emergence cages which were checked every 2 days and shifted every 2 weeks.

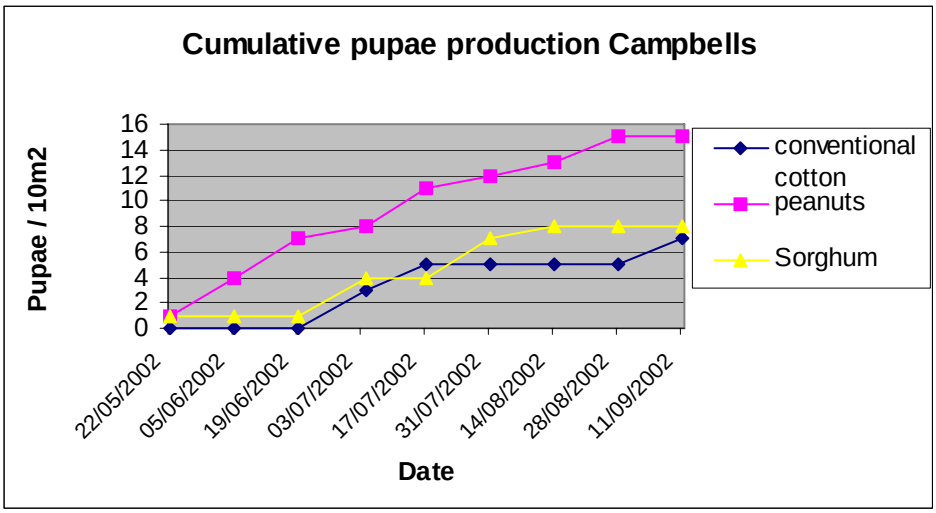


Fig. 11: Cumulative pupae production at Campbells in Peanuts, sorghum and unsprayed conventional cotton. Sampling was conducted fortnightly by hand.

5. Collaborative research projects

5.1 Northern Australia Mirid compensation trial in collaboration with Dr Tom Lei

Aim: This study was initiated to address the prevalence of sucking pest damage on bolls in northern Australia. To assess the degree of yield compensation from simulated green mirid damage to bolls damage was imposed on bolls at two levels comparable to low and high mirid infestations at 3 time periods during the fruiting period (early, mid, and late). Using this design, we assessed the relative degree of yield recovery from low and high damage to early, mid season and late bolls.

Methods:
Sicot 289i was planted in Tape 2 on 3 April 2002 at a rate aimed at achieving a plant density of 10 – 12 plants/m. The crop was managed according to the standard management tools used on bulk cotton at KRS and was irrigated using subsurface drip irrigation. There were seven treatments replicated 4 times in a complete randomised block design. The treatments are given in the table below. Two levels of boll damage were used: 5 bolls per m (low) and 20 bolls per m (high). Bolls to be damaged were randomly chosen but spread roughly evenly across the 2 m row.

Each boll to be damaged received pectinase enzyme solution injected into 2 locks at 0.01 ml per lock. The bolls selected for damage were 2-3 cm in diameter. The solution was prepared by mixing one part pectinase (Sigma-Aldrich P4716 Pectinase from Aspergillus niger, solution in 40% glycerol) and 4 parts water. Since this enzyme may degrade in high temperature, the mixture was freshly prepared on the day of the application and kept in an eski with ice while in the field. Early damage took place 4 weeks after first flower (to ensure sufficient number of bolls of the appropriate age), mid and late damage treatments will occur at 3 and 6 weeks following the early damage.

Treatment	No. of bolls damaged per metre	
Control	0 [C]	
Early damage 27/6/02	5 [EL]	20 [EH]
Mid damage 18/7/02	5 [ML]	20 [MH]
Late damage 6/8/02	5 [LL]	20 [LH]

Each plot (i.e. treatment-rep) was 2m x 3 rows. There was at least a 10-m buffer to side and ends of the field to avoid edge effects on maturity. There were 2 buffer rows between plots and 2-3 m between plots down the row.

At harvest maturity date was estimated by sequential picks. The center 2m row was used for picking. At each pick, the number of bolls with tight lock (fail to fluff out) and the extent of staining on seed cotton was recorded

and rated on a scale developed by Tom Lei and Mozzam Khan. Seed cotton was ginned, weighed and fibre quality determined.

5.2 Registration of Bollgard 2

To collect data to facilitate the registration of Bollgard 2, trials were conducted in collaboration with Monsanto in both 2001 and 2002. In each season comparisons were made between DP50, DP50b and DP 50bx. As one has come to expect from Monsanto no results have ever been forth coming.

5.3. Spiders Bio-diversity and abundance with Dr Mary Whitehouse

Aim: To determine the bio-diversity and abundance of spiders in cotton at Katherine Research station

Methods: Spider abundance and bio diversity were monitored in both unsprayed conventional cotton (Sicala 40) irrigated using LEPA irrigation and sprayed Ingard cotton (Siokra V16I) irrigated using a center pivot fitted with overhead sprinklers. The sprayed cotton was sprayed 3 times. The sprays were:

11/7/02 62.5 ml Regent
25/7/02 800 ml Talstar
5/9/02 2l larvin + 1l Rescue.

Sampling was undertaken five times throughout the season (Table 10) using both beat sheets to sample plant dwelling spiders and pitfall traps to sample ground dwelling spiders. Following sampling all spiders were forwarded to Dr Mary Whitehouse for identification. At the time of writing this report no results had been received

Table 10: Timing of spider sampling in the pivot and unsprayed conventional cotton.

Sample time	Pivot V16i		Unsprayed conventional	
	Beat sheet	Pit fall trap	Beat sheet	Pit fall trap
Planting date	9/4/02		24/4/02	
first square	3/5/02	3/5/02	3/6/02	3/6/02
first flower	3/6/02	3/6/02	8/7/02	8/7/02
peak squaring	8/7/02	8/7/02	9/8/02	9/8/02
peak boll	15/9/02	15/9/02	15/9/02	15/9/02
60% bolls open	1/10/02	1/10/02	1/10/02	1/10/02

6.0 Insect Management

Pest issues and observations pertaining to their management at Katherine.

The insect sampling data and results obtained over the last two years suggest a number of things.

Although transgenics provide good control of Helicoverpa the longevity of the protection provided by Ingard appears to be much shorter than that in southern Australia. Ingard expression appears to fall below critical levels within 75 days of planting. The reason for the early failure of ingard is unclear but may be related to the rapid early growth of the crop or the cold weather that is experienced from June onwards. It remains to be seen whether this is the same in Bollgard II. However, one spray for Helicoverpa was required in the Bollgard II grown in 2001. A possibility is that with the longer expression of Bollgard II, low residual populations of Helicoverpa may remain late in the season when the Bt expression falls, making control unnecessary.

Resistance levels are in general low. This is to be expected considering the limited history of agricultural production and hence the reduced selection pressure in the area. Despite this the susceptibility of Helicoverpa at Katherine to Bt is significantly less than that of the susceptible reference strain. However, Helicoverpa at Katherine are still more susceptible to Bt than larvae from Kununurra.

Spodoptera damage has been apparent early in the season in both 2001 and 2002 but seems to disappear after about May. The damage is very patchy and was only controlled in one field in 2001. Despite this it remains to be seen whether the move to earlier planting results in an increase in their pest status. Damage from other lepidotera appears to be insignificant.

As a result of the shift to Bollgard II® early season sucking insect damage is likely to become the most significant insect problem. Experience to date suggests that the immigration of sucking insects into cotton starts soon after planting. Unlike southern areas, tipping out does not occur regularly and as a result they don't start to become a problem until the cotton starts squaring. At this stage the amount of damage that can be sustained without yield penalty is unclear. However, in the replicated sucking insect trial conducted in 2002 populations of 1.5/m did not adversely affect yields when left unchecked up until first flower. A second trial conducted after first flower demonstrated significant yield losses with populations as low as 0.5/m. Further work is required to clarify the robustness of these results. Although 0.5 sucking insect / m appears to be a low threshold it is questionable how much of a problem they will be after the initial population is removed from the crop.

At this stage the pest status of red banded shield bugs remains unclear. It has been reported in the literature that RBSB go into a semi dormant state in the cool months (Applied Entomology and Zoology 29: 585-592). If this is the case their pest status may be limited in cool seasons. This should be investigated to see if it is the case in Katherine.

Aphids have remained a minor pest. However, pest numbers were higher in 2002 than in the previous year. On the whole, hover fly numbers were able to increase to levels sufficient to control them when left undisturbed. Despite this control was required in 2 fields. In the first, Pirimor was used at 750 g / ha. Control was excellent suggesting that rates at the lower end of the label recommendations may be more appropriate.

In general beneficial populations have been low and have shown a tendency to disappear early in the season possibly in response to the onset of the cold weather. In many cases and inparticular Trichogramma, this disappearance has been before chemicals have been used.

Pest densities in crop

Although the aim of the cotton production system being developed at Katherine is to produce cotton with minimal reliance on insecticides, insecticide applications are required. In 2001 this amounted to an average of 5.5 sprays per management unit and in 2002, 5.3 sprays / management unit (Tables 11 and 12). A complete outline of all sprays applied in 2001 and 2002 is outlined in Tables 13 and 14

Although insect pressure was anecdotally lower in 2002 than in 2001, this was not reflected in reductions in the number of sprays applied. There were a number of reasons for this including:

The thresholds for heliothis were lowered considerably in 2002 due to the extremely poor fruit set experienced as a result of the cold weather encountered.

The heliothis pressure experienced extended considerably longer in 2002 than in 2001 with sprays still being applied well into October
Aphid sprays were required in 2002, which were not required in 2001.

Table 11: Summary of insecticide applications in 2001

Pest	Range of sprays	Average Sprays
Brown mirids	1-2	1.75
GVB and RBSB	1-2	1.25
Heliothis	2-4	3
Total sprays	4-7	5.5

Table 12: Summary of insecticide applications in 2002

Pest	Range of sprays	Average Sprays
Sucking insects	0-2	1.1
Aphids	0-2	0.7
Heliothis	2-5	3.8
Total sprays	3-7	5.3

Table 13: Insect control requirements 2001

Date	Field and product	Insects	cost
	T1		
30-Apr	125 ml Regent	Brown Mirids	39.5
16-May	125ml Regent	Brown Mirids	39.5
04-Jun	800 ml bulldock	Mirids, Spodoptera, heliothis, RBSB, GVB	21.52
15-Jun	4l predator	heliothis	42.88
28-Jun	150ml tracer	heliothis	52.8
19-Jul	800ml talstar + 400ml pbo	heliothis	65.22
			261.42
	T2		
30-Apr	125 ml Regent	Brown Mirids	39.5
16-May	62.5 ml Regent	Brown Mirids	19.25
04-Jun	800 ml Bulldock	Mirids, Spodoptera, heliothis, RBSB, GVB	21.52
15-Jun	4l predator	heliothis	42.88
17-Jul	150ml Tracer	heliothis	52.8
			175.95
	B1		
04-May	125 Ml regent	Brown Mirids	39.5
22-May	125 Ml regent	Brown Mirids	39.5
08-Jun	150ml Confidor	Mirids, RBSB, GVB	60
19-Jun	800ml Bulldock	Mirids, Spodoptera, heliothis, RBSB, GVB	21.52
29-Jun	4l predator	heliothis	42.88
04-Jul	800ml talstar	heliothis	52.8
07-Aug	1 l Rescue + 2 l larvin		71.35
			327.55
	Bollgard 2		
16-May	62.5 ml Regent	mirids	19.25
15-Jun	400 ml Dimethoate	mirids	3.42
02-Jul	125 ml regent	RBSB, mirids, gvb	39.5
10-Aug	1 l Rescue + 2 l larvin	heliothis	71.35
			133.52
	B2/3		
10-May	62.5 ml Regent	brown mirids	19.25
06-Jun	125 ml Regent	brown mirids	39.5
03-Jul	150ml tracer	heliothis	52.8
10-Jul	800ml talstar + 400ml pbo	heliothis	65.22
			176.77

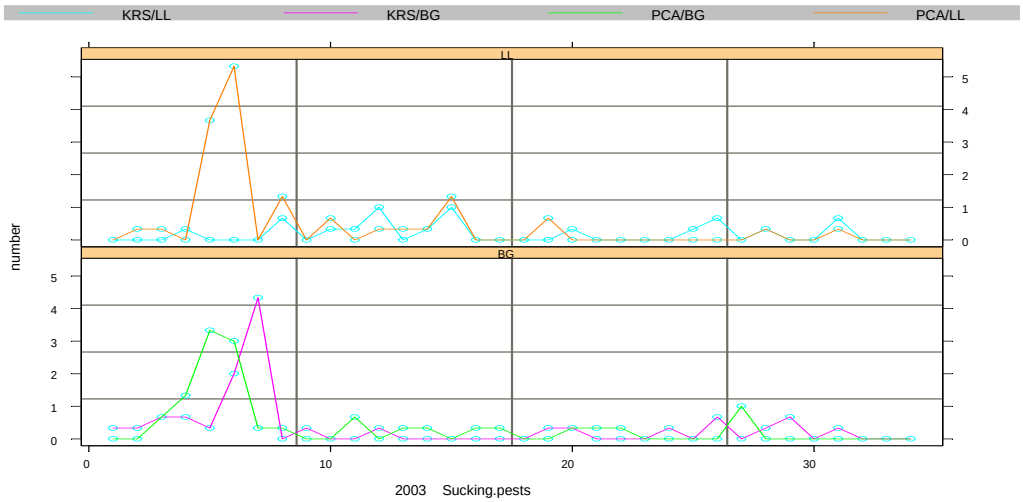
Table 14: Insect control requirements 2002

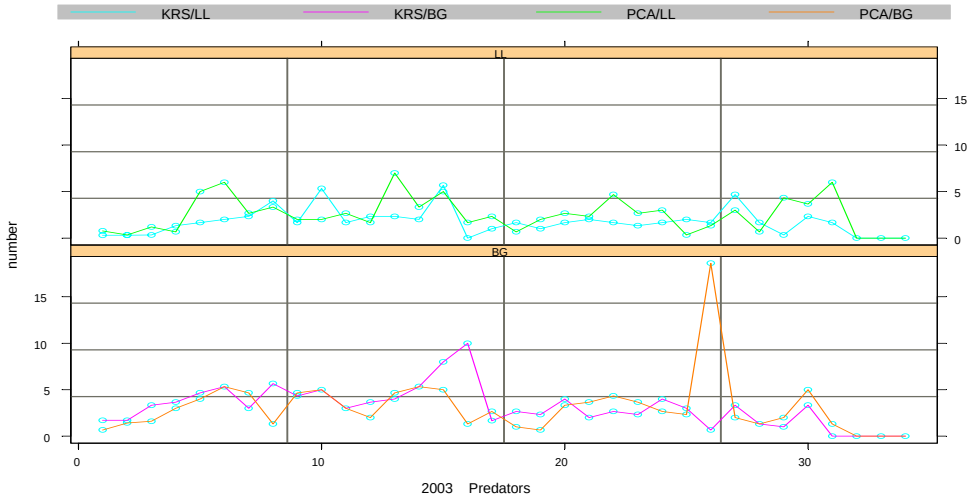
Date	Field and Product	Insects	cost
	T1		
23/05/2002	40 ml Regent+salt	RBSB and GVB	11
07/06/2002	65ml Regent +salt	RBSB, GVB and Mirids	18
02/07/2002	700 ml Affirm	Heliothis	71
25/07/2002	750 g Pirimor	Aphids	38
06/08/2002	800 ml Talstar	Heliothis	50
22/08/2002	1 l Rescue + 2 l larvin	Heliothis	71
23/09/2002	1 l Rescue + 2 l larvin	Heliothis and aphids	71
			330
	Tape 2		
23/05/2002	40 ml Regent + salt	RBSB and GVB	11
13/06/2002	750 ml Steward	heliothis	62
21/06/2002	850 ml Steward	Heliothis	70
20/08/2002	800 ml Talstar + 400 ml PBO	Heliothis	62
23/09/2002	1 l Rescue + 2 l larvin	Heliothis and aphids	71
			276
	A1		
17/05/2002	62.5 ml Regent	Mirids, RBSB and GVB	18
30/05/2002	40 ml Regent + salt	RBSB and GVB	11
07/06/2002	650 ml Steward	Heliothis	53
02/07/2002	700 ml Affirm	Heliothis	71
11/07/2002	850 ml steward	Heliothis	70
22/08/2002	2l larvin	Heliothis	51
16/09/2002	250 ml Folimat	Aphids	9
			283
	A2/3		
24/06/2002	62.5 ml Regent + salt	Mirids, RBSB and GVB	18
17/07/2002	800 ml Steward	Heliothis	66
25/07/2002	800 ml Talstar	Heliothis	50
20/08/2002	800 ml Talstar + 400 ml PBO	Heliothis	62
23/09/2002	1 l Rescue + 2 l larvin	Heliothis	71
			267
	P16i		
11/07/2002	62.5 ml Regent + salt	Mirids, RBSB and GVB	18
25/07/2002	800 ml Talstar	Heliothis	50
05/09/2002	1 l Rescue + 2 l larvin	Heliothis	71
			139
	P 289i		
11/02/2002	850 ml Steward	Heliothis	70
06/08/2002	800 ml Bulldock + 400 ml PBO	Heliothis	32
20/08/2002	75 ml gemstar	Heliothis	6
19/09/2002	1 l Rescue + 2 l larvin	Heliothis	71
12/10/2002	850 ml Steward	Heliothis	70
			249

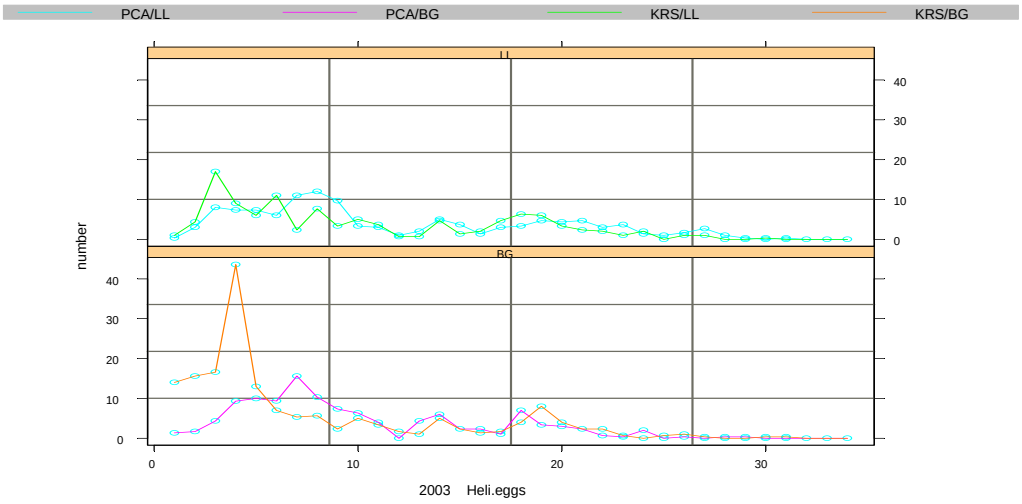
Appendix 2a. Probabilities (*P*) and *t* values generated from a linear mixed effects model examination of relative insect densities (grouped) in cotton grown with and without lablab companion crop at KRS and PCA in 2003 and 2004.

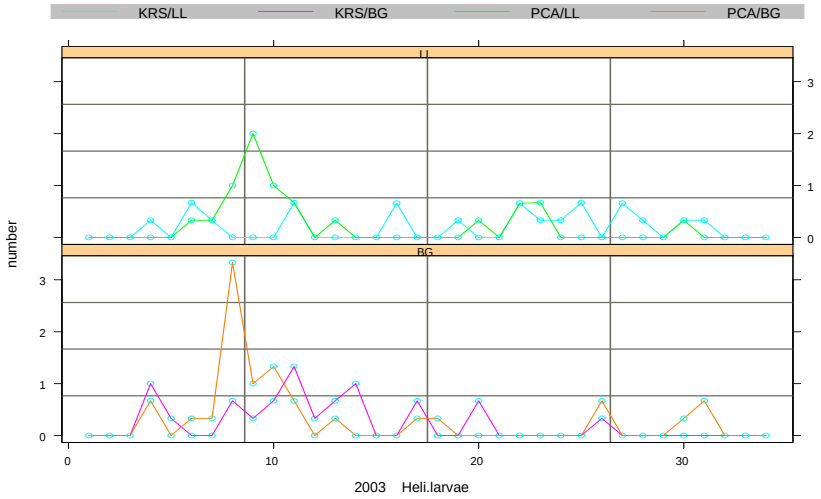
Year	Group	*t* value	*P*
2003	Sucking pests	-0.27	0.83
	Predators	-2.55	0.24
	Helicoverpa eggs	-0.10	0.94
	Helicoverpa larvae	-0.77	0.58
2004	Sucking pests	1.14	0.40
	Predators	0.95	0.52
	Helicoverpa eggs	-1.10	0.47
	Helicoverpa larvae	-1.28	0.43

Appendix 2b. Graphs of insect group densities over time in cotton grown with and without lablab companion crop at KRS and PCA in 2003 and 2004. LL = cotton grown with lablab companion, BG = cotton grown with no companion.






PCA/LL
PCA/BG
KRS/LL
KRS/BG
LL
BG
number
0
10
20
30
40
2003 Heli.eggs


KRS/LL
KRS/BG
PCA/LL
PCA/BG
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BG
number
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2
3
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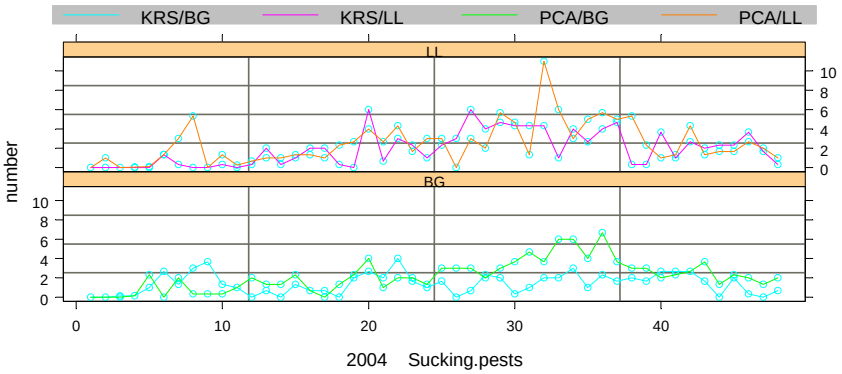
2003 Heli.larvae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KRS/LL
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LL
BG
number
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2
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6
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10
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10
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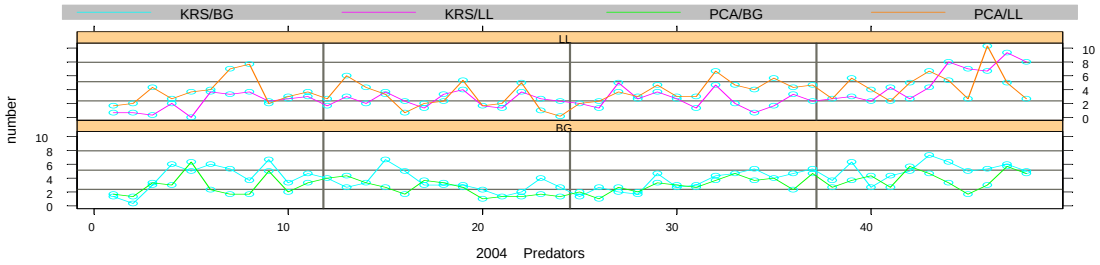
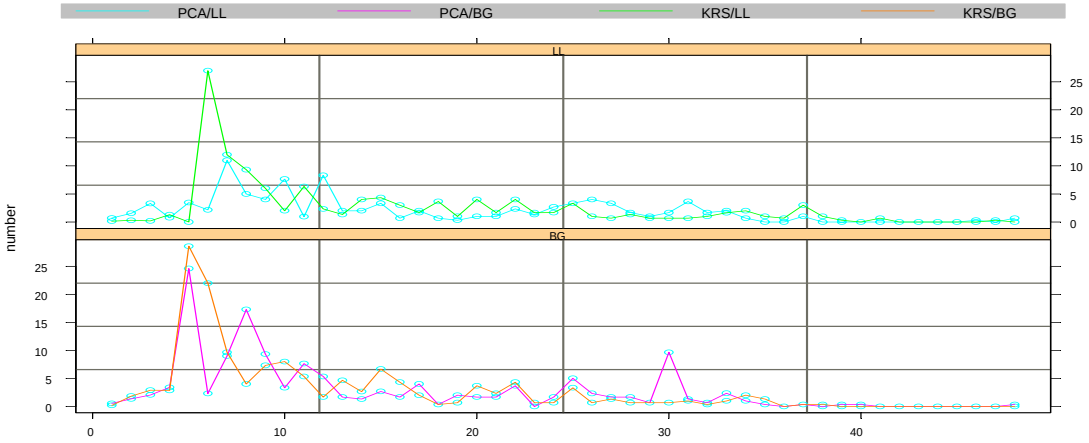
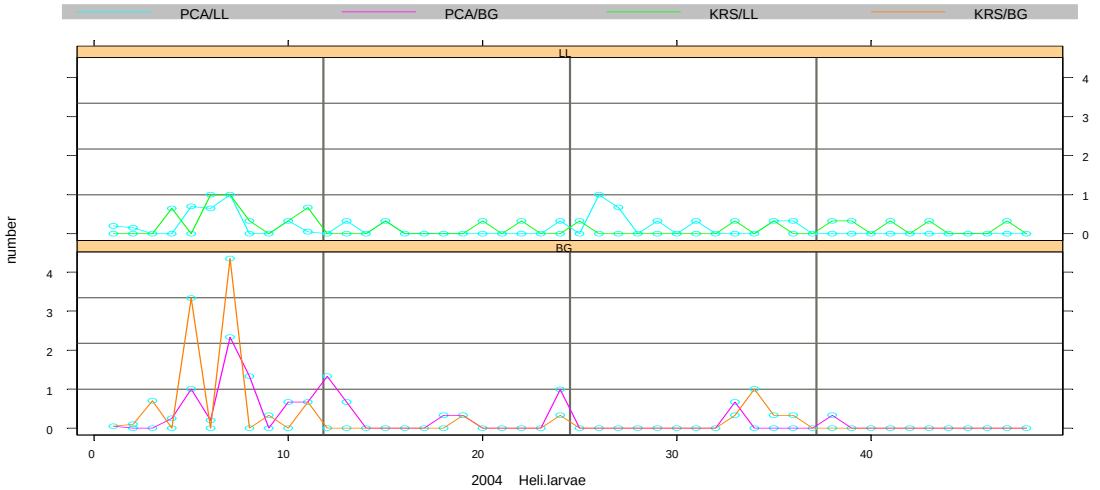
30
40
2004 Sucking.pests



2004 Predators

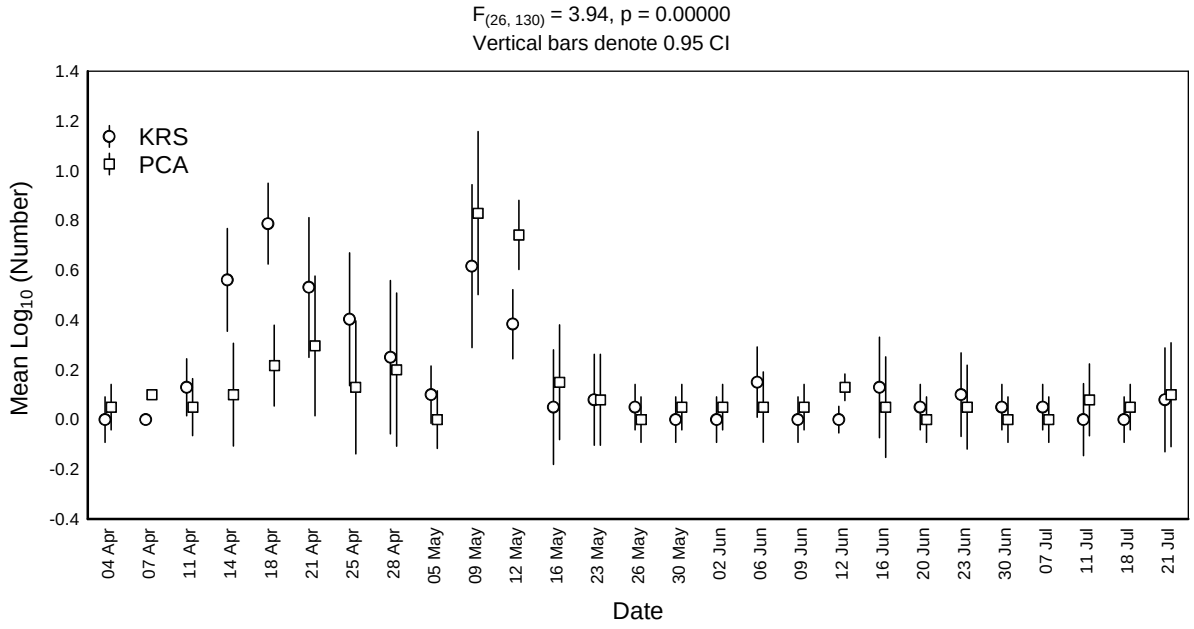


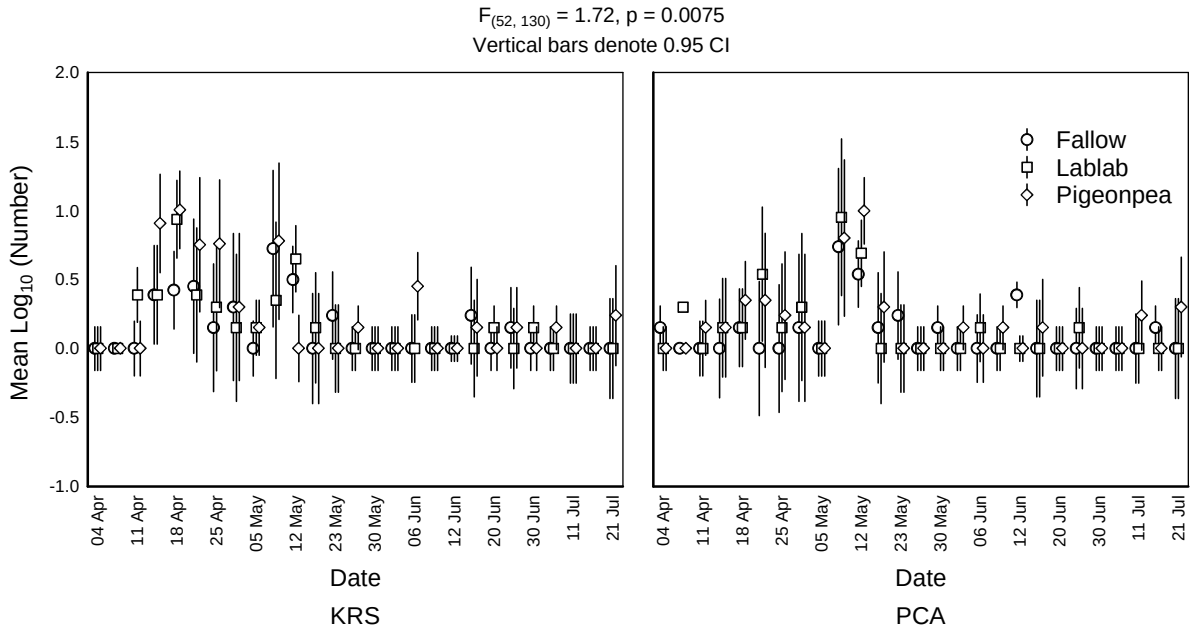
2004 Heli.eggs

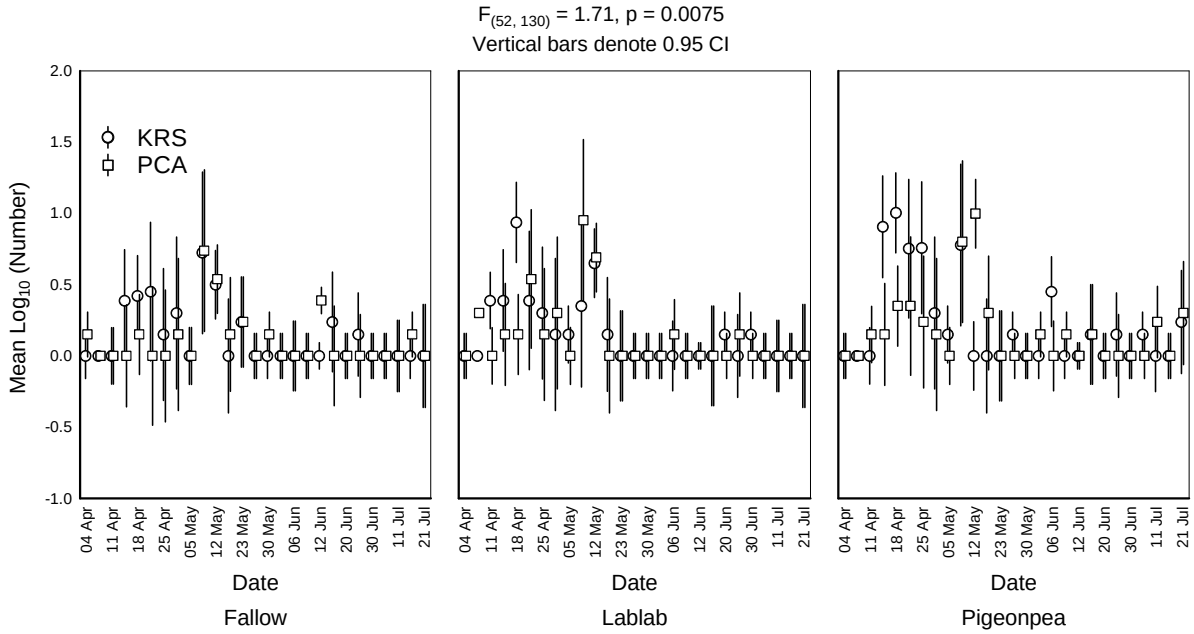


2004 Heli.larvae

Appendix 3. The relative density of total *Helicoverpa* larvae in cotton over time compared between pivot irrigation fields and treatments grown at KRS and PCA in 2005.







Appendix 4. *F* values, degrees of freedom (df) and probabilities (*P*) generated from repeated measure ANOVA analysis of relative insect numbers between companion crop treatments grown in field B1 at KRS in 2005. The relative status of insect groups are given.

Group	Status	*F* value	df	*P*
Aphids	Pest	0.69	52, 156	0.94
Green vegetable bugs	Pest	1.08	20, 60	0.39
Helicoverpa eggs (white)	Pest	0.67	48, 144	0.94
Helicoverpa eggs (brown)	Pest	0.75	52, 156	0.88
Helicoverpa eggs (total)	Pest	0.65	52, 156	0.96
Very small larvae	Pest	0.74	34, 102	0.84
Small larvae	Pest	0.91	14, 42	0.56
Medium larvae	Pest	1.68	20, 60	0.06
Total larvae	Pest	0.98	52, 156	0.53
Hoverfly larvae	Predator	1.37	14, 42	0.21
Lacewing eggs	Predator	1.22	20, 60	0.27
Ladybeetle adults	Predator	1.14	62, 186	0.26
Ladybeetle larvae	Predator	0.38	32, 96	0.99
Leafhoppers	Pest	0.47	62, 186	0.99
Mirid adults	Pest	0.77	32, 96	0.79
Mirid nymphs	Pest	0.85	50, 150	0.74
Mirid total	Pest	0.96	56, 168	0.56
Parasitised eggs	Predator	0.89	16, 48	0.58
Predatory bugs	Predator	0.61	56, 168	0.98
Redbanded shield bugs	Pest	0.93	58, 174	0.62
Spiders	Predator	0.79	62, 186	0.85
Spodoptera larvae	Pest	0.89	50, 150	0.69
Thrips	Pest	0.43	56, 168	0.99
Whiteflies	Pest	0.45	54, 162	0.99

Appendix 5. Insecticide (and one defoliation) applications for cotton trials conducted at KRS and PCA during 2003.

Date	Field	Plot	Zone	Chemical	Rate	Reason	Recorded	Comments
30-Apr-03	A 2/3	Reg		Regent	62.5ml	Trial	No application record	P2 sprayed with regent today
08-May-03	A 2/3	Reg				Trial	Yes	Sprayed A2/3 South with Ovasyn 2 lt/ha
09-May-03	P2			Regent		Trial	Yes	A1 sprayed with 125ml regent
15-May-03	A 2/3	Reg	North	Regent		Trial	Yes	P2 sprayed with regent today at 100mls
19-May-03	A 2/3		South	Ovasyn	2l/Ha		Yes	H2 sprayed with 80mls/ha regent 1500
21-May-03		Reg, 1M	North	Regent		Trial	Yes	grams of salt
	A 1			Regent	125ml	Trial	Yes	P1 sprayed with 70mls/ha regent 1350
29-May-03	A 2/3	Reg	South	Regent	125ml	Trial	Yes	grams of salt
05-Jun-03	P2			Regent	100ml/Ha	Trial	Yes	H1 sprayed with regent @70 mls/ha
	A 2/3	Reg			125ml/Ha	Trial	Yes	
06-Jun-03	H2			Regent	80ml/Ha + Salt 1500g	Trial	Yes	
	P1			Regent	70ml/Ha + Salt 1350g	Trial	Yes	
12-Jun-03	A 2/3	Reg	South			Trial	No application record	
18-Jun-03	A 2/3	Reg, 0.5, 1M	South	Regent	125ml	Trial	No record	
25-Jun-03	A 2/3	Reg	South		125ml/Ha	Trial	Yes	
02-Jul-03	A 2/3	Reg	South		125ml	Trial	No application record	
04-Jul-03	H1			Regent	70ml/Ha	Trial	No application record	
10-Jul-03	A 2/3	Reg		Regent	125ml	Trial	Yes	
16-Jul-03	A 2/3	Reg	South	Regent	125ml	Trial	Yes	
18-Jul-03		1.5M		Regent	125ml	Trial	Yes	
31-Jul-03				Regent	125ml/Ha	Trial	Not in diary	
15-Aug-03	refuge			Crop	90g WP	Defoliation	Yes	

Appendix 6. Results from paired *t* tests examining predatory bug densities prior to and following insecticide applications in cotton grown under pivot irrigation in Katherine during 2005. *t* = *t* value, df = degrees of freedom and *P* = probability.

Field	Insecticide	*t*	df	*P*
Both	fipronil	2.12	11	0.052
KRS	indoxacarb	0	5	1
PCA	indoxacarb	0.54	5	0.61
KRS	pirimicarb	-0.79	5	0.47
KRS	fipronil plus salt	-0.85	5	0.43

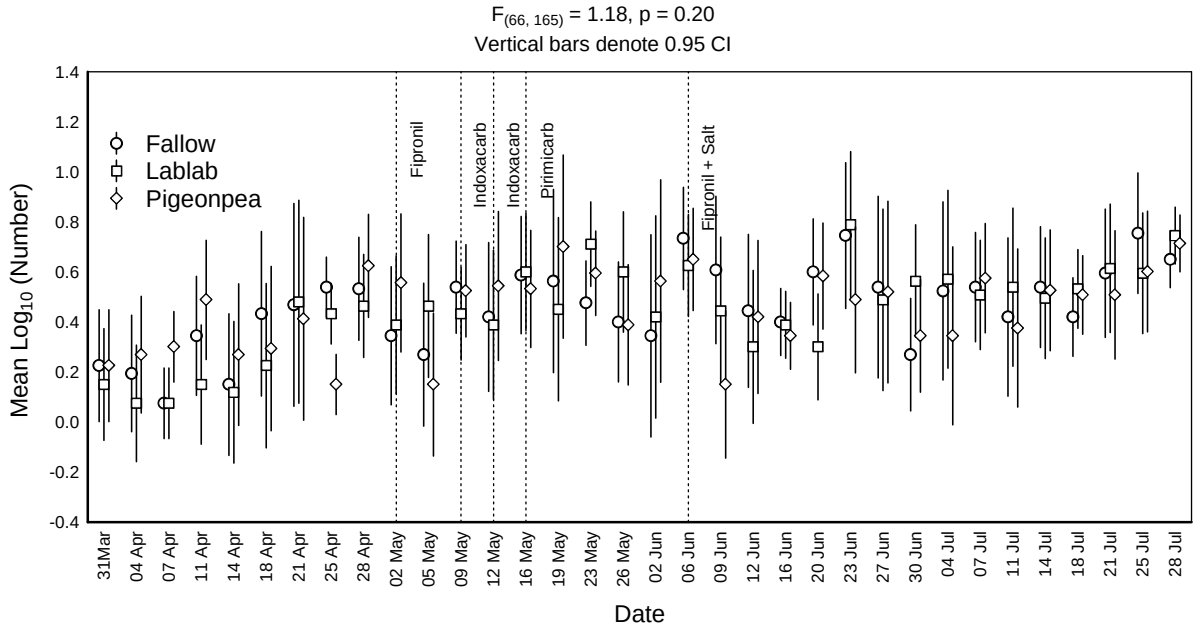
Appendix 7. Results from paired *t* tests examining predatory bug densities prior to and following insecticide applications in cotton grown under lateral irrigation in B1 during 2005. *t* = *t* value, df = degrees of freedom and *P* = probability.

Insecticide	*t*	df	*P*
fipronil	1.39	11	0.19
fipronil	1.00	11	0.34
fipronil plus salt	-0.38	11	0.71
dimethoate plus salt	-0.51	11	0.88

Appendix 8. Results from paired *t* tests examining spider densities prior to and following insecticide applications in cotton grown under pivot irrigation in Katherine during 2005. *t* = *t* value, df = degrees of freedom and *P* = probability.

Field	Insecticide	*t*	df	*P*
Both	fipronil	1.52	11	0.16
KRS	indoxacarb	0.81	5	0.46
PCA	indoxacarb	0.19	5	0.86
KRS	pirimicarb	1.81	5	0.13
KRS	fipronil plus salt	-1.45	5	0.21

Appendix 9. The relative density of spiders in cotton over time compared between companion crop treatments (see legend) grown under pivot irrigation at KRS and PCA in 2005. The dates and active constituents of insecticide applications are given.



Appendix 10. *F* values and probabilities (*P*) generated from analyses of hand and machine harvested yield estimates comparing treatments in early and late season sucking pest trials conducted under lateral irrigation in A2 and A3, respectively, in 2003. df = degrees of freedom.

Trial	df	Harvest method	*F* value	*P*
Early season	5, 15	Hand	0.91	0.50
		Machine	0.64	0.67
Late season	4, 12	Hand	2.04	0.15
		Machine	1.65	0.23

Appendix 11. *F* values and probabilities (*P*) generated from analyses of plant mapping data comparing treatments in early and late season sucking pest trials conducted under lateral irrigation in A2 and A3, respectively, in 2003. df = degrees of freedom.

Trial	df	Variable	*F* value	*P*
Early season	5, 15	Height	0.58	0.71
		Nodes	0.56	0.73
		Fruiting branches	0.41	0.83
		First fruit branch	0.44	0.81
		Vegetative branches	0.2	0.96
		Vegetative bolls	0.44	0.82
		Total bolls	0.59	0.71
		Fruit position 1	1.18	0.36
		Fruit position 2	0.75	0.60
		Fruit position 3	0.68	0.65
		Proportion vegetative bolls	0.79	0.57
Late season	4, 12	Height	0.67	0.62
		Nodes	0.03	0.99
		Fruiting branches	0.53	0.72
		First fruit branch	1.58	0.24
		Vegetative branches	1.86	0.18
		Vegetative bolls	0.81	0.54
		Total bolls	1.91	0.17
		Fruit position 1	0.51	0.73
		Fruit position 2	0.63	0.65
		Fruit position 3	0.17	0.95
		Proportion vegetative bolls	1.24	0.34

Appendix 12. *F* values and probabilities (*P*) generated from analyses of net variation pre- and post-exposure in plant mapping variables between treatments in redbanded shield bug field cage trials conducted under pivot irrigation at KRS in 2005. All analyses have 3 and 12 degrees of freedom. Significant probabilities are shaded.

Variable	*F* value	*P*
Height	*0.59*	*0.63*
Fruiting nodes	0.17	0.91
Fruiting bolls	0.35	0.79
Vegetative bolls	0.61	0.62
Total bolls	0.35	0.8
New fruit position 1	0.48	0.7
new fruit position 2	0.81	0.51
New fruit position 3	0.43	0.73
New fruit position 4	0.24	0.87
Proportion vegetative bolls	2.33	0.13
Retention position 1	3.91	0.037
Retention position 2	0.79	0.52
Retention position 3	2.2	0.14

Appendix 13. Manuscript entitled, “Development of a treatment threshold for sucking insects in determinate Bollgard II transgenic cotton grown in winter production areas”, by Dr Andrew Ward.

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Development of a treatment threshold for sucking insects in determinate Bollgard II transgenic cotton grown in winter production areas

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Abstract Little is known about the impact of sucking insects on cotton grown in tropical production areas. To examine this, an experiment was conducted at Katherine in northern Australia to determine the impact of mirids (*Creontiades dilutus* (Stål) and *Creontiades pacificus* (Stål), green vegetable bug (*Nezara veridula* (L.)) and red-banded shield bug (*Piezodorus hybneri* (Gmelin)) on the yield and fibre quality of the transgenic cotton variety DP50bx containing genes for the expression of Cry1A(c) and Cry2A(b) δ-endotoxins of *Bacillus thuringiensis* Berliner variety *kurstaki*. The trial examined the impact of sucking insect populations in the range 0.5–2.0/m^2. Yields of unsprayed plots were approximately 30% of those obtained in plots sprayed on a threshold of 0.5/m^2 which did not differ significantly from plots sprayed regularly to exclude sucking insects. The increase in yield in the low pest density treatments was the result of improved fruit retention in the middle part of the plant. Fibre quality was similar in all treatments. However, damage ratings to individual bolls did differ between treatments. Bolls in the high insect density treatments received more damage than those in the low density treatments. As a result of this study, a tentative treatment threshold of 0.5 sucking insect/m^2 is recommended in determinate Bollgard II cotton varieties grown in winter production areas.

Key words *Creontiades dilutus*, *Creontiades pacificus*, *Nezara veridula*, *Piezodorus hybneri*.

INTRODUCTION

In northern Australia, research is investigating the production of cotton in the winter (dry season) between March and November. This period represents a move away from summer production and has been proposed to allow a number of major cotton insect pests including *Spodoptera litura* (F.) and *Pectinophora gossypiella* (Saunders) to be avoided (Yeates 2001). Although this has been successful, sucking insects remain a problem. Species that are commonly encountered in the Northern Territory include mirids (*Creontiades dilutus* (Stål) and *Creontiades pacificus* (Stål)), green vegetable bug (*Nezara veridula* (L.)) and red-banded shield bug (*Piezodorus hybneri* (Gmelin)) (Ward 2002). The damage caused by each of these species is similar and results from feeding on squares (flower buds) and bolls (developing fruit). Feeding on squares and young bolls results in abscission, whereas feeding on older bolls results in warty growths on the inside carpel and discoloured lint (Bundy *et al.* 1999; Khan & Bauer 2001, 2002), which results in both loss of yield and reductions in lint quality.

In eastern Australia, sucking insects such as mirids and shield (stink) bugs have in the past presented a limited problem to cotton, with most being controlled with broad spectrum insecticides targeting *Helicoverpa* spp. larvae (Fitt 2000). However, sucking insects have become an increasing problem in the USA (Greene *et al.* 2001) and Australia. Reasons for this include the production of transgenic cotton containing a gene for the expression of the Cry1A(c) δ-endotoxin of *Bacillus thuringiensis* Berliner variety *kurstaki* and a shift towards production systems with minimal reliance on insecticides. The introduction of 2 gene Bt cotton (Bollgard II) containing the additional δ-endotoxin of *B. thuringiensis*, Cry2A(b) is likely to reduce the requirement to control *Helicoverpa* spp. and *Heliothis* spp. larvae with the net effect of further increasing the sucking insect problem. This problem is likely to also be encountered in northern Australia if a cotton industry based on the production of 2 gene Bt cotton (Bollgard II) ever established.

Numerous authors have reported action thresholds for stink bugs and shield bugs in both Australia and the USA. In the USA, nominal thresholds have been proposed of one pentatomid bug/2 m of row when sampling is undertaken using a ground cloth (Greene *et al.* 1997, 2001). In southern Australia, Khan and Bauer (2002) proposed a threshold of one green vegetable bug/m^2 and three red-banded shield bugs/m^2 when sampling using a similar technique. Thresholds for mirids have been set at between 0.5 and 1/m^2 depending on whether the cotton is being grown in a warm or cool production area (Mensah *et al.* 2003). Despite these studies, there has been limited work reported on Bollgard II cotton and no work

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relating to sucking insects in winter cotton production systems.

A major difference between the summer production systems of southern Australia and the winter production system being researched in northern Australia is the temperature regimes under which the crop is produced. In a summer production system, temperatures are cool at the beginning and end of the season and warm in the middle, while in a winter production system the opposite is the case. This is likely to affect the plant's physiological response to damage, in particular how the plant compensates for fruit loss; when the demand for assimilate peaks in mid-season, the plant's ability to produce photosynthate is reduced as a result of short days and cool temperatures.

In this paper I report on a trial examining the yield losses resulting from sucking insects in a determinate Bollgard II cotton variety produced in a winter production area of northern Australia.

MATERIALS AND METHODS

A crop of DP50bx Bollgard II cotton was planted on 23 April 2002 at Katherine Research Station, Katherine, Australia. No treatments were applied to the seed at planting. The crop was fully irrigated using a lateral move irrigator fitted with low energy precision application (LEPA) nozzles and fertiliser (nitrogen, phosphorus, potassium) was applied throughout the season as part of the irrigation process (fertigation) according to crop demand. As a result of high square (bud) retention (>95%), no pre-flowering insect control was required.

On 18 June 2002 (3 d before first flower) all plots were sampled to ensure that each treatment had similar fruit loads; the number of plants/m^2, number of fruiting branches, number of squares and number of abscised squares was measured in five, 1 m^2 sections per plot.

The trial consisted of five treatments arranged in a randomised complete block format. Each treatment was replicated four times. The treatments were an unsprayed control, an insect-free plot maintained by making applications of 125 mL/ha of Regent (200 g/L of fipronil) every 9 d (insect exclusion), and plots sprayed when the density of sucking insects (sum of mirids, mirid nymphs, red-banded shield bugs and green vegetable bugs) exceeded 0.5, 1 and 2/m^2. These plots were sprayed with 62.5 mL/ha of Regent whenever the predetermined treatment threshold was exceeded. Each plot was 18 m (rows) wide and 30 m long.

Intensive monitoring of the crop commenced on 21 June 2002 when the crop reached first flower. First flower was defined as one flower/m^2. Sampling continued until cut-out (3 September 2002). During this period the trial was sampled twice weekly (Tuesday and Friday). On each sampling occasion, four 0.5 m^2 areas were sampled visually in each plot and the number of mirids, mirid nymphs, green vegetable bugs and red-banded shield bugs was recorded. As the crop was Bollgard II, the number of *Helicoverpa* spp. larvae remained low throughout the trial and did not require control.

To test the impact of sucking insect populations on crop maturity, the number of open bolls was recorded in each plot twice weekly commencing on 30 September 2002 and continued until in excess of 60% of the bolls were open, at which point the crop was defoliated. On each occasion bolls were removed from a defined 2 m^2 section of crop and were retained to determine how damage changed over time. To determine the impact of sucking insects on the appearance of the fibre and mature bolls, damage ratings were made on each boll. Each boll was rated on a categorical scale of 0–4 with 0 being bolls showing no damage and 4 being bolls showing severe damage including a failure to open properly and severe staining of the lint (Lei *et al.* 2002).

Yield was assessed by hand-picking three randomly selected, 2 m^2 sections of crop in each plot between 1 and 4 November 2002, when the crop had reached commercial maturity. The seed cotton from each section of row from each plot was weighed separately and then bulked. The seed cotton was ginned using a hand-operated gin to remove the lint from the seed. Fibre quality assessments were made on the resulting lint using an HVI 900 semi-automatic from Zellweger-Uster by CSIRO Division of Plant Industry, Narrabri. Properties measured included turnout (the percentage of the seed cotton weight comprising lint), fibre length, uniformity, fibre strength and micronaire (fibre diameter).

In each plot, three 1 m^2 sections of crop were mapped immediately before harvest. Mapping involves recording the distribution of fruit on the cotton plant and was done and recorded using a standard format included in the Cotton Logic V4.1 Decision Support Program (CSIRO 2001).

Data analysis

All data were analysed using Statistica V6 (Stat Soft Inc. 2002) and SPLUS V6.1 (Insightfull Corp. 2002). The pre-trial measurements, taken in each plot to determine whether all plots were the same at the start of the experiment, and the yield data were analysed using ANOVA with a nested randomised complete-block model structure. When required, Tukey's honestly significant difference (HSD) test was used to separate means. The sucking insect density data and the boll damage rank data were analysed using restricted maximum likelihood estimation (REML). To determine whether the composition of sucking insects present in each treatment was similar, the proportion of each species was analysed using a multivariate analysis of variance with insect density (mirid adults, mirid nymphs, red-banded shield bugs and green vegetable bugs) as the response vector, and treatment applied as the independent variable. Differences in fibre quality were tested using ANOVA.

The date of crop maturity was estimated by fitting a simple quadratic expression ($y = \beta_1 x + \beta_2 x^2$) to the maturity pick data and determining the point where 60% of bolls were open.

RESULTS

There were no differences between treatments or blocks for plant population, number of fruiting branches or number of

squares at the start of the experiment ($P > 0.05$) (Table 1). However, there were both treatment and block effects in the analysis examining the number of abscised squares (treatment: $F = 4.3$; d.f. = 4, 88; $P < 0.01$, block: $F = 23.2$; d.f. = 3, 4; $P < 0.01$). Square abscission was higher in the insect exclusion treatment than the other four treatments (Table 1). Numbers of abscised squares was low (2–5/m^2) compared with numbers of retained squares (114–122/m^2) and, total square retention was still more than 95% at first flower. The reasons for the differences in square abscission observed are unclear. However, square abscission is common in pre-flowering cotton and has many causes including insect feeding and plant physiological factors. Square loss often exceeds 30% at first flower in commercial cotton crops with no yield loss, making the differences observed in this experiment insignificant.

During the experiment nine applications of Regent were made to the insect exclusion treatment. To maintain the predetermined thresholds for the remaining treatments, five sprays were applied to the 0.5/m^2 treatment and three were made to the 1/m^2 treatment. The 2/m^2 treatment did not reach threshold effectively making it an unsprayed control.

A complex of three species of sucking insects was encountered during the experiment. These comprised 8% mirid adults, 58% mirid nymphs, 27% red-banded shield bugs and 7% green vegetable bugs. As expected the sucking insect populations differed between treatments ($F = 16.71$; d.f. = 4, 407; $P < 0.001$). Populations were higher in the unsprayed control and 2/m^2 treatments than in the remaining treatments (Table 2). There was also a significant interaction between time and treatment suggesting that the population trends differed between the treatments ($F = 7.01$; d.f. = 4, 407; $P < 0.001$). Although the populations encountered in each of the treatments differed, their composition did not, with similar proportions of each species in each treatment ($F = 0.51$; d.f. = 12, 34.6; $P = 0.90$) (Table 2).

At the conclusion of the experiment there were highly significant differences ($P < 0.001$) in both the yields obtained in each of the treatments and the number of bolls in each treatment (Table 3). Both yield and the number of bolls were highest in the insect exclusion treatment and lowest in the unsprayed treatments.

The results of the plant mapping demonstrated that fruit retention was similar in all treatments up until the fifth fruiting branch after which retention fell sharply in the unsprayed and 2/m^2 treatments (Fig. 1). The reduction in fruit retention on the upper part of the plant led to earlier maturity in the high insect density treatments with the unsprayed control reaching maturity 9 d earlier than the insect exclusion plots (Table 3).

Table 1 Plant population, number of fruiting branches, number of squares and number of abscised squares per meter of row 3 d before the commencement of the experiments. Numbers in brackets represent the standard error of the mean. For abscised squares, numbers in the same row followed by the same letter are not different at $P = 0.05$. Significant differences did not exist for all other parameters

Variable	Plants/m^2	Squares/m^2	Fruiting branches/m^2	Abscised squares/m^2
Unsprayed control	12.4 (0.53)	120.7 (3.8)	83.3 (3.4)	3.2 (0.5)a
Insect exclusion	12.2 (0.49)	118.9 (4.3)	79.4 (2.6)	5.5 (0.8)b
0.5/m^2	11.8 (0.47)	122.4 (4.3)	81.6 (3.0)	2.8 (0.3)a
1.0/m^2	11.6 (0.72)	118.7 (4.5)	79.2 (3.7)	4.2 (0.5)a
2.0/m^2	11 (0.39)	114.7 (3.4)	78.4 (2.8)	3.4 (0.3)a

Table 2 Mean (SE) sucking insect populations (sum of mirid, red-banded shield bug (RBSB) and green vegetable bug (GVB)) recorded in each treatment between 21 June 2002 and 3 September 2002 and the composition of each population

Treatment	Mean sucking insects/m^2 (SE)	% Mirid adults	% Mirid nymphs	% RBSB	% GVB
Unsprayed control	1.17 (0.17)	9.18	57.14	24.49	9.18
Insect exclusion	0.4 (0.08)	2.94	50.00	38.24	8.82
0.5/m^2	0.37 (0.07)	9.68	54.84	29.03	6.45
1.0/m^2	0.68 (0.13)	10.53	61.40	22.81	5.26
2.0/m^2	1.19 (0.13)	8.00	60.00	29.00	3.00

Table 3 Yield, number of bolls and the delay in maturity relative to the control (60% open bolls) recorded in each treatment. Numbers in brackets represent the standard error of the mean. Numbers within each row followed by the same letter do not differ significantly at $P = 0.05$

Variable	Yield (g of seed cotton/2 m^2)	Bolls/2 m^2	Delay in maturity relative to control (days)
Unsprayed control	119.4 (16.5)d	48 (3.2)a	–
Insect exclusion	306.8 (16.5)a	70.9 (5.8)b	9
0.5/m^2	252 (16.8)b	70.6 (5.7)b	6
1.0/m^2	200 (19.2)c	59.8 (6.4)c	5
2.0/m^2	59.8 (6.4)ab	46.3 (5.2)a	1

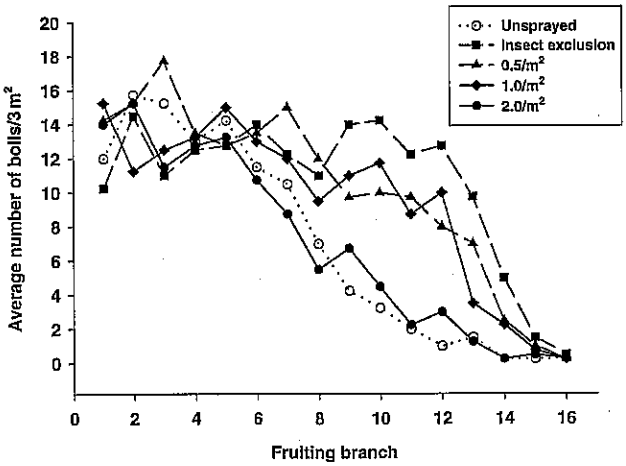

Fig. 1. Average number of bolls on each fruiting branch at picking in each treatment.

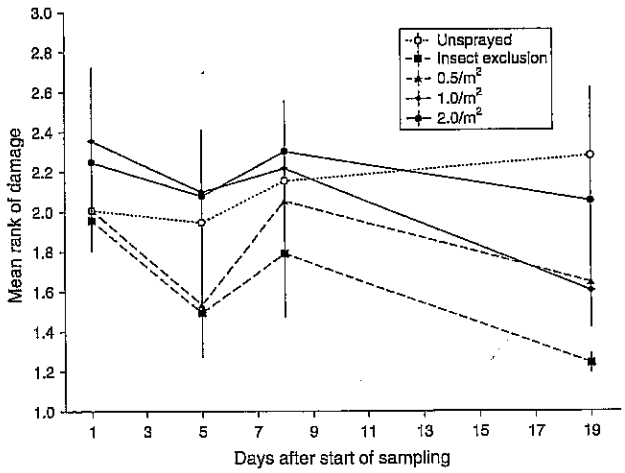

Fig. 2. Mean damage rank of bolls picked from each treatment. Picking commenced on 30 September and concluded on 18 October. Vertical bars represent the standard error of the mean.

The regressions used to estimate the maturity date had R^2 values ranging between 0.9 and 0.94.

In addition to the impact of sucking insects on fruit retention, there was also an increase in the amount of boll damage sustained ($P < 0.01$). The average boll damage rank was higher in the unsprayed control and the 2.0/m^2 treatment than the insect exclusion treatment (Fig. 2). Despite the differences in damage between treatments the damage sustained was constant over time with similar damage ranks being recorded on bolls on the upper and lower branches (as indicated by when they opened) ($P < 0.05$). There was also no interaction between time and treatment ($P > 0.05$). There were no differences in any of the fibre quality measurements taken ($P > 0.05$) with the exception of micronaire, which was only just significant ($P = 0.05$). However, Tukey's HSD test failed to detect differences between the micronaire means.

DISCUSSION

In this experiment, the presence of mirids, red-banded shield bugs and green vegetable bugs resulted in significant yield reductions. At the highest densities recorded using visual checks, which were less than two sucking pests/m^2, yield reductions in the vicinity of 60–70% were observed.

Feeding by sucking insects results in both the abscission of squares and young bolls as well as damage to developing bolls (Khan & Bauer 2001). Although both fruit abscission and boll damage were apparent in this experiment, the plant-mapping data suggested that fruit abscission had the largest impact on yield. Boll retention on the first five fruiting branches was similar in all treatments. However, after the fifth fruiting branch the number of bolls recovered on each node was much lower in the unsprayed and 2/m^2 treatments relative to the other three treatments (Fig. 1). Boll damage was also evident with higher average damage ranks recorded on bolls taken from the higher pest density treatments than in the lower pest density treatments. Despite these differences, the fibre qualities were similar in each treatment. The similarity in fibre quality between treatments reflects the observations made by Lei *et al.* (2002) who observed similar fibre qualities irrespective of mirid density. They hypothesised this to be the result of damaged cotton being discarded in the ginning process. Surprisingly, the damage ranks remained relatively constant throughout the season despite changing populations of sucking insects.

Damage similar to that imposed by sucking insects has been reported to delay crop maturity (Brook *et al.* 1992a). However, in this experiment the opposite was apparent, with the unsprayed control reaching maturity (60% of bolls open) 9 d before the insect exclusion plots. This was probably the result of low fruit retention on the higher nodes of the treatments with high sucking insect populations. As a result, maturity was reached sooner in these plots than in those where more fruit was set on top of the plant.

Cotton has been widely reported as having the ability to compensate for early season fruit loss (pre-first flower) (e.g. Brook *et al.* 1992b; Sadras 1995; Stewart *et al.* 2001). The main mechanisms by which this is thought to occur are by the substitution of damaged squares by other squares that would have been lost as a result of physiological shedding or by the production of more fruit. As a result, the ability of the crop to compensate for the loss of fruit has become an important component in the development of action thresholds for the pre-flowering and early fruit-set period. However, in this study there was no evidence of compensation, as boll production did not increase in response to sucking insect damage. In part, this may be because fruit retention was very high at first flower with over 95% of squares still present at this time. However, despite this, these results mirror those obtained by Lei *et al.* (2002) who also observed no evidence of compensation in response to sucking insect damage in field trials in south-east Queensland.

The ability of the plant to compensate for fruit loss following first flower may have also been compromised in this exper-

iment because of the mismatch between assimilate production and assimilate demand that occurs under winter production systems such as that being investigated in northern Australia. In winter production systems photosynthesis is severely reduced as a result of short day lengths and cool temperatures at the time when demand for photosynthate is highest (peak flowering and fruit development). At temperatures below 12°C cotton plants go into cold shock with net photosynthesis ceasing at temperatures below this point. The effect of cool temperatures would have been particularly important in the season during which this experiment was conducted as it was exceptionally cold during the peak flowering and fruit development period. During the season, 73 nights below 11°C were recorded with a minimum temperature of 2.3°C reached. This compares with the median number of nights below 11°C of 17 for Katherine (Yeates 2001).

A difficulty encountered when researching sucking insects in field studies is how to rate the relative impact of different instars and species and hence what action threshold should be used. This is particularly relevant in cotton where a complex of pest insects representing a range of species of different ages is often present. To counteract this problem Khan and Bauer (2002) proposed a scale of equivalency, rating different pest insects based on laboratory studies. In contrast, Greene *et al.* (2001) considered all sucking pests equally in their action threshold. For simplicity, all sucking insects were considered equal in this experiment, irrespective of instar or species. The results of this study suggested that given the conditions under which this experiment was conducted, a tentative threshold for the complex of sucking insects present in this experiment was 0.5/m^2 when sampling is conducted using visual techniques; populations in excess of 0.5/m^2 resulted in very high yield losses. This result is similar to the action thresholds proposed by Khan and Bauer (2002) and Mensah *et al.* (2003) for cool regions in eastern Australia, but half that of the threshold for warm regions for green vegetable bug and mirids, in southern Australian summer cotton production areas. Greene *et al.* (2001) proposed a similar threshold for the complex of sucking insects encountered in South Carolina, which included green vegetable bug.

The crop grown in this trial was lower yielding than is normally the case for Katherine. In favourable seasons yields in excess of 420 g of seed cotton/m^2 are consistently recorded compared with the 153 g of seed cotton/m^2 recorded in the highest yielding treatment in this experiment. Probable reasons for the low yield were threefold; it was not a commercial variety, the exceptionally cool growing conditions under which it was produced and the highly determinate nature of the variety grown. As a result, the action threshold proposed in this study may only apply to determinate Bollgard II varieties grown in cool conditions. Despite the differences in the conditions encountered in this trial and those encountered in the trials conducted by Khan and Bauer (2002) and Greene *et al.* (2001), the results obtained in all three studies were similar, suggesting that an action threshold of 0.5 sucking insects/m^2 is relatively robust across a range of species compositions and conditions. As a result, a threshold of 0.5 sucking insects/m^2 is tentatively proposed once cotton starts flowering when using visual sampling techniques.

The results of this experiment provide a starting point on which future work relating to sucking insect thresholds in winter production systems can be based and indicate that relatively low populations of sucking insect pests have the potential to inflict considerable damage to determinate Bollgard II cotton varieties when left untreated in winter production systems in northern Australia. Areas requiring further work include quantifying the damage inflicted by different species and instars, the impact of sampling methods on sampling efficiency, the impact of seasonal conditions on the plants response to sucking insect damage and the need to research alternative techniques to manage or avoid sucking pest build up and hence reduce the requirement to spray.

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Sicot 289B

Sicot 80B

CSX57B

CHQX21B

20410-30B

20410-48B

20458-8

20458-26

20458-34

20458-36

20458-57

20458-74

20458-75

20458-80

20463-33

61401B

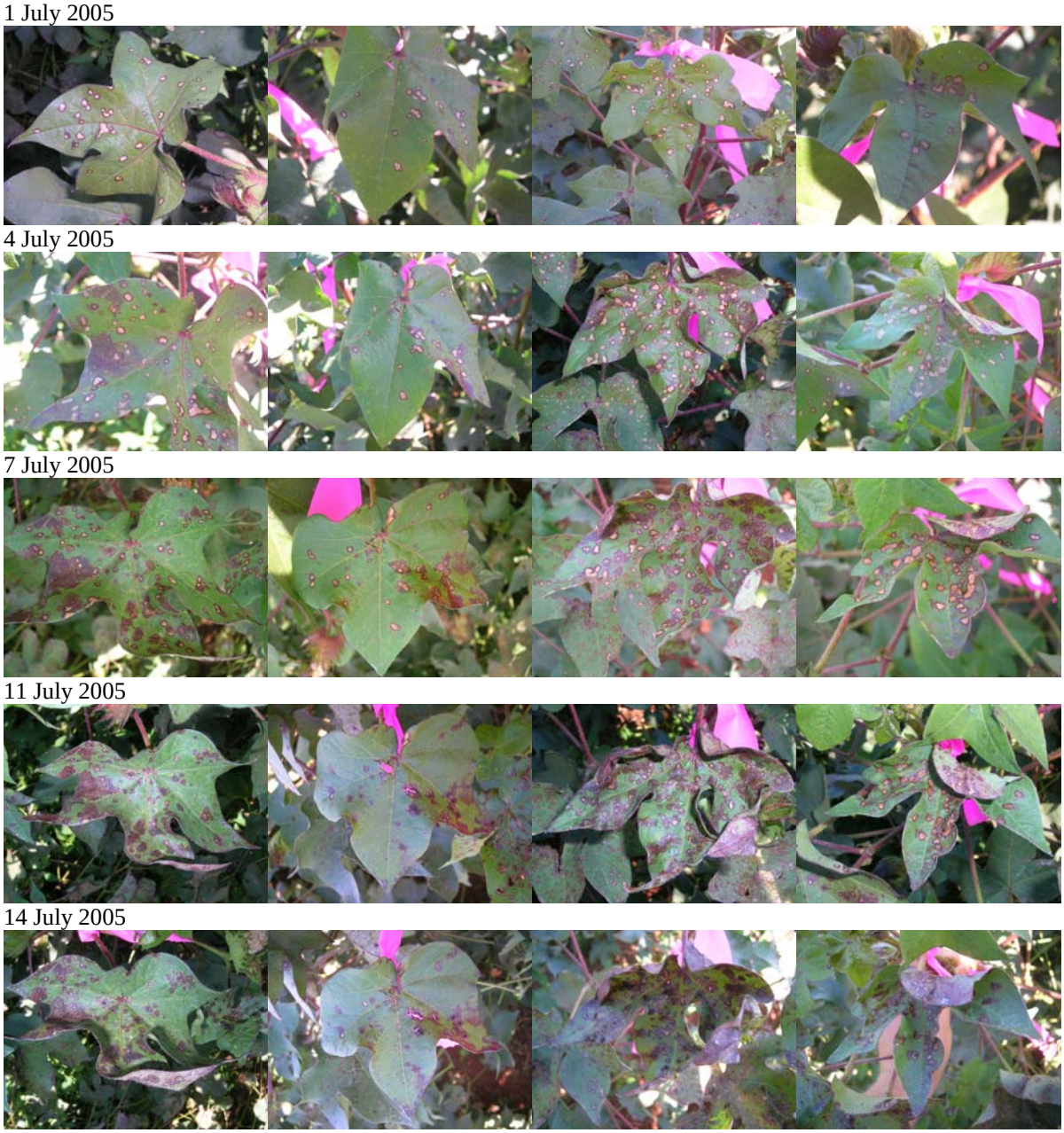
62401B

62404B

Diagnostic shots

Lower leaf surface | Lower surface damage | Upper leaf surface | Upper surface damage

	Leaf 1	Leaf 2	Leaf 3	Leaf 4
1 July 2005				
4 July 2005				
7 July 2005				
11 July 2005				
14 July 2005				



18 July 2005

21 July 2005

26 July 2005

28 July 2005

5 August 2005

9 August 2005