CSIRO Entomology
Cotton Research Unit
and
CRC for Sustainable Cotton Production

FINAL REPORT

Project title: **Identifying key groups of soil fauna in cotton agroecosytems.**

Project code: **CSE69C**

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A final report prepared for the CRC for Sustainable Cotton Production and the Cotton Research and Development Corporation

FINAL REPORT — CSE69C

BACKGROUND

Interest amongst cotton growers in utilising the contribution of beneficials in pest management is gaining momentum due to a recent squeeze on profit margins (increasing pesticide costs and decreasing cotton prices), environmental pressures (ie endosulfan) and the emphasis placed on IPM by key research, extension and industry groups. Research to quantify the effectiveness of beneficials and to facilitate prediction of their impact on insect pests is required for the development of effective IPM systems and for continued fostering of grower interest. Research on beneficial soil fauna in Australian cotton agroecosystems is very much in its infancy. Feeding preferences and the contribution of soil-surface inhabiting predators (referred to hereafter as soil predators) to cotton pest management are virtually unknown. Similarly, little is known of absolute population densities for these groups. This project quantified the densities of soil fauna, including soil predators, in commercial irrigated cotton fields under sprayed and unsprayed conditions. Direct field and laboratory studies were used to establish the feeding links between common soil predator species and key pests (e.g. *Helicoverpa* spp.) during the cotton-growing season. Preliminary calibrations of qualitative enzyme–linked immunoassay (ELISA), used to detect prey protein in the gut of predators, were explored for the common brown earwig, 3 ladybird species and the red and blue beetle.

OBJECTIVES

- Quantify and compare the population densities of beneficial soil faunal groups in commercial irrigated cotton fields that are managed using conventional, Ingard®, and Envirofeast® technologies.
- Determine the feeding links of key soil–dwelling predator species on selected key insect pests of cotton using direct observational approaches (field, glasshouse or laboratory studies) and complementary qualitative techniques (screening of predator gut contents using ELISA).
- Develop a research sampling protocol for estimating the densities of key soil faunal groups in commercial irrigated cotton.

EXTENT PROJECT OBJECTIVES WERE ACHIEVED

- An effective research sampling protocol was developed for estimating absolute densities of soil predators.
- Early into the project we decided that the most useful comparison for the cotton industry was between sprayed and unsprayed cotton. This provides information on potential densities of soil predators and subsequent densities following insecticide applications. Population densities were therefore compared in sprayed and unsprayed commercial cotton at Doreen (97/98) and Auscott Narrabri (98/99). Experiments were also planned at Norwood to make comparisons between densities in commercial Ingard® and Envirofeast® cotton, but unfortunately the advent of *Fusarium* at this farm in 1997/98 season prevented the continuation of this work. Instead, an opportunity was taken to link up with experiments at ACRI and investigate the effects of early season insecticides on soil predators

found in the cotton canopy and on the ground floor. Ants were the most abundant soil predators on early season cotton during both 97/98 and 98/99 and so this predator group was studied in detail.

- An effective protocol for studying predation on early season cotton was developed using ants and *Helicoverpa armigera* eggs as a model. This protocol utilises the Observer® software package and can be adapted to study other predator–prey interactions in the field.
- Detailed feeding experiments on the consumption rates of *H. armigera* larvae by the earwig, *Labidura truncata*, and *H. armigera* eggs by three common ladybird species and the red and blue beetle were conducted in the laboratory. A protocol for the ELISA gut assays was developed and then used to determine the detection interval of larvae in the guts of *L. truncata* and eggs in the guts of striped, three banded, & transverse ladybirds and the red and blue beetle. Further refinements to the ELISA technology are required before the large scale screening of predators collected in the field can be performed.

METHODOLOGY

Soil predator densities on the soil surface in sprayed and unsprayed commercial cotton (/ ha)

Large field tents (3 rows x 3m x 2m tall) were used to 'fence' off areas of cotton. Nine pitfall traps were permanently installed in the beds of each tent–area for the duration of the study period. Tents were erected and sealed at their base on each sampling occasion to prevent soil predators escaping or entering the enclosed area. Baits or preservatives were placed in the pitfall traps which were then opened for 7–10 days to exhaustively remove all the soil predators present within the confined area of the tents. This procedure provided an estimate of the absolute density of soil predators in a known area of cotton. Data were collected using this technique in sprayed and unsprayed treatments during January 1998 at Doreen (Block 2) (west of Wee Waa) and during November, December and January at Auscott–Narrabri in 1998/99 (Field 16). The fields selected for this work were part of the large scale field trials run by the Australian Cotton CRC to investigate the efficacy and non–target effects of Ingard® and 2–gene Bt cotton on target and non-target fauna. Six insecticide sprays had been applied to the sprayed cotton treatment in each field prior to the January sampling period.

Ant densities on plants (/ m)

Densities of ants on plants were assessed at ACRI using suction sampling in 97–98 and 98–99, and whole plant sampling in 99–00. In the first two seasons, suction samples were collected using mini garden 'blower vacs' from a complete row (i.e. 20 m) in each plot immediately before the first insecticide was applied, and at approximately twice weekly intervals thereafter. Four suction samples were taken per insecticide treatment. Whole plant sampling was used during 99–00 and involved the counting of ants on sets of 50 plants in unsprayed cotton. Twelve to sixteen random sets of whole plant samples were taken on each sampling occasion.

Impact of insecticides

Field experiments investigating the effects of new and currently registered insecticides on early season (late November to mid January) abundance of ants on the soil surface and in the crop canopy were conducted at ACRI during 1997/98 and 1998/99 in collaboration with Dr Lewis Wilson. A randomised block design was used during each season, with four replicates of five or seven insecticide treatments and an unsprayed control. Strips of 4 rows of safflower and/or sunflower were sown between cotton blocks to serve as nurseries for beneficial insects and green mirids and to help reduce drift between plots. A total of thirteen insecticides were tested during at least one growing season. Insecticides were applied 3 times in 97–98 and 4 times in 98–99 at 7–10 day intervals. D–vac suction samples were collected before and after each spray to compare ant densities on plants between the different treatments. Pitfall traps were also included in several treatments (control, thiodicarb and fipronil) during 97-98 and 98–99 to investigate the effects of insecticides on the abundance of ants on the soil surface. All ants were identified to species.

Egg mortality on plants

Experiments were conducted at ACRI during December 1998–99 and December—early January in 1999–00 to investigate the foraging behaviour and prey diets of ants, and their consumption rates of *Helicoverpa* eggs. Predation of *Helicoverpa* eggs by other predators present in the canopy was also determined. Areas of 1m x 1m were marked out in unsprayed irrigated cotton and then artificially stocked with *H. armigera* eggs. Fresh cotton leaves were placed in buckets containing laying female *Helicoverpa* moths overnight. Leaf discs (5 mm diameter) containing a *Helicoverpa* egg were then cut out and glued singly on the upper surfaces of terminal leaves at a density of 30 eggs / m.

Detailed observations of egg mortality and the foraging behaviour of individual ants were recorded as soon as the leaf disks were in place on the plants using tape recorders in 1998 and using lap–top computers running 'The Observer' ® software in 1999. Both systems allowed us to quantify the proportion of time individual ants spent on the ground versus different parts of the cotton plant, and performing different foraging activities (e.g. feeding on nectar from nectaries or attacking eggs). Each egg–infested area was observed for 20 minutes at least once per day between 8–11 AM and 4–7 PM. Missing eggs were replaced on new leaves so each 1m x 1m had a density of 30 eggs / m at the beginning of the observation period. Fifty–one hours of observation were completed during both 98–99 and 99–00, giving a total of 102 hrs. Ground densities of ants were estimated before and after each 20–min observation period and the data recorded on the Observer® program. The presence of secondary pests (e.g. aphids, whitefly, jassids) and other predators on plants within the marked areas was also recorded.

Gut assays

<u>1. Preliminary assays with *H. armigera* MAb</u>

Cell lines of the monoclonal antibody (MAb) for *H. armigera* were successfully cultured in Canberra with the assistance of Dr Steven Trowell and members of his technical team. A laboratory at ACRI was renovated and equipped for running ELISA assays. These assays use a monoclonal antibody that is specific to immature life stages of *H. armigera*. Hence, only those predators that have eaten an egg or larva of *H. armigera* within a certain time period should be positive. This technique has the potential to be a very powerful qualitative tool for identifying which predators are eating *Helicoverpa* eggs or larvae and therefore complements the direct observation studies described above. I then undertook a series of assays to calibrate all the components involved with the ELISA technology. The most suitable assay involved using an indirect ELISA, neat dilutions of primary antibody, a Dako ® rabbit anti–mouse secondary antibody diluted at 1:2000 in 1% milk, and a HRP substrate. Indirect ELISA's involving all insects found in cotton were then assayed so as to confirm the absence of any cross–reactivity between prey species and the *H. armigera* MAb. This tests for 'false-positives' that might occur should the protein not be truly unique to *H. armigera*.

2. Detectability of II instar *H. armigera* larvae in the guts of *Labidura truncata* (Common brown earwig)

To effectively use the ELISA technology it is important to know the retention time of a given prey in the gut of the predator since this determines the length of time that a prey item can be reliably detected. It is also important to determine the effect of temperature on the retention interval since prey may be digested faster at higher temperatures, thereby shortening the time which prey can be detected. Initial experiments to derive this information were begun using the common brown earwig. This species was an ideal candidate because it could be readily collected in the field and in the laboratory it consumed *Helicoverpa* larvae very quickly. Earwigs were collected in cotton fields at ACRI using baited pitfall traps. Male and female adults of *L. truncata* were then placed in separate living chambers, made from small plastic boxes and given water. A total of 528 adult individuals were used to investigate the interaction between three temperature settings (25, 30, and 35 °C) and five time intervals after feeding (T_0, T_1 hr, T_2 hr, T_3 hr, & T_{24} hr) on the detectability of *H. armigera* larval protein in the earwigs gut. All adults were starved overnight in their respective growth chambers prior to feeding and randomly allocated to a temperature and time interval after feeding. Second instar *H. armigera* larvae were then fed to 16 male and 16 female earwig individuals for each temperature by time interval combination and a further 16 were left unfed as controls for each temperature (176 individuals per temperature). Weights of each earwig and *Helicoverpa* larva were recorded. After feeding, earwigs were placed in a growth chamber at the appropriate temperature for the appropriate time period after which they were quickly transferred to a freezer set at –80 ° C for storage. All earwigs were later dissected on dry ice and the frozen digestive tract removed and assayed for the presence of *H. armigera* protein using the indirect ELISA in (1).

3. Detectability of *H. armigera* egg protein in the guts of three ladybirds and the red and blue beetle

This work followed the same rationale as that outlined in 2 above. Ladybirds and red and blue beetles were collected on sunflowers at ACRI during November and December, 1999. All beetles were maintained in culture in the lab for at least a week, and then deprived of food 48 hrs prior to feeding. A total of 160 striped ladybirds, 72 transverse ladybirds, 72 three–banded ladybirds, and 72 red and blue beetles were used. In this study *H. armigera* eggs were used instead of larvae. Striped ladybirds were each fed 1, 5 or 10 *H. armigera* eggs and frozen at 8 time intervals after feeding (T_0, T_1 hr, T_2 hr, T_3 hr, T_4

hr, T_6 hr, T_{12} hr, & T_{24} hr). Transverse ladybirds, 3–banded ladybirds and red and blue beetles were each fed either 1, 5, or 10 eggs and frozen at one of three time intervals after feeding (T_0, T_{12} hr, & T_{24} hr). All ladybirds were fed at 25 ° C. Weights of each beetle were recorded as in (2). Whole body homogenates were assayed using the indirect ELISA in (1), rather than the gut dissection method used for larger predators.

Consumption rates of Helicoverpa spp. eggs

Data on the consumption rates of *H. armigera* eggs by striped, three–banded & transverse ladybirds and the red & blue beetle were also collected during 1999–2000 as an additional component to the assays related to detectability in (3) above. These data together with the frequency of field–collected predators showing positive for the *H. armigera* protein can be used, with care, to compare the potential effectiveness of different predator species. All beetles were collected in the field and maintained in the lab as described in (3) above prior to feeding. A total of 367 beetles were offered meal sizes of either 1, 5, or 10 eggs (total eggs fed = 1845). The number eggs remaining at 1, 2, 3, 4, 5, 6 & 24hrs after feeding was recorded.

RESULTS

Densities on the soil surface in commercial cotton

Data on ant densities on the soil surface suggest there is potential for large populations (• 800,000 ants / ha) to be maintained in commercial furrow–irrigated cotton fields. For example, ground densities of ants in unsprayed cotton in the lower Namoi, ranged from 4,815 individuals / ha prior to first square at Auscott Narrabri to 812,222 individuals / ha in January at Doreen (Table 1). Ground densities of ants in unsprayed cotton at 'Doreen' and 'Auscott Narrabri' in the Namoi valley were between 3.6 and 25 times greater than sprayed cotton, suggesting insecticides caused significant reductions at both field sites. In contrast, densities on the soil surface of commercial fields not treated with insecticides increased as the growing season progressed. We have assumed that those fields with the highest densities of ants on the soil surface in early season cotton are also likely to have the highest ant densities on cotton plants.

Early season densities of earwigs, predaceous beetles, and spiders were variable between sprayed and unsprayed treatments at Auscott–Narrabri. Earwigs had higher densities in sprayed cotton at both commercial farms and this is consistent with earlier studies. However, the finding that the black field earwig, *Nala lividipes*, was the dominant species collected during all four sampling periods was unexpected. Densities of predaceous beetles and spiders were significantly greater in unsprayed treatments (c.f. sprayed) during the single sampling period in January at Doreen. However, at Auscott Narrabri they were only significantly greater in the unsprayed treatment during the mid-January sampling period. Spider densities in the unsprayed cotton at Auscott–Narrabri were more than 7.5 times greater than those in sprayed cotton during mid–January.

Table 1: Absolute densities of soil fauna at Auscott Narrabri and Doreen.

Site – Season	Taxa	Density / ha Sprayed		Unsprayed	
Doreen (97/98)					
Jan 22–26, 1998	Earwigs	14,444		10,833	
	Nala lividipes		11,111		7,778
	Labidura truncata		833		833
	Ants	226, 944		812,222	
	Pheidole		200,000		744,167
	Iridomyrmex vicinus		26,944		68,056
	Predaceous Beetles	2,500		20,556	
	Microlestes macleayi		556		6,944
	Spiders	5,000		10,833	
	Wolf spiders		5,000		10,833
Auscott (98/99)					
Nov 16–26, 1998	Earwigs	6,667		3,333	
	Nala lividipes		6,296		3,333
	Labidura truncata		370		0
	Ants	741		4,815	
	Pheidole		0		370
	Iridomyrmex vicinus		741		4,444
	Predaceous Beetles	34,444		9,630	
	Ladybird larvae		4,074		370
	Spiders	48,889		27,037	
	Wolf spiders		38,519		8,889
Dec 10–17, 1998	Earwigs	5,556		5,556	
	Nala lividipes		4,815		4,815
	Labidura truncata		370		0
	Ants	741		5,926	
	Pheidole		370		741
	Iridomyrmex vicinus		370		5,185
	Predaceous Beetles	11,852		7,407	
	Ladybird larvae		0		0
	Spiders	13,333		32,222	
	Wolf spiders		8,889		9,630
Jan 11–22, 1999	Earwigs	7,407		9,259	
	Nala lividipes		6,667		4,815
	Labidura truncata		0		1,852
	Ants	1,852		46,296	
	Pheidole		0		370
	Iridomyrmex vicinus		1,852		45,926
	Predaceous Beetles	12,963		35,926	
	Ladybird larvae		741		24,074
	Spiders	31,852		247,407	
	Wolf spiders		7,778		23,333

Ant densities and behaviour on plants

In contrast to densities on the soil surface, the abundance of ants on unsprayed plants was highest early in the season and declined sharply after Christmas. The soil surface of cotton fields is exposed (only 10–40% plant cover) and largely devoid of prey early in the season, and so it is at this time that ants spend a significant proportion of their foraging activities climbing small cotton plants to visit extrafloral nectaries and search for prey. As the cotton approaches first flower, densities on plants seem to decline with the greater availability of prey (both live and dead) on the soil surface. The mechanisms causing this change in ant foraging behaviour were not determined.

At ACRI, we found ants to be amongst the most abundant predator group found on squaring cotton prior to Christmas. Four groups of ants are commonly found on cotton plants in NSW (*Australian Cottongrower*, **20(5)**: 38–41). These are *Iridomyrmex vicinus, Pheidole, Rhytidoponera metallica and Paratrechina.* All four groups visit extrafloral nectaries, whilst only two groups, *Pheidole* and *I. vicinus*, commonly take *Helicoverpa* spp. eggs. *Pheidole* spp. accounted for between 77–84% of the total ants found on plants during the last three growing seasons (1997–1998 to 1999–2000). Density estimates of *I. vicinus* and *Pheidole* from suction samples averaged between 0.4–0.8 individuals / m during 1995–96 to 1998–99, although we have found suction sampling to underestimate ant densities on plants. During December 1999, densities from individual plants, estimated using direct visual counts, averaged 2.7 *Pheidole* / m of row (Figure 1A), with an average of 10.6% of plants at the time of sampling being infested with *Pheidole* (Figure 1A). However, even at the highest densities, no more than approximately 25% of plants were infested with *Pheidole* on any one sampling occasion (Figure 1B). Ant densities on cotton plants after Christmas averaged less than 0.5 individuals / m and 1.25% of plants infested (Figure 1A). This decline was consistent with previous studies conducted in collaboration with Lewis Wilson.

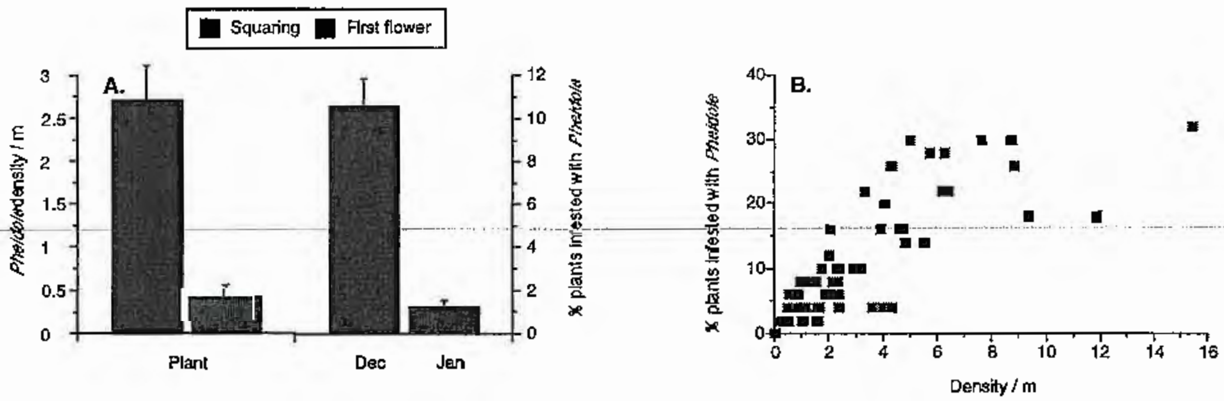


Figure 1: Density and percent plants infested relationships for *Pheidole* found on plants before (squaring) and after (first flower) Christmas in unsprayed cotton at ACRI during 1999–2000.

Nectar obtained from extra–floral nectaries is a key food source for ants, especially *Pheidole*, in early season cotton prior to Christmas (both pre–squaring and squaring cotton). Apart from our observation areas we noticed that other small areas of cotton plants were heavily infested with cotton aphid during the last week before Christmas. These plants were regularly checked for ants and the tending of aphids was only observed occasionally. *Paratrechina, I. vicinus* and *Pheidole* were all seen tending aphids on the undersides of leaves on at least one occasion.

Egg predation on early season cotton

Ants were the only predators observed attacking eggs on early season cotton during a total of approximately 102 person hours of direct observation during 1998–99 and 1999–00. Only *I. vicinus* and *Pheidole* took eggs during December 1998. On December 22, *I. vicinus* were observed removing 11 eggs with a further 50 eggs missing between observation periods. The greenhead ant, *R. metallica*, found eggs on several occasions but was never observed attacking eggs. All four of the common species–groups of ants found on cotton collected jassids as prey.

The smallest species–group of ants, the *Pheidole*, removed 97% of all eggs taken by ants during December 1999 (*Australian Cottongrower*, 21(2)). Other species–groups observed taking 1 or 2 eggs were *Paratrechina* and *I. vicinus*. *Pheidole* removed eggs from terminal leaves during 40% of the occasions they were observed on plants. Having found an egg on a leaf, *Pheidole* workers took about 2.3 minutes to remove the egg. Other workers were consistently observed on the same leaf the egg was found within 5 minutes of an egg–laiden worker commencing its return journey down the side–branch. This process is commonly known as recruitment, a foraging behaviour whereby the deposition of pheromone trails by food–laden workers ensures that other nest mates efficiently exploit rich food sources. *Pheidole* workers removed a total of 59 eggs during twenty observation periods, giving an average of 2.95 eggs removed / 20–minute observation period. However, *Pheidole* failed to find and remove eggs placed on the terminal leaves of taller squaring and flowering plants in January.

Pheidole were present on plants during at least 75–80% of the total observation hours, whilst *I. vicinus* and *R. metallica* climbed plants less than 30% of the time. Each ant species spent the largest proportion of their time in the plant canopy on leaves and regularly visited the extra–floral nectaries. *Pheidole* ants for example, spent similar proportions of their total time (~ 8%) sucking on nectaries and attacking eggs (Figure 2).

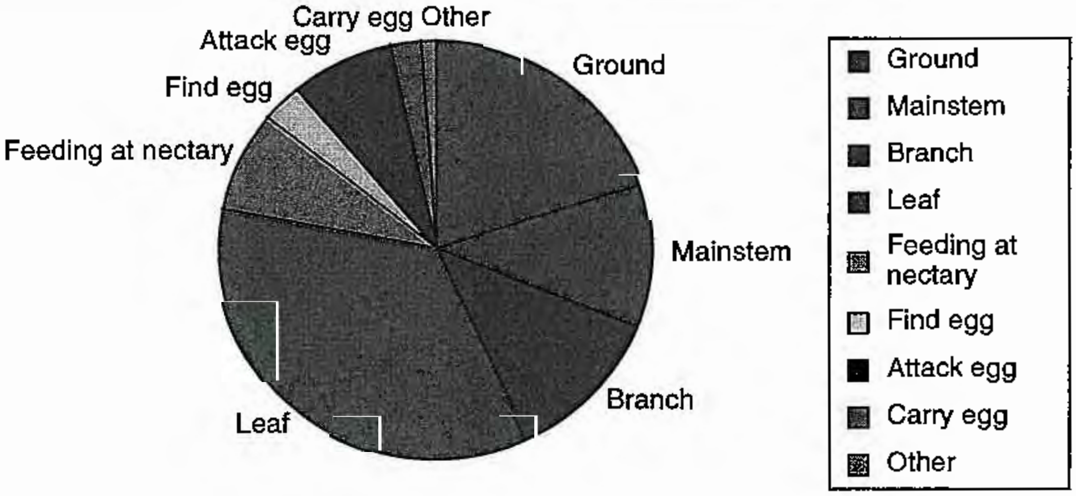


Figure 2: Example of the foraging behaviour of *Pheidole* ants as recorded by the Observer ®.

Efficacy of gut content assays

Results from the cross reactivity testing confirmed that the monoclonal antibody used for the Lepton® kit is specific to *H. armigera* and has no cross reactivity with any of the other insects found in cotton that were assayed.

(1) Detectability of 2nd instar larvae in the guts of *Labidura truncata* (common brown earwig).

Laboratory studies have shown that the common brown earwig is a voracious larval predator of Lepidoptera, false wireworms and armyworms. In our studies, adults had an average handling time of approximately 46 seconds for second instar *H. armigera* larvae (mean of 160 males & 157 females) and survived for long periods, in excess of 20 days, without food. ELISA results showed that further refinements to the gut assay protocol are required if the *H. armigera* MAb is to be effective for detecting the presence of larvae in the gut of an earwig. Data for 0–3 hrs after feeding were considerably less than the desired 100 % positive for each of the three temperature settings (Figure 3).

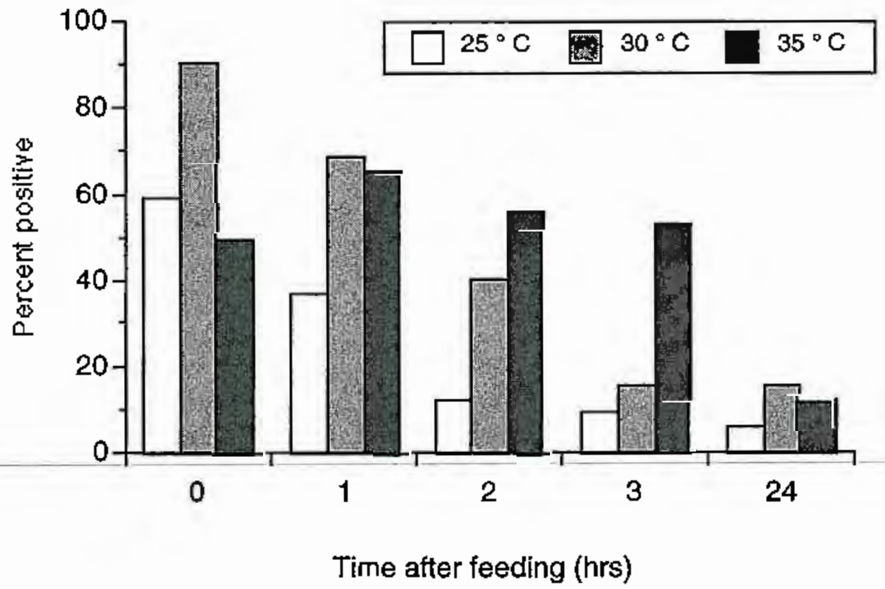


Figure 3: Prey detectability (percent positive) over 5 time intervals after feeding at 3 temperatures in the digestive tracts of the earwig, *Labidura truncata* (adult males & females), after consuming a single 2nd instar *H. armigera* larva (n = 16 males & 16 females / time interval).

(2) Detectability of *H. armigera* eggs in the guts of 3 lady beetles and the red and blue beetle.

Striped and 3–banded ladybirds consumed eggs more readily than the transverse ladybird and the red and blue beetle. Data for egg detectability were variable (Table 2) and so further labwork is required to successfully calibrate and validate the *H. armigera* MAb used in the Lepton® kit for the purpose of predator gut assays.

Table 2: Prey detectability (percent positive) at different time intervals after feeding (TAF) in four beetle species after consuming 3 meal sizes of *H. armigera* eggs (– = no data).

TAF	Meal size (# eggs)	Percent positive for each beetle species			
		Striped	3–banded	Transverse	Red & Blue
0	1	87.5	50	75	62.5
	5	100	100	87.5	62.5
	10	100	75	100	50
1	1	0	–	–	–
	5	100	–	–	–
	10	100	–	–	–
2	1	0	–	–	–
	5	87.5	–	–	–
3	1	12.5	–	–	–
	5	100	–	–	–
4	1	37.5	–	–	–
	5	87.5	–	–	–
6	1	0	–	–	–
	5	25	–	–	–
12	1	0	25	12.5	12.5
	5	0	12.5	0	0
	10	0	50	25	40
24	1	12.5	37.5	12.5	0
	5	0	12.5	0	0
	10	0	12.5	0	0

n = 8 beetles / meal size

Impact of insecticides

Use of many insecticides early in the growing season presents a major impediment to the conservation and management of ants in cotton (*Australian Cottongrower*, **20(5)**: 67–71). This is important given the significance that ants seem to play as an early season predator of *Helicoverpa*. Only 2 of the 13 foliar insecticides tested at ACRI during the last four seasons had a low impact on predacious ants. Ten of the thirteen insecticides used in our experiments at ACRI reduced ant densities (individuals / m) in cotton foliage by more than 40% with respect to the control, and were given a high or very high impact rating (Table 3). Fipronil, dimethoate, and endosulfan caused the greatest reductions in ant abundance across seasons (Table 3). Even at the lower application rate of 12.5 g ai / ha, fipronil reduced densities of egg predator ants by 95% during 98/99. Chlorfenapyr applied at high and low rates, and spinosad also caused significant reductions in ant densities during both growing seasons they were tested. Thiodicarb caused significant reductions to ants in 3 of the 4 seasons. Indoxacarb, amitraz, abamectin, and imidacloprid all had a high or very high impact rating (Table 3), but the available data for these insecticides showed statistically significant effects in only one growing season. Emamectin benzoate and pirimicarb were the only two foliar insecticides to have minimal effect on ants in the canopy and be given a very low impact rating. In fields where ants are abundant, reliance on endosulfan, fipronil, dimethoate, chlorfenapyr and even spinosad during stage I may prevent this predator group contributing to the suppression of pest populations.

Table 3: Impact of insecticides applied as foliar sprays on ant densities in early season cotton at ACRI.

Target spp. & insecticide	Rate	% reduction with respect to control					Impact rating[4]
	(g ai / ha)	95/96[1]	96/97[1]	97/98[2]	98/99[2]	Average	
Helicoverpa spp.							
Endosulfan	735	71	nd	nd	88	80	very high
Indoxacarb	127.5	nd	nd	nd	74	74	very high[5]
Chlorfenapyr (also mites)	200–400	nd	nd	47	86	67	very high
Methomyl	169	45	nd	nd	nd	45	High[5]
Spinosad	96	nd	30	59	nd	45	high
Thiodicarb	750	44	37	0	71	38	moderate
Emamectin benzoate	8.4	nd	<1	11	nd	6	very low
Mites							
Amitraz (also *Helicoverpa* spp.)	400	45	nd	nd	nd	45	high[5]
Abamectin[3]	5.4	nd	nd	29	53	41	high
Sucking pests							
Fipronil (mirids)	12.5–25	nd	71	71	95	79	very high
Dimethoate (aphids)	140	81	nd	nd	nd	81	very high[5]
Imidacloprid (mirids & aphids)	49	nd	nd	25	92	59	high
Pirimicarb (aphids)	250	nd	<1	nd	nd	<1	very low[5]

[1] all ant species collected & [2] egg predator ants.

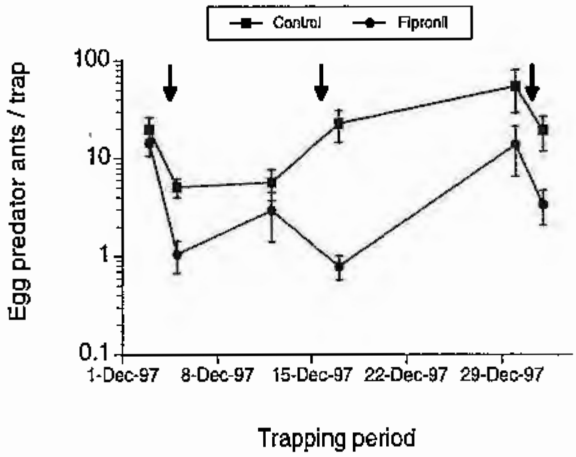
[3] 5.4 g ai / ha is the recommended rate of Abamectin to control spider mites whilst 10.8 g ai / ha is recommended to control *H. punctigera*.

[4] Impact rating: very low = < 10%; low = 10–20%; moderate = 20–40%; high = 40–60%; very high = > 60%.

[5] Impact rating based on a single season of data and hence requires caution with interpretation.

nd: Insecticide not tested during growing season (no data).

The effects of both fipronil and thiodicarb on the relative abundance of ants on the soil surface, as indicated by pitfall trapping, during 97–98 and 98–99 were consistent with those for the crop canopy. Fipronil caused significant reductions to pitfall trap catches during both seasons, whilst the impact of thiodicarb was only significant during 98–99 (Figure 4).



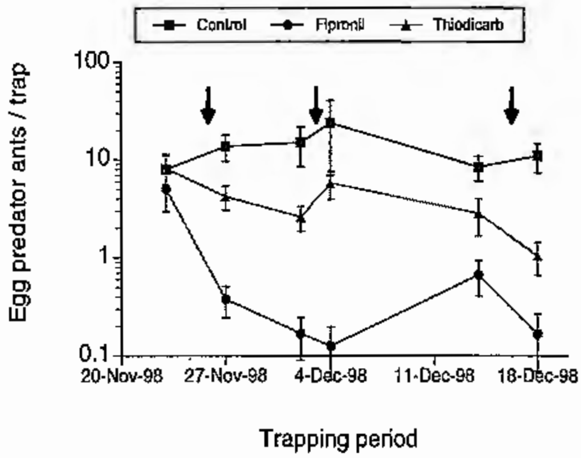


Figure 4: Effect of fipronil and thiodicarb on the abundance of *Pheidole* and *I. vicinus* on the soil surface during 97–98 (left) and 98–99 (right) at ACRI. Vertical arrows show spray dates.

APPLICATION OF RESULTS AND FUTURE RESEARCH NEEDS

Densities of soil fauna in cotton

The approach of using large field tents to quantify the absolute densities of predatory insects in commercial cotton worked extremely well. Several outcomes from the results of these data are worthy of mention here. Firstly, the variability of early season data from Auscott–Narrabri clearly highlighted the importance of future research addressing the question of how growers can effectively increase and maintain high beneficial numbers early in the growing season. For instance, though *Pheidole* were abundant at Doreen and ACRI, they were extremely scarce at Auscott Narrabri. Secondly, the data from Doreen suggest that there is potential for large population densities of ants to be maintained in furrow–irrigated cotton, and where this scenario is achieved, *Pheidole* and *I. vicinus* ants are likely to be amongst the most important predator groups in early season IPM. It is important to note that the small size of *Pheidole* means that this species will often go unnoticed in cotton unless crop scouts are educated and specifically requested to look for them. Thirdly, efforts to quantify the significance of spiders in Australian cotton must be recognised as a priority in future research involving predators. This group was relatively abundant in both the absolute density studies and in D–vac sampling in the insecticide comparison experiments. Little is known about the foraging strategies or prey preferences of this group in Australian cotton (nothing since A.L. Bishop's work in SE Qld during early 1980s). Fourthly, the large field tent system provides other researchers investigating predator-prey interactions and other topics requiring exclusion or enclosure of insect groups with a very effective research tool. For example, the field tents allowed Dr Lewis Wilson to obtain excellent data on the impact of aphids on cotton growth during 1999/2000 that would have been difficult to achieve with other approaches.

Density results for ants on unsprayed cotton highlight the opportunity for growers to manage some predator groups specifically as 'early season' predators. In the case of ants, numbers on cotton seem to drop off very sharply after Christmas, and similar experiences often occur with ladybirds and red & blue beetles. In contrast, several key groups of spiders might best be managed as 'mid–season' predators. Having recognised this distinction, it is also important that strategies be developed that promote and maintain stable and abundant early season populations of natural enemies *per se*.

Predation studies

Having established that ants were amongst the most abundant predator group found on cotton at ACRI during the month of December across several seasons, it is important to recognise that ants were also the only predators observed taking *H. armigera* eggs during more than 100 hours of direct observation. Whilst *Pheidole* was clearly the most effective predatory species–group in B18 at ACRI during 1999/00, the relative importance of *Pheidole* versus *I. vicinus* and these two species–groups of ants versus other predator species is likely to vary significantly between fields, farms and regions. It is important that quantitative studies on the predation of eggs and immature stages of key pests like *Helicoverpa* spp. be continued in future research efforts. The Observer® system offers researchers an effective tool to achieve this goal for the entire spectrum of predator–prey interactions. Quantitative data of this kind is urgently required for inclusion in the Industry's IPM guidelines and the subsequent ranking of predator groups for their likely impact on pest species. Recent experience at field days clearly indicated that the need for predator groups to be 'weighted' according to their relative impact is gaining increasing support amongst leading growers and consultants wanting to use predator data in control decisions.

Results from direct field observations and day and night suction sampling indicated that the common brown earwig is rarely found on cotton. Despite this earwig species being an abundant and aggressive predator of *Helicoverpa* spp. larvae, this finding implies it is unlikely to be a key predator of *Helicoverpa* spp. in Australian cotton. Preliminary laboratory studies on consumption rates of *Helicoverpa* spp. eggs by ladybirds and red and blue beetles showed the striped ladybird to be the 'star performer'. Having generated this preliminary data, it is recommended that a detailed and comparative study on prey preference and predator behaviour be completed for the spectrum of common predator species and the different life stages of *Helicoverpa* spp., spider mites, aphids, thrips, green mirids, and jassids. The observer® could be used to quantify the feeding behaviour of each predator species.

Gut assays

Further work and consultation with Dr Stephen Trowell in Canberra is required to pinpoint the problems associated with the efficacy of the *H. armigera* MAb. I have achieved excellent results using the same methodology for earwig dissections whilst on exchange in Dr James Hagler's laboratory in Phoenix, Arizona. The first step would be to run a series of assays using different buffer and predator sample volumes. The seriousness of the problem associated with detectability of eggs in the guts of ladybirds and the red and blue beetle is also not yet known. However, since the MAb was specially designed for the egg life stage, this problem should be relatively simple to solve.

Impact of insecticides

Research findings on the impact of insecticides on ant densities on cotton have complemented Lewis Wilson's generalist data on the common predator groups. Upon registration, new products like Emamectin benzoate (Affirm®) could fit nicely into stage I to control *Helicoverpa* spp. whilst causing minimal impact on predators like ants. However, reliance on endosulfan, fipronil, dimethoate, chlorfenapyr and spinosad during stage I may prevent ants contributing to the suppression of pest populations in fields where they are abundant.

The effects of insecticides applied at planting on specific predator groups like ants, ladybirds, and red and blue beetles are also poorly understood, and this might be an opportunity that warrants further research. Similarly, selective removal of predators like ants and ladybirds that are egg predators might be useful to compare in more detail the rates of *Helicoverpa* spp. survival and comparative impact of different predator groups.

PUBLICATIONS

- *Magazine articles*:

Lytton–Hitchins, J. (1999). What do we know about our predator friends down under? *Australian Cottongrower*, July–August: 54–58.

Lytton–Hitchins, J. (1999). Identifying ants in cotton. *Australian Cottongrower*, September–October: 38–41.

Lytton–Hitchins, J. and Wilson, L. (1999). Impact of insecticides on ants. *Australian Cottongrower*, September–October: 67–71.

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- *Conference Proceedings*:

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- *Scientific publications*

Lytton–Hitchins, J.A. (2000). Species composition and ecology of soil–dwelling Coleoptera, Collembola, Dermaptera, and Formicidae in cotton. *CSIRO Division of Entomology Technical Papers Series*, (submitted).

Lytton–Hitchins, J.A. (2000). Prey diets of soil–dwelling Coleoptera, Dermaptera, and Formicidae in cotton. *CSIRO Division of Entomology Technical Papers Series*, (submitted).

Lytton–Hitchins, J.A., Greenslade, P., & Longstaff, B.C. (2000). Relative and seasonal abundance of Collembola (Insecta, Apterygota) in irrigated cotton fields of New South Wales, Australia. *Applied Soil Ecology*, (submitted).

Miscellaneous

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Lytton–Hitchins, J.A. (March 1999). Gut content assays and their application to cotton. *Lower Namoi valley cotton field day*, 3 pp.

March 1999. A 'gut feeling' for biological control of insect pests. *Cotton Magazine*. p. 10.

Interview with Dianne Tyson during 'cotton news segment' on 2WE Radio, Bourke on July 1 1999.

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Lytton–Hitchins, J. (March 17, 2000). Ants can be useful predators in early season cotton. *Lower Namoi Valley Field Day* – Living in the GMO Era.

What do we know about our predator friends down under?

By James Lytton-Hitchins, CRC for Sustainable Cotton Production, CSIRO Cotton Research Institute

Cotton fields often harbour a significant diversity of parasitoids and predators. Two groups can be distinguished within the complex of predators.

First, 'plant-based' species which capture their prey within the canopy of the cotton plant during both their immature and adult life stages.

Second, 'ground-based' predators which use or depend upon the soil for at least one stage of their life cycle and may have either 'climbing' or 'non-climbing' life habits.

Very little is known about the role of soil predators in the suppression of key pests in Australian cotton. This is mainly because of the nocturnal habits of most soil predators, the problematic and laborious nature of sampling, and difficulties associated with species identification.

Many soil predators hide within the

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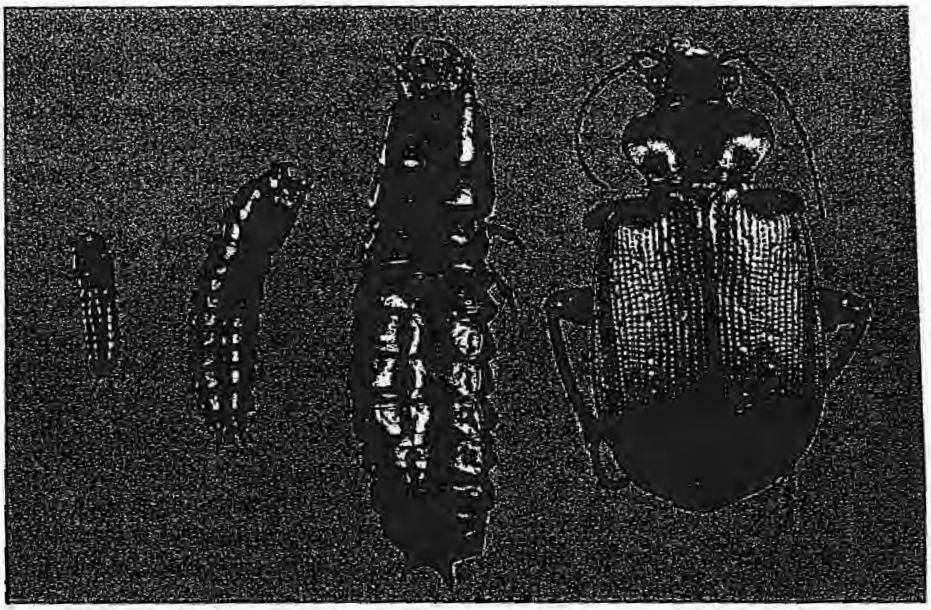

Adult and larvae of the Green Carab Beetle, *Calosoma schayeri*: predator of lepidopteran larvae. (Photo by John Green, CSIRO Entomology)

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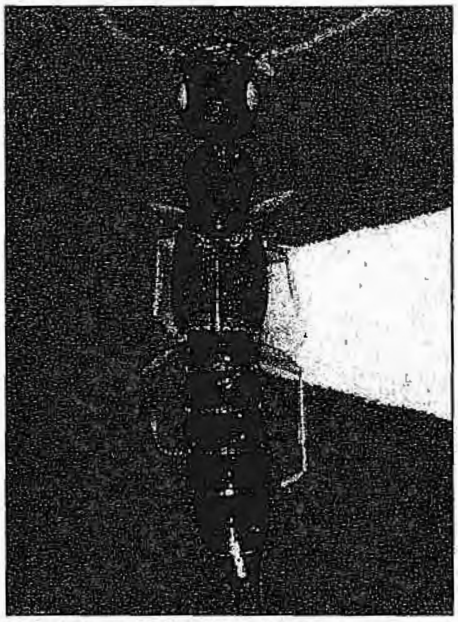
ground floor habitat during the day and can be very active on the ground or in the plant canopy at night. So they are not collected in routine suction sampling, sweep or visual whole plant samples collected during the day. It was only after pitfall traps were used during a recent baseline study that the abundance of these predators in our cotton fields was brought to the attention of researchers.

GROUPS FOUND IN COTTON

The predominant soil predators found in cotton include certain species of earwigs, spiders, beetles (for example carabids and staphylinids) and ants (see photos). But little is known about the biology, life habits or predatory efficiency of individual species. A summary of what is known about individual species is given in Table 1.

Of the two earwig species present, the Common Brown Earwig appears to be more abundant and ideally suited to even the most extreme cotton management practices. Wolf spiders are perhaps the most significant group of predaceous spiders found on the ground floor, but little is known about their species diversity.

Approximately 50 predaceous species of carabids and staphylinids have been recorded in cotton and at least five species identified as worthy candidates for ongoing research.



Adult staphylinid beetle, *Astenus* sp.: Potential egg predator of *Helicoverpa* spp. Note: All staphylinid beetles have shortened wings that expose their abdominal segments.
(Photo by John Green, CSIRO Entomology)

Earwigs, wolf spiders and certain species of carabids are present in the majority of cotton fields at planting. Ants are also often observed at this time in fields that are well drained and managed with minimum tillage practices. Increases in the abundance and diversity of soil predators often coincide with the initiation of squares and/or first flower in fields where no broad spectrum insecticides have been used.

PREY SOURCES AND FORAGING ACTIVITIES

Most soil predators found in cotton fields are polyphagous — that is they consume a wide variety of food sources — and are likely to capture live prey or scavenge dead carcases at least some of the time. But detailed prey records from day and night field observations are not yet available for any soil predators found in Australian cotton.

The Common Brown Earwig (*Labidura truncata* — see photo page 57) is a voracious predator of lepidopteran larvae, false wireworms and armyworms. While in the laboratory, the smaller Black Field Earwig consumed large numbers of *Helicoverpa* spp. eggs. The Black Field Earwig is occasionally collected in day-time

TABLE 1: Examples of soil predator species with a 'known' status in Australian cotton

Taxa	Species	Common Name	Life Habit	Predator status
Dermaptera — Earwigs	2			
Labidura truncata		Common Brown Earwig	Nocturnal and non–climbing	Larvae and pupae predator
Nala lividipes		Black Field Earwig	Nocturnal and climbing	Egg predator
Araneida — Spiders	Many			
Lycosa godeffroyi		Garden Wolf Spider	Nocturnal and climbing	Larvae predator
Carabidae — Carabids	≥22			
Calosoma schayeri (adults)		Green Carab Beetle	Nocturnal and climbing	Larvae and pupae predator
Geoscoptus laevissimus		—	Nocturnal and non–climbing	Larvae and pupae predator
Formicidae — Ants	≥19			
Iridomyrmex vicinus group		—	Day–active and climbing	Egg predator
Pheidole spp.		—	Day–active and climbing	Egg predator
Rhytidoponera metallica group		Greenhead Ant	Day–active and climbing	Leafhopper predator

dvac samples, but the extent to which either species forages in cotton foliage at night is unknown. The diets of ground-dwelling spiders, carabids and staphylinids in cotton are the least known of all.

Of the carabid beetles, the aggressive Green Carab Beetle (see photo page 54) is perhaps the best known predator of *Helicoverpa* spp. larvae and can be observed in the top terminals both at night and on overcast days. Certain species of carabids, staphylinids and ground-based spiders have been collected in dvac samples and observed in the crop canopy at night.

Field experiments at ACRI during 1998–99 indicated that the ants, *Pheidole* spp. and *Iridomyrmex vicinus* group spp., could be important egg predators of *Helicoverpa* spp. in fields where they are abundant. These two groups, together with the Greenhead Ant (*Rhytidoponera metallica*) were also frequently observed capturing live vegetable leafhoppers on cotton foliage and scavenging dead individuals on the soil surface.

DOES MANAGEMENT AFFECT THEIR POPULATIONS?

Soil predators spend a significant proportion of any 24 hour period resting or foraging on or beneath the soil surface. So the abundance of soil predators which also climb cotton plants can be influenced by management practices targeted at both the cotton foliage and the soil (for example soil insecticides, irrigation and cultivation).

Of all the soil predators that climb cotton plants, recent field studies suggest it is the ants that spend the largest amount of time foraging both on the soil surface and in the cotton foliage.

During irrigations, adult workers will transport all larvae contained within their saturated nests into the plant canopy. Larvae are first carried to the top terminals

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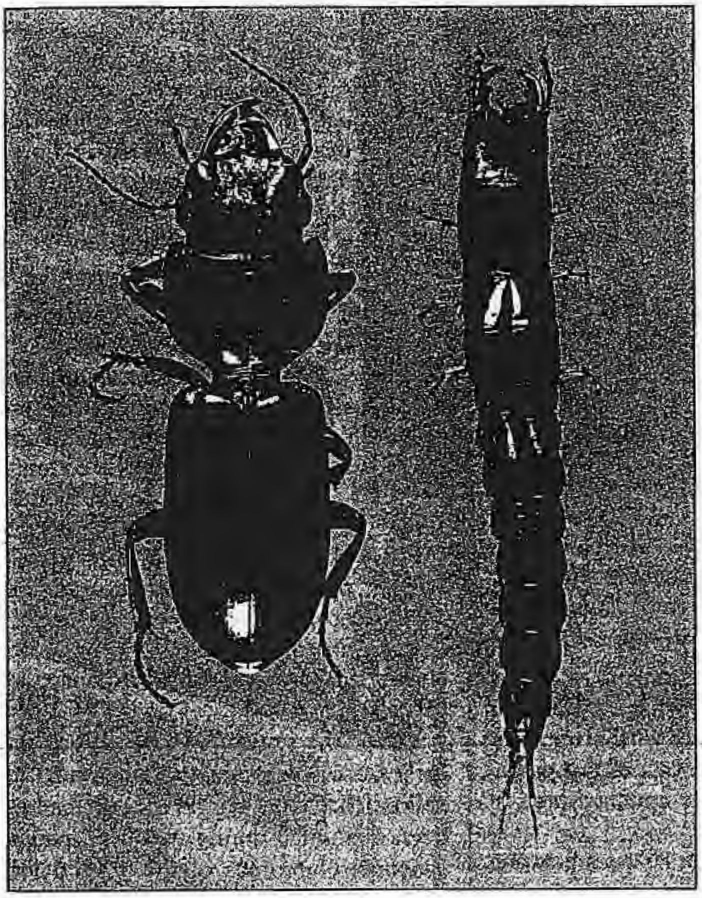
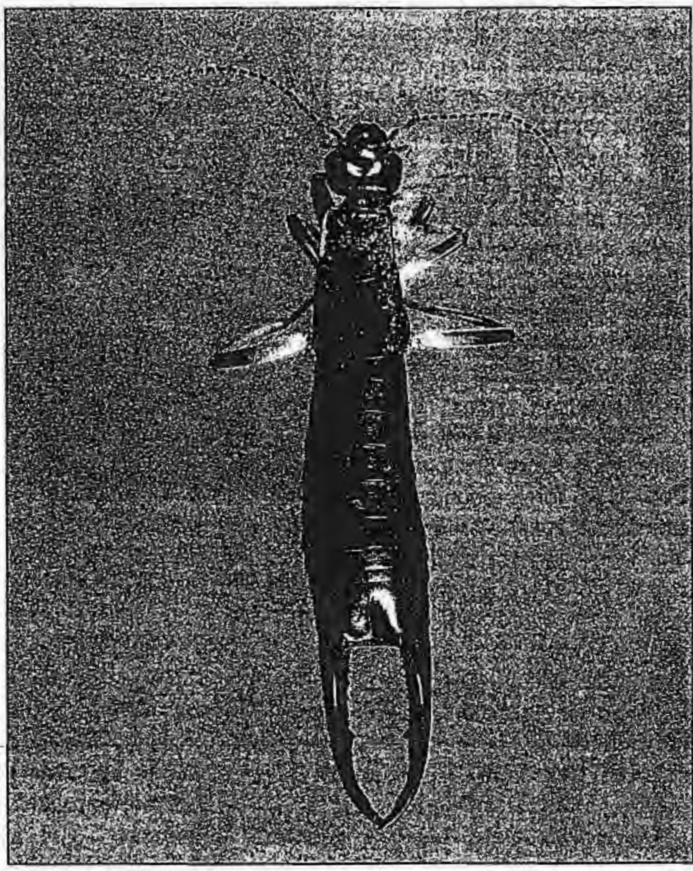


Adult and larva of *Geoscaptus laevissimus*: a carabid predator of wireworm-armyworm larvae. (Photo by John Green, CSIRO Entomology)



Adult male of the Common Brown Earwig, *Labidura truncata*: predator of wireworm-armyworm larvae and lepidopteron larvae and pupae. (Photo by John Green, CSIRO Entomology)

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and placed in clumps on the upper leaf surfaces, then carefully placed inside the bracts of squares (see photo). They are returned to the reconstructed nest after the water stops flowing in the furrows. So ants are one group of soil predators whose population densities are significantly affected by insecticides, cultivation, and irrigation.

HOW DO WE STUDY SOIL PREDATORS?

Reliable estimates of population density are vital when studying predators in cotton. But few sampling methods are truly compatible with the foraging times and nocturnal habits of soil predators. Ants often forage on cotton plants during the morning and afternoon hours and so they are one group whose relative abundance can be obtained using the conventional approach of suction sampling of the cotton foliage.

Wolf spiders are best sampled using head lamps (for example a Petzl zoom torch mounted above operators eyes) at night. This technique is most effective when cotton plants are relatively small and the soil surface is well exposed (that is until first flower). The eyes of wolf spiders reflect a blue light similar to 'blue diamonds' and so individuals within a defined area (for example 10 rows by 100 metres) can be quickly found and collected.

Density estimates of carabids, staphylinids and earwigs are more difficult and time consuming to obtain. Both suction and pitfall trap samples can be collected at night on a weekly basis to compare relative abundances across different treatments or fields. Pitfall traps are installed so they sit level with the soil surface in the plant line of raised beds for the entire duration of the season.

The activity of these soil predators on the soil surface during nominated nights is then assessed by setting attractive baits in the traps. But to obtain absolute density estimates a more complicated system is required.

We have been using large field tents (three rows by three metres by two metres tall) to 'fence' off areas where pitfall traps are permanently installed in the beds. Tents are erected and sealed at their base on each sampling occasion. Baits or preservatives are placed in the traps for seven to 10 days to collect all soil predators present within the confined area of the tents.

***Iridomyrmex vicinus* group sp. ants and larvae inside the bract of a cotton square during irrigation: Effective egg predators of *Helicoverpa* spp. on young cotton plants. Notice the workers climbing the mainstem with larvae in their mandibles and descending empty.** (Photo by Cheryl Mares, CSIRO Entomology)

To study the feeding linkages between soil predators and key insect pests, we are using both direct observational and indirect, post-mortem techniques. The direct approach involves quantifying the feeding preferences, diets and consumption rates of chosen species in the glasshouse and field.

The indirect technique is known as enzyme-linked immunoassay (ELISA) or more simply, gut content assays. ELISAs provide researchers with a precise and rapid way to identify 'who eats what' in the field throughout the season. The technique uses highly specific monoclonal antibodies to screen large numbers of predatory insects for the presence of a nominated pest species in their gut.

Monoclonal antibodies (MAbs) must first be developed by immunising mice with pure protein samples of a given life stage (for example eggs or second instar larvae) of a particular pest species. Purified samples of the stage-specific monoclonal antibodies are grown in culture and then validated and calibrated for different 'potential' predators. This process is currently underway at ACRI using the *H. armigera* MAb developed for the Lepton kit.

The long-term goal of this ELISA work is to be able to collect predators from cotton fields throughout a given growing season, screen their gut contents for the presence of *H. armigera* eggs and/or larvae, and then use this field data on density and predation frequency to derive an 'on-the-go' predator index specific to individual fields.

RESEARCH IN PROGRESS

Three key areas of research are currently being undertaken.

First, absolute population densities are being quantified in commercially grown conventional, Ingard and unsprayed cotton.

Second, studies on the mortality of *H. armigera* eggs on plants by ants, prey diets of soil predators, and the efficacy of gut content assays using the *H. armigera* MAb were initiated during 1998–99 and will be expanded in 1999–00.

Third, the impact of insecticides on early season abundance of ants found in cotton foliage and on the soil surface has been quantified during two consecutive seasons. Outcomes of this insecticide research will feature in a future article.

Research currently being undertaken is in the early stages of determining the role soil predators might play in the suppression of insect pests in cotton. Guidelines from the outcomes of this research will be progressively incorporated into IPM.

Genuine appreciation is extended to Peter Glennie, Kylie May, David Blows, Kym Armytage, Harvey Gaynor, Stefan Henggeler, Ben Stevens and associated farm staff for their assistance during field work and interest in this research. The CRC for Sustainable Cotton Production and Cotton Research and Development Corporation provided generous funding for this research. Thanks also to Drs Lewis Wilson, James Hagler and Ted Wilson for their encouragement.

Identifying ants in cotton

By James Lytton-Hitchins, Australian Cotton CRC, CSIRO Cotton Research Unit, Narrabri

Ants are an important group of predators in cotton throughout the southern states of the US, Brazil and Kenya.

In New South Wales, four groups of ants are commonly found on cotton plants. These four groups can easily be distinguished in the field by crop scouts, consultants and growers.

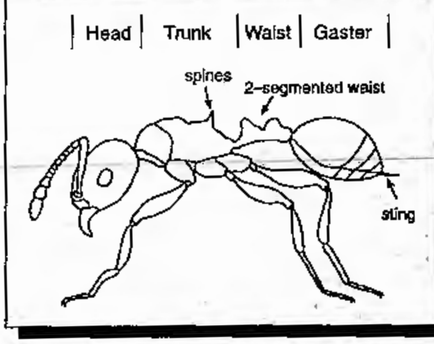
Each group utilises a wide variety of food sources within a cotton field. As fluid feeders, foraging worker ants do not directly ingest solid food, but instead capture live and scavenge dead prey and return it to the colony.

Ant larvae digest the prey which is then redistributed to adult workers as liquid secretions. So the needs of the colony dictate the foraging activities of workers in a cotton field. In this article the characters that allow each species-group to be easily identified are described, and what is known about the diets of each group in cotton outlined.

FIGURE 1: Overview of the four common groups of ants found in cotton

Characters for identification		Known pest prey	Group
body large or tiny with sting (microscope)	always black with blue–green shine large body (≥ 6 mm long) waist 1–segmented	jassids	*Rhytidoponera metallica*
	tan, black or yellow tiny body (≤ 2 mm long) waist 2–segmented pair of spines on trunk minors and majors	*Helicoverpa* eggs jassids thrips	*Pheidole*
body medium size without sting (microscope)	pointed black gaster 3 mm long long and stout hairs circlet of hairs on tip of gaster	— (nectar feeder)	*Paratrechina*
	tan, brown, or black 3–4 mm long often follow pheromone trail plain body with few hairs	*Helicoverpa* eggs jassids	*Iridomyrmex vicinus*

FIGURE 2: Basic body structure of a Pheidole worker



HOW TO IDENTIFY ANT GROUPS

Each ant group found in cotton may include several different species. But only those characters that distinguish one group from another are mentioned here since it is not necessary for scouts/consultants/growers to differentiate between individual species in any given group. Important characters distinguishing each group are summarised in Figure 1.

Pheidole

Pheidole are the smallest (less than two mm long) and often most common ants found on cotton plants. Workers have two distinct body size classes called majors and minors. Majors or 'soldiers' as they are also called, defend the inhabitants and food in the nest. So they are usually found in or near their nest and have heads and mandibles that are twice the size of the minors. The small minors are primarily responsible for the foraging activities of the colony.

Pheidole ants are tan, black or yellow in colour; and are distinguished by their two segmented waist, spines and sting (see Figure 2; photos 1a & 1b). Workers are effective scavengers of dead carcasses and seeds; and have been observed carrying *Helicoverpa armigera* eggs, adult tomato thrips, jassids, and springtails.

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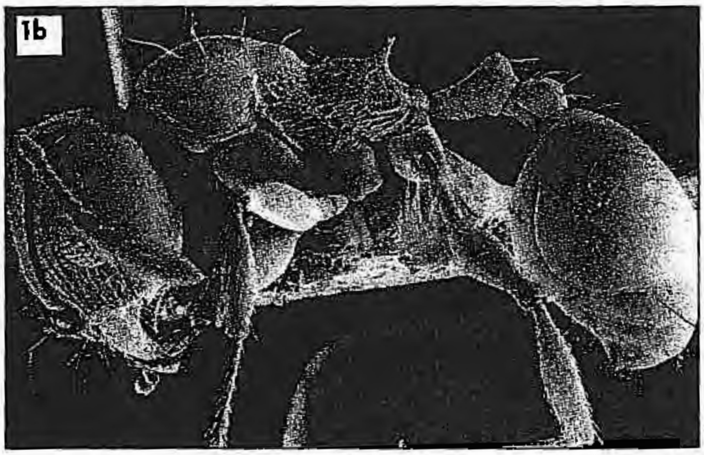

Pheidole sp.: Predator of Helicoverpa spp. eggs. LEFT (1a): Characteristic tan colour of worker and soldier [photo Cheryl Mares, CSIRO]. RIGHT (1b): Scanning Electron Micrograph (SEM) showing spines and two segmented waist (see Figure 2) (x94) [photo Eric Hines, CSIRO].

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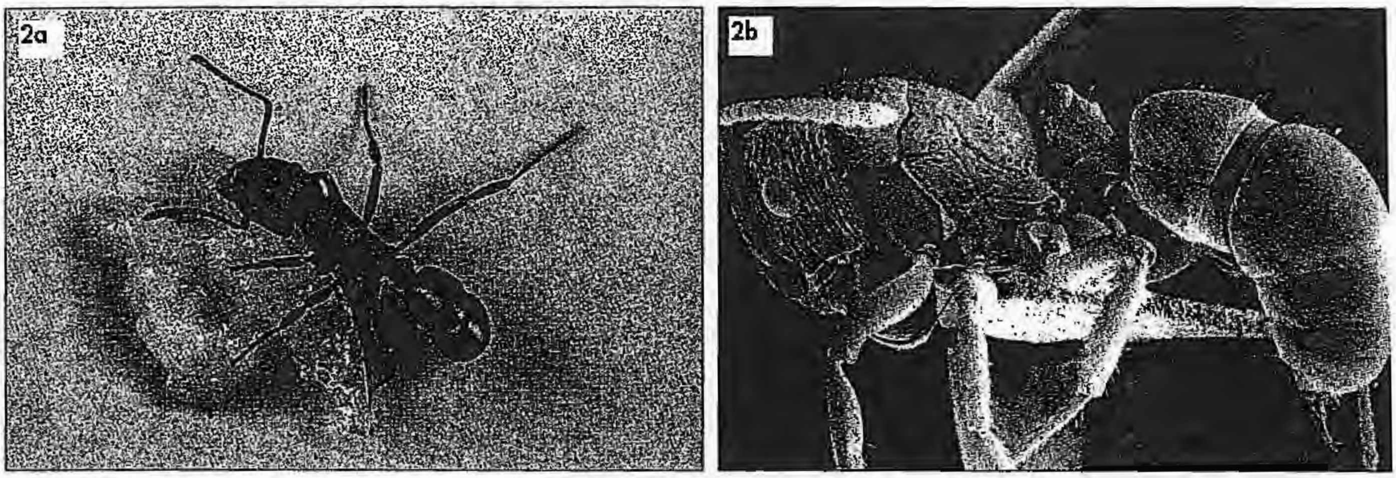
Apart from being generalist predators, some ant species may also be associated with pests. For example, a yellow Pheidole species has been observed 'tending' bean root aphids on seedling cotton. Nests have a low mound of soil particles around the entrance and are often seen in both hills and furrows after rain or irrigation.

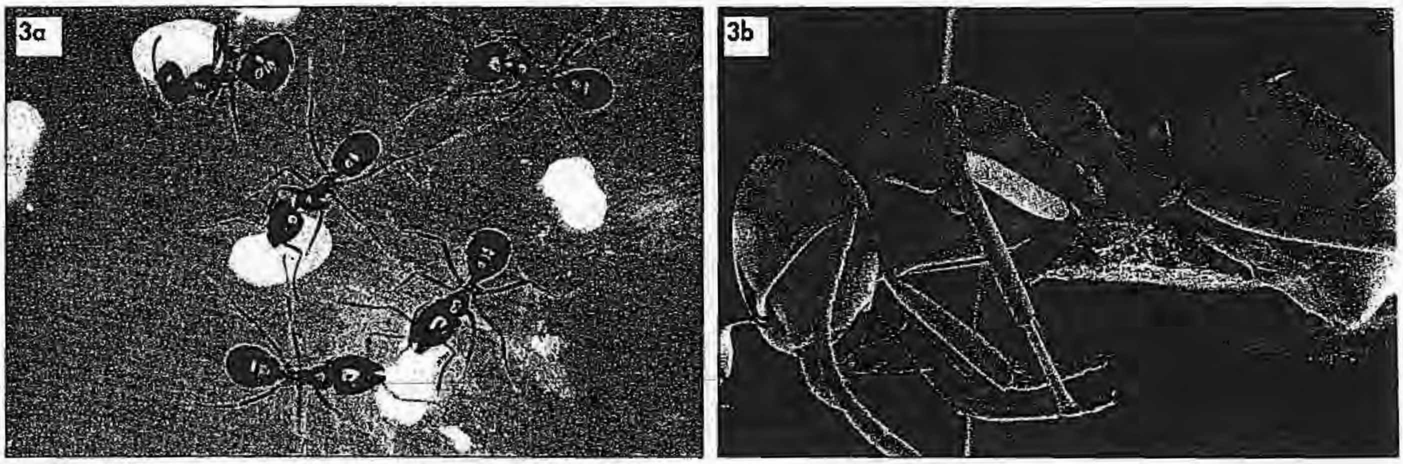
Rhytidoponera metallica: Greenhead ant

The Greenhead ant is approximately six mm long and has the largest body of the four groups. Workers are black and probably best known for their blue-green metallic shine and their sting (photo 2a). Under the microscope their sting, pronotum tooth, and one-segmented waist a distinguishing characters (photo 2b).

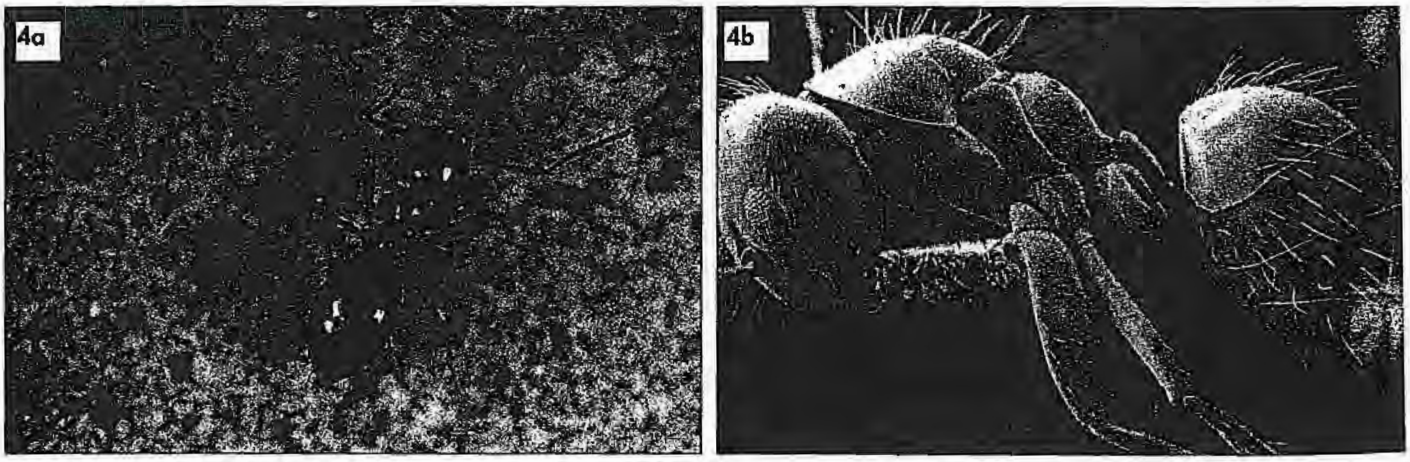
Greenhead ants are opportunisi predators that were frequently observ capturing jassids during 1998–99 fie experiments at Narrabri. But at no sta were they observed taking *Helicover* spp. eggs. Workers can be very abunda in fields newly developed for irrigation a



Rhytidoponera metallica **group: Predator of jassids. LEFT (2a): Metallic shine of a worker [photo by David McClenaghan, CSIRO Entomology]. RIGHT (2b): SEM showing sting and pronotum tooth (x30) [photo by Eric Hines, CSIRO Entomology].**



Iridomyrmex vicinus **group: Predator of Helicoverpa spp. eggs.**
LEFT (3a): Workers and their larvae [photo by Cheryl Mares, CSIRO Entomology]. RIGHT (3b): SEM showing plain body surface (x54)



Paratrechina sp.: Nectar feeder. LEFT (4a): Shiny black and cone-shaped gaster (abdomen) of a worker [photo by David McClenaghan, CSIRO]. RIGHT (4b): SEM showing stout upper body hairs and circlet of hairs on tip of gaster (x66) [photo by Eric Hines, CSIRO].

often forage for long periods during the day and at night.

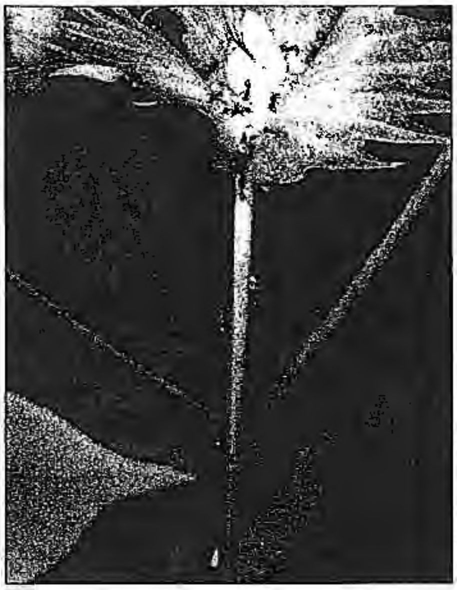
Nests require good drainage and can be found in the raised beds of fields, on head ditch and roto-buck areas, and in field borders. Pupae are elongated, leathery, orange-brown in colour and approximately seven mm long.

Iridomyrmex vicinus

Species belonging to the *Iridomyrmex vicinus* group are three to four mm long (photo 3a). Workers are fast and aggressive foragers on cotton plants and are often observed travelling along pheromone trails or highways in single file ('tandem-running') on the soil surface.

Once a worker finds multiple prey on a cotton plant, it will capture a single item and deposit a pheromone trail during its return to the nest. This process recruits other workers in the colony so that the entire food source is efficiently harvested.

Field observations during 1998–99 at Narrabri showed that *I. vicinus* workers frequently took *Helicoverpa spp.* eggs and jassids in plots where these ants were abundant. *I. vicinus* ants are dark brown, uniformly black, or tanned in colour; often have a characteristic odour when crushed; and at least one species has a blue-green shine on its gaster.



Iridomyrmex vicinus **group ants in a cotton square.**

Under the microscope you can see that *I. vicinus* are without a sting, have a 1-segmented waist, and plain body surface with few hairs (photo 3b). Nests within cotton fields are confined to the plant line in raised beds and workers are sometimes seen transporting their white larvae into the plant canopy when irrigation water is flowing down furrows.

Paratrechina

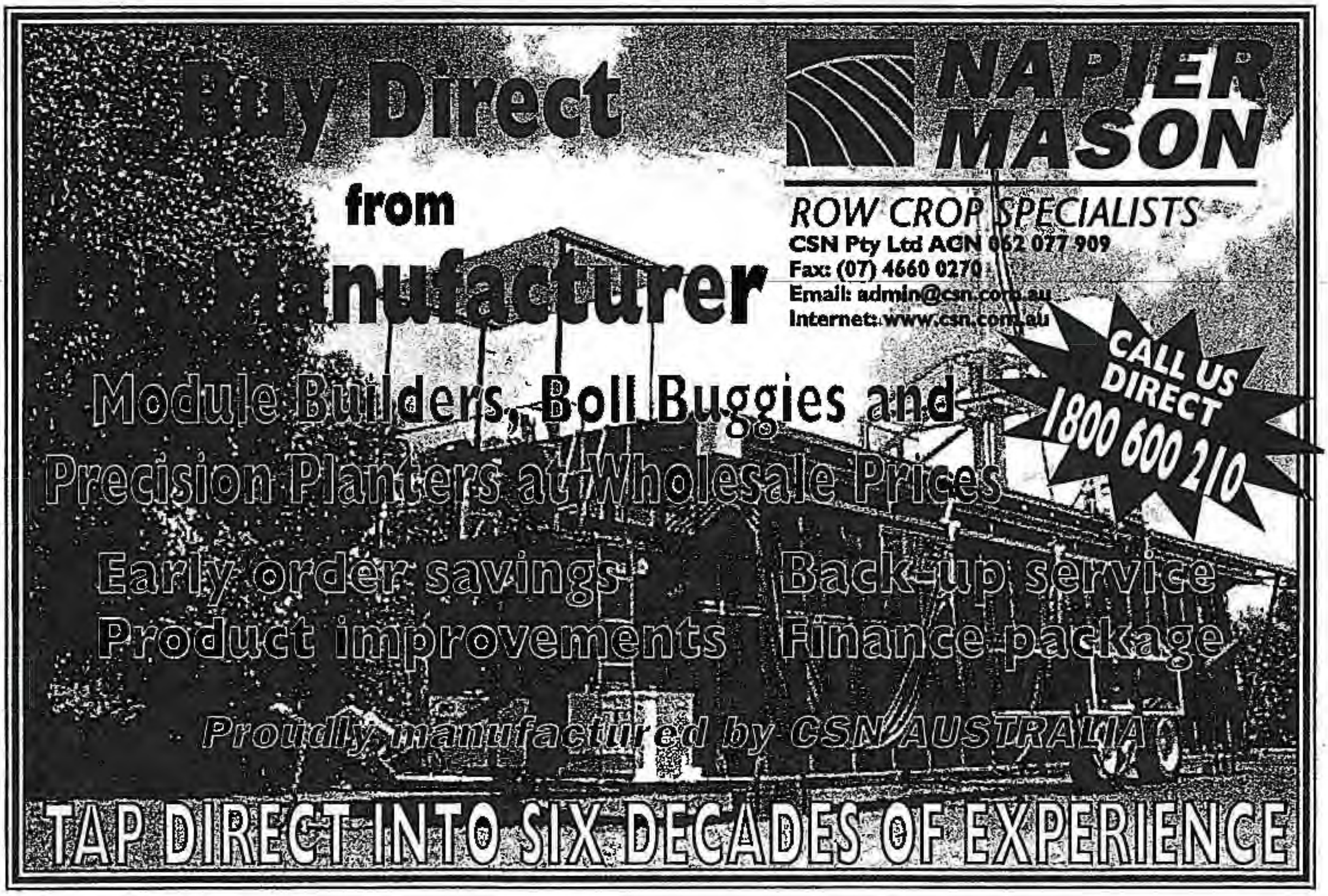
Paratrechina ants are three mm long and their foraging activities on cotton foliage are almost entirely confined to the visiting of extrafloral nectaries.

Workers can be distinguished with the naked eye from *I. vicinus* by their cone-shaped (pointed) and shiny black gasters (photo 4a). Under the microscope, distinguishable characters include long, stout, black or dark coloured hairs on their upper body surfaces; no sting; and, a circlet of hairs around the tip of the cone-shaped gaster (photo 4b).

CONCLUSION

- It is easy to identify the four common groups of ants found in cotton; and,
- Whilst the importance of ants as predators has not yet been quantified in Australian cotton, Pheidole and *Iridomyrmex vicinus* both take *Helicoverpa spp.* eggs on cotton plants.

I am most grateful to Cheryl Mares and Eric Hines from CSIRO Entomology in Canberra who provided the photos (visual) and scanning electron micrographs (SEM's: magnified), respectively. The CRC for Sustainable Cotton Production and Cotton Research and Development Corporation provided funding for this research.



Impact of insecticides on ants

By James Lytton-Hitchins and Lewis Wilson, Australian Cotton CRC, CSIRO Cotton Research Unit

Ants are a distinctive group of predators in undisturbed habitats and grazing land throughout cotton districts in New South Wales and Queensland. In cotton, they are recognised as important generalist predators of key insect pests in the southern states of the US, Brazil and Kenya.

But the predatory activity of ants is not currently incorporated into IPM guidelines for Australian cotton because very little is known about their role in the suppression of *Helicoverpa spp.* or other pests. Recent field experiments at Narrabri showed that two groups of ants are predators of *Helicoverpa spp.* eggs in squaring cotton (see *Identifying ants in cotton*, page 38, in this issue).

Given the potential significance of ants as predators of key pests in Australian cotton, it is also important to understand which management factors affect their abundance. One of the most obvious factors is likely to be the use of insecticides to control insect pests. We have used two approaches to investigate the impact of insecticides on the abundance of ants in cotton.

Firstly, absolute ant densities (per hectare) have been estimated in sprayed and unsprayed cotton on commercial farms. Here we collect all the ants present within field tents (area of nine square 69 ▷

TABLE 1: Absolute densities of ants in commercially sprayed and unsprayed cotton

Site — Season	Species	Density (per hectare) Sprayed	Density (per hectare) Unsprayed
Doreen — 1997–98			
January 22–26	Pheidole	200,000	744,167
	Iridomyrmex vicinus	26,944	68,056
	Total	226,944	812,222
Auscott Narrabri — 1998–99			
November 16–26	Pheidole	0	370
	Iridomyrmex vicinus	741	4,444
	Total	741	4,815
December 10–17	Pheidole	370	741
	Iridomyrmex vicinus	370	5,185
	Total	741	5,926
January 11–22	Pheidole	0	370
	Iridomyrmex vicinus	1,852	45,926
	Total	1,852	46,296

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metres and sealed at their base) over a period of seven to 10 days (see *What do we know about our predator friends down-under?* in July–August, 1999 issue).

Secondly, relative ant densities (per metre) on cotton plants treated with different insecticides have been estimated using D-vac suction samplers at Narrabri. Suction samples provide comparative estimates of ant density on cotton plants and have the advantage of being substantially easier to obtain than the 'field tent' method. In this article we discuss the impact of insecticides on ant densities using both these measures of abundance.

ABSOLUTE DENSITIES IN COMMERCIAL FIELDS

Data on absolute densities of soil predators were collected in sprayed and unsprayed treatments during January 1998 at Doreen (west of Wee Waa) and during November, December and January at Auscott Narrabri in 1998–99. The fields selected for this work were part of the field trials run by the Australian Cotton CRC to investigate the efficacy and non-target effects of Ingard and two gene Bt cotton on pest and beneficial insects. Six insecticide sprays had been applied to the sprayed cotton in each field prior to the January sampling period.

Insecticides caused significant reductions in the absolute densities of ants in sprayed cotton at both field sites. Ant densities in unsprayed cotton during January at Doreen and Auscott Narrabri were 3.6 and 25 times greater, respectively, than those in sprayed cotton (Table 1). Densities in sprayed cotton at Auscott Narrabri were almost negligible (that is less than 2000 individuals per hectare) during all three sampling months.

Pheidole spp. comprised between 88–92 per cent of the ant fauna at

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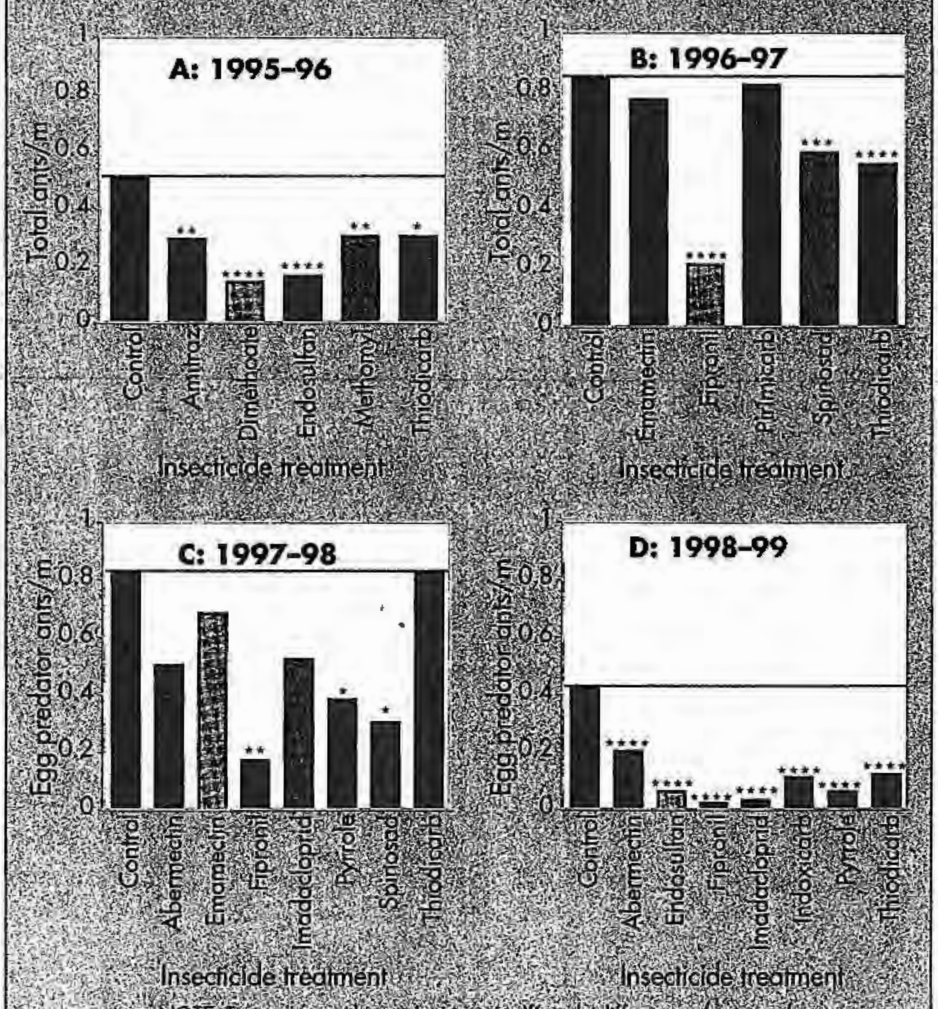
FIGURE 1: Effect of insecticides on the abundance of egg predator ants in cotton foliage at Narrabri over the past four seasons



NOTE: Treatments with asterix were significantly different to the control (****p<0.001, ***p<0.005, **p=0.01, *p=0.05)

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Doreen. During January, this species-group had a density of 744,167 individuals per hectare in unsprayed cotton (Table 1). Whilst the need to irrigate the Doreen field restricted the sampling period to five days, total densities in the cotton treated with insecticides (226,944 per hectare) in this field were still five times greater than those in unsprayed cotton (46,296 per hectare) at Auscott Narrabri. Irrigations clearly did not eliminate ants from unsprayed cotton.

WHICH INSECTICIDES CAUSE THE GREATEST MORTALITY?

Field experiments investigating the effects of new and currently used insecticides on early season (late November to mid January) abundance of ants in the crop canopy have been conducted at Narrabri over the past four growing seasons. A randomised block design was used during each season, with four replicates of five or seven insecticide treatments and an unsprayed control. Strips of four rows of safflower and/or sunflower were sown between cotton blocks to serve as nurseries for beneficial insects.

A total of 13 insecticides have been tested during at least one growing season (Table 2). Insecticides were applied five times in 1995–96 and 1996–97, three times in 1997–98, and four times in 1998–99 at seven to 10 day intervals. D-vac suction samples were collected from a complete row (20 metres) in each plot immediately before the first insecticide was applied, and at approximately twice weekly intervals thereafter. During 1997–98 and 1998–99 seasons, all ants were identified to species and pitfall traps also used before and after each spray to compare the relative abundance of ants on the soil surface.

TABLE 2: Impact of insecticides applied as foliar sprays on ant densities in early season cotton at Narrabri

Target pest spp. and insecticide	Rate applied (gai/ha)	Reduction with respect to control (%)[4] 95–96[1]	96–97[1]	97–98[2]	98–99[2]	Average	Impact rating[5]
Helicoverpa spp.							
Endosulfan	735	71	nd	nd	88	80	V. high
Indoxicarb	127.5	nd	nd	nd	74	74	V. high[6]
Pyrrole (also mites)	200–400	nd	nd	47	86	67	V. high
Methomyl	169	45	nd	nd	nd	45	High[6]
Spinosad	96	nd	30	59	nd	45	High
Thiodicarb	750	44	37	0	71	38	Mod
Emamectin benzoate	8.4	nd	<1	11	nd	6	V. low
Mites							
Amitraz (also Helicoverpa spp.)	400	45	nd	nd	nd	45	High[6]
Abamectin[3]	5.4	nd	nd	29	53	41	High
Sucking pests							
Fipronil (mirids)	12.5–25	nd	71	71	95	79	V. high
Dimethoate (aphids)	140	81	nd	nd	nd	81	V. high[6]
Imidacloprid (mirids & aphids)	49	nd	nd	25	92	59	High
Pirimicarb (aphids)	250	nd	<1	nd	nd	<1	V. low[6]

[1]All ant species collected; [2]Egg predator ants; [3]5.4 gai per hectare is the recommended rate of Abamectin to control spider mites whilst 10.8 gai per hectare is recommended to control *H. punctigera*; [4]Data for each season shown in Figure 1; [5]Impact rating: very low = less than 10 per cent; low = 10–20 per cent; moderate = 20–40 per cent; high = 40–60 per cent; very high = greater than 60 per cent; [6]Impact rating based on a single season of data and hence requires caution with interpretation; nd: Insecticide not tested during growing season (no data).

Ten of the 13 insecticides used in our experiments reduced ant densities (individuals per metre) in cotton foliage by more than 40 per cent with respect to the control, and were given a high or very high impact rating (Table 2). Fipronil, dimethoate, and endosulfan caused the greatest reductions in ant abundance across seasons (Table 2 and Figure 1).

Even at the lower application rate of 12.5 gai per hectare, fipronil reduced densities of egg predator ants by 95 per cent during 1998–99. Pyrrole applied at high and low rates, and spinosad also caused significant reductions in ant densities during both growing seasons they were tested (Figure 1).

Thiodicarb caused significant reductions to ants in three of the four seasons. Indoxicarb, amitraz, abamectin, and imidacloprid all had a high or very high impact rating (Table 2), but the available data for these insecticides showed statistically significant effects in only one growing season (Figure 1). Emamectin benzoate and pirimicarb were the only two foliar insecticides to have minimal effect on ants in the canopy and be given a very low impact rating.

The effects of both fipronil and thiodicarb on the relative abundance of ants on the soil surface during 1997–98 and 1998–99 were consistent with those for the crop canopy. Fipronil caused significant reductions to pitfall trap catches during both seasons, whilst the impact of thiodicarb was only significant during 1998–99 (Figure 2).

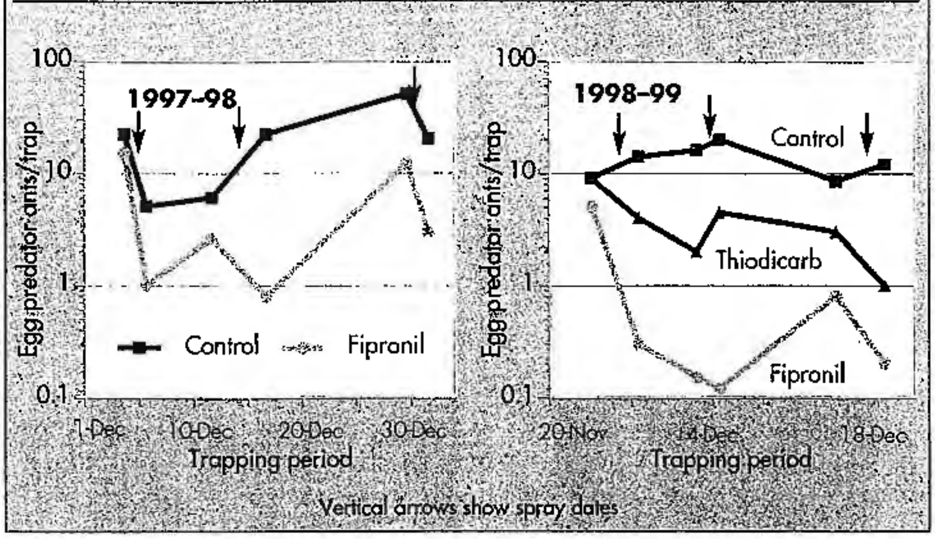
CONCLUSIONS

- Two groups of ants take *Helicoverpa spp.* eggs on cotton plants. However, the significance of ants as predators of *Helicoverpa spp.* eggs has not be quantified. This will be a major focus of research at Narrabri during 1999–2000.
- Preliminary data on absolute densities suggest there is potential for large ant populations (greater than 800,000 per

hectare) to be maintained in commercial furrow-irrigated cotton fields.

- Use of many insecticides presents a major impediment to the conservation and management of ants early in the cotton growing season. Only two of the 13 foliar insecticides tested at Narrabri during the past four seasons had a low impact on predacious ants. In fields where ants are abundant, reliance on endosulfan in conventional cotton and fipronil or dimethoate in transgenic cotton during Stage I may prevent this predator group contributing to the suppression of pest populations.
- While the contribution of ants as predators of key pests in Australian cotton has not yet been determined, increased grower adoption of integrated pest management practices (IPM) and associated reduced use of broad spectrum insecticides early in the growing season will help conserve beneficial insects generally, including ants.
- The period up to first flower offers a window of opportunity to utilise predacious ants in IPM. Firstly, it is during this period that cotton in most regions can tolerate quite heavy early season damage with little, if any, effect on either yield or crop maturity. Secondly, the effectiveness of the Bt-gene in Ingard cotton in suppressing Helicoverpa is highest early in the season. Finally, irrigated cotton is usually not watered prior to first flower, so any potential disruptions to ant foraging activities caused by irrigation should be minimal.
- The use of seed treatments and insecticides applied at planting may offer a more selective approach to early season pest management. The effects of these strategies on soil predators like ants are not known in Australian cotton and this might be an opportunity that warrants further research.

FIGURE 2: Effect of fipronil and thiodicarb on the abundance of egg predator ants on the soil surface during past two seasons at Narrabri



Genuine thanks to David Blows and Kym Armytage at Doreen and Stefan Henggeler and Ben Stephens at Auscott Narrabri for their cooperation during field work and interest in this research. The CRC for Sustainable Cotton Production and CRDC provided generous funding for this research.

Small *Pheidole* ants can be important egg predators in early season cotton

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The period from pre–squaring to first flower offers a window of opportunity to utilise ground predators like ants in cotton IPM. Research at the Australian Cotton Research Institute (ACRI) indicates that ants are often the most abundant predators found in squaring cotton prior to Christmas. In Australia, two species–groups of ants, *Iridomyrmex vicinus and Pheidole*, commonly take *Helicoverpa* spp. eggs in cotton. *Iridomyrmex vicinus* are easily recognised in the field whereas *Pheidole* can be missed during routine crop scouting exercises due to their small body size (≤2 mm). Quantitative data on the significance of ants as egg predators in early season cotton are not yet available in Australia. Evidence from cotton growing regions like the southern states of the USA, Brazil and East Africa suggests that some ant groups or species can be important *Helicoverpa* spp. egg predators. This article follows earlier articles on identifying ants in cotton (*Australian Cottongrower*, **20(5)**: 38–41) and the impact of insecticides on ants (*Australian Cottongrower*, **20(5)**: 67–71). Data from the 1999–2000 season on the mortality of *Helicoverpa armigera* eggs caused by *Pheidole* ants are presented.

ASSESSING EGG MORTALITY, ANT FORAGING BEHAVIOUR & ABUNDANCE

Egg predation was assessed in unsprayed cotton in Block 17 at ACRI during December when plants were squaring and in early January when plants were producing their first flowers. Areas of 1m x 1m were marked and then artificially stocked with *H. armigera* eggs. Leaf discs with an egg layed on them were glued singly on the upper surfaces of terminal leaves at a density of 30 eggs / m. Detailed observations of egg mortality and the foraging behaviour of individual ants were then recorded using lap–top computers and 'The Observer' ® software. This system allowed us to quantify the proportion of time each ant species spent on the ground versus different parts of the cotton plant, and performing different foraging activities (e.g. feeding on nectar from nectaries or attacking eggs). Each egg–infested area was observed for 20 minutes at least once per day between 8–11 AM and 4–7

PM. Ground densities of ants were estimated before and after each 20–minute observation period. Plant densities of ants were assessed separately by counting the number of each species on 12–16 random samples each consisting of 50 plants per sampling occasion. The presence of secondary pests (e.g. aphids, whitefly, leafhoppers) and other predators was also recorded.

PHEIDOLE DENSITIES

Ants were the most abundant predator group found on squaring cotton plants during December. *Pheidole* comprised 77% of the total ants found on plants during the last week before Christmas and were between 5–25 times more abundant than any other ant species. Density estimates prior to Christmas averaged 2.7 *Pheidole* / m of row (Figure 1), with an average of 10.6% of plants infested with *Pheidole* (Figure 2A). The maximum percentage of plants infested with *Pheidole* on any one sampling occasion was approximately 25% (Figure 2B). However, *Pheidole* densities on cotton plants dropped sharply by early January (Figure 1). This trend for reduced ant abundance in January is consistent with previous studies at ACRI (eg *Australian Cottongrower*, **20(5)**: 67–71).

EGG MORTALITY

Ants were the only predator group observed to find, attack and carry *Helicoverpa* eggs on early season cotton plants in this study. *Pheidole* removed 97% of all eggs taken by ants during December. *Pheidole* removed eggs from terminal leaves during 40% of the observation periods they were found on plants. Having found an egg on a leaf, *Pheidole* workers took about 2.3 minutes to remove the egg. About 3–5 minutes after an egg–laiden worker commenced its return journey down the plant other workers would appear on the same leaf and begin searching. This process is commonly known as recruitment, a foraging behaviour whereby the deposition of pheromone trails by food–laden workers ensures that other nest mates efficiently exploit rich food sources. *Pheidole* workers removed a total of 59 eggs during twenty observation periods, giving an average of 2.95 eggs removed / 20–minute observation period. No eggs were taken by *Pheidole* during January.

FORAGING BEHAVIOUR

Pheidole were present on plants during 75–80% of the 51 person hours of direct observation, whilst *I. vicinus* and *R. metallica* climbed plants less than 30% of the time. Within the plant canopy, each ant species spent the majority of their time on leaves where they regularly visited the extra–floral nectaries. *Pheidole* ants for example, spent a similar proportion of their total time (~ 8%) feeding at nectaries and attacking eggs (Figure 3).

ALTERNATIVE FOOD SOURCES FOR ANTS

Nectar obtained from extra–floral nectaries appeared to be an important food source for ants, especially *Pheidole*, during December when they spent much of their time foraging on plants. Patches of cotton plants were also heavily infested with the cotton aphid during the last week before Christmas. In this study ants were only occasionally observed tending aphids on young cotton. However, the presence of large numbers of ants on young cotton plants has often been observed where aphids are present (Forrester, N., & Wilson, L.J.; pers. comm.). In this study *Paratrechina, I. vicinus* and *Pheidole* were all seen tending aphids on the undersides of leaves on at least one occasion. As in previous seasons, jassids were the most common prey items observed in the mandibles of *I. vicinus* and *R. metallica* workers travelling on the soil surface.

CONCLUSIONS

(1) Ants were the most abundant predator group found on early season cotton plants during this study. At this stage in the growing season the soil surface of cotton fields is exposed (only 10–40% plant cover) and largely devoid of prey. Hence, it is at this time that ants spend a significant proportion of their foraging activities climbing small cotton plants to visit extrafloral nectaries and search for prey.

(2) *Pheidole* were the most abundant predators on cotton during December, and comprised 77% of the total ants found on cotton. Densities averaged 2.7 individuals / m of row which was equivalent to nearly 11% of plants being infested at any point in time. However, during January only 1.25% of plants were infested with *Pheidole* and densities fell below 0.5 individuals / m. Whilst the active foraging behaviour of ants on young cotton suggests a greater percentage of plants than reported here would be infested for greater time scales (eg 24 hrs), the extent and effectiveness of their foraging with respect to finding prey are yet to be quantified.

(3) The small size of *Pheidole* requires that care be taken during routine checks of early season cotton.

(4) In this study ants were the only predator group to find, attack and carry *H. armigera* eggs on squaring plants during the observation periods of 8–11 AM and 4–7 PM. *Pheidole* were effective predators of *H. armigera* eggs on cotton during December, and removed an average of 2.95 eggs / 20 minutes. However, *Pheidole* failed to find and remove eggs placed on the terminal leaves of taller squaring and flowering plants in January.

(5) Further research is needed to determine the impact of *Pheidole* and *I. vicinus* as predators of *Helicoverpa* spp. eggs during November from plant establishment to first square. The value of ants in IPM and the impact of management practices on their populations must then be validated at several locations within different cotton growing regions.

ACKNOWLEDGMENTS

I am grateful to Tony Bird, Fiona Rayner and Shiming Liu for their excellent technical assistance during this study. The CRC for Sustainable Cotton Production and Cotton Research and Development Corporation provided generous funding for this research.

Figure Captions

Figure 1: *Pheidole* densities before (squaring) and after (first flower) Christmas.

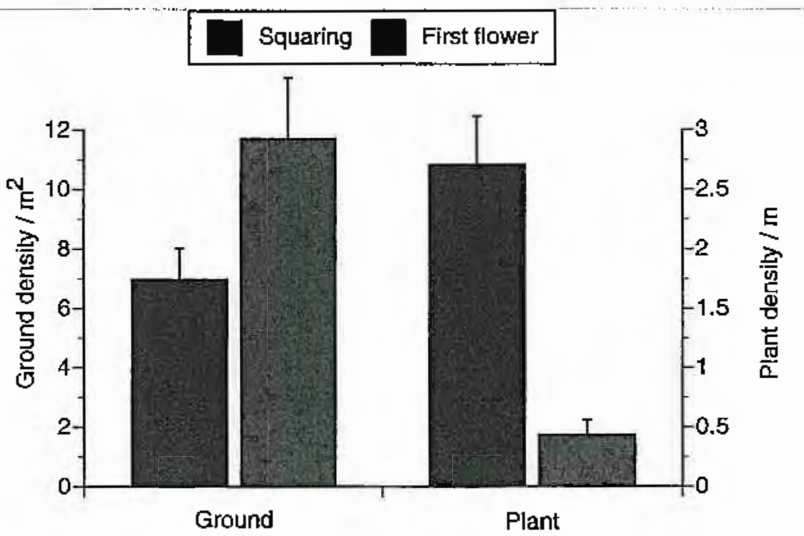


Figure 2: Percent plants infested with *Pheidole* (A) and the numerical relationship between *Pheidole* density and percent plants infested (B).

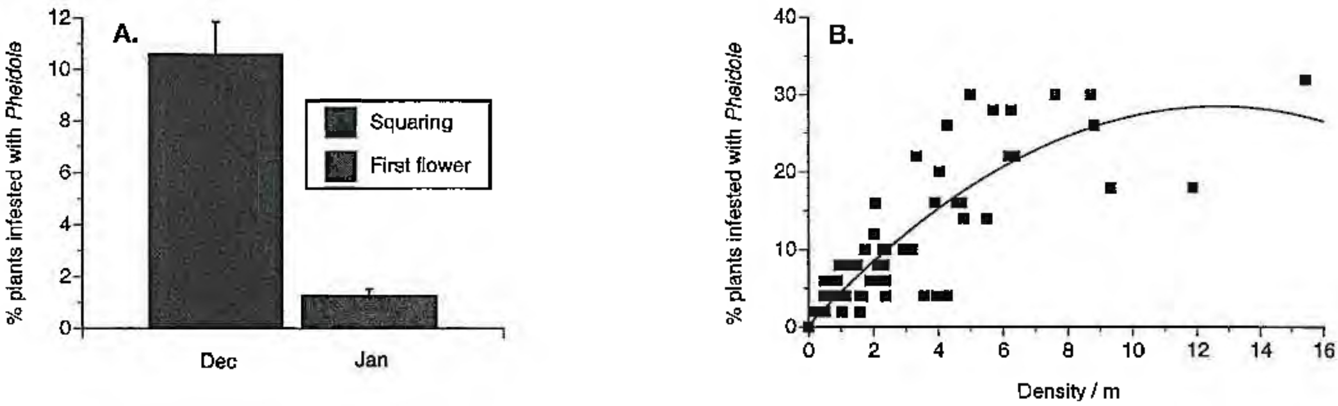
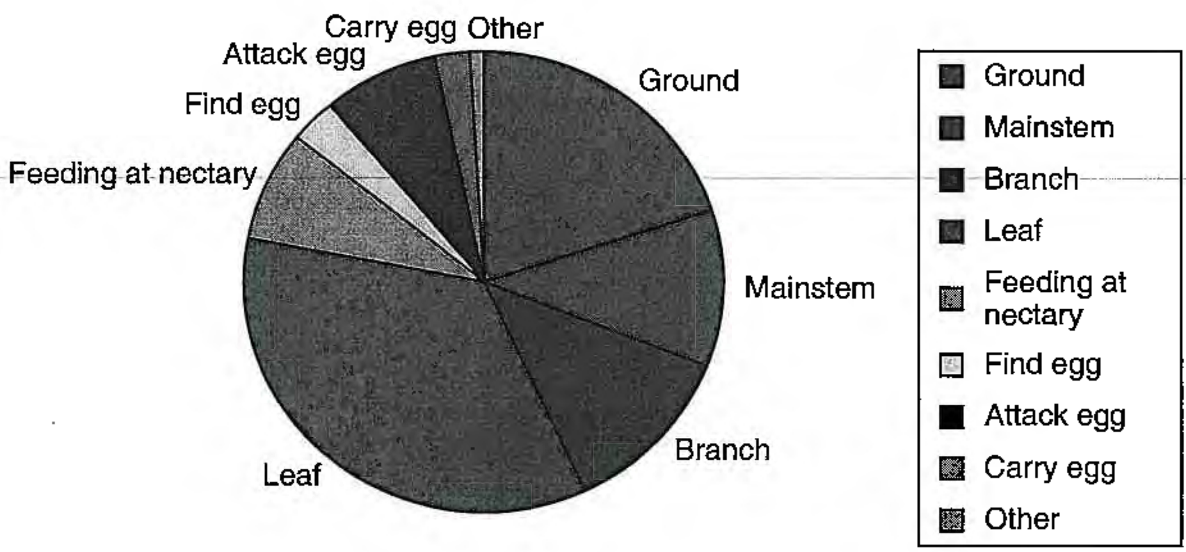


Figure 3: Foraging behaviour of *Pheidole* ants during December 1999.



Impact of Insecticides on Ant Abundance in Cotton at ACRI

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Introduction

Interest amongst cotton growers in incorporating beneficial insects into pest management is at an important stage in its development. Growers need reliable and cost effective tools to incorporate beneficials into IPM systems used on their own farms. However, while it is clear that beneficials are important in managing insect pests, we have little understanding of their quantitative contribution to the suppression of pest species. Research is still needed to quantify the effectiveness of beneficials, predict their impact on key insect pests, and where appropriate, investigate the impact of current management tactics.

Historically, lady beetles, red and blue beetles, and large predacious bugs appear to have attracted the most attention amongst researchers working on beneficials in Australian cotton. However, in other large cotton producing countries like the United States, other predator groups like fire ants and spiders are considered to be equally important (e.g. López *et al.*, 1996). Ants are generalist predators that have chewing mouthparts. In East Texas for example, red imported fire ants (RIFA), *Solenopsis invicta* Buren, are currently considered to be key predators of immature cotton fleahoppers, bollworm / tobacco budworm eggs and larvae, and immature boll weevils (López *et al.*, 1996). However, repetitive insecticide sprays are reported to cause significant reductions in RIFA densities (Sterling, 1978). In undisturbed habitats and grazing land where cotton is grown in NSW and QLD, the ant fauna is often very distinctive, and have been recognised as 'ecosystem engineers' of the ground strata (Greenslade & Greenslade, 1983). Whilst the common groups of ants found in irrigated cotton in NSW have been documented (Lytton–Hitchins, 1998), little is known about: (i) their potential role as predators of immature stages of *Helicoverpa* spp., (ii) the impact of farming operations (e.g. tillage and irrigation), and (iii) the effect of insecticides on their abundance.

The objectives of our study were twofold. First, to quantify the impact of new and currently used insecticides on the survival of ants found in the cotton canopy. Second, and for the first time, to investigate the impact of two insecticides, an industry standard, thiodicarb, and a new insecticide, fipronil, on numbers of ground predators, especially ants (the latter formed part of the third authors' fourth year University thesis). This is the first study in Australian cotton where the effects of insecticides on ants, have been studied at the level of 'species–group' and morphological–species. All comparisons were made with untreated cotton. Data presented for insecticides used in 1997/98 are considered preliminary. Additional data on the impact of a range of insecticides on total ants is given for 1995/96 and 1996/97.

Materials and Methods

The field experiment was conducted from December 1997 until early January 1998, in Block 18 at the Australian Cotton Research Institute. Sicala V2 seed treated with quintizone and apron (QAP) was sown on October 17. A randomised block design was used, with four replicates of seven insecticide treatments and an unsprayed control. The seven insecticide treatments included avermectin (5.4 g ai /ha), emamectin benzoate (8.4 g ai/ha), fipronil (25 g ai/ha), imidacloprid (49 g ai/ha), Pyrrole (200 g ai/ha), spinosad (96 g ai/ha), and an industry standard, thiodicarb (750 g ai/ha). Plots were 12 rows (1m beds) by 20 m, and arranged in four blocks of eight plots. Strips of safflower and sunflower, each consisting of 4 rows, were sown between the cotton blocks to serve as nurseries for beneficial insects. The middle six rows of each cotton plot were artificially infested with two–spotted mite, *Tetranychus urticae* Koch, in November, whilst the central 8 rows were treated with insecticide on December 3, 16, and 31.

D–vac suction samplers were used to sample ants crawling on the cotton foliage in all plots, and pitfall traps (baited and unbaited) used to assess ant abundance on the soil surface in control, fipronil, and thiodicarb treatments. Sampling began immediately before the first insecticide was applied; and at approximately twice weekly intervals thereafter for D–vacs, and before and after each spray for pitfall traps. D–vac samples were collected in the morning on each sampling date and taken from a complete row (i.e. 20 m) of each plot. The number and position of ant mounds and direction of ant trails was also visually mapped. Throughout the sampling period, regular field observations were made of large *Iridomyrmex vicinus* grp sp. 1 mounds to determine the impact of cultivation on ant survival and behaviour, and to observe their behaviour during and after irrigation. Ant abundance can be influenced by their proximity to undisturbed or uncultivated areas, such as the sunflower and safflower strips, and pasture areas adjoining Block 18. We therefore also made comparisons between plots that contained active ant colonies and those that were visited by large ant trails from other plots, from the sunflowers, or from the pasture strip adjoining the taildrain. One standard and two large plastic pitfall traps were placed in the plant line of rows six and seven at 5, 10, and 15 m from each plot edge. Standard traps were filled with 95% ethanol preservative, whilst attractant baits were used in the plastic traps (Lytton–Hitchins, 1998). Baits were tuna or a special ant food (Bhatkar and Whitcomb, 1970). Traps were opened between 1800–1900 hrs and collected between 0700–0800 hrs the next morning.

Analysis of variance (ANOVA) with Fischer's protected least significant difference (P = 0.05) was used on log transformed data to determine if insecticide treatments applied during the duration of the experiment had a significant effect on ant abundance in the cotton canopy or on the soil surface in comparison to the untreated control.

Results and Discussion

Ants on ground floor (97–98)

Species–groups recorded

Four ant genera including *Iridomyrmex vicinus* grp, *Pheidole*, *Rhytidoponera metallica* group, and *Paratrechina* were consistently observed on the soil surface and within the cotton canopy during the sampling season. Tuna proved to be the most effective of the three additives (either preservative or bait) used for catching *I. vicinus* grp spp. and *Pheidole* spp. Only *Pheidole* spp. were attracted more to 95% ethanol than the specialised ant diet (P=0.02).

Ant trails

The abundance of ants within the cotton and sunflower – safflower strips was strongly influenced by the presence of foraging trails of *I. vicinus* group sp. 1 from nests situated beyond the experimental area. On clear evenings and mornings, trails of *I. vicinus* grp. sp. 1 would often travel from the native pasture that adjoined Wee Waa Road, across the taildrain, into the cotton plots, and then across to the sunflowers; or even enter the sunflower strips directly. These trails often continued to be active throughout the night in November and December. However, dew droplets were often present on the upper leaf surfaces of cotton plants at night, and in no instance were workers observed climbing on wet cotton leaf surfaces.

Effects of cultivation and irrigation

A single cultivation on December 4 caused significant reductions to catches of ants across all treatments; with full recovery of abundances in control plots for *I. vicinus* grp and *Pheidole* spp. taking 4 and 2 weeks, respectively. Irrigation also had a profound and immediate effect on the behaviour of some ant species that had immature stages in their nests. *I. vicinus* grp larvae and *R. metallica* pupae were both present in the cotton plots. During each irrigation, larvae from entire nests of *I. vicinus* grp were rapidly transported by their workers to the top terminals where they were placed in clumps on the upper surfaces of the cotton leaves. On a few occasions larvae were placed in the V–shaped notches between the node branches and cotton mainstem. Larvae were then carefully placed inside the bracts of squares located in the top third of the canopy. Once the nest in the cotton hill was re–constructed and the flow of irrigation water had stopped, workers carried the larvae down the mainstem and back into the repaired nest. Similar transport exercises were observed in the sunflowers, with the only exception being that soil aggregates were carried into the V–shaped notches and larvae placed within these soil 'shelters'. At no time were workers observed catching or carrying prey whilst engaged in these transport exercises to rescue their larvae.

Feeding activities of ant groups

Paratrechina sp. 1 appeared to be the most frequently observed workers in the cotton canopy during late December and January. Careful and repeated observations using field cages showed that these workers were visiting extrafloral nectaries on the undersides of leaves and adjoining sites where aphids were present. In no instance did they take eggs laid at night on upper leaf or outer square surfaces by caged female *Helicoverpa armigera* moths.

Trails of *I. vicinus* grp spp. were also very busy at this time in some cotton plots, mainly at the taildrain end of the field, but all trails ended up in the sunflower strips. Large numbers of workers climbed the sunflower plants and many travelled up to the flower heads. Workers collected sap exudates at various locations along the mainstem where the phloem had been damaged by cockatoos. Sunflowers have also been reported to be particularly attractive to other ant species, such as *Myrmicaria* sp. and *Pheidole* sp., in western Kenya, where sunflower plants were visited more frequently and in greater numbers than either maize or sorghum (van den Berg *et al.*, 1997).

Distribution of mounds

I. vicinus grp spp., *Pheidole* spp., and *Paratrechina* sp. 1 had mounds present in most plots. In a small number of cotton plots, mounds of *R. metallica* grp sp. were also present. Mounds of *I. vicinus* grp spp., *Paratrechina* sp. 1, and *R. metallica* grp sp. were always confined to the plant line, and usually between cotton plant stems; whilst *Pheidole* spp. sometimes built their mounds in furrows after rain or irrigation. Several *I. vicinus* grp spp. mounds were also situated, either on bare soil in the cotton hills between plots, or in the raised beds of sunflowers.

Effect of insecticides on ground floor abundance

Across the duration of this experiment, fipronil caused significant reductions ($P < 0.001$ in each case) in the mean pitfall trap catches of *I. vicinus* grp, *Pheidole* spp., and total ants (e.g. left graph in figure 1 for total ants). Abundance of these same ant groups in cotton treated with fipronil remained lower than the control from after the application of the first spray till the end of the experiment in early January (e.g. right graph in figure 1 for total ants). However, thiodicarb appeared to have no detectable effect on pitfall trap catches of these ant groups.

It is significant that during this experiment, ants were the only ground–based predators that were in large abundances shortly after sowing and actively foraged on the cotton foliage up until just prior to first flower (beginning of stage II), as researchers in East African field crops have also reported (van den Berg, 1993; cited in *et al.* 1997). Whilst prophylactic in–furrow insecticides were not used in this experiment, it is also of potential interest that chlorpyrifos 15 G (15% granular) applied at 2.2. kg ai / ha at flowering in peanut fields in Alabama, caused the greatest reductions in soil predators like earwigs, ants and spiders of five granular insecticides used to control lesser cornstalk borer, *Elasmopalpus lignosellus* (Zeller) (Mack, 1992).

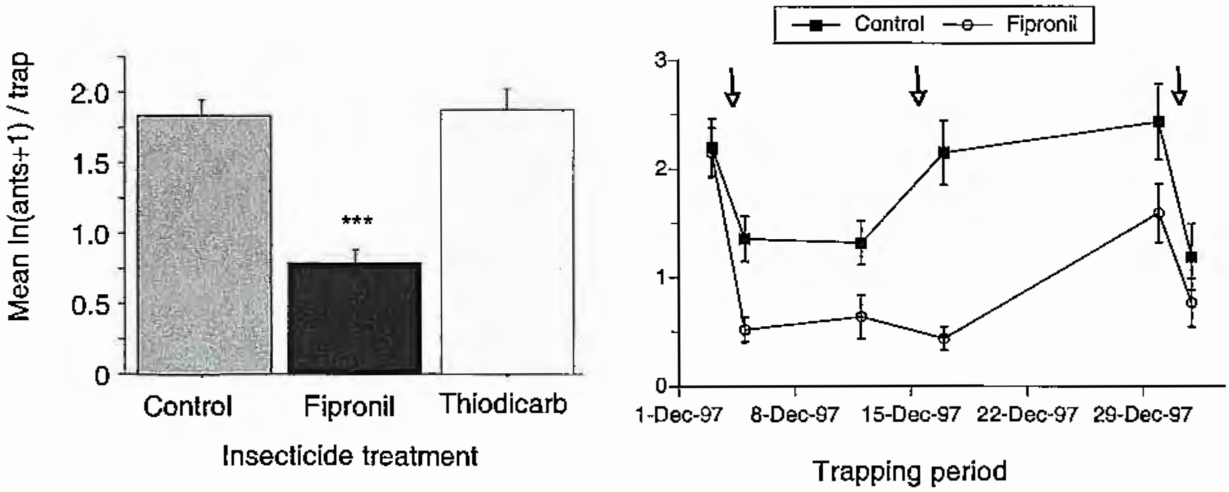

Figure 1: Effect of fipronil and thiodicarb on abundance of total ants caught in pitfall traps across the sampling season (left) and individual trapping periods (right) at ACRI during 1997/98. Cotton sprayed with fipronil (shaded black) was significantly different to the control (*** $P = 0.001$) whilst thiodicarb was the same. Vertical arrows in the right graph show spraying dates.

NOTE: (i) Y axis is for log transformed mean counts; and (ii) the initial decline in ant abundance caught in the untreated control cotton was caused by an inter–row cultivation.

Ants in cotton canopy (97–98)

Species–groups recorded

Ant genera collected in D–vac samples across ten sampling dates, in descending order of abundance, included *Pheidole* (79%), *I. vicinus* grp (17%), *Paratrechina*, *R. metallica* group, and *Cardiocondyla*. Workers of *I. vicinus* grp sp. 1 were observed removing artificially placed *Helicoverpa* spp. eggs on the upper leaves of young cotton plants in November and early December. Morphological species from each of the four ant genera found on the ground floor were also observed climbing in 'trail formation' to the top terminals of the adjoining sunflower plants.

Patterns of abundance in untreated cotton

Ant abundance on foliage was highest during the early part of the experiment (up to December 17), with mean densities of total ants in control plots ranging between 0.48 ± 0.04 to 2.66 ± 0.72 individuals / m. Ant abundance was generally lower thereafter with mean densities in control plots ranging between 0.09 ± 0.04 to 0.39 ± 0.09 individuals / m for the five sampling dates after December 17. Whilst it is not known exactly why these sharp declines in ant densities occurred so early in stage II in untreated cotton, there is a need to trial other methods, such as the shake–bucket technique (Pyke *et al.*, 1980), for sampling ants in stages II and III.

This would determine whether the decline is real or partially due to the sampling inefficiency of the D–vac method on larger plants.

Effects of cultivation

As with pitfall catches on the ground floor (see above), the single cultivation following the first sampling date caused significant reductions in ant abundance on the foliage across all treatments.

Effects of insecticides on ants in the cotton canopy (95–98)

Fipronil, spinosad, and Pyrrole treatments caused significant reductions in seasonal abundances of total ants / metre of cotton row with respect to the control (Figure 2). For the two ant genera comprising 96% of the total seasonal catch in cotton foliage, only densities of *Pheidole* spp. were significantly reduced by these three insecticides. *I. vicinus* grp spp. preferred to forage in the foliage of the sunflowers, and so the small densities present on cotton plants might best explain why insecticides caused no apparent reductions.

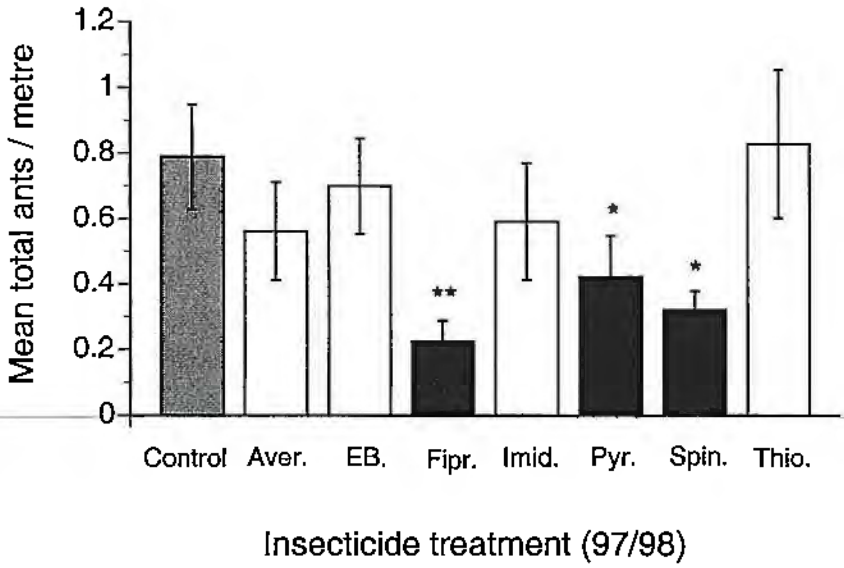

Figure 2: Effect of insecticides on the abundance of total ants in the canopy of cotton treated with (Av. = Avermectin; EB. = Emamectin benzoate; Fip. = Fipronil; Imid. = Imidacloprid; Pyr. = Pyrrole; Spin. = Spinosad; Thio. = Thiodicarb) and without (Con. = Control) insecticides at ACRI during 1997–98. Treatments shaded in black were significantly different to the control (** and *, $p = 0.01$ and $p = 0.05$ respectively) whilst those in white were the same. Data included 10 sampling dates from December 3 – January 5.

Results from 1997/98 are in general agreement with those from similar experiments conducted during 1995/96 (Figure 3; Wilson *et al.*, unpublished) and 1996/97 (Figure 4; Wilson and Lally, unpublished). First, in both 1996/97 (Figure 4) and 1997/98 (Figure 2) seasons, fipronil was the insecticide causing the greatest mortality to ants. Second, five applications of fipronil and spinosad both caused significant reductions in total ant densities (Figures 2 and 4). However, total ant abundance in the foliage was also significantly reduced by five applications of thiodicarb in both 1995/96 (P=0.05; Figure 3) and 1996/97 (P=0.001; Figure 4). Hence, thiodicarb caused reductions in ant densities on cotton foliage during 95/96 and 96/97, but not 97/98. Two factors were unique to 1997/98 that may have influenced the data presented. First, only 3 sprays of each insecticide were applied in comparison to 5 in both 1995/96 and 1996/97. Second, frequent and effective rain during December 1997 prevented use of the tractor driven spray–rig, and so insecticides were applied using a hand boom on 2 of the 3 spraying dates.

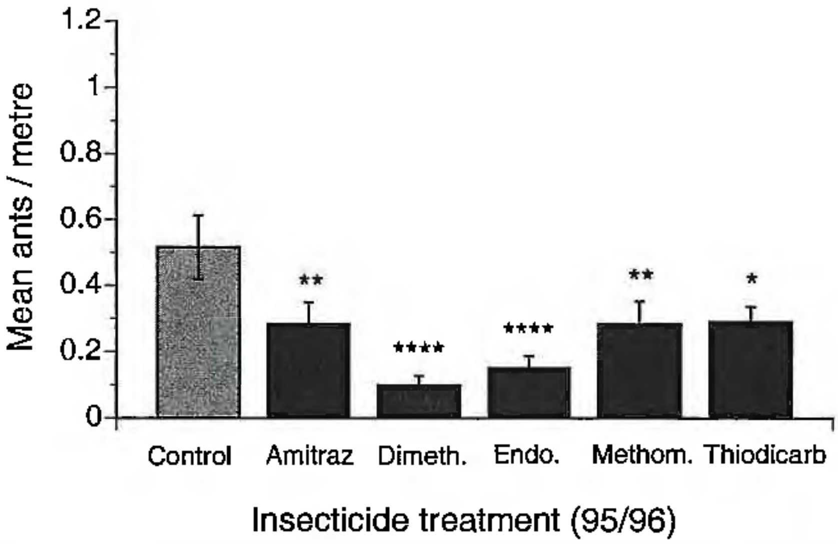

Figure 3: Effect of insecticides on the abundance of total ants in the canopy of cotton treated with (Amitraz @ 400 g ai/ha; Dimeth. = Dimethoate @ 140 g ai/ha; ; Endo = Endosulfan @ 735 g ai/ha; Methom. = Methomyl @ 169 g ai/ha; Thiodicarb @ 750 g ai/ha) and without (Control) insecticides at ACRI during 1995–96. Treatments shaded in black were significantly different to the control (*, **, ****; p = 0.05, 0.01, and 0.001 respectively).

It is significant that all insecticides used during the 1995/96 experiment, representing a range of chemical groups, all caused reductions on the abundance of ants in the cotton canopy (Figure 3). Of the five insecticides used, dimethoate (140 g ai/ha) and endosulfan (735 g ai/ha) had the greatest impact. In 1996/97, only cotton treated with emamectin benzoate and pirimicarb had similar densities of ants on the foliage in comparison to the control (Figure 4). Results presented across the three cotton growing seasons (95/96, 96/97, and 97/98) can therefore be used to distinguish insecticides that had no impact on ant abundance with those that caused significant reductions. Insecticides that had no effects on ant abundance, and may therefore be compatible with conserving ant populations in IPM systems include: (i) emamectin benzoate (96/97, 97/98), (ii) pirimicarb (96/97), (iii) avermectin (97/98), and imidacloprid (97/98). In contrast, a range of insecticides appeared to reduce densities of ants on cotton. Those insecticides that caused the greatest reductions include: (i) fipronil (96/97, 97/98), dimethoate (95/96), and (iii) endosulfan (95/96).

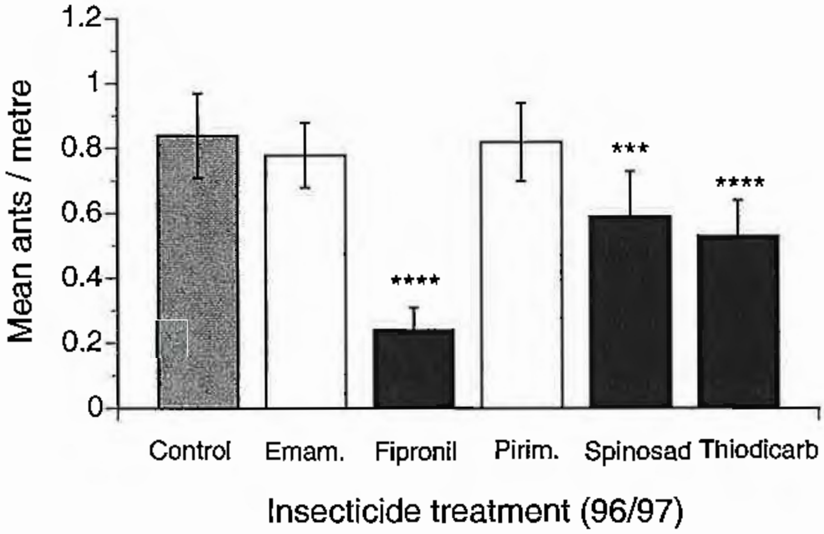

Figure 4: Effect of insecticides on the abundance of total ants in the canopy of cotton treated with (Emam = Emamectin benzoate @ 8.4 g ai/ha; Fipronil @ 25 g ai/ha; Pirim. = Pirimicarb @ 250 g ai/ha; Spinosad @ 96 g ai/ha; Thiodicarb @ 750 g ai/ha) and without (Control) insecticides at ACRI during 1995–96. Treatments shaded in black were significantly different to the control (***, ****; $p = 0.005$, and 0.001 respectively).

Conclusions

(1) *Pheidole* spp. and *Iridomyrmex vicinus* grp spp. were the dominant ants found on cotton foliage.

(2) Ants appeared to be the most abundant ground–based predators in stage I, and were often seen searching for prey on young cotton plants.

(3) Should ants prove to be key predators of *Helicoverpa* spp. eggs, it is possible that their impact will either be confined to, or most pronounced, during stage I. Preliminary evidence suggests that ant densities in cotton foliage during stages II and III are insignificant, but this may in part be due to difficulties in sampling ants on larger plants.

(4) Irrigation and heavy rains caused ant workers to rescue their larvae from drowning by carrying them into the canopies of nearby cotton and sunflower plants. Ants are less likely to contribute to predation of *Helicoverpa* spp. eggs on cotton during these periods.

(5) Inter–row cultivation after planting caused significant reductions in ant abundance (2–4 weeks). Preservation of ant densities during stage I would require changes in soil and weed management practices.

(6) Insecticides can have a significant effect on ant abundance, both on the soil surface and on foliage. Fipronil, dimethoate, and endosulfan caused the greatest reductions in ant abundance; whilst spinosad, thiodicarb, methomyl, amitraz, and pyrrole caused significant, but less severe reductions. Emamectin benzoate, pirimicarb, avermectin, and imidacloprid had no effect on ant abundance in these experiments.

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Acknowledgments

This project was funded by the Co–operative Research Centre for Sustainable Cotton Production and the Cotton Research and Development Corporation. Sincere thanks is extended to Ms Toni Bird for valuable technical assistance.