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).

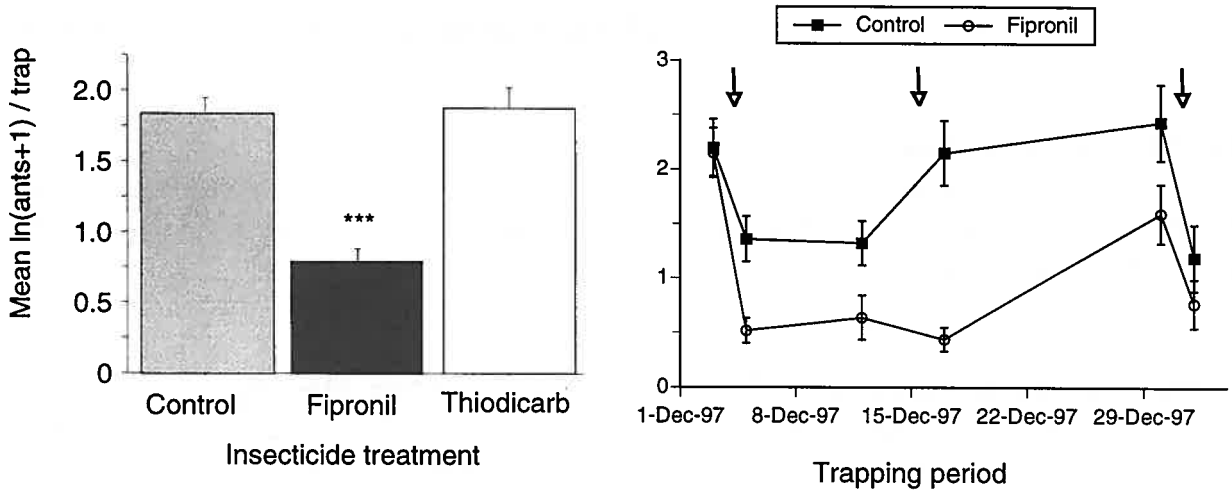

Figure 1: Effect of fipronil and thiodicarb on abundance of total ants caught in pitfall traps across the sampling season (left) and individual trapping periods (right) at ACRI during 1997/98. Cotton sprayed with fipronil (shaded black) was significantly different to the control (*** $P = 0.001$) whilst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

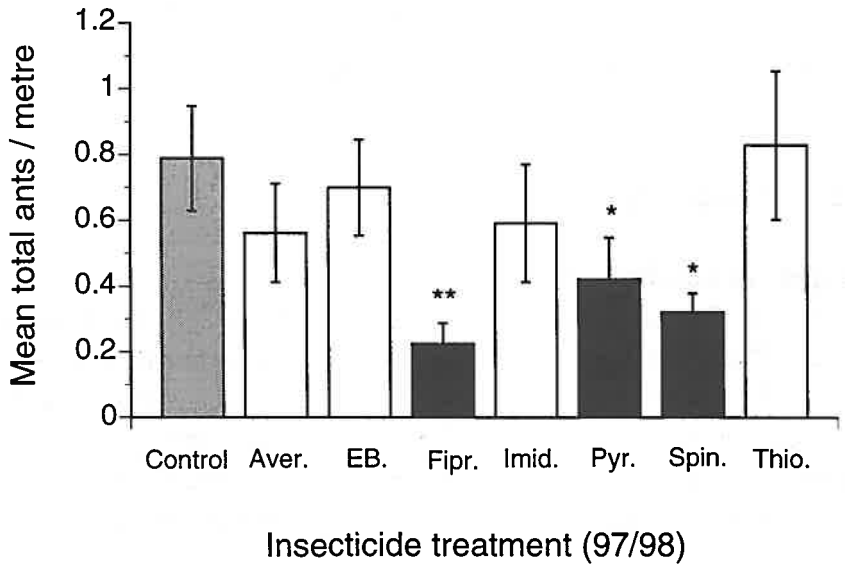

Figure 2: Effect of insecticides on the abundance of total ants in the canopy of cotton treated with (Av. = Avermectin; EB. = Emamectin benzoate; Fip. = Fipronil; Imid. = Imidacloprid; Pyr. = Pyrrole; Spin. = Spinosad; Thio. = Thiodicarb) and without (Con. = Control) insecticides at ACRI during 1997–98. 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.

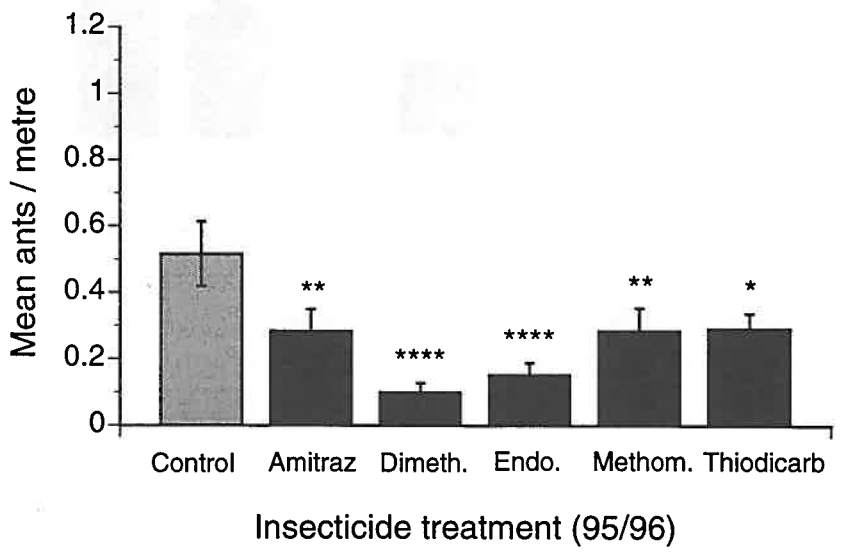

Figure 3: Effect of insecticides on the abundance of total ants in the canopy of cotton treated with (Amitraz @ 400 g ai/ha; Dimeth. = Dimethoate @ 140 g ai/ha; ; Endo = Endosulfan @ 735 g ai/ha; Methom. = Methomyl @ 169 g ai/ha; Thiodicarb @ 750 g ai/ha) and without (Control) insecticides at ACRI during 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).

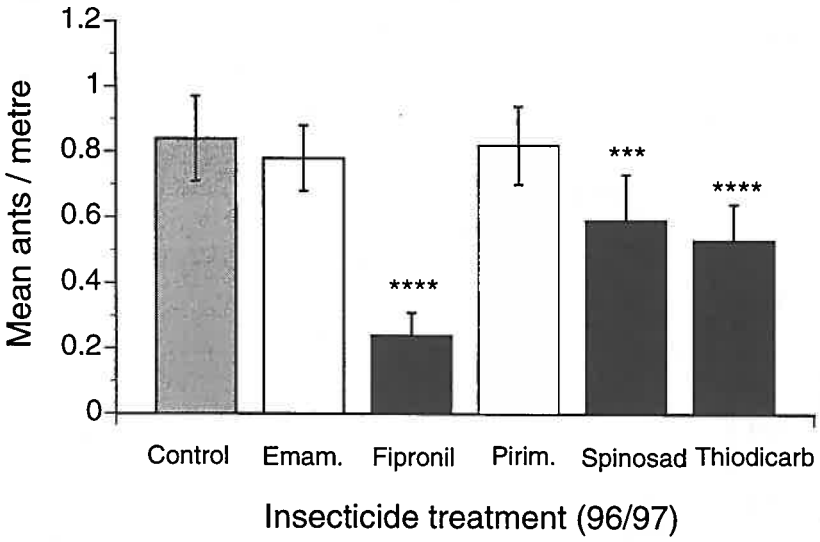

Figure 4: Effect of insecticides on the abundance of total ants in the canopy of cotton treated with (Emam = Emamectin benzoate @ 8.4 g ai/ha; Fipronil @ 25 g ai/ha; Pirim. = Pirimicarb @ 250 g ai/ha; Spinosad @ 96 g ai/ha; Thiodicarb @ 750 g ai/ha) and without (Control) insecticides at ACRI during 1995–96. 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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