CHAPTER FIVE

Non-target effects of carbaryl baited–trench and fipronil barrier spray treatment on the ant, *Anoplolepis custodiens* (Hymenoptera: Formicidae).

ABSTRACT

A study was conducted to determine the effects of a fipronil barrier spray and carbaryl bran bait applied in a baited trench - two methods recommended for control of armoured bush crickets (ABC) - on the non-target insect fauna. The pugnacious ant, *Anoplolepis custodiens* (Smith) (Formicidae), which forms a conspicuous biological component of the field and ground layer in sorghum fields, was selected as an indicator organism for evaluating the impact of insecticides on non-target fauna. Effects of bait and barrier treatments were evaluated using direct counting of active *A. custodiens* active burrows as a means of monitoring ant abundance. The experimental plots were monitored for eight months, at two weekly intervals, following application of carbaryl insecticide bait or fipronil barrier spray. Baited-trench and targeted barrier strip spraying strategies both had a relatively transient impact on the active burrows. A return to active burrow levels similar to that of the control treatment was observed four to six weeks after baited-trench and barrier strip spraying. It is concluded that both barrier spraying and the baited trench are relatively benign in terms of impact on non-target organisms. Applied in the right place at the correct time, both of these ABC control strategies present insecticide in such a way that it selectively targets armoured bush crickets as they invade crop fields, and so minimal amounts of insecticide needs to be applied and there is minimal ecological disrupted.

Key words: *A. discoidalis*, *Anoplolepis custodiens*, barrier spray, Botswana, bran bait, fipronil, non-target fauna.

INTRODUCTION

Cereal fields provide important habitats for species of invertebrates and vertebrates. These organisms visit fields to feed on plant material and the abundant invertebrate fauna within the agro-ecosystem (Potts & Vickerman 1974). Pesticides are often applied to cereals in order to control pest species. However, the use of insecticides in cereal crop production has also been shown to bring about a severe decline in the abundance and diversity of non-pest invertebrates (Potts 1970), including certain important beneficial arthropods (Vickerman & Sunderland 1977; Ba-Angood & Stewart 1980).

The need to examine the potential adverse impact of pesticides on non-target organisms is increasingly being recognised. Ripper (1956), Newsom (1967), Ware (1980) and Jepson (1989) all attest to the need for more selective insecticide agents or formulations that would provide suitable levels of pest control and yet be relatively safe for non-target species. In the United Kingdom and many other developed countries, evaluation of the risks posed by agrochemicals to non-target organisms is now an integral part of the pesticide registration process (Denholm *et al.* 1998). And this concern is by no means limited to developed countries. In the present project, subsistence farmers in Botswana showed great concern about possible negative environmental effects of control strategies involving the use of pesticides (Matsaert *et al.* 2000) against the ABC.

In Chapters four and five of this thesis, control strategies against the ABC were developed, evaluated and recommended. The barrier treatment measures that were developed involved spraying a 3-m wide barrier of fipronil 200 SC (Regent®) at a rate of 28.6 g a. i. ha^{-1} around fields to control the ABC. The baited-trench method involved selectively applying 90 g a. i. per 100 m of carbaryl 85 WP (Karbaspray®) as a bran bait in a trench surrounding a one-hectare field. The carbaryl rate used was 2.5 g a. i. mixed with 1 kg of bran and applied in 4 g bait heaps at 3 m intervals in the trench. However, despite these reduced volume rates, monitoring the non-target fauna needs to be done.

One means by which the environmental impact of pesticides on non-target fauna can be monitored is through studying the population response of particular bio-indicator organisms following chemical application. According to Fossi *et al.* (1994), an ideal bio-indicator for a particular environment should be an organism that is abundant in the study area, easily found at all times of the year, its preferred habitat being associated with the soil system and its diet exclusively found in that environment.

Many different organisms have previously been used in ecotoxicological studies on effects of pesticides, as illustrated by the following examples. Fossi *et al.* (1994) used the lizard, *Gallotia gallotti*, as a bio-indicator of organophosphorus contamination in the Canary Islands. Termites (*Coarctotermes* spp.) were used to study the non-target impact from blanket application of fipronil for migratory locust control in Madagascar (Tingle & McWilliam 2001). Crickets and grasshoppers were used in non-target impact studies as part of locust control operations in Mauritania and Madagascar (Raveloson 2001; Tingle & McWilliam 2001). Stewart (1998) used non-target grasshoppers as indicators of the side effects of chemical locust control in the Karoo region of South Africa. Predatory beetles have been used extensively as bio-indicators in previous studies (Balança & de Visscher 1996; Peveling 2000).

A number of previous studies have shown that ants are reliable indicators of arthropod recovery tendencies after habitat disturbance (Majer 1983; Andersen 1990; Lambert *et al.* 1991; Tingle 1993; Chambers *et al.* 1997). Earlier research has also reported on ant susceptibility to insecticides (Takken *et al.* 1978; Ali 1980; Grant & Crick 1987). Ants have been recorded scavenging on a variety of sprayed or moribund insects following treatment with DDT (Hoffmann *et al.* 1949) and deltamethrin (Holloway 1990).

Ants are one of the most ubiquitous groups found in both natural and man-made terrestrial systems. They occupy a wide range of ecological niches, as predators, scavengers, graminivores, herbivores and honeydew feeders (Skaife 1979. Tingle *et al.* (1992) reported ants as dominant members of the epigeal invertebrate fauna in the woodland savannah in Zimbabwe. From a human perspective, the beneficial effects of ants almost certainly outweigh their nuisance value such as biting during weeding and harvesting. For farmers, the greatest benefit from ants is from their predation of insect pests. They also process dead organic matter (Prins *et al.* 1990). Other desirable activities of ants include seed dispersal, weed seed predation, nest building (which increases the heterogeneity of the area), scavenging and predation on other organisms (Chambers *et al.* 1997).

The common pugnacious ant, *A. custodiens* (Smith), is a fairly large ant species, which is indigenous to Southern Africa and belongs to the family of sugar ants, the

Formicidae. In Botswana the e ant is not a pest but can be a nuisance to farmers during weeding. Pugnacious ants however were also observed feeding on a great variety of insects such as termites and mealy bugs and even on ABC (Fig. 5.1). *A. custodiens* is the most abundant and widespread ant in Southern Africa and is widely distributed in woodland savannah and cropping areas (Smit 1964). The species is very common in farmers' fields in eastern Botswana. Its burrow openings are a conspicuous feature of the field surface, and therefore the species' abundance can be monitored in terms of ant activity around the burrows. Partly for this reason, *A. custodiens* was selected as the bio indicator species for the present study.

A. custodiens, as a predatory ant, can have a beneficial effect in pastures and crops by reducing herbivore populations. *A. custodiens* has been reported to destroy eggs of the sugarcane borer moth, *Eldana saccharina* Walker (Leslie 1982). *A. custodiens* was observed to be a common predator of aphids and other insects in maize and sorghum fields in South Africa (Du Toit 1995). The latter author observed these ants to feed on the sugar cane aphid, *Melanaphis sacchari* (Homoptera: Aphididae) in sorghum and to become abundant in the latter part of the growing season. Ants were also reported to feed on *Rophalosiphum maidis* (Homoptera: Aphididae) in maize. In a study by Louw (1968) it was observed that *A. custodiens* activity declined during the months of August and September.

There are few recorded natural enemies of ABC. Birds are the most apparent natural enemies, witnessed feeding heavily on the ABC. In a survey by Matsaert *et al*. (2000), farmers recognized the abdim stork (*Ciconia abdimii*) as one of their best allies in the control of ABC. Recent research by Mviha (PhD thesis, in prep.), has demonstrated that the subterranean driver ant *Dorylus helvolus* is the principal natural enemy of *A. discoidalis* in eastern Botswana, inflicting 50 % egg mortality on ABC egg pod populations. Parasitoid wasps (*Nixonia* spp., Scelionidae) also contributed to egg mortality, but at a much lower level of approximately 1%. This provides further justification for using an ant as a bio-indicator for non-target impact in the present investigation.

The objective of this study was thus to determine the immediate and possible long-term impact of carbaryl bait and fipronil barrier sprays on selected non-target invertebrates. The ant used was the pugnacious ant as the bio-indicator because of its high abundance in the crop ecosystem, its high sensitivity to the acute effects of pesticides and because several ant species are recognised as being important natural enemies of *A. discoidalis* in eastern Botswana.

MATERIALS AND METHODS

Study site and treatment

Field trials were undertaken at Sebele (24^0 34S, 25^0 57E) during the 2000/2001and 2001/2002 cropping seasons. The trials were conducted in sorghum fields planted in mid-December of each season.

Sampling of insect fauna

The aerial fauna of each plot was sampled using a sweep net, sampling both during the day and also around camping Gaz® N 206 lamps at night. One lamp was placed in the middle of each block and set at a height of 1.5 m above ground (Fig. 5.2). A total of five light attractors were mounted in the experimental area. The night collection process, which normally started immediately after sunset, lasted for three hours. During the daytime, sweep nets were used to sample both flying and stationary insects within the crop ecosystem. Ten sweeps were made in each plot. Samples were collected 48 hours before and after treatment. Samples from plots were placed in a honey jar, which contained a few drops of ethyl acetate adsorbed onto a cotton wool to serve as killing agent.

To sample geophilic fauna, five pitfall traps were sunk in each plot. The plot was sub-divided into five, 5 m x 2 m sub-plots. A pitfall trap was sunk at the center of each sub-plot. Each pitfall trap consisted of two empty paper cartons (22 cm x 7.5 cm x 7 cm), one placed within the other. The outer carton was permanently sunk into the soil, while the inner carton was only inserted during sampling. Samples were collected 48 hours before and after treatment. When not in use, the inner carton was inverted on to the outer carton to seal it. This trap design limited habitat disturbance, allowed for easy removal of the samples and also prevented the pitfall hole from collapsing. Each trap was half filled with a weak detergent solution. The solution caused insects falling into the trap to drown rapidly. Presence/absence assessments were then made.

The insects collected from aerial and geophilic fauna were combined and then preserved in the laboratory for counting and identification. Insects were identified to species level using the insect reference collection of the research department in Sebele Agricultural Research Station.

Pugnacious ants in particular were used as bio indicator to monitor the impact of carbaryl bait applied uniformly in the trial plot and fipronil cover spray. Activity of pugnacious ants in the trial plots was monitored over an eight-month period using the total number of active ant burrows as an indicator of ant colony well being. An active ant burrow was defined as a burrow with at least five ants entering or leaving the burrow at the time of observation (Fig. 5.3).

Impact of the baited-trench and barrier spray treatment on pugnacious ants

This study assessed the impact of carbaryl baits and fipronil spray applied at 29 g a.i./ha to target ABC – namely as a baited trench and as a barrier spray. Experimental plots (10 m x 5 m) were divided into five equal sub-plots (5 m x 2 m). The bait was applied at 3-meter interval inside a 300 mm deep trench that surrounded each plot. A teaspoon was used to apply 4 g of bit per bait station to give a total of 0.45 g a.i./plot. Untreated bran carrier was applied in the control trench. In the barrier spray treatments, fipronil was applied low volume around the periphery of the crop with a knapsack sprayer. A 3-meter strip was applied around each sub-plot at a rate of 0.15 g a.i./plot (see appendix 2). The impact of the baited-trench and barrier spray treatments on non-target invertebrates was monitored in each case using *A. custodiens* as the bio-indicator as previously described. Sampling for active ant burrows took place 48 hours before treatment and on nine occasions at weekly intervals after treatment. During each count, all active ant burrows in each transect were counted and recorded. The sub-plots were permanently marked out for future counts. The experiments were terminated when the numbers of ant burrows in plots that received insecticide treatments were again similar to those of control plots.

RESULTS

Impact of the baited-trench and barrier spray treatments on numbers of active pugnacious ants burrows

Pugnacious ants (*A. custodiens*) and a number of ground dwelling tenebrionid beetles formed the bulk of the pitfall trap and sweep net catches before insecticide treatment commenced. The commonest beetle species were: *Psammodes scrobicollis*, *Zophosis* spp. and *Somaticus beachuanas* (Coleoptera: Tenebrionidae). Other insects collected were *Gryllus* spp. (Orthoptera: Grylllidae) and *Zonocerus elegans* (Orthoptera: Pyrgomorphidae). The abundance of insects sampled 48 hours before and after cover application of carbaryl bait and fipronil spray is illustrated in figs. 5.4 and 5.5 respectively. A total of 650 ants were caught before treatment. The number of ants caught was reduced to 10 in fipronil plot and to 620 in the carbaryl bait plot. Fipronil killed several other non-target species immediately after its application in the trial plots. A severe reduction in the numbers of *A. custodiens*, *Gryllus* spp. and *Z. elegans* occurred in plots treated with fipronil, whilst tenebrionid beetles were completely eliminated from these plots. Insect abundance after bait application was not significantly different ($p < 0.05$) from that of the control for any of the indicated species.

Neither of the two targeted application techniques had a prolonged impact on the numbers of active ant burrows. After an immediate reduction in ant populations following treatments, a return towards levels similar to those of the control treatments was observed after four to six weeks (Fig. 5.6).

DISCUSSION

The reduction in abundance of arthropods observed in baited plots can be attributed to consumption of the carbaryl bait by these arthropods. The insects may be attracted to the bait by its fermenting smell and to its moisture. The population decline in invertebrates in those plots treated with fipronil, on the other hand, was due to body contact and persistence of the insecticide. The results are in agreement with previous findings on the impact of fipronil on non-target invertebrates (Tingle *et al*. (in press). Grout *et al*. (1997), in leaf–disc bioassays with the predatory mite (*Euseius addoensis*), listed fipronil amongst the most detrimental of insecticides used in the citrus industry in South Africa. Comparing the impacts of blanket treatments of fipronil and chlorpyrifos on non-target invertebrates, Sokolov (2000) concluded that fipronil had more severe effects than chlorpyrifos for most (but not all) non-target invertebrates.

The recovery of ant populations, as indicated by the number of active burrows a few weeks after application would seem to be partly due to the decline in activity of the carbaryl, depletion of bait and partly as a consequence of the bait having dried out, making it less attractive to the insects. The present study has demonstrated that the adverse effects of fipronil on non-target invertebrates are reduced, if fipronil is applied as a barrier strip spray rather than applied as a full cover spray. This finding is in agreement with Tingle & McWilliam (1999) who also demonstrated that barrier spraying significantly reduced the adverse effects of fipronil on termites known to be highly sensitive to this insecticide. Previous studies using several other sprayed insecticides have shown similar effects on ants. The long term effect of DDT on epigeal ants was implicated in causing lower numbers of foraging workers following four years of DDT treatment, at very high application rates to cowpea plots in Nigeria (Critchley *et al.* 1980). Lambert *et al.* (1991) recorded high mortality levels of *Camponotus* spp. and the arboreal ant, *Crematogaster* spp., immediately following ground-spraying operations with deltamethrin. Aerial application of deltamethrin to control tsetse in Botswana, caused post-spray decline in the numbers of *Camponotus* spp. caught in pitfall traps in mopane woodland (Ali 1980). Similarly, *Camponotus* spp. was severely affected by carbaryl spraying in the USA (Murphy & Croft 1990). These reports raise concern over area-wide use of insecticide cover spraying *per se*, but especially so in the case of highly persistent compounds like fipronil. The striking finding here, so far as carbaryl bait use is concerned, is that cover application with bait had a relatively small impact on *A. custodiens* populations, which recovered almost as quickly as when the bait was applied in a trench. This finding supports Price and Brown (1997), who argued that targeted (surface) baiting offers a practical and ecologically attractive alternative to ultra low volume sprays for direct crop protection by farmers against locusts. However, whilst the application of bait inside a trench may offer little extra protection for non-target invertebrates, it probably does provide additional safety to non-target vertebrates, since many potential consumers such as guinea fowl, storks or chickens, which might prey on ABC would not readily access the 300 mm bait trench.

To conclude, it can be said that the effects of the baited trench and barrier spray on ants were relatively short-lived and may in both cases have occurred through reduction in worker numbers rather than by colonies being completely destroyed. Thus, on the basis of the present evidence, discriminative use of both these techniques for ABC control appears unlikely to have a major effect on ant populations. A concern, which remains, however, is the possible impact of bait-killed ABC consumed by scavengers and predators, since the above field trials were conducted during recession years for ABC, with very few dead bodies present.

The use of the pugnacious ant, *A. custodiens*, as a bio indicator to determine the impact of barrier spraying and the baited trench on field invertebrates in general appears to have been an appropriate choice. *A. custodiens* not only has the features of an ideal bio-indicator: it is abundant and predominant in the crop ecosystem, while its scavenging behaviour brings it into contact with bait or ground sprayed insecticides.

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1:0.5

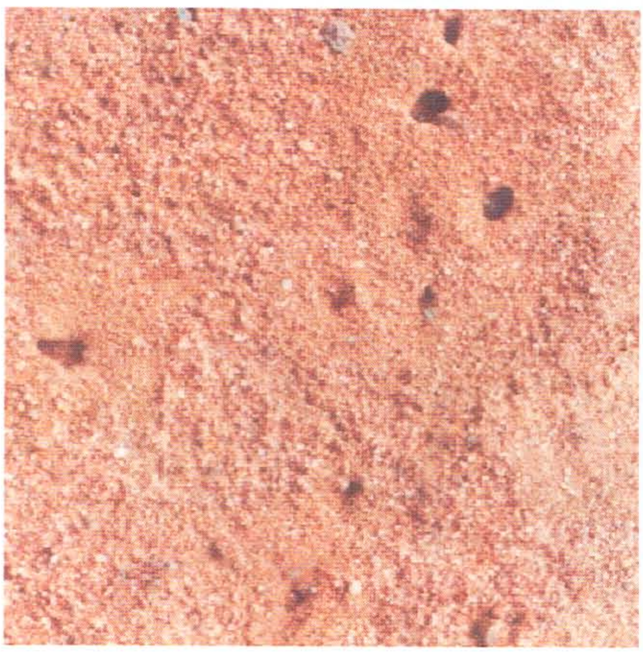
Fig 5.1. Crickets killed and dragged to nests by *Anoplolepis custodiens*. Ants killed the crickets when they fell into a newly constructed trench.

Table 5.1. Chemical name, formulation, dose and volume of application for carbaryl and fipronil insecticides.

Common name	Chemical name	Formulation	Application rate (g a.i.ha^{-1})
Carbaryl	1-Naphthyl methyl carbamate	850 g kg^{-1}	90.0
Fipronil	(±)-5-Amino-1-(2,6-dichloro-4-trifluoro-methylphenyl)-4-trifluoromethyl-sulfinylpyrazole-3-carbonitrile	200 g l^{-1}	28.60

1:0.5

Fig 5.2. Light attractor used to facilitate collection of nocturnal invertebrates.



1:0.8

Fig 5.3. Active burrows of *A. custodiens* at Sebele, Botswana.

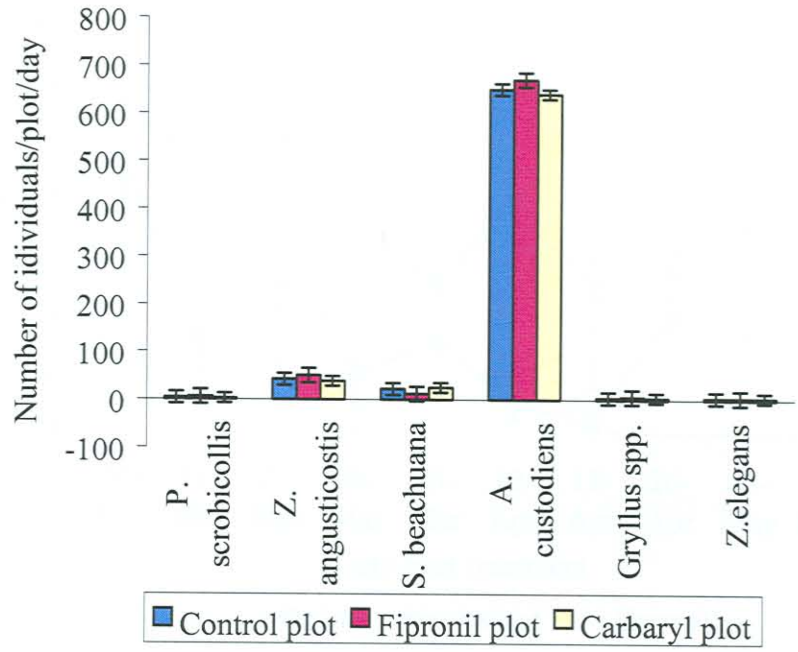


Fig. 5.4. Abundance of species recorded on intended trial plots before treatment. Bars represent standard errors (SE).

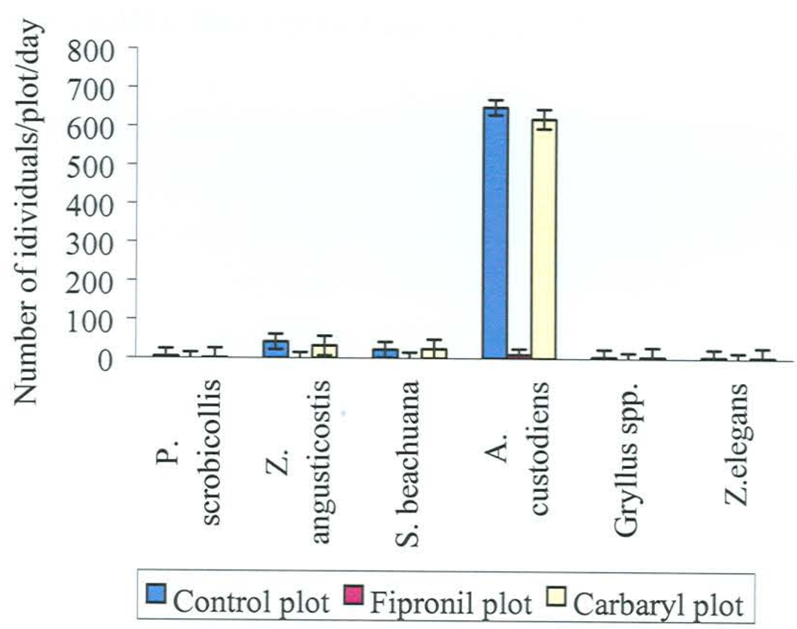


Fig. 5.5. Abundance of species recorded on trial plots after treatment. Bars represent standard errors (SE).

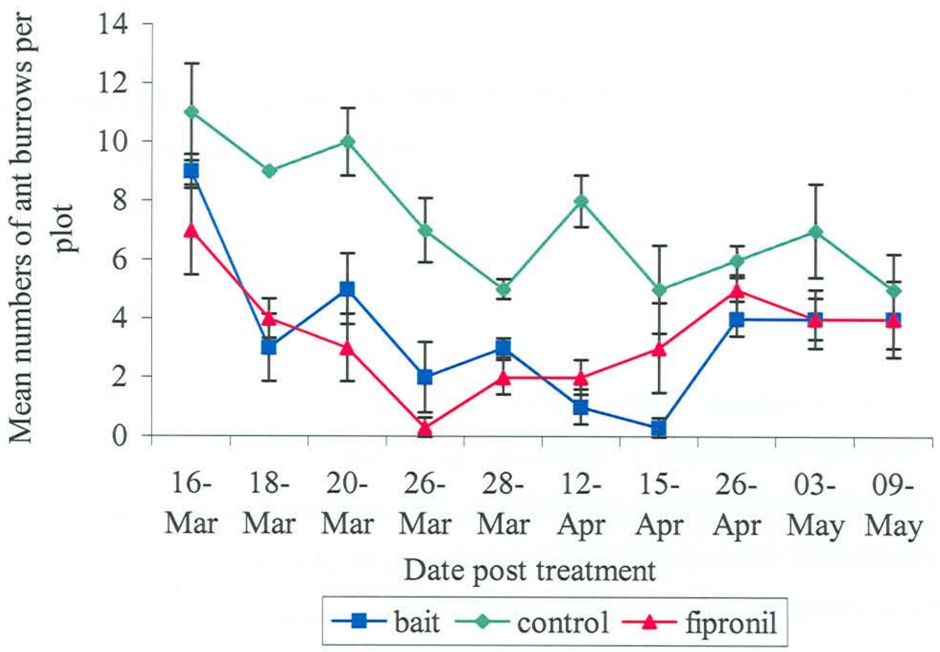


Fig 5.6. The mean numbers of active ant burrows in plots at Sebele between March and May 2002 after application of the carbaryl baited-trench and fipronil targeted barrier spray strategies on 16 March 2002. (Treatment $p < 0.001$; Time $p < 0.001$). Bars represent standard error (SE).