IMPACT OF INSECT GROWTH REGULATORS ON NON-TARGET SPECIES, WITH AN EMPHASIS ON COCCINELLIDS ON CITRUS, IN SWAZILAND

by

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ABSTRACT

This study investigated effects of insect growth regulators (IGRs), recommended for use on citrus in southern Africa, on non-target organisms, particularly species of Coccinellidae in and around three citrus estates in the lowveld region of Swaziland, i.e. Tambuti, Tambankulu and Tunzini estates. Some of the species are important predators of citrus pest insects. The distribution of coccinellids within an agricultural land mosaic and factors affecting this distribution pattern were also investigated. Results showed that coccinellid densities and species diversity were lowest in the natural veld surrounding the orchards. In contrast, citrus orchards had the highest coccinellid densities and diversity. River borders, along the Great Usuthu river, had intermediate densities and diversity. While temperature, relative humidity and ground insolation had no significant effect on coccinellid population densities, the presence / absence of prey (host plant), on the other hand, was an important factor. This was the case both for phytophagous and predatory coccinellid species. These showed close patterns of distribution with their respective host plants or prey species. Economically important species, such as *Rodolia* spp., were restricted to the orchards, while other species, especially those whose economic role is uncertain, such as *Cheilomenes lunata* and *Scymnus* spp., were found in most habitat types.

Coccinellid population densities were assessed during four treatment programmes: 1) an integrated pest management (IPM) programme where no IGRs were used, 2) a programme where any of the recommended IGRs were to be applied, 3) conventional pesticide programme, and 4) an orchard which did not receive any chemically treatment over the last four years (at Tambankulu estate only). Significant differences between treatment programmes were observed at all three estates, when only economically important coccinellid species were assessed. Orchards under an IPM programme (with no IGRs) were found to have significantly higher population densities compared to those in IGR-treated or under a conventional pesticide regime. The untreated orchard at Tambankulu, however, had significantly higher coccinellid densities of economically important species and higher overall population densities than any of the managed orchards. Of the three estates assessed, Tunzini had significantly higher population densities of all beneficial coccinellids, (excluding the untreated orchard at

Tambankulu). Natural vegetation around Tunzini and Tambuti contributed to the higher coccinellid densities compared to Tambankulu, which was surrounded by other agricultural land.

Laboratory and field experiments on two non-target species, the ladybird *Chilocorus nigritus* (Fabricius) (all stages) and the moth *Bombyx mori* (Linnaeus) (larval stage) assessed specific IGR effects, if any. Three IGRs, buprofezin, teflubenzuron (both chitin synthesis inhibitors) and pyriproxyfen (a juvenile hormone analogue) were used. All three pesticides are currently recommended for use on citrus in southern Africa and were tested at the recommended dosages. Laboratory experiments showed that all three IGRs have a negative impact on both non-target species. When *B. mori* received direct applications, there was larval mortality as a result of the failing to complete moulting or dying immediately after moulting. In addition, no larvae were able to pupate after having fed on contaminated leaves. In the case of *C. nigritus*, larvae fed IGR-treated scale or sprayed with buprofezin suffered significantly higher mortality than controls, while IGR effects on those sprayed with, or fed scale-treated with, pyriproxyfen or teflubenzuron were not significant. No adults however, emerged from any pupae in any of the treatment groups. All three IGRs had ovicidal activity on *C.nigritus* eggs. Adult fecundity in both field and laboratory experiments was not affected significantly after exposure to any of the three IGRs. In field experiments, the proportion of larvae of the moth and ladybird that developed up to the reproductive adult stage, after exposure to buprofezin, was not significantly different from the control. This was not the case for pyriproxyfen and teflubenzuron. Buprofezin was therefore found to be the least detrimental of the three IGRs tested.

Minimal drift was observed when a knapsack sprayer was used. However, there was spray drift up to 32m (the furthest distance assessed here) where commercial sprayers were used. This suggests that pesticide drift from orchards to adjacent areas would have serious implications for biological diversity in the river borders and rivers adjacent to the estates. The sensitivity of the non-target species to the IGRs tested needs serious consideration, as it indicates a broader spectrum of activity for the compounds than what is promoted. Additionally, natural control may be affected. This is because the timing of IGR applications and increasing coccinellid populations coincide, resulting in a reduced pool of coccinellid predators.

This study emphasised the importance of an appropriately heterogenous landscape to maximise habitat availability for the coccinellids. Although the economic role of the multihabitat coccinellid species recorded here is unknown, they nevertheless clearly contribute to citrus pest control. Such a mosaic landscape, in conjunction with IPM, with no IGR use, promotes ecological diversity and controls pests with minimum disruption to biodiversity. The use of IGRs in citrus thus needs careful reconsideration in light of the non-target effects observed on the two species, especially the valuable predatory species, *C. nigritus*.

PREFACE

This study represents original work by the author and has not been submitted in any form to another university. Where use was made of the work of others, it is duly acknowledged in the text.

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CHAPTER 1

INTRODUCTION

Coccinellid biology and distribution in landscape

Species of the Coccinellidae vary in size from 0.8mm to 18mm (Hodek, 1973; Majerus and Kearns, 1989). They also have a range of feeding habits, with some genera, such as *Epilachna* and *Subcoccinella* being phytophagous and while others, such as *Micraspis* being mycophagous. Nevertheless, the main economic value of this family arises from the predaceous species many of which feed on plant pests of economic crops. These may be predators of aphids, e.g. *Hippodamia* spp., coccids, e.g. *Rodolia* spp., mites, e.g. a number of *Stethorus* spp., psyllids e.g.*Calvis* spp. and chrysomelid larvae, e.g. *Neoharmonia* spp. (Hagen, 1962; Hodek, 1973; Majerus and Kearns, 1989; Drea and Gordon, 1990).

Predatory coccinellids usually establish in cultivated crop systems when prey populations have reached levels high enough to sustain the predators. Species feeding on sedentary prey species like scale insects and mealybugs, e.g., *Rodolia cardinalis* (Mulsant), tend to show close synchrony with their prey species in the absence of other detrimental factors. This synchrony is, however, absent or less pronounced in aphidophagous species, where the prey have a very high reproductive potential, and build up to high population levels at the beginning of the season. The coccinellid predators then establish themselves only later in the season and their populations lag behind their prey (Hagen, 1962; Fazer, 1988). This may explain why aphidophagous predators are less successful as the sole biological control agents of aphids, while better success is experienced with the scale / coccid predators.

Eggs, which are yellowish to reddish-orange in colour, are laid in batches, usually protected under tree barks, leaves and scale insects, within the vicinity of their essential prey. Some genera, e.g. *Halmus,* deposit their eggs on exposed substrates in small, erect clusters (Hodek, 1967; Fazer, 1978; Drea and Gordon, 1990). To maximise their reproductive success, females tend to cease ovipositing at a particular site once the critical number of eggs has been laid or when there is a critical number of larvae present (Ferran and Dixon, 1993). Larvae emerging from eggs usually spend a day or two at the hatching site, during which time they feed on the egg shells, nonviable or unhatched eggs (Hodek, 1973; Fazer, 1978; Majerus and Kearns, 1989). The habit of cannibalism enables the survival of at least some of the newly emerged larvae, especially when natural food is scarce or unavailable (Drea and Gordon, 1990). The first-instar larvae search for food near the hatching site.

Almost all Coccinellidae species have three larval moults to separate the four instars (Nadel and Biron, 1964; Dorge *et al*, 1972, Hodek, 1973) although five instars were observed by Vesey-FitzGerald (1953) in *Chilocorus nigritus* (Fabricius). Newly-emerged larvae feed by piercing and sucking their prey, with periodic regurgitation of the prey contents. As the larvae mature, they develop a chewing action so that they are able to lift off the protective cover in prey, like scale insects, or chew through the cover to expose the prey within. The larvae of most species feed on almost all stages of the prey insect. While the mature scale insects may present problems for some coccinellid species, leading to the development of preference for particular age groups of prey (Hagen, 1962; Samways, 1988; Drea and Gordon, 1990), predatory species like *Chilocorus nigritus* are able to feed on the hard, mature scale insects (Samways and Wilson, 1988; Samways *et al.*, 1997).

Before each moult, larvae normally stop feeding and attach by their anal organ onto a substrate so as to anchor themselves for moulting. Aggregation of larvae during moults has been observed in some species (Hodek, 1973). The larval period ranges from one to three weeks depending on species, prey availability and environmental conditions. The last larval instar may have a non-feeding period, e.g. in the tribe *Chilocorini*, during which time it remains immobile. Some species however, do not have this stage.

Pupation usually occurs in a protected area. The pupae may occur singly or congregate in small groups. *Chilocorus nigritus* is known to congregate both on citrus trees and bamboo trunks in small groups in the lowveld of southern Africa (Samways, 1984). During this period, pupae respond to external stimuli by upward jerking movements of the body and this may be an attempt at defending themselves. The pupal stage lasts about one week (Hodek, 1973; Majerus and Kearns, 1989).

On emergence, coccinellid adults are usually soft, matt and light coloured with none of the patterns on elytra characteristic of most coccinellids. The normal colouring and patterning are acquired gradually over the weeks (Fazer, 1988). Adults mate a few days after emergence and a mating pair may remain in copulation for several hours. Prolonged mating periods seem to promote the production of spermatozoa or their transfer, although it may also serve to prevent insemination of the female by other males. A single copulation may be enough to ensure the female's fertility throughout her reproductive lifespan. Females, however, tend to have repeated matings during their adult life if males are present (Hodek, 1967; Fazer, 1988; Drea and Gordon, 1990).

Depending on prey availability, climate and time of the year, some species may have more than one generation a year. Their longevity may be extended, especially if they overwinter as adults. Most species have newly-emerged adults that undergo a period of dormancy during the cooler winter months when food is scarce. These periods of dormancy are a means for the adults to pass unfavourable climatic conditions and prey scarcity. During this time, development is also arrested. Dormant individuals usually do not feed, which means that their digestive tracts are empty, and food is reserved in their enlarged fat bodies, e.g. *Cryptolaemus montrouzieri* Mulsant, *Rodolia cardinalis* and *Exochomus flavipes* Thunberg. In females, the ovaries become inactive, while in the males, spermatogenesis is arrested. As conditions become more conducive for growth, individuals regain activity and development (Hodek, 1967; Samways, 1984).

During these dormancy periods, some coccinellid species disperse to suitable overwintering habitats and may form aggregations, e.g. *Hippodamia convergens* Guérin, although some species merely spend these periods individually or in small groups under tree bark, litter, etc., e.g. *Stethorus* species. Aggregating groups are usually those associated with ephemeral prey species and not the sessile Homopteran species, e.g. all members of the tribes *Hippodamini* and *Anisostictini* aggregate in temperate regions. Aggregations are probably a means of bringing the sexes together to facilitate mating, which takes place at the end of the dormancy period before dispersal. After the dormancy period, adults become engaged in search of their ephemeral prey, resulting in dispersal of the population and reduced chances of encountering mating partners. Adult coccinellids associated with the armoured scales often do not congregate (Hagen, 1962; Hodek, 1973; Drea and Gordon, 1990), although some do, e.g. *C. nigritus* (Samways, 1988).

In addition to dispersal to find suitable hibernation sites, some coccinellids also move within and between habitats in search of food. Adult members of Coccinellidae have high mobility and thus may occur in a variety of habitats. Their habits range from the stenotopic species whose occurrence is restricted to a particular habitat, e.g. *Anisosticta novemdecimpunctata* Linnaeus is a hygrophilous species which occurs only in wetland areas where reed mace (*Typha latifola* Linnaeus) and reeds (*Phragmites australis*) grow, to the eurytopic species whose habitat-preference is not restricted. Intermediate species may have preference for a particular habitat although not necessarily restricted to it (Hodek, 1973; Majerus and Kearns, 1989). Habitats utilised by these beetles may be cultivated agroecosystems or natural, uncultivated land. Cultivated or managed habitats often provide prey species at high densities during favourable weather conditions. Alternatively, natural (uncultivated) habitats provide plant and physical shelter sites during periods of dormancy and also act as alternative prey reservoirs when their target prey species become scarce in the cultivated areas (Van Emden, 1981; Hattingh and Samways, 1991; Burel and Baudry, 1995).

The various habitats exploited by coccinellids may be considered as ecological units which interact with one another, and these interactions affect their dynamics (Baudry, 1989). At the landscape level, intensively managed agroecosystems like fruit orchards may be considered as distinct anthropogenic land mosaics embedded within a matrix of distinct habitat types. The distance between the various landscape elements created is very important, since coccinellids rely on two to three habitats during their development or for overwintering (Holt *et al*, 1995; With and Crist, 1995; Forman, 1995).

However, the colonization of a particular habitat is largely determined by habitat quality rather than mere ability of coccinellids to reach that habitat (Duelli, 1988). Criteria important for colonization are usually species-specific requirements, including the presence of essential prey, oviposition sites, shelter and mating partners. In addition to habitat quality, survival of these populations is also dependant on the quality of the surrounding landscape (Hodek, 1967; Duelli, 1988; Burel and Baudry, 1995).

In the presence of a variety of ecological niches within the land mosaics, more species are likely to colonise and survive within the area. Creation of a mosaic of different crop systems promotes stability within the landscape due to an increase in its heterogeneity, thus providing habitats for all life-stages of the coccinellids (Duelli, 1988). Different coccinellid species require different ecological niches, both temporally and spatially for the various life stages and move from one habitat to another as they develop (Stubbs and Fry, 1991; Burel and Baudry, 1995). For example, the first generation of *Coccinella septempunctata* Linnaeus, *Adalia bipunctata* (Linnaeus) and

Propylea quatuordecimpunctata (Linnaeus) oviposites on nettle, *Urtica dioica* Linnaeus after emergence from hibernation, where as the adults and newly-emerged larvae fed on *Microlophium evansi* Theobald. As adults, the second generation then moves onto bean-cultivated land infested with *Aphis fabae* Scopoli. The second generation also breeds on the bean crop (Hodek, 1973). To promote earlier establishment in the cultivated crop, the nettles can be managed, e.g. they are cut down early in the season, to promote early migration of the coccinellids into the pest-infested crop thus effecting pest control much earlier (Burel and Baudry, 1995).

Habitat fragmentation has a major influence on a suite of ecological processes, both at the individual and ecosystem level. Fragmentation usually results in the decrease of a habitat suitable for insects (Pyle *et al.*, 1981; Fry, 1995). The presence of natural habitats near to cultivated land can have a favourable effect on the general abundance of beneficial coccinellids, especially mobile aphidophaga. Diversity of this agricultural mosaic, determined by its plant heterogeneity and spatial arrangement, plays a role in the ability of these predators to control pests (Keller and Duelli, 1988; Burel and Baudry, 1995).

The reliance of the beneficial insects on a variety of habitats and the resultant interaction between the different habitat types is important for pest control (Volkl, 1988). An agricultural mosaic interwoven with a network of natural and semi-natural areas provides alternative habitat sites for beneficial coccinellids when the cultivated land is disturbed, e.g. after pesticide applications, mowing, harvesting, etc. and prey populations are low. This mosaic landscape with different habitat elements, e.g. wind breaks, natural forest / vegetation, river banks and adjacent orchards supports the maintenance of a rich fauna (Duelli, 1988; Ryszkowski *et al.*, 1993) and this promotes a permanent flow of entomophagous coccinellids between the various mosaics (Duelli, 1988). In an extensively managed area like citrus orchards, there is a mosaic of habitat patches. Highly mobile adult entomophagous coccinellids exploit these various habitats as and when the need arises. Adjacent orchards may also serve the same function as alternate habitats when prey becomes scarce in a previously colonised orchard, or if the orchard is disturbed by insecticide applications, mowing, cutting down, etc. (Hodek, 1967, Duelli, 1988).

Survival of the first instar larvae is the most critical link in the coccinellid life-cycle, so essential prey need to be readily available to ensure predation success and ultimate development and growth of the larvae (Baumgaertner *et al.*, 1981). Relative distance between habitat patches and subsequent interchange of individuals between these patches is dependent on the dispersal ability, size of the species and life stage being considered. Immature coccinellid have low mobility and so it is important to have suitable habitat patches within short distances. The permeability of the interconnecting mosaic also needs to be taken into consideration, e.g. road corridors, stripped down orchards cause a decline in mosaic permeability sometimes resulting in the decrease in colonisation rates, further isolating the patches. This usually occurs in agroecosystems where the land is fragmented, so that habitat patches are reduced in size and may be separated from each other by a matrix not suitable as a habitat (Kalkhoven, 1993). If the landscape has been so fragmented that there is resource patchiness, then the survival of coccinellid populations may be compromised. In such systems, this may ultimately result in the coccinellid population occupying isolated patches, where the resident population has a very low probability of survival in the long term (Fahrig and Merriam, 1985) and may also be reduced in size depending on the quality of the patch as suitable habitat (Kalkhoven, 1993).

Agricultural intensification is one of the major causes of the loss of insect populations due to the removal of small but essential landscape elements (Pyle *et al.*, 1981; Forman, 1995). The resulting land mosaics form distinct habitats with alteration of spatial patterning of the ecosystem, with possible loss of connectivity between the mosaics formed. This further leads to increased isolation of the patches (With and Crist, 1995). To promote the preservation of essential habitats, both natural and anthropogenetically disturbed, there needs to be preservation of habitat heterogeneity, thus lending stability and promoting species diversity within the same managed agroecosystem (Duelli, 1988).

In addition to land fragmentation, insecticides used to maintain agroecosystems also have detrimental effects on beneficial insects like coccinellids. Integrated pest management promotes the utilisation of agrochemicals only when it is absolutely essential. When the regulative agrochemical treatment is necessary, it is important to know the effects of the pesticides as well as its possible impact on non-target species such as coccinellids (Finney, 1971). According to Fry, (1971) many insects affected by agrochemicals are non-target species, which however, serve an important functional role within the community structure. In the presence of alternative habitats within the landscape, these insects may colonise these patches when cultivated areas are sprayed with insecticides. In this way, the predators, usually generalists, can survive on alternative prey in untreated habitat patches, until prey populations recover sufficiently to be exploited in the treated areas, where the applied chemical may have degraded to harmless components.

Such movement and colonisation of habitats would be maximised by suitable proximity of the patches as well as provision of suitable alternative habitats within the agricultural landscape. Beneficial, predatory species would be able to maintain relatively high population levels and be able to colonise cultivated areas earlier than they would normally (Andow and Risch, 1985). This minimises the predators' failure to synchronise with their prey species. For example, planting of bamboo, *Dendrocalamus giganteus* Munro, next to citrus orchards provides overwintering sites for *Chilocorus nigritus*, enabling it to colonise red scale infested citrus orchards earlier in the season. The spatial and temporal arrangement of the landscape elements may thus be used as contributory factors towards maximising the activity of beneficial coccinellids within agricultural land mosaics.

Insect growth regulators

With the increased need for higher agricultural production, the first synthetic insecticides were made available during the 1940s. Insecticide use enabled the generation of huge benefits in increased food production (Van der Werf, 1996). By the 1960s, organophosphates had gained widespread use in agricultural ecosystems. This class of insecticides was fast acting with broad-spectrum activity and outstanding efficiency. They were utilised in a wide range of ecosystems due to their affordable price and ability to maintain their efficiency in mixture and alternation pesticide programs (Bulhozer, 1991). By the mid-1980s, 2×10^6 tons of pesticides were applied to agricultural crops worldwide (Pimentel and Levitan, 1986).

In agroecosystems, pesticide use however tends to alter the interrelationships of closely-associated species and therefore alters the function of the ecosystem, whereby the number of arthropod species present and / or the arthropod population may be reduced (Pimentel and Edwards, 1982). Most insecticides used in agroecosystems are nonselective, resulting in mortality of both pests and their natural enemies, further upsetting the biological equilibrium (Ripper, 1956; Peleg, 1982). In general, pesticides target a particular stage in the pest's life-cycle and kills the most susceptible stage, resulting in the formation of a uniform pest age structure (Waage, 1989). Due to the synchronised pest populations, natural enemies are seriously affected since their preferred target stages may not be available. This results in the further decline of natural enemy populations (Pimentel and Levitan, 1986). These first and second generation insecticides thus affect natural enemy populations more than they do the target pests. Pests and secondary pest species proliferate to higher population levels, increasing the need for more pesticide applications and maintaining the pesticide treadmill. Since the introduction of neurotoxic insecticides and acaricides, tetranychid mites became annual pests in all fruit growing regions of North America while predatory species, such as *Typhlodromus* species were eliminated (Biddinger and Hull, 1995).

The sole reliance on conventional pesticides for arthropod pest control led to the development of resistance among pest populations, further increasing economic losses due to pest damage (King and Bennett, 1989; Power and Karieva, 1990; Soule *et al.*, 1990). Resistance has been observed in all the major classes of pesticides, e.g. carbamates, pyrethroids, dichlorodiphenyltricholoethane (DDT) and organosphophates. By 1986, 447 arthropod species were resistant to more than one insecticide of the major classes, where 59% of these were agricultural pests (Brattsen *et al.*, 1986, Pedigo, 1989), e.g. the tufted apple bud moth, *Platynota idaeusalis* (Walker) developed resistance against organophosphates (Biddinger and Hull, 1995). Organophosphates, carbamates and synthetic pyrethroids were used extensively to control lepidopteran pests and bugs. However, cotton pests such as *Heliothis virescens* (Fabricius), developed widespread resistance to these compounds and their use invariably triggered pest outbreaks causing pest resurgence and secondary pest replacement (Cremlyn, 1978; Masner *et al.*, 1987; Pedigo, 1989).

Most synthetic pesticides developed during the last five decades target the nervous system, e.g. organophophorous esters and N-methylcarbamates inhibit acetylcholinesterase, an enzyme which regulates synaptic transmissions while DDT and pyrethroids disrupt the sodium sensitive channels of the nervous system (Ehrenfreund, 1990). The sites targeted by these compounds are also present in all higher animals, so these compounds also have undesirable effects on mammals and fish (Pedigo, 1989; Ehrenfreund, 1990). In addition, concerns about pesticide effects on the environment and human health were also voiced. Pest control came under the scrutiny of the media, environmental groups and regulatory agencies (King and Bennett, 1989; Van der Werf, 1996). Due to the loss of efficacy by the synthetic pesticides as well as environmental concerns, an impetus was created for the development of pesticides which would manage pests populations more efficiently as well as being environmentally acceptable (King and Bennett, 1989). These compounds were developed to target or interfere with processes which are specific to invertebrates, ideally only members of a specified insect order. One possible target was the insect hormone system (Ehrenfreund, 1990).

This led to the development of the third generation insecticides, the insect growth regulators (IGRs), e.g. diflubenzuron ('Dimilin'), Buprofezin ('Applaud', teflubenzuron ('Nomolt'), pyriproxyfen ('Nemesis'), diofenolan ('Aware') and triflumuron ('Alsystin'). IGRs are insecticides whose activity is based on their disturbance of normal activity of the insect's endocrine system, so interfering with the moulting process and interrupting growth. Alternatively, they may block chitin synthesis whereby they inhibit the deposition of chitin in the integument (cuticle). As a result, the mechanical properties of the new cuticle are impaired, thus affecting the integrity of the new cuticle (Cremlyn, 1978; Hassall, 1982; Pedigo, 1989).

Insect growth regulators act at the end of the larval / pupal development and may induce transovarian sterility (Masner *et al.*, 1987, Ascher and Eliyahu, 1988). They are highly specific to arthropods with low mammalian and fish toxicity (King and Bennett, 1989; Voss, 1992). IGRs are important and effective in pest management programmes where economic pests have developed resistance to conventional insecticides, like the pyrethroids, carbamates and organophosphates or in situations where the pests inhabit ecologically sensitive environments such as water bodies (Capinera *et al.*, 1991; Drabek, 1991), e.g. pyriproxyfen provides effective control of organosphophate-resistant citrus red scale, *Aonidiella aurantii* (Maskell), while treatment of ponds with fenoxycarb led to significant reduction of OP- resistant *Culex quinquefasciatus* Linnaeus populations due to high pupal mortality and emergence of abnormal adults (Schaeffer *et al.*, 1987; Hattingh, 1996). This class of insecticides was formerly believed to be selective and thus compatible with integrated pest management programmes.

The cuticle and moulting process

Insect growth is a cyclical event, where periods of growth alternate with periods of cuticle formation. Alternation of these events is under hormonal control. The cuticle is an integral part of the epidermis, and in rigid skeletal structures like the appendages and head, the cuticle has to be shed to enable growth. In preparation for the moulting process, the epidermis is detached from the old cuticle during apolysis and a new cuticle is deposited by mitotic cell formation. The old endocuticle is digested by the moulting fluid and is absorbed by the epidermal cells. The undigestible exocuticle and epicuticle are then shed off. By contraction of the intersegmental muscles in the abdomen, the haemolymph is driven forward and expands the new cuticle, resulting in rupture of the old cuticle along preformed lines of weakness. The insect withdraws from the undigested old cuticle (Wigglesworth, 1970; Borror *et al.*, 1989; Drabek, 1991).

Moulting hormone (MH), also called ecdysone, from the prothoracic gland is responsible for the growth and moulting process. When the moulting hormone is secreted, the production of a hormone, the juvenile hormone, from the corpora allata is suppressed. In order to change form, i.e., from egg to larva to pupa to adult, the juvenile hormone levels decline or are completely suppressed. High levels of the juvenile hormone prevent the development into the next stage so that its persistence results in further larval / nymphal development with the insect maintaining juvenile characteristics (Wigglesworth, 1970; Drabek, 1991).

A new cuticle is soft and colourless and over the next few hours or days, sclerotin, protein and chitin are laid down and this darkens and hardens the new cuticle. Chitin is a polysaccharide which serves to support extracellular structures, especially the exoskeleton (Cohen, 1987; Borror *et al.*, 1989). In insects, it is a major component of chitoprotein structures such as the peritrophic membrane and cuticle, whereby it lends tensility and hardness to these structures. Chitin synthesis and its degradation are also under the control of the moulting hormone and this process involves an orderly sequence of events, with N-acetyl-D-glucosamine molecules polymerised and incorporated into chitin chains (Cohen, 1987; Borror *et al.*, 1989).

Since the exoskeleton does not allow continuous growth, all insects need to moult. Even during embryonic development, insects undergo a similar moulting process in the egg, so insecticides interfering with the moulting process have the potential to act not only on the larvae but on the egg as well, either through the female or directly (Ehrenfreund, 1990). Chitin is universally present in the insect cuticle, so interference with its formation may also be a target. The moulting process and chitin formation are highly differentiated and synchronised events and are thus susceptible to selective intervention (Cohen, 1987; Drabek, 1991).

Types of IGRs and their activity

IGRs are compounds capable of disrupting this synchronised sequence of events by interfering with the moulting process. Juvenile hormone analogues (JHAs) mimic the activity of the natural juvenile hormone. JHAs maintain artificially high levels of the juvenile-hormone mimicking compound. They inhibit moulting through interference with ecdysone biosynthesis, e.g. in *Blatella germanica* (Linnaeus), *Galleria mellonella* (Linnaeus) and *Ephestia cautella* (Walker). Alternatively, JHAs may have a suppressive effect on the corpora allata resulting in the possible inhibition of natural JH synthesis, e.g. in *Oncopeltus fasciatus* (Dallas), *Diploptera punctata* and *Manduca sexta* (Linnaeus) (Masner *et al.*, 1987). In this way, insects tend to maintain their larval form and not develop into the next stage. Supernumerary larvae may also develop, so that the treated larvae maintain their larval stage for longer than normal and possibly fail to develop further, i.e. pupate. Last instars of some totrix moths, (*Pandemis* spp., *Adoxophyes* spp. and *Archips* spp.) were not able to transform into adults after treatment with fenoxycarb (Drabek, 1991). Fenoxycarb is active against holometabolous and hemimetabolous insects, including Hymenoptera, Coleoptera, Dictyoptera, Orthoptera and Thysanoptera. Topical treatment of orthopteran *Melanoplus sanguinipes* (Fabricius) and *M. differentialis* (Thomas) nymphs with fenoxycarb resulted in twisted and misshapen wings, increased developmental time and a significant reduction in egg production (Capinera *et al.*, 1991). Similar results were obtained during the treatment of *B. germanica* (King and Bennett, 1989). Fenoxycarb is also effective in the control of a variety of economically important homopteran pests on citrus, e.g. *Chrysomphalus aonidium* (Linnaeus), *Aonidiella aurantii* (Maskell) and *Saissetia oleae* (Olivier) (Peleg, 1982).

Pyriproxyfen is another JHA used against economic pests. This compound affects the hormonal balance of insects and disrupts the normal process of insect development, resulting in pupal mortality and abnormal adults (Schaeffer and Mulligan, 1991). Pyriproxyfen also has strong ovicidal activity. For example, egg hatch in *Bemisia tabaci* (Gennadius) and *Spodoptera littoralis* (Boisduval) was suppressed after exposure to pyriproxyfen (Ishaaya and Horowitz, 1992; Ascher and Eliyahu, 1988). Effect on eggs was either direct ovicidal activity or through transovarian activities in the female. In agroecosystems, pyriproxyfen is used against pests such as *Cydia* *pomonella* (Linnaeus), *Grapholita molesta* (Busck), *A. aurantii* and *Ceroplastes floridensis* Comstock, among many others.

Against hymenopteran pests such as *Solenopsis invicta* Buren and *Reticulitermes flavipes* (Kollar), JHA treatment resulted in a shift in caste differentiation, intercaste production, lethality to immature stages and degeneration of the queen's reproductive organs. Such developments compromise the integrity of the colonies, resulting in brood decline and eventual colony death (Su and Scheffrahn, 1989; Banks and Lofgren, 1991).

Another class of IGRs acts as chitin synthesis inhibitors (CSIs), where moulting is inhibited by the disruption of chitin synthesis. CSIs act by disrupting the activity of chitin synthetase, an enzyme involved during cuticle formation, or disrupting the polymerisation of N-acetyl-D-glucosamine units during chitin formation (Cohen, 1987). This results either in the disruption of cuticle deposition or alteration of its composition (Ishaaya, 1981; Kobayashi *et al.*, 1989; Konno, 1990). One of the first IGRs developed was diflubenzuron, a CSI which alters cuticle composition. Its activity is mainly through the disruption of chitin formation, affecting the elasticity and firmness of the endocuticle. Eggs of the codling moth, *Lasperyresia pomeonella* (Linnaeus) and *Diaprepes abbreviatus* (Linnaeus) treated with diflubenzuron had reduced viability with emerging larvae failing to reach adulthood (Hoying and Riedl, 1980, Schroeder *et al.*, 1980). Similar effects were observed with *Musca domestica* Linnaeus, where treated larvae failed to reach adulthood, although egg viability was not affected (Ables *et al.*, 1975).

Buprofezin, another CSI, is known to inhibit the incorporation of N-acetyl-D-glucosamine into the chitin fraction and the resulting cuticle deposition. Symptoms of its effects include the formation of lesions in the cuticle, malformations of the new cuticle, disrupted production of the peritrophic membrane, ovicidal and chemosterilant effects (Nagata, 1986; Cohen, 1987; Kobayashi *et al.*, 1989, Hattingh and Tate, 1995). In the homopteran *Nilarpavata lugens* Stal nymphs, the deposition of new cuticle and digestion of the old cuticle were severely inhibited after buprofezin treatment. Nymphal mortality occurred during delayed moults and adults had lower reproductive capacity (Cohen, 1987; Kobayashi *et al.*, 1989).

A wide range of homopteran pests in orchards are controlled using buprofezin, including *A. aurantii*, *S. oleae*, *Trialeurodes vaporarium* (Westwood), *B. tabaci* and *Unaspis citri* (Comstock) (Yarom *et al.*, 1988; Roditakis, 1990; Smith and Papacek, 1990, Gerling and Sinai, 1994). Mortality in immature stages usually occurred at the end of the instar or end of larval development (Heinrichs *et al.*, 1984; Konno, 1990). Teflubenzuron also inhibits chitin formation, subsequent cuticle formation and moulting. It produces symptoms similar to buprofezin and is active against *Plutella xylostella* Linnaeus, *Sitophilus oryzae* (Linnaeus), *S. oleae* among other pests (Ismail and Wright, 1991; Lampson and Morse, 1992; Eisa and Ammar, 1992).

The activity of IGRs is not immediate like conventional insecticides. These compounds elicit symptoms of poisoning a few days after treatment, usually during or immediately after the moulting period (Nagata, 1986; Cohen, 1987). Symptoms are thus apparent at the end of larval / pupal development and at the end of larval formation during embryonic development (Masner *et al.*, 1987; Drabek, 1991). In larval stages, mortality is due to their inability to shed off the old cuticle, while eggs are unable to hatch (Cremlyn, 1978; Hassall, 1992). This results in significant reduction in oviposition rates as well as suppression of progeny in pest species (Cremlyn, 1978; Abo-El-Ghar, 1992; Nasseh *et al.*, 1992).

While having a broad-spectrum activity against agricultural pests, e.g. on pears, apples, peaches, citrus and vegetables, IGRs supposedly have low environmental impact (Zungoli *et al.*, 1983, Hassall, 1992, Schreurer, 1994). Various studies indicate that there is no accumulation of these compounds in the tissue of vertebrates like fish (Apperson *et al.*, 1978) and have low toxicity, if any, against common beneficial insects. Fenoxycarb, diflubenzuron, buprofezin and pyriproxyfen were reported to have no effect on beneficial parasitoids such as *Metaphycus bartletti* Annecke and Mynhardt, *Muscidifurax raptor* Girault and Saunders; *Comperiella bifasciata* Howard, *Aphytis holoxanthus* DeBache and *A. lingnanensis* Compere, where the rate of parasitism and parasitoid development were not affected (Ables *et al.*, 1975; Peleg, 1982; Ishaaya *et al.*, 1991/92). Economic predators like *Typhlodromus rhenanoides* Athias-Henriot and *Orius* species were not affected by buprofezin and pyriproxyfen respectively (Nagai, 1990; Ragusa Di Chiara *et al.*, 1993). Brood colonies of the honey bee, *Apis mellifera* Linnaeus, were not affected by diflubenzuron exposure (Schroeder *et al.*, 1980). In addition to this, IGRs are not phytotoxic, have been claimed to have negligible drift effects when sprayed at the recommended wind conditions, and degrade rapidly into harmless compounds in the soil (Davis *et al.*, 1991; Hassall, 1992; Nicholson, 1994).

Contrary to the above-mentioned positive aspects, IGRs are now appearing to have some detrimental effects on non-target species, especially beneficial arthropods (Ware, 1982, Hattingh, 1996). Since most IGRs are chitin synthesis inhibitors, they have the potential to affect other non-target arthropods as chitin is a major component of arthropod exoskeletons. Toxic effects may be manifested as death of exposed larvae, or failure of eggs to hatch. Various studies have indicated that non-target and beneficial insects are affected by IGRs. Coccinellid predators like *Chilocorus bipustulatus* Linnaeus, *Cryptolaemus montrouzieri* Mulsant, *Stethorus punctum* (LeConte), *Rodolia cardinalis* and *C. nigritus* had larval mortality, reduced egg viability and reproduction and reduction in the number of late instars completing their development, following exposure to IGRs like diflubenzuron, buprofezin, fenoxycarb and methoprene (Peleg, 1982; Smith and Papacek, 1990; Loia and Viggiani, 1992; Biddinger and Hull, 1995, Hattingh and Tate, 1995). Predatory mites such as *Typhlodromus athiasae* Porath and Swirski, *Phytoseiulus persimilis* Athis-Henriot and *T. rhenanoides* Athias-Henriot suffered reduced fecundity after exposure to triflumuron and fenoxycarb (Mansour *et al.*, 1993; Ragusa Di Chiara *et al.*, 1993). Other non-target species affected were *Forficula auricularia* Linnaeus and *Encarsia luteola* Howard when treated with diflubenzuron and buprofezin respectively (Sauphanor *et al.*, 1993; Gerling and Sinai, 1994). Overall IGR effects were a reduction in predator and parasitoid populations within a few generations and increased pest populations, e.g. the phytophagous mite *Tetranychus cinnabarinus* (Biosduval) had increased fecundity after exposure to triflumuron and fenoxycarb (Mansour *et al.*, 1993).

Nontarget species in areas adjoining treated agroecosystems, as well as water bodies adjacent to these treated areas and receiving runoff from them, are also affected by pesticide applications (Pimentel and Levitan, 1986; Grout, 1998). Arthropods observed in water bodies adjacent to sprayed agroecosystems suffered significant mortality of all developmental stages due to decreased emergence of larvae (from eggs) and adults (from earlier stages) in populations of *Apocyclops* spp., *Baetis* spp., chironomids and littoral species (Sainz-de-los-Terreros and Garcia-de-Jalon, 1991; Sundaram *et al.*, 1991). In diflubenzuron treated lakes, crustacean populations were suppressed temporarily but then recovered (Apperson *et al.*, 1978). Fenoxycarb and diflubenzuron also accumulated in the bluegill sunfish, *Leponus macrochirus* Rafinesque. The residues however, did not persist and declined when fish were placed in untreated water (Apperson *et al.*, 1978; Schaeffer *et al.*, 1987). These results however, indicate that arthropod and non-target organisms in IGR-contaminated ecosystems are susceptible to inadvertent, detrimental non-target effects.

The emergence of studies indicating that IGRs also have undesirable effects and are not as specific as previously thought should serve as an indication that this insecticide class also needs to be treated with the same caution as the previous generations of synthetic insecticides. In the 1980s, outbreaks of *Icerya purchasi* in Israel increased in frequency. These outbreaks were thought to be linked to increased IGR applications, such as fenoxycarb applied for soft and armoured scale control (Mendel and Blumberg, 1991). Recent developments in southern Africa also indicate that IGRs could lead to the disruption of the ecological balance established through integrated pest management of economic pests (Hattingh, 1996). These warnings that this new class of insecticides may not be as innocuous to biological control agents suggests that further investigation is needed, especially in the field.

AIMS OF THE PRESENT STUDY

The aims of the study are :

(i) To determine the assemblage composition of coccinellid species in selected citrus orchards in Swaziland.

(ii) To assess and compare the effects of insect growth regulators on this assemblage.

(iii) To assess specific effects of insects growth regulators on selected non-target species.

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CHAPTER 2

COCCINELLID DISTRIBUTIONAL RESPONSE TO AN AGRICULTURAL LAND MOSAIC.

ABSTRACT

Tambuti estate, Swaziland, is an agricultural mosaic consisting of citrus orchards, pine windbreaks, river borders and natural veld. The distribution of coccinellids within the habitat types and the influence of temperature, relative humidity, ground insolation and prey presence was assessed over a two year period. The highest coccinellid densities and species richness occurred within the orchard habitats. Habitats on the southern and western boundaries of the estate, which bordered the Great Usuthu river were closer to the orchard habitat in species composition than the natural veld habitat. The presence/absence of prey was apparently the most important environmental variable which determined coccinellid distribution in the agricultural landscape. A significant correlation was observed between prey presence and coccinellid densities. Relative humidity, temperature and ground insolation had no significant effect on coccinellid densities, although temperature and ground insolation were negatively correlated with coccinellid densities.

Cheilomenes lunata (Fabricius), *Scymnus* species and *Nephus* species were the most widely dispersed species, occurring in all the habitats sampled while the economically important species, *Rodolia cardinalis* (Mulsant) was observed only in the citrus orchards. The coccinellid species sampled were not good indicators of habitat type. All habitat types appear to be essential for continued presence and survival of coccinellid species, especially those restricted to particular habitat types like the economically important species. The heterogenous landscape mosaic provides for maximum plant and coccinellid diversity thus increasing the potential for natural control of pest species in the citrus orchards.

INTRODUCTION

Insect distribution and habitat loss in agricultural landscapes

In an undisturbed natural ecosystem, the landscape usually consists of an extensive and connected area which has a dominant role in the functioning of the landscape. Such an area is referred to as the matrix and for most organisms like insects, this constitutes a continuous habitat where the favourable habitat of the species is more extensive than their dispersal range (Forman and Gordron, 1986; Majerus, 1994). With the increasing utilisation of land, anthropogenic effects on natural matrices have become evident as a result of the breakdown of such landscape elements into smaller units, creating a mosaic of habitat patches within the natural vegetational matrix. Habitat fragmentation results from the division of natural and semi-natural habitats into smaller patches. Agriculture is the basic mechanism by which humans manipulate the natural landscape and is thus one direct cause of the loss of natural habitats (Gilpin *et al.*, 1992; Fry, 1995). The resultant landscape is usually a combination of habitat patches in the matrix, with possible connection by corridors and is referred to as a land mosaic. The variety of patches means that the land mosaic is highly heterogenous, with varying types and suitability of habitats for the organisms occurring within the patches (Forman, 1997).

The disturbance of a landscape for agricultural use inevitably results in habitat destruction and loss for organisms occurring within that biotope. Due to their small size and dispersal ability, especially apterous species and juvenile stages, insects are one of the main groups which can lose their habitat as a result of agricultural activity. Insects are sensitive to ecosystem changes or disruptions and their distribution is affected by changes in the availability of suitable habitats, i.e. habitats where the organisms can reproduce, oviposit, find shelter and essential feeding material. If such habitats are reduced by anthropogenic activity, there is a reduction in the total amount of habitat available to the insects. This poses a threat to their survival since habitat destruction is one of the major factors leading to insect species scarcity (New, 1984; Collins and Thomas, 1991; Samways, 1994).

In the resultant agricultural mosaic, the insect habitats may be patchy with the insects' favourable patches still close enough for the insects to be able to move from one to the other. Alternatively, the insects' favourable habitats may be so fragmented as to be completely isolated from each other, so that the favourable patches are too far from one another for the insects to migrate to. The formation of isolated habitat patches may pose a threat for the continued survival of an insect population. Insects need a variety of habitats at various stages of their life-cycle and as such, if habitat patches are too far apart as a result of habitat destruction in the surrounding area, insects may not be able to reach the required habitat. This would ultimately result in population decline as species would be unable to perpetuate themselves. Additionally, the agricultural landscape is highly dynamic with various disturbances, e.g. pesticide applications, mowing, harvesting, etc. so that even if a population managed to survive abundantly in a particular patch, it is still vulnerable to local extinction due to the management practises (Duelli, 1988; Fry and Lonsdale, 1991; Moore, 1991).

While insects are considered as man's most important competitor for plant resources in an agricultural landscape, their preservation within this landscape is still of great importance. Insect communities are a balanced network and fully dynamic. When one species is decimated due to some anthropogenic intervention, other component species may be adversely affected through direct or indirect linkages (New, 1984; Gilpin *et al.*, 1992). This is especially true for insects predator/prey (parasitoid/host) relationships. Due to their predaceous (parasitoid) activity, many insects are considered important natural control factors of agricultural pests. The activity of natural predators and parasitoids is thus considered to be an effective means for controlling economically damaging insects (Pyle *et al.*, 1981; Kromp, 1989). Their efficacy is maximised if the predators are able to attack the prey (pests) on all the prey's host plants. Since their prey may not be restricted to one single habitat, these predators will also tend to migrate between favourable and unfavourable habitats within the landscape in search of prey, e.g. *Adalia bipunctata* (Linnaeus) subsists on pollen in bushy vegetation when aphids are rare on herbaceous plants in order to survive. However, the effectiveness of these predators may be compromised if the prey occurs in separate habitats and the predators are unable to reach these habitats to find prey (Banks, 1956; Hemptinne and Dempster, 1986; Jimenez, 1986; Booij and Noorlander, 1992), as may happen in a fragmented landscape.

Importance of spatial heterogeneity

The spatial pattern of the various habitats within the agricultural mosaic is important for the mobility of insects, especially the predators. The presence of habitat corridors or favourable habitat patches would ensure continued migration between the various habitat patches. In addition to providing passage between habitats in search of feeding material, these areas would also serve as reservoir habitats, whereby they would enable the maintenance of breeding populations thus minimising fragmentation of the population gene pool after habitat fragmentation. The elements within these patches would provide for predator recovery after a disturbance, e.g. pesticide application, deterioration of crop quality, etc. in the managed habitat (New, 1984; Duelli, 1988; Booij and Noorlander, 1992).

The maintenance or creation of a mosaic of natural and semi-natural habitats thus offers a heterogenous spectrum of habitats and provides for higher insect diversity. The preservation of these insects, especially predaceous species, increases the potential for natural enemy control. The activity of predaceous insects like carabids, staphylinids and coccinellids in pest reduction is well documented. In addition, predator conservation also results in increased faunal diversity of the agricultural landscape, which can have certain species confined in the arable environment, e.g. 25% of carabids in Europe are confined to arable fields (Murdoch *et al.*, 1972; Booij and Noorlander, 1992). Predator conservation cannot be achieved only through the reduction of pesticide use, the spatial pattern of natural and semi-natural habitats also has a major impact on the maintenance of these populations.

In agricultural ecosystems, coccinellids are one of the most important predatory groups of insects, and preservation of all coccinellid life stages is important. The adults feed in a variety of habitats before finding suitable oviposition sites, e.g. *Propylea quatuordecimpuncatata* (Linnaeus) is an abundant and economically important species in stone fruit orchards infested with *Hyalopterus pruni* (Geoffroy) and *Anuraphis persicae* (Passerini) in early summer before moving onto herbaceous plants in meadows during midsummer to build up their populations. The juvenile stages contribute to natural control with their voracity and abundance (Mills, 1982). Coccinellids are known to provide natural control of a variety of agricultural pests and their preservation through the maintenance of an array of habitats is undoubtedly beneficial to agriculture. Members of this group have varying habitats and range from the stenotopic to the eurytopic species (Hodek, 1973; Majerus, 1994).

Occurrence of coccinellids in specific habitats seems to be determined by various environmental variables, e.g. the occurrence of prey and physical conditions of the environment (Hodek, 1967; 1973). Various studies and reviews do not provide a clear indication of the effect of these variables on coccinellid distribution. While Majerus (1994) and Hodek (1973) are in agreement about the importance of prey availability on coccinellid distribution, Banks (1956) concluded that coccinellid distribution was influenced by the presence of adults rather than prey. Coccinellid species also respond differently to similar environmental variables, e.g. while *Coleomegilla maculata* (Der Geer) had a positive response to relative humidity in cornfields, *Hippodamia convergens* Gue'rin was insensitive to the same variable (Hodek, 1973).

In addition to the uncertainty about coccinellid response to environmental conditions, very few ecological studies have been done on this family of insects in the southern African region. Tambuti estate is an agricultural mosaic consisting of anthropogenic and natural habitats, i.e. citrus orchards, pine windbreaks, river borders and natural veld. The study was thus carried out in order to investigate the distribution of coccinellids in this land mosaic as well as their response to physical environmental conditions.

MATERIALS AND METHODS

Sites and sampling

1. Managed and natural habitats - Field work was carried out at Tambuti estate, Swaziland (26.43S 31.46E) (Fig. 1). Transect lines were drawn from the north to south and east to west through the estate. Within the estate, eight orchards were selected along these directions, with four orchards along each direction. At the edge of the orchards, transects were extended into the natural veld. Sampling points were at 100m intervals and at each interval, three sites were sampled. Towards the northern and eastern directions, transects extended up to 400m into the natural bushveld. In the south the transect extended up to about 180m while in the west it only extended up to approximately 80m. The southern and western sides of the estate were bordered by the Great Usuthu river, thus the limitation in sampling distances.

Sampling was carried out once a month using a beating tray supplemented by visual sampling. At each sampling site, a tree or shrub and surrounding grass were sampled for any coccinellid species, with the adult, pupal and larval stages counted. Each interval thus had three sampling sites and sampling was carried out from February 1996 to January 1998, making up a sampling period of twenty-four months.

Initially all coccinellids were collected and sent for identification but as the study progressed with increasing familiarity with the coccinellids, easily identifiable species were released after

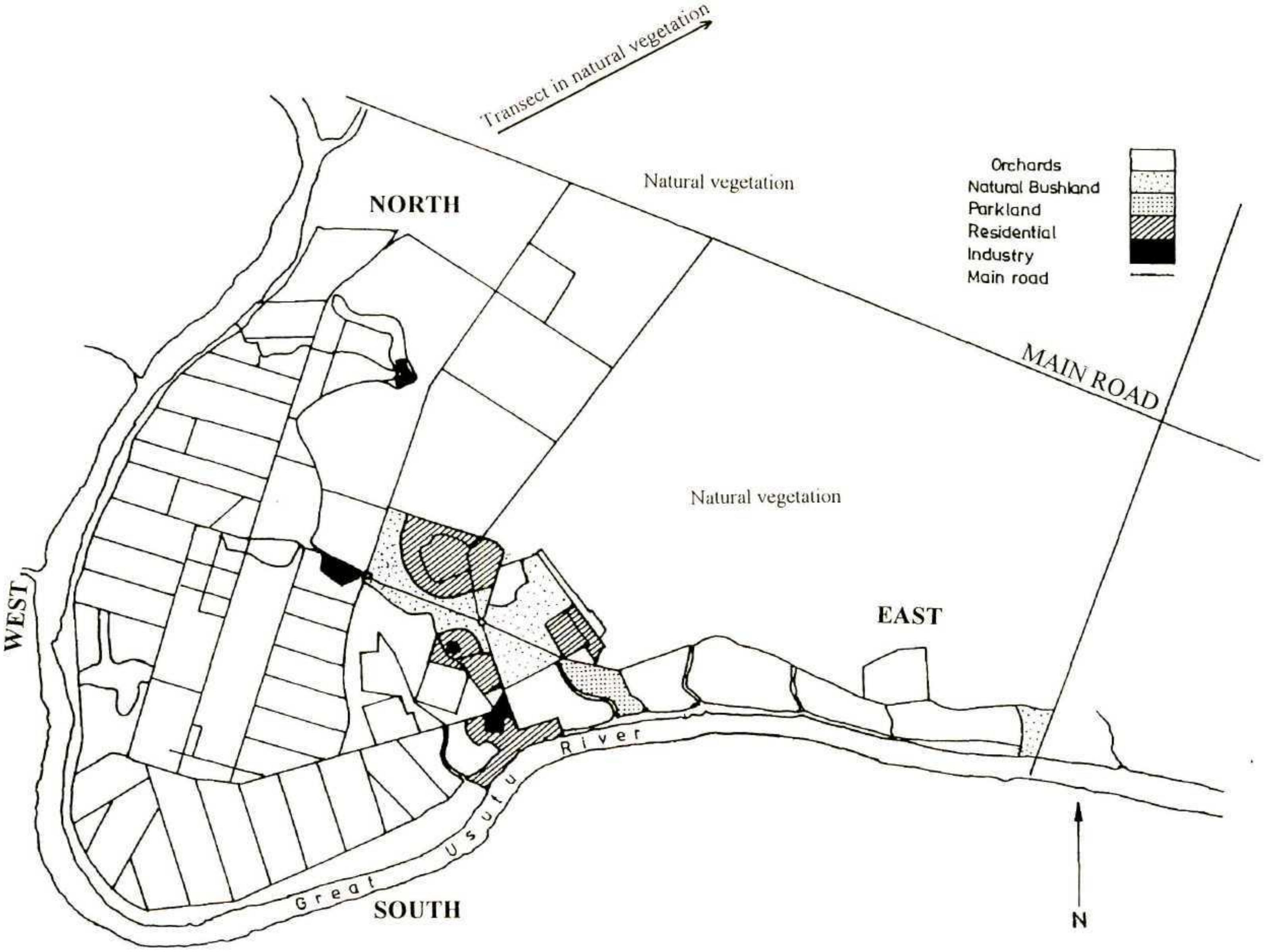

Fig. 1 Map of Tambuti estate. North, east, west and south indicate direction of transects extending into the natural habitats. 1 : 26 000 approx.

identification and counting.

2. Natural vegetation in bushveld - In order to investigate the species composition of coccinellids along a natural vegetation type, sampling was carried out within the bushveld. The site was in Tambuti estate, away from the orchards. Sampling was carried out along a transect extending 100m from the main road up to 500m into the veld. Each interval also had three sampling sites, where coccinellids were sampled on vegetation at each site. This habitat was also sampled monthly for twenty-four months.

Environmental variables - At each of the sampling sites, air temperature, relative humidity and percentage ground insolation were measured. Percent ground insolation was used as a measure of the amount of sunlight penetrating through the vegetation, to which the organisms on vegetation are exposed. This was measured as a percentage of a $1m^2$ quadrat exposed to sunlight under the vegetation. The presence/absence of prey as well as the vegetation type on which the coccinellids were found were also noted.

Data analyses - The coccinellid counts were log (x+1) transformed, while the percentage data, i.e. relative humidity and ground insolation were arcsin transformed. Temperature data was untransformed. Statistical analyses were carried out using Primer, a multivariate analysis statistical package and SPSS packages. In order to minimise the effect of vagrant or rare species on the results, species which contributed 3% or less to the total coccinellid densities were excluded from the final analyses (Clarke and Warwick, 1994).

RESULTS

1. Total population analyses - A total of 1645 coccinellids was collected from all sampling sites, representing 35 species (Table 1). Monthly counts from all habitats sampled during the whole sampling period were used to calculate the cumulative species counts for the overall coccinellid population (Fig. 2). There was a steady increase in the number of coccinellid species collected over the first year. After February 1997, there were fewer species additions. A plateau was reached in October 1997 which was maintained to the end of the sampling period.

Monthly variations - The highest number of individuals was collected during the months of February in both years. Coccinellid populations subsequently declined after this month, with the lowest levels observed during the months of November/December. The 1996/97 season showed an increase in population densities during spring (August). This pattern was not evident in the second year (Fig. 3). Despite monthly variation in the number of individuals, species richness was relatively stable throughout the year (Fig.4). Although not statistically significant, the first year had a relatively higher and uniform species richness compared to the second year, which was notably lower in July.

The managed orchard habitat had significantly higher population densities than the natural habitats (ANOVA, $p = 0.004$). Further analyses indicated that there were no significant differences in the coccinellid population densities between the two years (ANOVA, $p = 0.191$). Data were thus combined in all subsequent analyses.

Table 1. Total coccinellid densities and species collected at Tambuti estate during 1996/98. 1 = orchards, 2 = eastern transect, 3 = northern transect, 4 = southern transect, 5 = western transect, 6 = habitat in bushveld. Numbers in **bold** represent species percentage in each habitat.

SPECIES	1	2	3	4	5	6
COCCINELLINAE						
Cheilomenes lunata (Fabricius)	183	67	30	21	10	47
	16.15	**51.54**	**36.14**	**21.88**	**16.95**	**32.41**
Cheilomenes propinqua (Mulsant)	16	1	0	0	0	0
	1.41	**0.77**	**0**	**0**	**0**	**0**
Declivata hamata (Schoenherr)	4	0	0	0	2	0
	0.35	**0**	**0**	**0**	**3.39**	**0**
Dysis decempunctata (Sicard)	0	1	0	0	0	0
	0	**0.77**	**0**	**0**	**0**	**0**
Hippodamia variegata (Goeze)	242	0	0	4	0	0
	21.36	**0**	**0**	**4.17**	**0**	**0**
Psyllebora variegata (Fabricius)	5	0	0	0	0	3
	0.44	**0**	**0**	**0**	**0**	**2.07**
Dysis sp.	72	6	0	0	0	13
	6.35	**4.62**	**0**	**0**	**0**	**8.97**
COCCIDULINAE						
Rodolia cardinalis (Mulsant)	127	0	0	0	0	0
	11.20	**0**	**0**	**0**	**0**	**0**
Rodolia sp.	6	0	0	0	0	0
	0.53	**0**	**0**	**0**	**0**	**0**
Novius sp.	4	0	1	0	0	0
	0.35	**0**	**1.20**	**0**	**0**	**0**
CHILOCORINAE						
Platynaspis capicola Crotch	11	2	0	2	0	0
	0.97	**1.54**	**0**	**2.08**	**0**	**0**
Chilocorus distigma (Fabricius)	4	0	0	0	0	0
	0.35	**0**	**0**	**0**	**0**	**0**
Brumus sp.	1	0	0	0	0	0
	0.09	**0**	**0**	**0**	**0**	**0**
EPILACHNINAE						
Epilachna bifasciata (Fabricius)	11	0	0	0	1	0
	0.97	**0**	**0**	**0**	**1.69**	**0**
Epilachna paykulli Mulsant	2	0	0	3	10	0
	0.18	**0**	**0**	**3.13**	**16.95**	**0**
Epilachna hirta (Thunberg)	1	0	0	0	1	0
	0.09	**0**	**0**	**0**	1.69	0

N.B. Table split across pages 49 - 50

SPECIES	1	2	3	4	5	6
SCYMNINAE						
Ortalia ochracea Weise	6	0	0	17	0	5
	0.53	**0**	**0**	**17.71**	**0**	**3.45**
Scymnus prob. *levaillanti* (Mulsant)	89	2	0	19	12	0
	7.86	**1.54**	**0**	**19.79**	**20.34**	**0**
Scymnus sp.	283	42	44	21	8	54
	24.98	**32.31**	**53.01**	**21.88**	**13.56**	**37.24**
Nephus sp.	32	5	4	2	2	8
	2.82	**3.85**	**4.82**	**2.08**	**3.39**	**5.52**
STICHOLOTINAE						
Pharoscymnus exiguus Weise	4	1	2	0	0	4
	0.35	**0.77**	**2.41**	**0**	**0**	**2.75**
Pharoscymnus uncosiphonalis Fursch	2	0	0	0	0	1
	0.18	**0**	**0**	**0**	**0**	**0.69**
Serangium sp.	7	0	1	0	0	0
	0.62	**0**	**1.20**	**0**	**0**	**0**
Unidentified larvae	7	0	0	0	2	2
	0.62	**0**	**0**	**0**	**3.30**	**1.38**
Unidentified pupae	4	0	1	2	3	5
	0.35	**0**	**1.20**	**2.08**	**5.08**	**3.45**
Unidentified adults	9	2	0	5	0	3
	0.80	**1.54**	**0**	**5.20**	**0**	**2.06**
TOTAL NO. COCCINELLIDS	1133	130	83	96	59	145
TOTAL NO. OF SPECIES	31	11	7	12	12	13

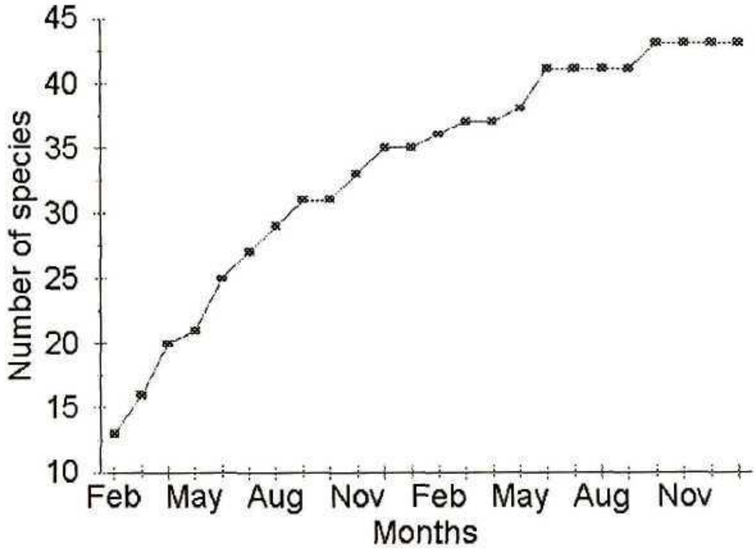

Fig. 2 Cumulative number of species collected from Tambuti estate over two year period.

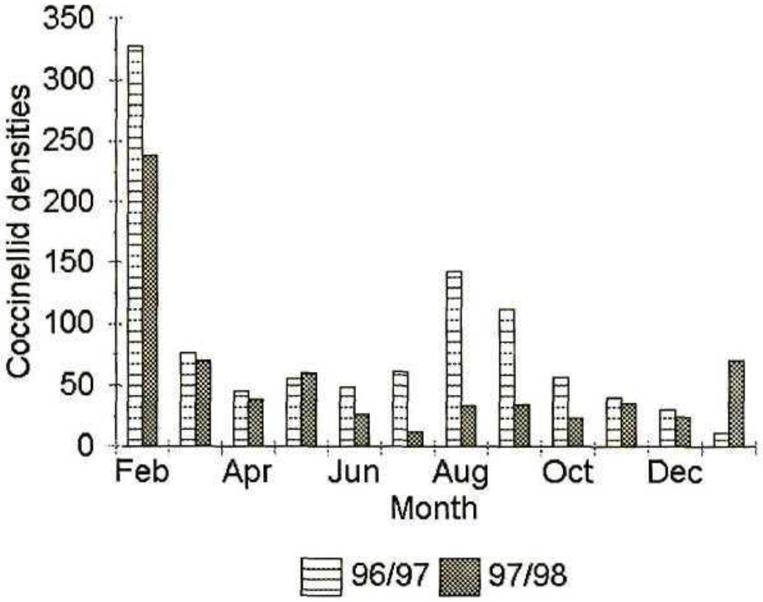

Fig.3 Total number of coccinellids collected per month over the sampling period.

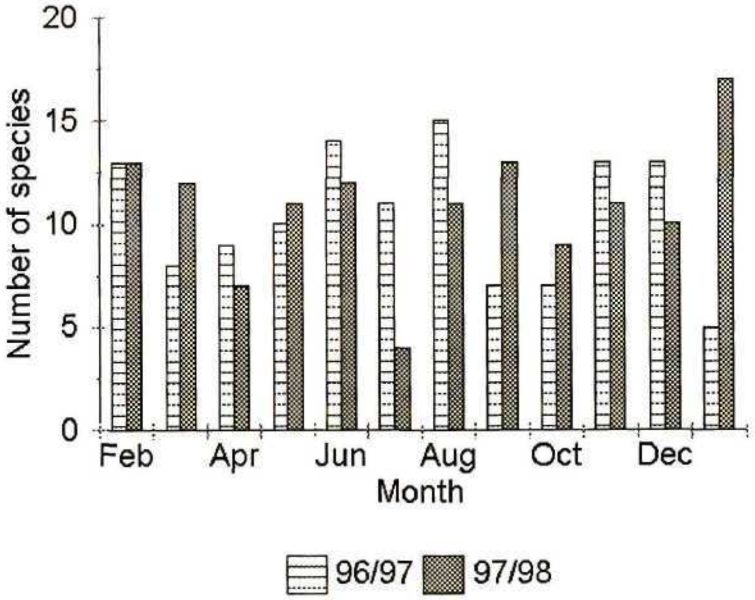

Fig.4 Species richness for each month over the sampling period.

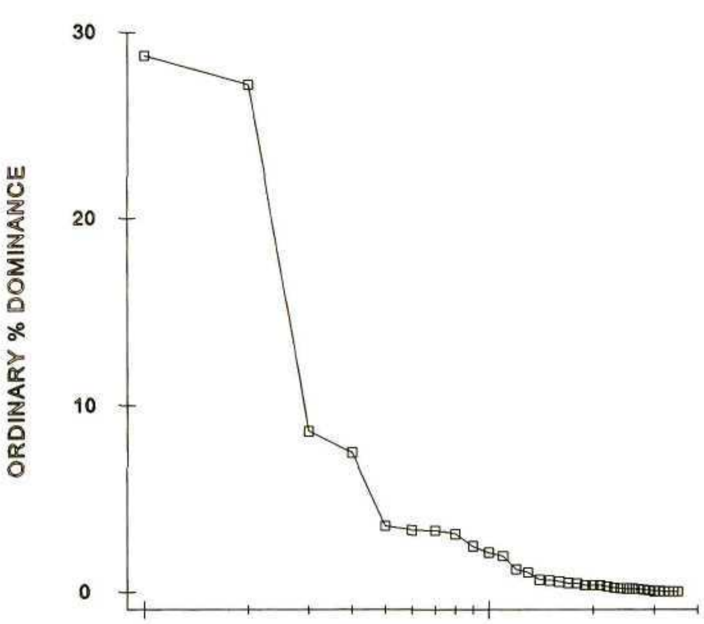

Fig. 5 Rank abundance curve for all sites at Tambuti estate.

The species rank abundance curve (Fig. 5) indicates that the estate was characterised by two species represented by a high number of individuals and a few with intermediate densities. A number of coccinellid species had only a few individuals sampled.

2. Managed and natural habitats - The highest coccinellid densities were observed in the managed orchard habitats, while densities in the natural habitats were significantly lower (Fig. 6) ($p = 0.009$). This habitat also had the highest number of species, with a total of 31 species collected. The natural habitat along the transects in the southern and western directions had higher species diversity compared to those in the eastern and northern habitats (Fig.7). The former transects were closer to the orchards and were bordered by the river.

Analyses of species distribution were carried out relative to location (north, east, west, south) and distance of sampling interval from the orchard habitat (100m, 200m, 300, 400m).

Location - The habitats with the most even species distribution were the orchards, and those in the south and west of the orchards, the last two being natural habitats. Habitats in the northern and eastern sides of the estate had few species represented by many individuals, few others with low numbers and none with intermediate densities as in the orchards (Fig.8).

Distance - Sampling sites at 300m and 400m from the orchards were characterised by at least one dominant species and low numbers of other species (Fig.9). At 200m, dominance by a species was still evident. At 100m, there was a more even distribution of species, with most species represented by intermediate values compared to the sites further away. The orchards still had the most even species distribution.

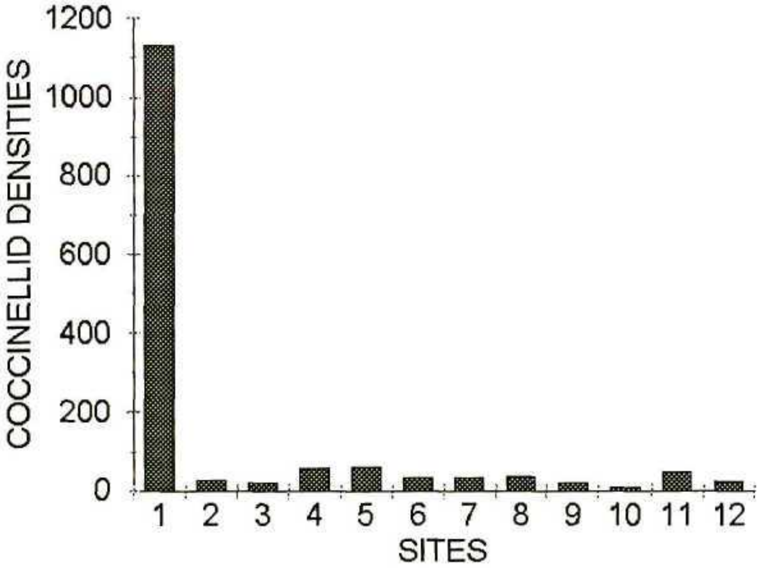

Fig.6 Coccinellid densities at each site. 1=orchards, 2=100m east, 3=100m north, 4=100m south, 5=100m west, 6=200m east, 7=200m north, 8=200m south, 9=300m east, 10=300m north, 11=400m east, 12=400m north.

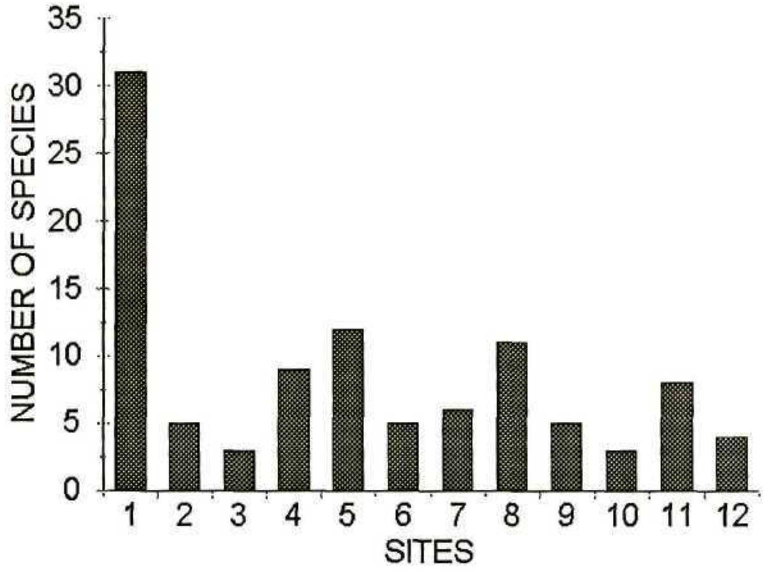

Fig. 7 Species richness at each of the sites sampled. 1=orchards, 2=100m east, 3=100m north, 4=100m south, 5=100m west, 6=200m east, 7=200m north, 8=200m west, 9=300m east, 10=300m north, 11=400m east, 12=400m north.

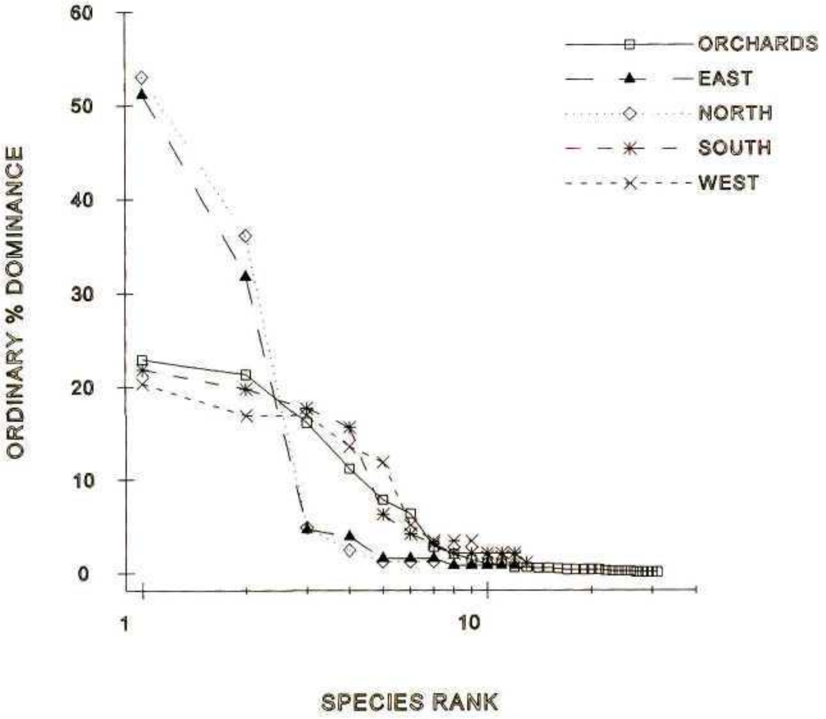

Fig. 8 Rank abundance curves for sites according to direction of transect intervals from orchards.

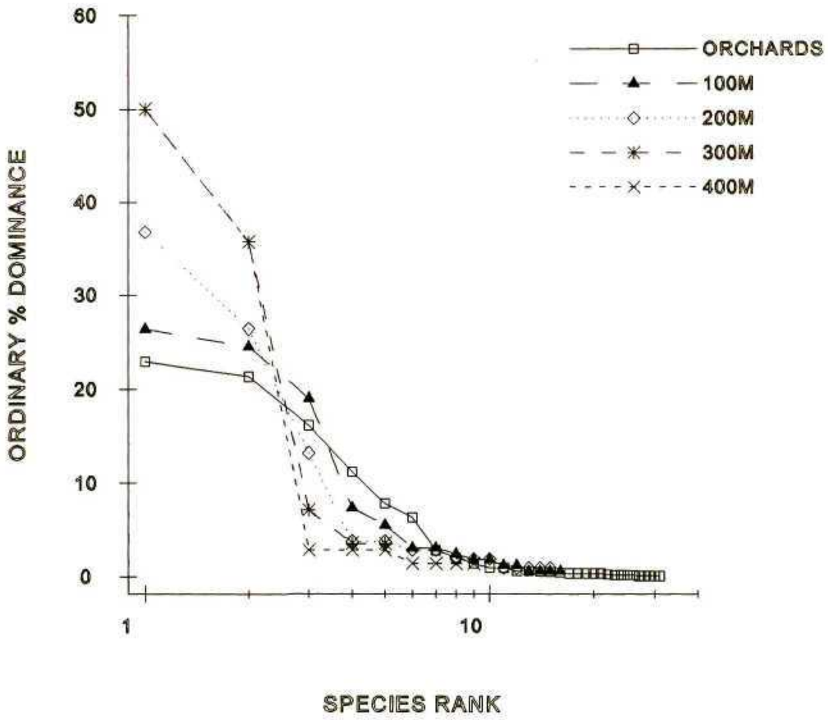

Fig. 9 Rank abundance curves for sites according to distance interval from orchards.

Cluster analyses and ordination by nonmetric multidimensional scaling (NMDS) - The total average densities from each sampling interval, rather that individual sampling sites, were used in order to make the data more interpretable (Ludwig and Reynolds, 1988; Clarke and Warwick, 1994). The dendogram produced indicated that the sites could be divided into three distinct groups at the 50% level of similarity (Fig. 10). The first group consisted of the orchards and sites at 100m from the orchards in the southern and western boundaries. Species common to sites in this group were *Scymnus* prob. *levaillanti* (Mulsant), *Cheilomenes lunata* (Fabricius), *Epilachna paykulli* Mulsant, *Scymnus* and *Nephus* species. The group divided further so that the sites within the orchards were separated from the two sites at the river borders. The orchards were characterised mainly by the presence of certain species, e.g. *Rodolia cardinalis* (Mulsant), *Chilocorus distigma* (Klug) , *Brumus* sp., *Hippodamia variegata* (Goeze).The southern sites at 200m formed the second group and species common in this group were *Platynaspis capicola* Crotch, *Ortalea ochracea* Weise, *C. lunata*, *E. paykulli*, *Scymnus* and *Nephus* species. The third group comprised all sites at the intervals in the northern and eastern habitats. Species common to sites in this group were *Pharoscymnus exiguus* Weise, *C. lunata*, *Scymnus* and *Nephus* species. This further divided so that the interval at 300m north was separated. The rest of the sampling sites in the northern and eastern transect formed their cluster. Differences between sites were also indicated by ANOVA ($p = 0.02$), where the orchard coccinellid densities were significantly different from those in the natural bushveld habitats, i.e. northern and eastern boundaries ($p = 0.05$). NMDS ordination also had the sites separated into groupings similar to those of the dendogram (Fig.11).

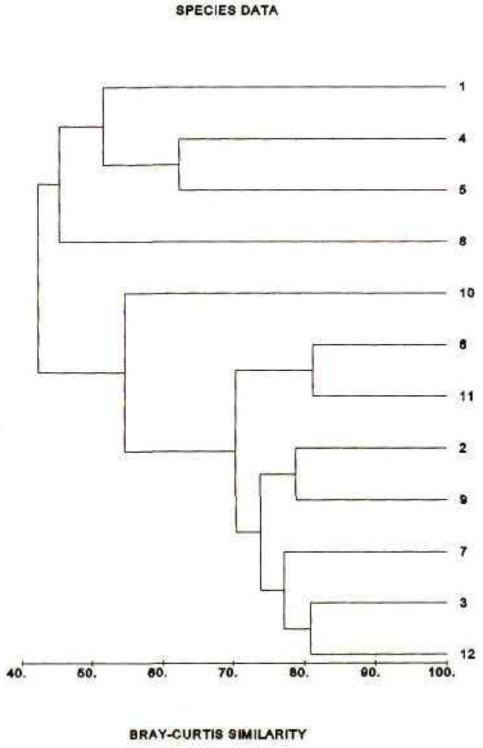

Fig. 10 Dendogram from cluster analysis of sites along transects. 1=orchards, 2=100m east, 3=100m north, 4=100m south, 5=100m west, 6=200m east, 7=200m north, 8=200m south, 9=300m east, 10=300m north, 11=400m east, 12=400m north.

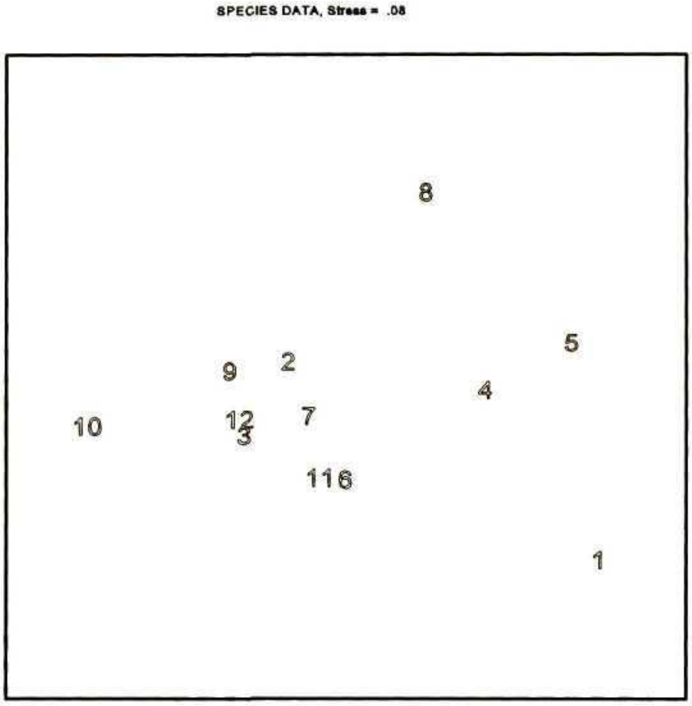

Fig. 11 Nonmetric multidimensional scaling (NMDS) ordination plot of sites sampled. See Fig.10 for site representation by numbers.

Environmental variables - A temperature gradient was observed where temperature was highest in the natural habitats (north and east) and lower in the sites near the river (west and south) while the orchards also experienced relatively high temperatures. Temperature was negatively correlated with coccinellid densities ($r = -0.312$, $p = 0.05$). Relative humidity had the highest values at sites nearest to the river. Lower values of this variable were observed in the northern and eastern habitats, which were furthest away from the irrigated orchards and the river. Correlation between coccinellid densities and %RH was 0.303 ($p = 0.054$). Ground insolation was lowest at sites with more vegetation cover, i.e. the orchards and river borders (western) habitats. The natural bushveld habitats had the highest insolation, especially in winter when there was minimal vegetation growth. Although not significant, coccinellid densities were negatively correlated with insolation ($r = -0.217$, $p = 0.174$) (Appendix 1).

Qualitative data were used for prey, where the presence or absence of prey were noted. The majority of sites examined had prey associated with coccinellids at some time during the year. However, no prey were observed at the sites on the west as well as on trees which were at least 300m from the estate in all directions. Prey were significantly correlated with coccinellid densities ($r = 0.379$, $p = 0.014$).

Simple multiple regression was also used to further test the significance of the environmental variables measured and the model was significant ($p = 0.025$), where the temperature, insolation and relative humidity had no significant impact on coccinellid densities, while the presence/absence of prey was significant ($t = 2.740$, sig. $t = 0.0095$).

3. Natural vegetation in natural bushveld. The natural habitat did not have an even distribution of species and was characterised by two species represented by a high number of individuals and several species with low representation (Fig. 12). This was also confirmed when the species abundance was analysed per sampling interval along the habitat (Fig. 13). All the sites at the various sampling intervals were characterised by one species with a high number of individuals. The first four distance intervals had species with intermediate representation and a number of species represented by low densities. The sites in this habitat therefore did not have a uniform distribution of species.

Species distribution did not vary significantly between the sampling intervals ($p = 0.920$), with the coccinellid densities and species richness being relatively uniform throughout the habitat (Fig. 14 and 15). None of the environmental variables, i.e. prey ($r = 0.228$, $p = 0.415$), %RH ($r = 0.197$, $p = 0.481$), temperature ($r = -0.197$, $p = 0.481$) and insolation ($r = -0.076$, $p = 0.787$), had any significant correlation with coccinellid densities. Although not significant, temperature and insolation were negatively correlated with prey density.

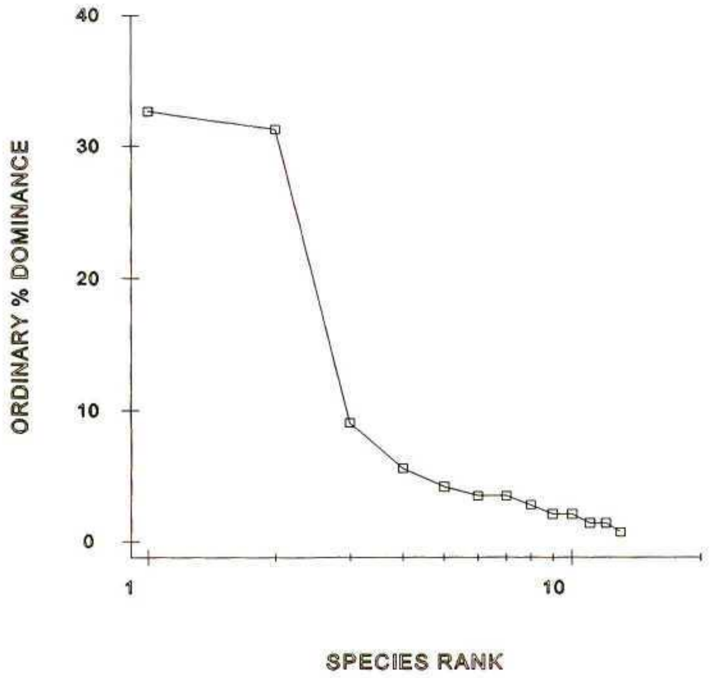

Fig. 12 Rank abundance curve for all sites in the natural vegetation habitat.

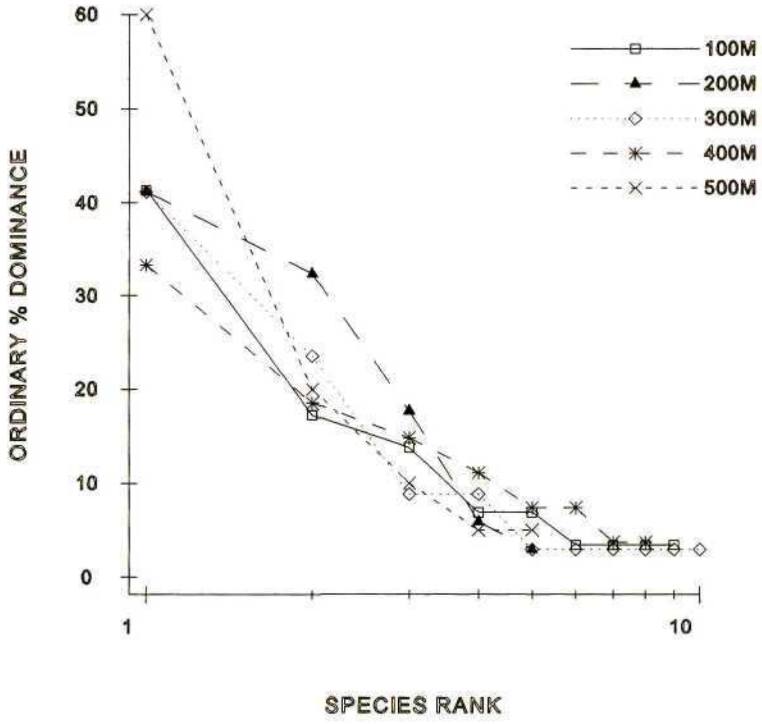

Fig. 13 Rank abundance curves for each distance interval in the natural vegetation habitat.

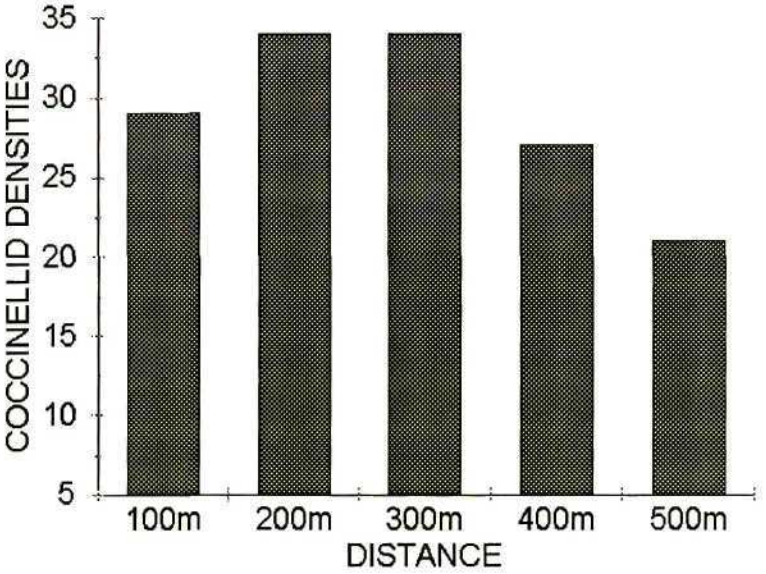

Fig. 14 Total number of coccinellids (coccinellid densities per site) at each sampling interval in the natural vegetation habitat.

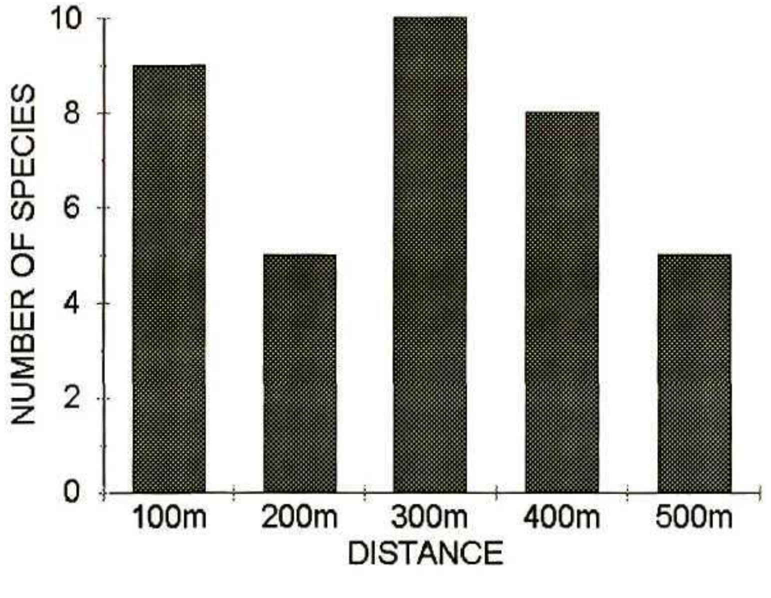

Fig. 15 Species richness at each of the intervals in the natural vegetation habitat.

DISCUSSION

Coccinellid distribution on land mosaic

These results indicate that the managed orchard habitat had the highest coccinellid densities and species richness compared to the natural habitats. This orchard habitat also had the most even species distribution, with no markedly dominant species. Duelli (1988) also observed that the highest diversity and density of coccinellid species were found within the managed crop system, followed by the semi-natural habitats, e.g. hedgerows and river borders and the lowest found in the natural habitats. This study, the first of its kind in southern Africa, corroborated the distribution pattern observed in Europe. The habitats bordering the river had the next highest coccinellid densities, especially since these had fewer sampling intervals compared to the other sites. These sites had good vegetational growth, e.g. shrubs, trees and reeds, probably due to their proximity to the river. They thus had continuous growth of vegetation, similar to the orchard environment where the citrus trees were present all year round. Unlike these sites, the sites at the eastern and northern side of the estate projected into the ranch. This has natural lowveld vegetation, characterised by thorny scrub, thorny shrubs and grass. During the dry winter months, the ranch experienced extremely dry conditions with minimal vegetational growth.

The presence of prey species, e.g. aphids, *I. purchasi* (Australian bug) , *A. aurantii (*red scale) for the predaceous species and weeds, grasses and trees for the phytophagous species in the orchards was probably a major factor contributing to the distribution pattern observed. Preferred coccinellid habitats are known to be determined by the presence of suitable prey (Hodek, 1973, Majerus and

Kearn, 1994). This could be an advantage for pest control, especially since coccinellids associated with sedentary prey species like red scale and Australian bug, e.g. *Rodolia* sp. and *C. distigma* at Tambuti, usually display close patterns of distribution with their prey (Hagen, 1962). Higher coccinellid densities in managed habitats may however, be detrimental to their populations since they are also likely to suffer from management effects, e.g. pesticide applications, mowing, harvesting, etc. carried out on the crop, making this habitat highly unstable for coccinellid populations present.

The occurrence of similar species in all the sampling sites could indicate that there is movement of coccinellids between the habitat patches sampled. The habitats are thus not too isolated to restrict coccinellid movement. The ability of species like *C. lunata* and *Nephus* sp. and *Scymnus* sp. to move between habitat patches may play a contributory role in pest control and coccinellid population reinforcement. In addition to the regulatory control these species may have on pests like aphids in the managed orchard habitat, their presence in the natural habitat would enable the reinforcement of coccinellid populations when they decline in the orchards, especially after pesticide application (Hodek, 1967, 1973). Since these species also reproduce in the natural and orchard habitats, as evidenced by the presence of larvae and pupae on vegetation at Tambuti, both habitat types may be considered to be favourable habitats for the species in the landscape mosaic assessed. According to Hodek (1967), an important criterion in the determination of suitable coccinellid habitat is the coccinellid species' ability to reproduce in that habitat. Adults are poor habitat indicators due to their high mobility and may thus occur in a non-typical habitat in its search for prey (Hodek, 1973).

The dominant species or species assemblages in each habitat type do not seem to be good indicators of a particular habitat type for the coccinellid species collected. In the orchards, species like *Hippodamia variegata* (Goeze), *Scymnus* sp. and *Cheilomenes lunata* dominated. *Hippodamia* sp. and *Cheilomenes* sp. are known to be aphid feeding species and thus occur in high numbers whenever there is high numbers of prey (Majerus and Kearn, 1989). These aphidophagous species were often found in the majority of sites when their prey were in abundance. Relatively higher population densities were found in the orchards where there was also higher plant diversity, e.g. weeds, grasses, citrus trees. Although not as populous as in the orchards, *C. lunata* also occurred on grass in the natural habitat and river borders, especially after rains. This pattern of widespread occurrence was, however, not consistent since few of these coccinellids were observed in the natural habitats during the second year of sampling, probably due to a variety of unknown environmental conditions. High population densities at a particular site were probably indicative of prey abundance rather than habitat specificity.

With the exception of the sites at the west of the estate, *Scymnus* sp. and *C. lunata* were also dominant in all the sites sampled and may be considered to be multihabitat species due to their utilisation of more than one habitat (Forman, 1997). Their distribution also seemed to be determined largely by the presence of their prey species, e.g. aphids for the aphidophaga, scale insects for *Scymnus* sp. This was also observed by Johki et al (1988) where the population densities of coccinellid species, e.g. *Harmonia axyridis* Pallas, *Coccinella septempunctata* Linnaeus, *Menochilus sexmaculatus* (Fabricius) and *Propylea japonica* (Thunberg) were positively correlated with prey density.

Throughout the sampling period, the economically important species observed at Tambuti, i.e. *Rodolia* sp. and *Chilocorus distigma*, were found only in the managed habitat. Restriction of economically important species to managed habitats was observed in Europe where 25% of carabid beetles occurred only in arable fields and the occurrence of 40 to 60 different species of carabids, staphylinids and spiders in the crop system is considered as normal (Booij and Noorlander, 1992). Being coccidophagous species, these coccinellid species tend to be less mobile than the aphidophagous species (Hodek, 1967). Besides the economically important species, a few other species were associated with specific habitats, e.g. *E. paykulli* was found only in the managed habitat and habitats bordering the river. This species was absent in the dry, hotter sites in the northern and eastern habitats, where their host plant was also not observed. While occurring in the orchards, *P. exiguus* and *Dysis* sp. also occurred on trees found in the natural veld, where there was less vegetational diversity and prey present.

Factors contributing to coccinellid distribution

While the coccinellids were not clearly indicative of a particular habitat, most were found on specific vegetation type. Species known to be phytophagous, e.g. *Epilachna* sp. were always found only on short curcubit weeds or solanaceous plants. Species observed in association with scale insects, e.g. *Scymnus* and *Nephus* spp. were found mainly on shrubs in the natural environment and on citrus in the orchards. In the natural habitats, the scale insects (unidentified species) occurred on stems of shrubs on which the coccinellids were found. Plant specialisation arises due to prey specialisation and is primarily influenced by prey choice. Association of a coccinellid species with a pest (prey) species however does not indicate its feeding on the pest

(Majerus, 1994). The aphidophagous species however, were not restricted to any particular vegetation type and occurred on trees, weeds, grasses in most of the habitats although restricted only to grasses in the natural habitat. Widespread occurrence indicates wider dispersal abilities and this was also observed in *Hippodamia* sp. by Hodek (1973). A difference observed however is that of these dominant aphidophagous species, only *C. lunata* was widespread while *H. variegata* was restricted only to the managed orchard habitat or close to them. This could indicate the possible restriction of this species to managed habitats within the region, in contrast to the Ukraine where it was found everywhere, including forests and only the larvae restricted to development in the fields (Hodek, 1967).

Not all the coccinellids collected were associated with prey, e.g. *Dysis* sp., *P. exiguus, Ortalia ochracea*, *Epilachna bifasciata* and *Epilachna paykulli*. Although species like *O. ochracea* were occasionally found on citrus trees, no prey (pests) were observed with them since they were always found on leaves with apparently no prey. Even in the natural habitat where they were in higher proportions, e.g. in southern habitat, no prey were observed although this species has been reported to feed on psyllids, Flatidae larvae and *Pheidole* ants (Samways *et al.*, 1997). *Epilachna bifasciata* was found on curcubit plants growing as weeds in the orchards while *E. paykulli* occurred on a solanaceous plant in the river border. Since these species are known to be phytophagous they would therefore not be associated with any prey. The importance of prey (host plant) on coccinellid distribution is also relevant to phytophagous species who were closely associated with their host plants.

The relationship between coccinellid distribution and prey was not apparent in the second survey where natural vegetation in the estate was sampled. In the managed habitat, predatory coccinellids are dependent on the presence and availability of their prey. In natural vegetation, the food supply is unreliable with little or no prey, e.g. aphids on the vegetation, probably because there is no management like irrigation, to promote diverse vegetational growth and attract phytophagous species. Vegetational diversity is correlated with insect species diversity (Duelli, 1988, Murdoch *et al.*, 1972). The coccinellids in the natural habitat are thus unlikely to be dependent on the unreliable prey presence. If they are not phytophagous, they will probably rely on their mobility to disperse to environments where prey are available. This may contribute to the lower coccinellid densities observed in the natural habitats.

The other environmental variables measured did not seem to have any apparent effects on coccinellid densities. Relative humidity was highest at sites nearest the river and in the orchards where there was irrigation. Influence of this variable on coccinellid densities is rather ambiguous as *Coleomegilla maculata* (Der Geer) had a positive response to it in a managed field while *Hippodamia convergens* Gue'rin was apparently insensitive to the same variable (Hodek, 1973). Temperature and ground insolation also has no apparent relationship with the species sampled. Both variables had species specific extremes, e.g. species like *C. lunata*, *H. variegata* and *Dysis* sp. were almost always found in well insolated areas since they were found on grass and weeds in direct sunlight, where temperatures were higher than in shaded areas. On trees in orchards or the bush, species found there occurred in cooler environment with very little insolation. It was noted, however, that the coccinellids were more active during the morning or afternoon while few were observed or found during the hot, midday hours of the day. Effects of environmental conditions are thus likely to be dependant on species specific requirements rather than the population as a whole.

Multiple regression also indicated the poor influence of the variables, except prey presence. Although not significant, temperature and insolation was negatively correlated with coccinellid densities. This was in apparent agreement with field observations where fewer coccinellids were observed during the hottest hours and in highly insolated areas.

Significance of habitat heterogeneity

Results suggest that the orchard environment forms favourable habitat patches within the less favourable natural veld habitat for coccinellids, especially for the economically important species. The occurrence of coccinellid species in both the natural and managed habitats emphasised the importance of maintaining a heterogenous array of habitats which are utilised by generalist species like *Cheilomenes* sp., *Scymnus* sp., *Nephus* sp. and promoting their beneficial use in the agricultural landscape. Although not economically important, the contribution of these species to the maintenance of low population levels of pests like aphids and scale insects is undoubtedly significant. While easily sampled, coccinellids apparently do not constitute good indicators, probably due to their distribution being dependant on prey availability rather than habitat fidelity. Habitats within the Tambuti land mosaic had coccinellid movement between them. The maintenance of a diverse plant cover at different spatial scales seems to be essential for coccinellid diversity and may ultimately contribute to pest regulation. Even though the economically important species were not observed to move between the various habitats, they also need complementary food sources, e.g. pollen and nectar from wild flowers and will thus need to utilise vegetation in the natural habitats (Burel and Baudry, 1995).

The multihabitat species also survive through the presence of an array of habitats. The land mosaic elements encourage the maximisation of coccinellid species, which contribute to natural pest management thus ensuring maximum production of fruits from the managed orchard habitats. The results emphasise the need to ensure passage of insects between the various mosaic elements, especially by minimising isolation of these habitats. Survival of coccinellid populations in the various mosaic elements ensures maximum faunal diversity within the agricultural landscape.

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CHAPTER 3

IMPACT OF INSECT GROWTH REGULATORS ON THE POPULATION DENSITIES OF COCCINELLIDS IN CITRUS ORCHARDS.

ABSTRACT

Predaceous coccinellids are one of the most frequently encountered predators within the citrus agroecosystem and the use of selective pesticides which promote activity of these arthropods is thus of great importance. A field experiment was carried out in three citrus estates (Tambuti, Tambankulu and Tunzini) in the Swaziland lowveld to assess the impact of insect growth regulators, recommended for use on citrus in southern Africa, on coccinellid population densities. On each estate, coccinellid population densities under various pesticide treatments were recorded within orchards exposed to the following treatment programmes. 1) under an integrated pest management (IPM) programme with no IGRs, 2) where buprofezin or pyriproxyfen were sprayed, 3) where only conventional pesticides with no IGRs were sprayed and 4) an untreated orchard on one estate (Tambankulu).

Results indicated that while there were no significant differences in population densities of the overall coccinellid populations due to pesticide treatments, significant differences were observed when only the economically important species were analysed. This was true when the estate populations were analysed per estate or combined ($p = 0.05$). The orchards under IPM had relatively higher population densities of the beneficial species compared to the IGR and non-IGR treated orchards ($p = 0.005$). When the untreated orchard was excluded, the population density of beneficial coccinellids was significantly higher at Tunzini citrus estate compared to Tambuti and Tambankulu estates ($p = 0.05$). The untreated orchard had significantly higher population densities of both the overall species and beneficial species ($p = 0.000$).

Coccinellid populations were highest during the month of February when all population data were combined. Tambankulu estate had the lowest species richness ($s = 10$) compared to Tunzini ($s = 22$) and Tambuti ($s = 23$) when only the managed orchards were compared. Tambankulu is an

extensively managed landscape with no natural vegetation in or around the estate while both Tambuti and Tunzini have large tracts of natural veld in and around the estates. Field evaluation of IGRs is a necessary prerequisite for informed decisions to be made about this class of pesticides, especially regarding their activity on beneficial coccinellids and for their incorporation into IPM programmes.

INTRODUCTION

Citrus is one of the leading cash and export crops in southern Africa and like most monocultures, the citrus agroecosystem has a wide range of pest species, e.g. citrus thrips, red scale, circular purple scale, citrus red mite (Bedford, 1968), fruit flies, aphids and mealybugs (pers. obs.). As a result, citrus orchards rely on pesticide applications for partial suppression of these pest species. While the citrus agroecosystem is a relatively stable habitat, widespread utilisation of non-selective pesticides may result in recurring infestations of primary and secondary pest species due to the suppressive effect of these pesticides on natural enemies (DeBach and Rosen, 1976; Bellows *et al.*, 1985; Morse *et al.*, 1986, Meyerdirk *et al.*, 1982; Waage, 1989).

With the realisation of the importance of natural enemies in agroecosystems, new methods of pest control, which incorporate the natural control of pests, were formulated. Orchard management tactics were changed to include pesticides that had minimal effect on natural control agents. In such integrated pest management (IPM) programmes, minimal use of pesticides is a priority. These programmes are ecologically based pest control strategies which promote the suppression of a pest complex below economic thresholds, through knowledge and understanding of the ecology of the crop system and its surroundings (Samways, 1981; Boneβ, 1983; Luck *et al.*, 1986; Waage, 1989). In an IPM programme, the awareness of the possible impact of pesticides on both target and non-target species is important so that pesticides are only used as a last resort, and only after careful monitoring of the system. Ultimately, pest control is achieved with minimum disruption to the environment (Luck *et al.*, 1986, Morse *et al.*, 1986; Poehling, 1989).

Since integrated control promotes the optimal utilisation of natural limiting factors, intensive research has identified natural enemies in and around the citrus agroecosystem. Within this system, arthropod predators from the coleopteran families Coccinellidae, Nitidulidae and Carabidae are of great importance in pest control (Clausen, 1940; Debach and Rosen, 1976). Of these predators, the predaceous coccinellids are the most commonly encountered, and have been observed to play an economically important role as natural control agents of major citrus pests, e.g. aphids, scale insects, mealybugs, phytophagous mites, whiteflies and occasionally eggs and young larvae of Lepidoptera and Coleoptera (Clausen, 1940; Hodek, 1973; Halperin *et al.*, 1995). Most of these pest species are amenable to control by natural enemies, with the majority of them having at least one coccinellid predator. Coccinellids can provide excellent biological control , e.g. *Rodolia cardinalis* (Mulsant), *Cryptolaemus montrouzieri* Mulsant and *Chilocorus nigritus* (Fabricius) are economically important control agents on citrus in southern Africa. Some coccinellids are so efficient in maintaining low pest population levels that they are sometimes mistakenly considered not to play an important role in the control of these pests, e.g. *Stethorus* species on their control of the pest mite *Tetranychus urticae* (Koch) (Clausen, 1940; Hagen, 1962; Hussey and Huffaker, 1976; Samways, 1984; Hattingh and Tate, 1995). Naturally occurring but less well known coccinellid species within the agroecosystem are just as important in pest control, since pest problems within the agroecosystem can be intense without their predatory contribution (Hagen, (1962).

As coccinellids provide natural control within citrus orchards, various cautionary measures are necessary to ensure their preservation and promote their use. One of these measures is the careful use of appropriate pesticides. Nevertheless, pesticide contact with both target pests and non-target natural enemies is unavoidable. Pesticide impact on non-target species, however, could be minimised with the use of compounds whose activity illustrates sufficient selectivity (Boneβ, 1983; Uygun *et al.*, 1994). One class of pesticides, i.e. the insect growth regulators (IGRs), has been promoted as compatible with IPM programmes in citrus orchards. IGRs have been considered to provide suitable selectivity and have been widely used in fruit orchards for control of a variety of pests (Grenier and Grenier, 1993; Sauphanor *et al.*, 1993) and have been considered suitable in IPM programmes (Biddinger and Hull, 1995). While the secondary effects of these pesticides on coccinellid predators were unobserved, IGRs were assumed to be effective in decimating pest populations with minimal harm to coccinellid predators. Various authors have, however, reported adverse activity of IGRs against coccinellids in the field environment (Mendel and Blumberg, 1991; Loia and Viggiani, 1993; Biddinger and Hull, 1995; Hattingh and Tate, 1995; Hattingh, 1996). In view of their reported toxicity to coccinellids, further evaluation of their activity was undertaken in the field.

In addition to selective pesticide use, the availability of a complex of favourable habitats maximises predator activity. The presence of a habitat mosaic around managed agroecosystems maximises diversity of prey and microhabitats, ensuring continuity of prey (Hodek, 1967; Root, 1973, van Emden, 1990). Presence of these habitat mosaics is therefore important in pest management through the conservation of beneficial arthropod species (van Emden, 1965; Duelli, 1988; Keller and Duelli, 1988).

The study was thus carried out to assess the impact of IGRs recommended for use on citrus in southern Africa on coccinellid populations within three citrus estates in Swaziland, i.e. Tunzini citrus estate, Tambuti and Tambankulu estates. The landscape around the orchards and within each estate was also taken into consideration.

STUDY SITES

The sites were in the lowveld of Swaziland. The lowveld has an average temperature of 22°C, receiving between 500mm and 900 mm of rain. This area is considered to be a drought risk area, and all the estates rely on the major perennial rivers of Swaziland which run close by. Natural lowveld vegetation is sparse acacia scrubland (Vilakati, 1986; Bowen, 1988).

Three estates were used in this study:

1) Tambuti Estate (26.43S 31.46E) has 931.6 ha under citrus, with the citrus harvested on 887.9ha in the 1995/95 season (Swaziland Citrus Board). The rest of the estate is unmanaged veld, parts of which are used as a cattle ranch. Tambuti thus has a variety of both natural and anthropogenic mosaics. Both the natural veld and citrus orchards were used as sampling sites as described in the relevant chapters (Figure 16). Tambuti is watered by the Great Usuthu river.

2) Tunzini Citrus Estate (IYSIS) (25.56S 31.39E) has 686ha under citrus, with 629ha harvested in the1995/96 season. IYSIS has both managed citrus orchards as well as huge tracts of a natural veld, parts of which are also used as a cattle ranch (Figure 17). IYSIS is watered by the Komati river.

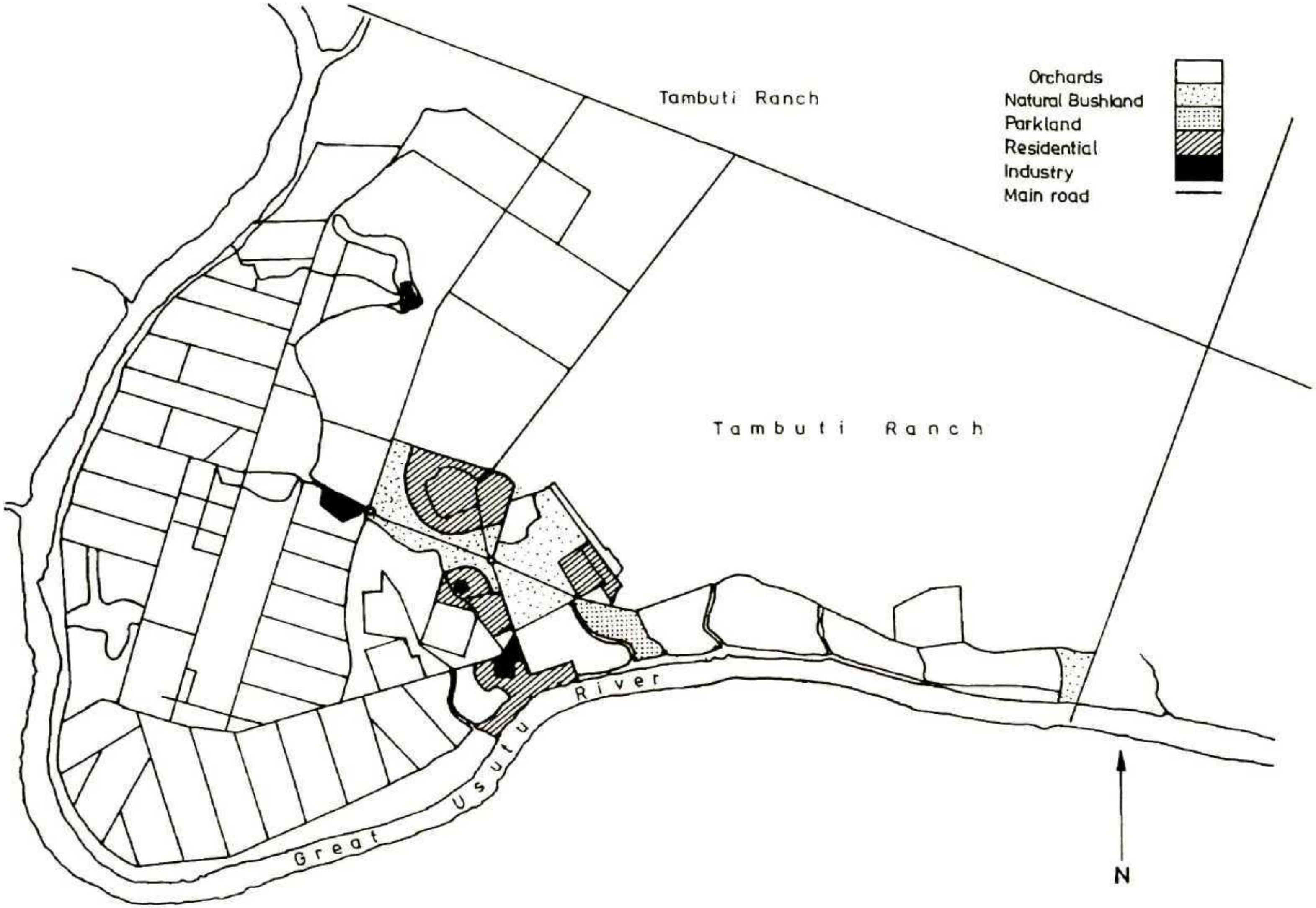

Fig. 16 TAMBUTI ESTATE 1 : 26 000 approx.

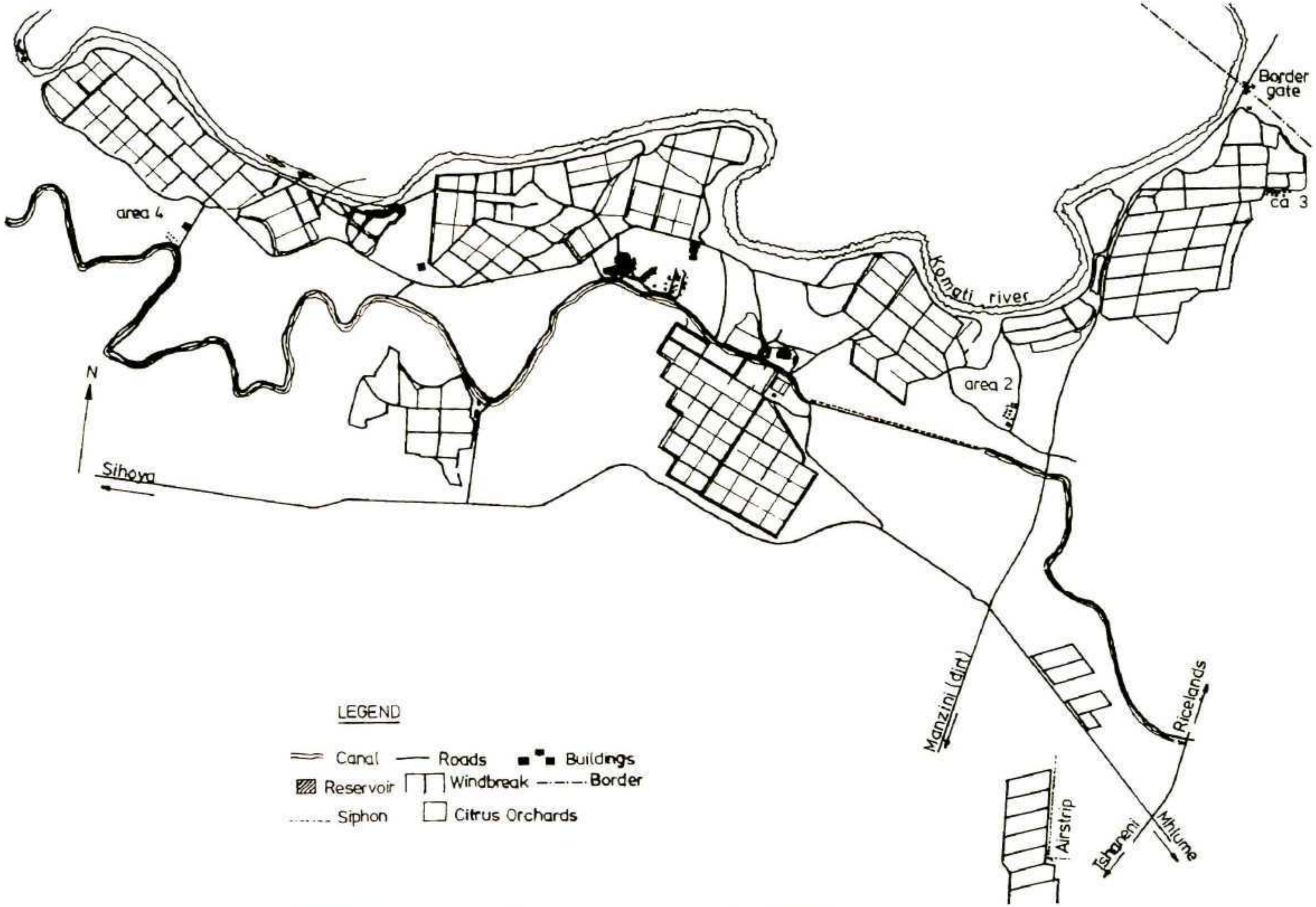

Fig. 17 TUNZINI CITRUS ESTATE 1 : 66 000 approx.

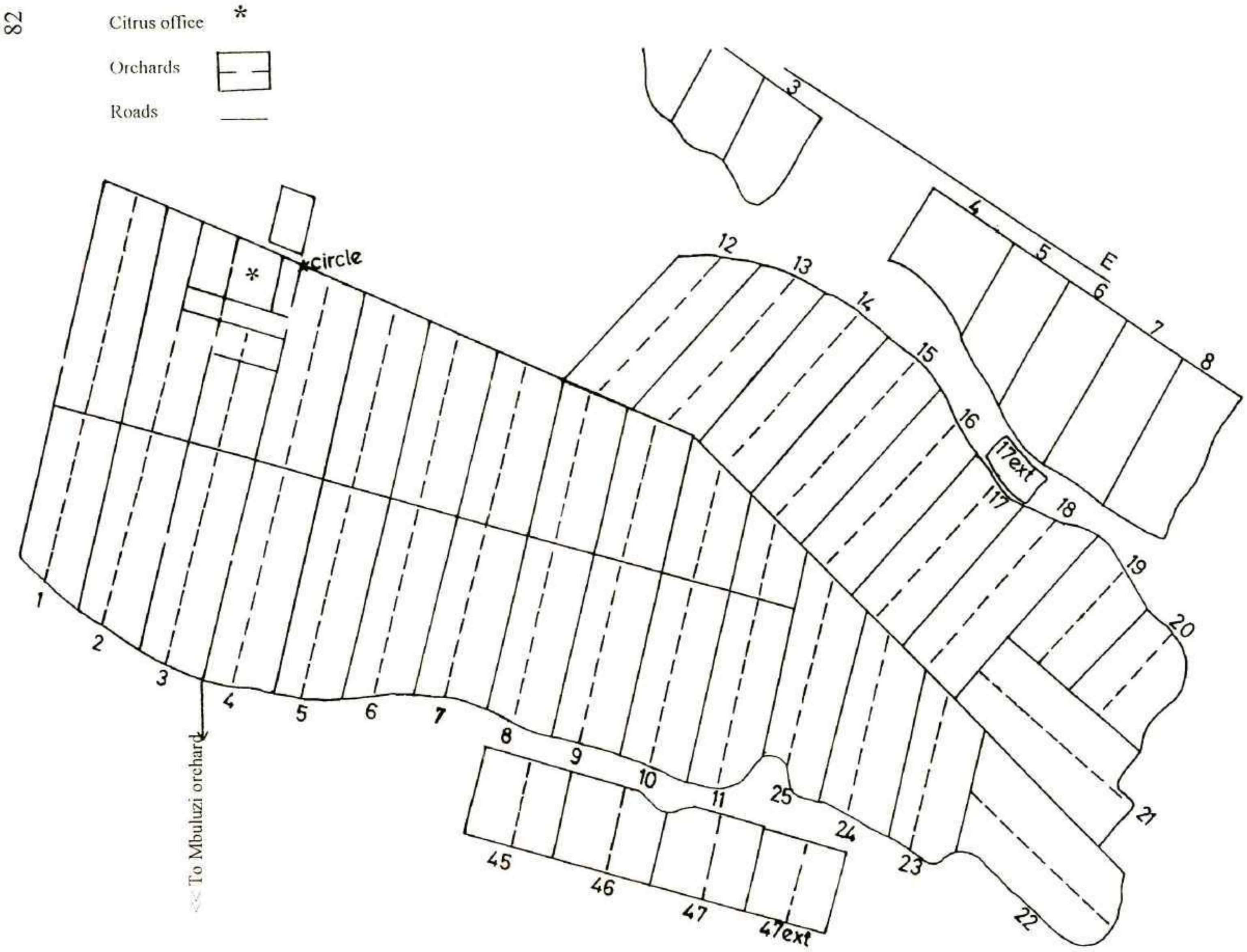

Fig. 18 TAMBANKULU ESTATE 1 : 20 000 approx.

3) Tambankulu Estate (26.07S 31.56E) has 550ha under citrus, with 327ha harvested. This estate is situated next to the Simunye sugar estate and is thus surrounded by sugarcane fields. It has therefore no substantial natural veld within or around it. The Tambankulu landscape is intensively managed either for citrus or for sugarcane growing (Figure 18). Tambankulu is watered by the Black Mbuluzi river.

MATERIALS AND METHODS

At each estate, at least three orchards were selected, one from each of the following treatment regimes:

1. Orchards where any of the insect growth regulators recommended for use on citrus were to be sprayed - ('IGR treatment').
2. An orchard where only selective chemicals were used under an integrated pest management programme - (IPM treatment').
3. An orchard under a normal chemical regimen where no insect growth regulators were to be used - (non-IGR treatment').

At Tambankulu, an additional orchard was added. This was the untreated Umbuluzi orchard and since the 1994/95 season, it had not received any chemical treatment. All the orchards were under citrus and producing fruit for commercial exploitation, except the Umbuluzi orchard which was not commercially exploited.

Replication of treatments was only possible between estates rather than within each estate. The estates all harvested for commercial purposes and as such, minimum disruption in the running of their programmes was a priority. Orchard management was subject to change without notice in keeping with their pest management programs. Large sample sizes were therefore used. The study would thus provide information on the existence of differences in coccinellid population densities due to treatments, if any (Hulbert, 1984; Brown, 1989).

Insect growth regulators used

During the experimental period, buprofezin (Applaud) was the only IGR sprayed in all three estates. In addition to this IGR, Tambuti also had pyriproxyfen (Nemesis) and teflubenzuron (Nomolt) sprayed in the experimental orchards. Data from the teflubenzuron-sprayed orchard however, could not be assessed, since buprofezin had also been sprayed in the same orchard earlier in the season.

Sampling method

Sampling began in early spring (August). Sampling commenced in the second row of each orchard, with sampling carried out on every tenth tree in a row. On completion of a row, the next row sampled was the tenth row from the last sampling row. This was carried out until twenty trees per orchard had been sampled. Within each tree, a beating tray was held under a randomly selected branch. The branch was vigorously beaten three times with a stick at arm's length towards the tree trunk. In addition to this, visual inspections were carried out for any coccinellid adults, pupae and larvae on the tree trunk or branch. Coccinellids were counted on all twenty sampling trees.

Voucher specimens were taken to the laboratory and later sent to the Plant Protection Research

Institute, Biosystematics Division (South Africa) for identification. Weedy vegetation and grasses surrounding the trees sampled were inspected for coccinellids.

Statistical analyses

Since the variances of the sample counts were greater than the mean, the data was log (x+1) transformed and ANOVA plus analyses of means were carried out using SPSS statistical package. Analyses were carried out for the overall coccinellid population in the estates.

RESULTS

Treatment effects within each estate

There were no significant differences between overall coccinellid populations due to the treatments applied ('IGR', 'IPM' and 'non'IGR' treatments) in any of the estates, $p = 0.178$ (Tambuti) (Fig.19), $p = 0.804$ (Tunzini) (Fig. 20) and $p = 0.379$ (Tambankulu, abandoned orchard excluded) (Fig.21). This indicated that the treatments had no significant effect on coccinellid population densities within the orchards. Significant differences due to treatment in orchards were only observed in Tambankulu when the 'abandoned' orchard was included ($p = 0.000$) (Fig. 22).

When only the beneficial species were analysed, significant differences due to pesticide treatment were observed at Tambuti ($p = 0.037$) (Fig. 23) and Tunzini ($p = 0.034$) (Fig.24), while none were observed at Tambankulu when the 'abandoned' orchard was excluded ($p = 0.379$) (Fig. 25). At Tambuti and Tunzini, the population of beneficial species in the IPM orchards was significantly

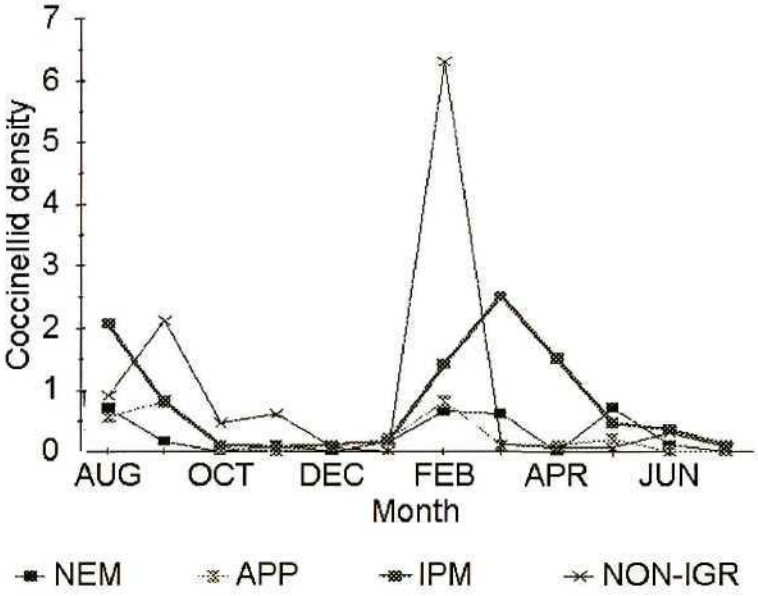

Fig. 19 Average number of coccinellids per tree (coccinellid density) at Tambuti estate. NEM=pyriproxyfen sprayed, APP=buprofezin sprayed, IPM=under IPM programme, NON-IGR=no IGRs sprayed.

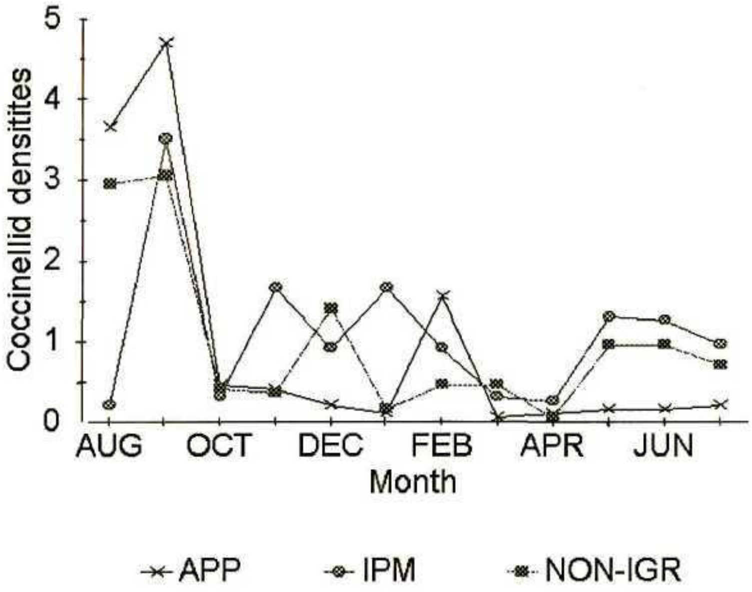

Fig. 20 Average number of coccinellids per tree (coccinellid density) at Tunzini. APP=buprofezin sprayed, IPM=under IPM programme, NON-IGR=no IGRs sprayed.

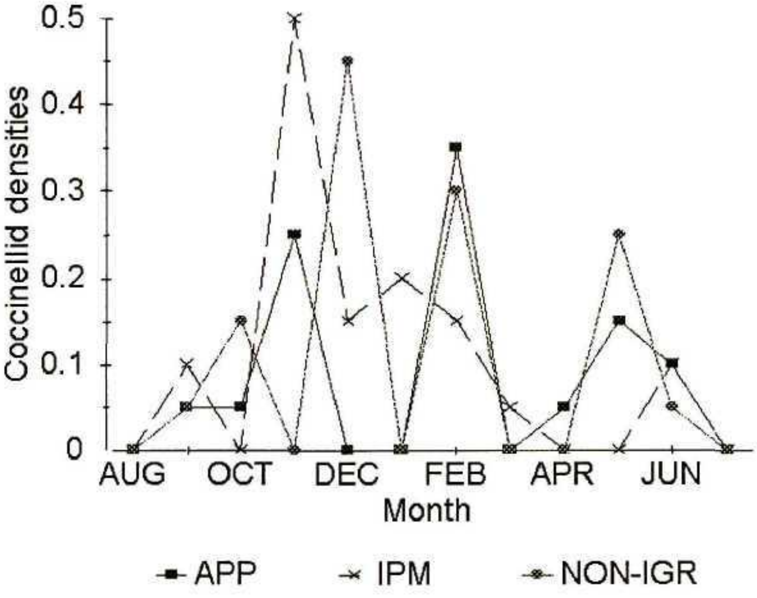

Fig. 21 Average number of coccinellids per tree (coccinellid density) at Tambankulu - untreated orchard excluded. APP=buprofezin sprayed, IPM=under IPM programme, NON-IGR=no IGRs sprayed.

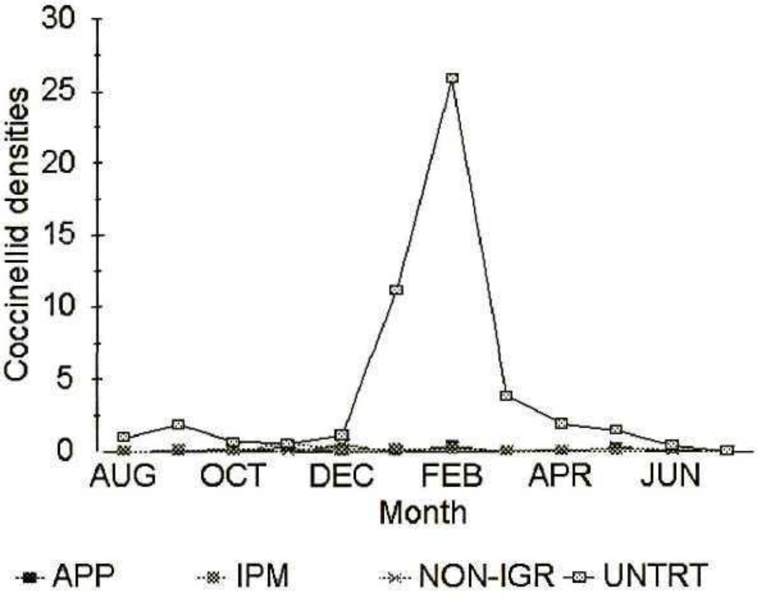

Fig. 22 Average number of coccinellids per tree (coccinellid density) at Tambankulu - untreated orchard included. APP=buprofezin sprayed, IPM=under IPM programme, NON-IGR=no IGRs sprayed, UNTRT=untreated orchard.

different from the non-IGR orchards. Fig.24 shows that the number of beneficial coccinellids declined steadily in the IGR orchard and was at its lowest three to six months after treatment with the IGR (i.e. in October). A highly significant difference in the population of beneficial species was observed at Tambankulu only when the 'abandoned' orchard was included ($p = 0.002$) with populations in this orchard being significantly higher than in the other orchards in the same estate ($p = 0.05$, Tukey HSD) (Fig. 26).

Treatment effects when all estates were combined

Total coccinellid counts for the estates were combined and further analysed (Table 2). These analyses were carried out when the 'abandoned' orchard was included and excluded. There were no significant differences in the average number of coccinellids per tree due to the pesticide treatment when the abandoned orchard was excluded ($p = 0.306$). A significant difference however, was observed when the 'abandoned' orchard was included ($p = 0.000$), with this orchard having significantly higher population levels of coccinellids compared to the other treatment groups (Tukey HSD, $p = 0.05$, Table 3).

There were highly significant differences in population densities of beneficial coccinellid species when these were analysed separately, with the IPM orchards having significantly higher populations levels of beneficial species than the IGR-treated and non-IGR orchards ($p = 0.005$) (Table 4). When the untreated 'abandoned' orchard was included, a highly significant difference was observed ($p = 0.000$), with the 'abandoned' orchard having much higher densities of

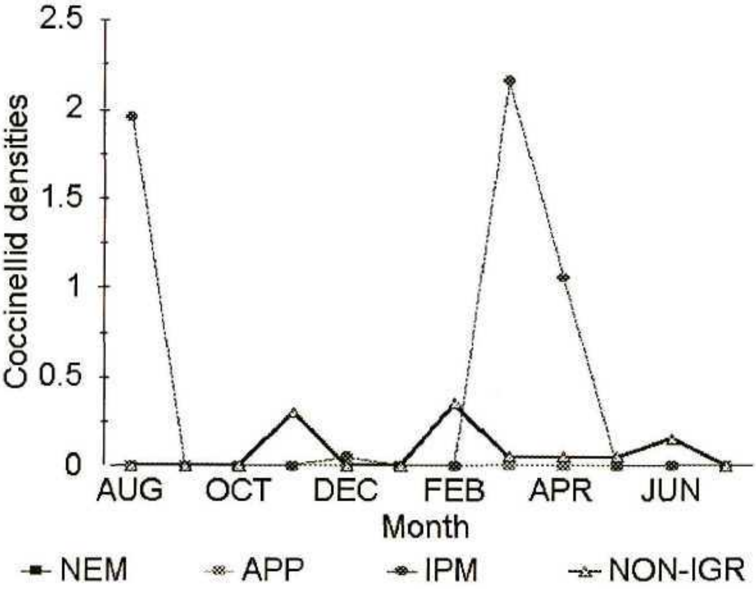

Fig. 23 Average number of beneficial coccinellids per tree (coccinellid density) at Tambuti estate. NEM=pyriproxyfen sprayed, APP=buprofezin sprayed, IPM=under IPM management, NON-IGR=no IGRs sprayed.

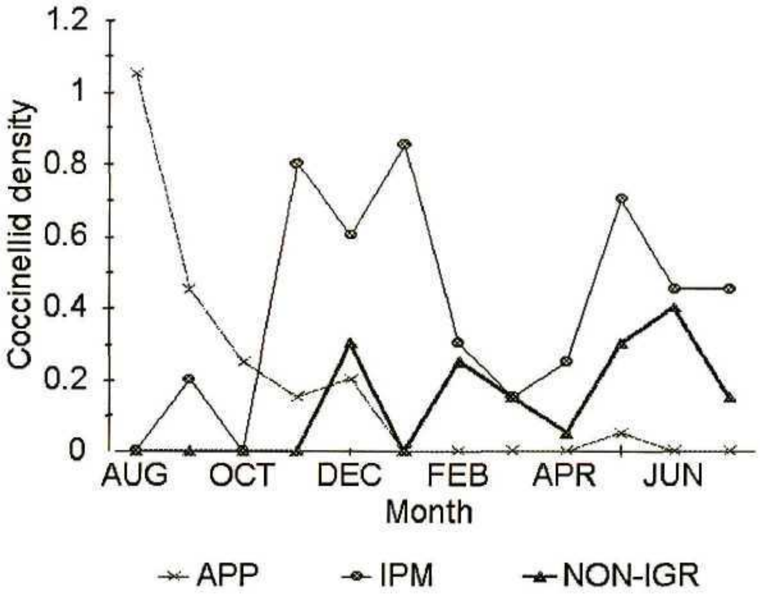

Fig. 24 Average number of beneficial coccinellids per tree (coccinellid density) at Tunzini. APP=buprofezin sprayed, IPM=under IPM programmes, NON-IGR=no IGRs sprayed.

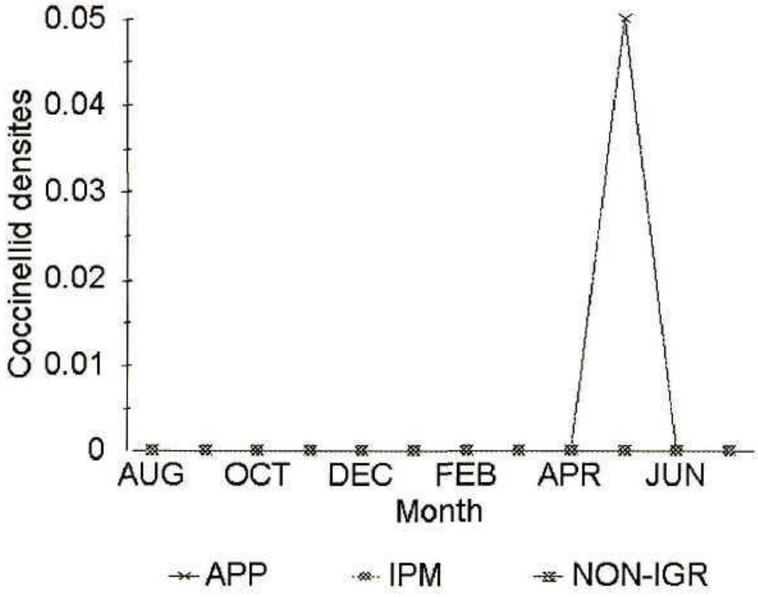

Fig. 25 Average number of beneficial coccinellids per tree (coccinellid density) at Tambankulu - untreated orchard excluded. APP=buprofezin sprayed, IPM=under IPM programme, NON-IGR=no IGRs sprayed.

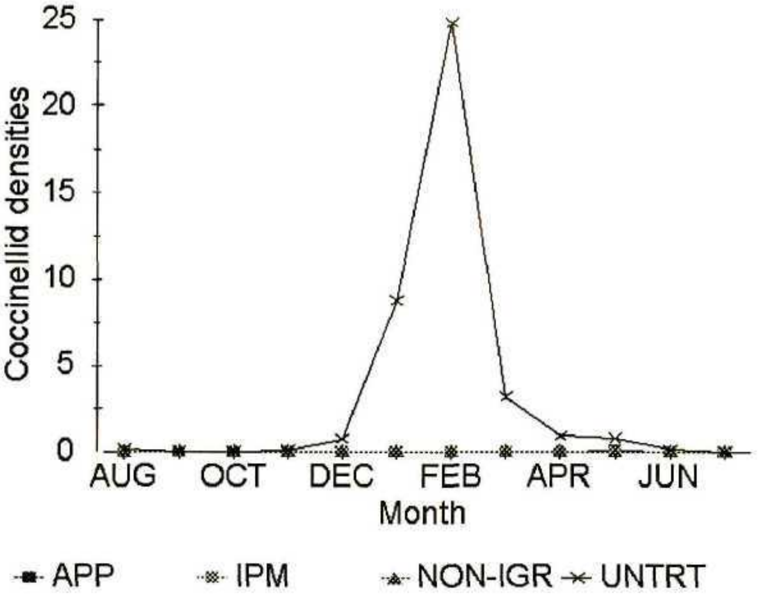

Fig. 26 Average number of beneficial coccinellids per tree (coccinellid density) at Tambankulu - untreated orchard included. APP=buprofezin, IPM=under IPM programme, NON-IGR=no IGRs sprayed, UNTRT=untreated orchard.

Table 2. List of all coccinellid species collected from each of the citrus estates during a twelve-month period.

Species	Tambuti	Tunzini	Tambankulu	
COCCINELLINAE			*	**
Cheilomenes lunata (Fabricius)	40	28	15	9
Cheilomenes propinqua (Mulsant)	4	4	10	0
Declivata hamata (Schoenherr)	0	4	2	2
Dysis sp.	14	5	3	3
Hippodamia variegata (Goeze)	159	21	9	9
Psyllebora variegata (Fabricius)	5	0	0	0
COCCIDULINAE				
Novius sp.	0	22	2	0
Rhyzobius lophantae (Blaisdell)	1	69	0	0
Rodolia cardinalis (Mulsant)	120	59	3	1
Rodolia sp.	1	16	0	0
CHILOCORINAE				
Brumus sp.	0	5	0	0
Chilocorus distigma (Fabricius)	1	0	1	0
Chilocorus nigritus (Fabricius)	0	26	778	0
Platynaspis capicola Crotch	6	6	9	0
EPILACHNINAE				
Epilachna bifasciata (Fabricius)	2	0	0	0
Epilachna paykulli (Mulsant)	1	0	1	1

N.B. Table split across pages 91 - 92

Species	Tambuti	Tunzini	Tambankulu	
SCYMNINAE			*	**
Nephus sp.	14	8	24	2
Ortalia ochracea (Weise)	3	6	10	0
Scymnus prob. *levaillanti* (Mulsant)	25	135	31	14
Scymnus sp.	112	221	34	3
Pharoscymnus exiguus Weise	5	36	14	0
Pharoscymnus prob. *uncosiphonatus* Fursch	1	1	3	0
Serangium sp.	0	29	56	0
Unidentified larvae	0	3	25	0
Unidentified adults	17	30	24	2
TOTAL COCCINELLIDS	531	734	1056	71
TOTAL BENEFICIAL COCCINELLIDS	123	170	784	2
NUMBER OF SPECIES	23	22	23	10

* - TOTAL NUMBER OF COCCINELLIDS WITH UNTREATED ORCHARD INCLUDED

** -TOTAL NUMBER OF COCCINELLIDS WITH UNTREATED ORCHARD EXCLUDED

Table 3. Total number of coccinellids sampled in orchards under indicated treatments. Numbers indicate treatment, where 1 =buprofezin, 2 = pyriproxyfen, 3 = IPM, 4 = non-IGR, 5 = Untreated orchard.

Estate	1	2	3	4	5	Total
Tambuti	58	63	191	219	*	531
Tunzini	234	*	263	237	*	734
Tambankulu	20	*	25	25	986	1056

* - treatment absent

Table 4. Total number of beneficial coccinellids sampled in orchards under the indicated treatments. Numbers indicate treatment, where 1 = buprofezin, 2 = pyriproxyfen, 3 = IPM, 4 = non-IGR, 5 = Untreated orchard.

Estate	1	2	3	4	5	Total
Tambuti	0	0	104	19	*	123
Tunzini	43	*	95	32	*	170
Tambankulu	1	*	0	0	783	784

* - treatments absent

beneficial coccinellids than the other treatment groups.

When the population densities of all coccinellid species collected were analysed relative to estate, there was a significant difference between the estates. Coccinellid population densities at Tunzini were significantly higher than both Tambuti and Tambankulu (untreated orchard excluded) population densities (Tukey HSD, $p = 0.05$). This was also true for the population densities of the beneficial species ($p = 0.004$) with Tunzini still having significantly higher populations (Table 4).

Monthly variations in coccinellid populations

Observations of the average monthly counts of coccinellids in the estates indicated that February had significantly higher population densities of coccinellids ($p = 0.011$). This month was significantly different from July when coccinellid densities from all three estates were combined (Tukey HSD, $p = 0.05$; figs. 19-22).

Beneficial coccinellid species were in low numbers at the beginning of the season, i.e. August and September, especially at Tambuti and Tambankulu. Tunzini had various population levels in the orchards, with the highest initial populations of beneficial species in the orchard subsequently sprayed with buprofezin. Presence of the beneficial species was clearly apparent from November onwards with a steady increase in their populations until February, after which there was a decline (Figs 23 - 26).

DISCUSSION

In the estates sampled, there were no significant differences in overall coccinellid densities due to pesticide treatment. This indicated that the coccinellid species were unaffected by the various treatment regimes. However, this was only true when all the species were sampled together, i.e. including non-economically important species. When only the species known to be economically important on citrus were included, there were significant differences due to the treatments.

At Tambuti estate, the orchards sprayed with IGRs, i.e. buprofezin or pyriproxyfen, had lower population densities of beneficial species than the one under an IPM programme while at Tunzini it was only the non-IGR orchard which differed significantly from the IPM orchard. Total coccinellid populations in the IGR orchard may have been inflated by the initial early season densities (prior to IGR application), which were notably higher than in the other orchards.

The differences in populations of beneficial species only, as opposed to all types of coccinellids, may have been due to beneficial species being the ones most likely to be exposed to any pesticides sprayed. Beneficial species are normally concentrated within the orchard environment where there is a higher density of prey (pest) species, e.g. red scale, mealybugs, aphids, etc. Non-economically important coccinellid species are not likely to be closely associated with citrus pests, and readily move in and out of the orchards in search of food. Species such as *Ortalia ochracea* (Weise) occurred in low numbers in the orchards at Tambuti and Tunzini but was also common in the natural vegetation outside the orchards in Tambuti (refer to chapter 2) as well as in the undisturbed, abandoned orchard. This,could indicate that exposure of such populations would be minimal due to their greater use of the natural habitat. Since pesticide applications are carried out when the pest populations are relatively high, the beneficial species are most likely to be exposed to any pesticides sprayed.

Field exposure of beneficial coccinellid species such as *Rodolia cardinalis*, *Stethorus punctum* (LeConte) to IGRs like fenoxycarb and pyriproxyfen led to a decline in their population densities. This decline was due to the detrimental effects of IGRs on mature larvae and pupae, in addition to their ovicidal activities (Loia and Viggiani, 1992, Biddinger and Hull, 1995). Such effects inevitably led to population suppression due to non-replacement of progeny. In southern Africa, the use of IGRs like pyriproxyfen, has led to the decline of beneficial coccinellid species such as *Rodolia cardinalis* and *Chilocorus nigritus* in and around citrus estates (Hattingh and Tate, 1995). Suppression of coccinellid species populations in IGR-treated orchards was also due to the sterilizing effect of this class of chemicals even some weeks after treatment (Loia and Viggiani, 1992).

Similar patterns appeared when results of all estates were combined. As in the case of individual estates, the total coccinellid populations showed no apparent differences due to treatment. However when only beneficial coccinellid species were considered, orchards under IPM had significantly higher population levels. This emphasises that IPM enables the pesticides to be used with minimum impact on non-target species (Morse *et al.*, 1986), especially after careful monitoring (Luck *et al.*, 1986).

These pesticides must have no long term effects. Yet IGRs were observed to persist for relatively long periods of time in the field, e.g. fenoxycarb sprayed in the field suppressed reproduction in *R. cardinalis* for about three months. Such effects are not peculiar to IGR-treated orchards, but also apply to conventional pesticides (Loia and Viggiani, 1992; Meyerdirk *et al.*, 1982). The study here clearly illustrates that IGRs are not as selective as is often claimed. Their use thus needs as careful consideration as conventional pesticides.

Comparison of coccinellid population densities in the commercially managed orchards with the 'abandoned' orchard illustrates how the use of any form of pesticide recommended for use on citrus results in disruption of coccinellid population densities and species richness (Table 2). An economically important species like *Chilocorus nigritus* thrived within the 'abandoned' orchard. Realistically however, such a situation cannot be tolerated in the commercial context as pests not subject to such coccinellid suppression blemish the fruit (DeBach and Rosen, 1976; Uygun *et al.*, 1994). The reduction in pesticide use thus needs the establishment of realistic economic thresholds as well as realistic marketing standards (DeBach and Rosen, 1976).

Analyses of the beneficial coccinellid populations for the three estates indicated that Tunzini had the highest population densities of these beetles. Tunzini has minimal utilisation of pesticides, including IGRs, whose use is also restricted to buprofezin and triflumuron (Technical Manager, pers. comm.). These two IGRs have not been associated with any negative repercussions in the field (Hattingh, 1996). Tambuti uses the most IGRs, with 33% of the estate being sprayed in 1994/95 (Technical Manager, pers. comm.). Despite the wider use of IGRs, Tambuti had a relatively higher and more diverse coccinellid assemblage compared to Tambankulu where the overall coccinellid populations were the lowest of the three estates (with the exception of the untreated orchard).

While Tunzini and Tambuti have large tracts of natural vegetation surrounding the orchards and estates, Tambankulu has no natural vegetation between the orchards and is surrounded by sugarcane fields. Orchards within this estate also have very little vegetation, e.g. grasses, weeds between the tree rows and some have only bare ground. At Tambuti and Tunzini, while the inter-tree rows were managed, these were sometimes left to grow quite densely before any mowing was done. This meant that opportunistic species like the aphidophagous species, e.g. *Cheilomenes* spp.and *Hippodamia variegata* on grasses and weeds and *Epilachna* spp. on weedy cucurbits, could also invade the orchard environment thus increasing the local species diversity.

The importance of natural or wild non-crop vegetation surrounding a monocultural agroecosystem as well as the presence of weeds within the system has been emphasised by various authors (Duelli, 1988; Keller and Duelli, 1988; Volkl, 1988; van Emden, 1990). Natural vegetation or the maintenance of a mosaic of different crop systems and uncultivated land is important for increasing species richness and maintaining population levels of the entomophagous fauna (Duelli, 1988; van Emden, 1990). Presence of a mosaic of habitats, as in Tunzini and Tambuti, ensures the continuity of food, including pollen and nectar, presence of refuge areas and suitable microhabitats for the predators and their long-term presence (Hodek, 1967; Root, 1973; Poehling, 1989; van Emden, 1990).

A more permanent entomophagous fauna thus maximises natural control, since favourable habitats are provided for the predatory species enabling them to complete their life-cycles when outside the orchards. At Tambuti and Tunzini therefore, the habitat mosaics promote the possible conservation of the beneficial coccinellids. For example, the predator *C. nigritus* is known to utilise *Dendrocalamus giganteus* clusters around citrus estates (Samways, 1984) which was observed at Tunzini. Van Emden (1965) also noted that coccinellid larvae and adults moved from uncultivated land into the crop at the beginning of the season.

Such movements may facilitate the reinvasion of an orchard by the beneficial coccinellids after the decimation of the population by pesticides applied, emphasising the importance of the wild/natural vegetation as reservoirs of natural enemies, further promoting host-natural enemy stability within the agroecosystem (Poehling, 1989). This, however, is only possible when the pesticides do not persist long enough to affect such incoming invaders. The lower population levels of coccinellids in the managed orchards at Tambankulu may be due to the absence of such refugia for the coccinellids. In the 'abandoned' orchard, there was no disturbance or perturbation of the system with resultant high numbers og beneficial coccinellids.

Results of this study indicate that while the overall coccinellid population may not be adversely affected by the IGR treatments, beneficial coccinellid populations are reduced by such treatments. Orchards under IPM where no IGRs were used, or, in the extreme case where the orchard received no chemical treatments, had the most persistent and highest population densities of the beneficial species. Although IGRs have been recommended for IPM, their continued use within these programmes needs to be reevaluated if the persistence of these important natural enemies is to be encouraged. The optimal timing of pesticide applications, using reliable economic thresholds may not achieve effective pest control if the pest's natural enemies are also not exploited (Poehling, 1989). Exploitation of these beneficial species is a self-perpetuating system which preserves these non-target species. A change in orchard management to include selective pesticides and to promote biological control would initially involve heavy crop losses caused by the initial high pest densities (Hattingh, 1996). However, this would ultimately enhance diversity and stability within the agroecosystem, enhancing biological control of the pest species with natural control agents (Poehling, 1989; Biddinger and Hull, 1995).

The high diversity and abundance of predator populations achieved through such programmes would be further enhanced by a mosaic of natural and semi-natural habitat refugia with alternative prey within and around the agroecosystem (Clausen, 1940; Duelli, 1988; Wyss, 1995). Coccinellidae species are important as predators of economically important species on citrus, e.g. mealybugs, phytophagous mites, scale insects, aphids, etc. (DeBach and Rosen, 1976), and their preservation within the citrus agroecosystem is worth promoting .

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CHAPTER 4

THE ACTIVITY OF INSECT GROWTH REGULATORS ON A NON-TARGET SPECIES, *CHILOCORUS NIGRITUS* (COLEOPTERA : COCCINELLIDAE) IN THE FIELD AND LABORATORY.

ABSTRACT

In southern Africa, *Chilocorus nigritus* (Fabricius) is one of the major coccinellid predators of the major citrus pest, *Aonidiella aurantii* (Maskell). Laboratory and field experiments were carried out on eggs, larvae and adults of this ladybird to determine the effects of three insect growth regulators (IGRs) normally used commercially against pests on citrus in the region. Two chitin synthesis inhibitors, buprofezin and teflubenzuron, and a juvenile hormone analog, pyriproxyfen, were applied to *C. nigritus* populations at the recommended dosages. Mortality and development of egg and larval stages as well as mortality and fecundity of the adults were recorded. Laboratory experiments indicated that, of the three IGRs tested, buprofezin was the most detrimental compound, especially to larval stages, whether the larvae were fed IGR-treated scale or whether sprayed with IGRs. Immediate larval mortality from pyriproxyfen and teflubenzuron was not significantly different from the control. However, none of the larvae fed with IGR-treated scale went into pupation. In contrast, pupation did take place in those that had only been sprayed with IGRs. Although pupation occurred, there was no adult emergence in all treatment groups. There was no mortality of adults either having eaten IGR-treated scale or having been sprayed with them. Adult fecundity was not affected by exposure to IGRs either in the laboratory or in the field. All eggs exposed to IGRs did not hatch.

Although in the field experiments larvae developed to the reproductive adult stage, effects on immature stages and the IGRs' ovicidal activity still had a detrimental effect on *C. nigritus* population levels. Adverse IGR activity on egg and larval development was still apparent at 32m, the furthest distance tested in the field. This calls for serious consideration for the possibility of

pesticide drift from citrus orchards. Due to the apparently detrimental effects on larval and egg development, spraying of IGRs is likely to impede population increase in orchards. This emphasises the need to avoid coincidence between the spraying period and *C. nigritus* population build-up, if the use of these compounds cannot be avoided. IGR impacts on non-target species thus still need further consideration, especially with regard to the incorporation of these chemicals into IPM programmes.

INTRODUCTION

Many species of the Coccinellidae are beneficial predators of homopteran pests. In the citrus industry, coccinellids have gained economic importance due to their activity against these pests. Red scale, *Aonidiella aurantii* (Maskell) is a serious homopteran pest in citrus, especially in southern Africa (Samways, 1988). Chemical control measures used against this pest became ineffective during the 1970s when the pest developed resistance against organophosphate compounds. A coccinellid predator, *Chilocorus nigritus* (Fabricius), was observed to have a major impact on red scale populations, especially in the southern African lowveld where the levels of red scale were often severe (Samways and Mapp, 1983, Samways and Tate, 1986).

Chilocorus nigritus is indigenous to India and the Far East (Ahmad, 1970). In Pakistan, this beetle is effective against *Aspidiotus destructor* Signoret, *Aonidiella aurantii* and *Quadraspiodiotus perniciosus* (Comstock). The beetle later established in the Seychelles, Mauritius, and East Africa where it fed on scale insects such as *Ischnaspis longirostis* (Signoret), *Chrysomphalus ficus* (Ashmmead) and *Pinnaspis buxi* (Bouche) (Vezey-Fitzgerald, 1941; Vezey-Fitzgerald, 1953; Greathead and Pope, 1970).This predator first appeared in the South African lowveld and later established in the Swaziland lowveld and Mozambique (Samways, 1984).

Chilocorus nigritus has appeared to be remarkably effective as a natural enemy against red scale populations, even in trees showing twig dieback due to extremely high levels of red scale infestations. Under such circumstances, the predator is capable of clearing the red scale and bringing it down to economically acceptable levels. (Samways and Mapp, 1983, Samways, 1988). *C. nigritus* control is maximised by its ability to feed on all sessile stages of the scale, including the gravid female, (Samways and Tate, 1986; Samways and Wilson, 1988).

Insecticides used to control economic pests are known to have deleterious effects on non-target organisms such as beneficial insect predators. These effects have presented unexpected problems for the agricultural industry, which is heavily dependent on agrochemicals to maximise profits. Despite being promoted as innocuous to non-target organisms, various studies have indicated that insect growth regulators (IGRs) also have a negative impact on non-target, beneficial species (Mansour *et al.*, 1993; Ragusa Di Chiara *et al.*, 1993; Sauphanor *et al.*, 1993; Gerling and Sinai, 1994; Butaye and Degheele, 1995).

More significantly for this study, IGRs seemed to have a detrimental effect on beneficial coccinellids as indicated by studies involving *Cryptolaemus montrouzieri* Mulsant (Smith and Papacek, 1990); *Rodolia cardinalis* (Loia and Viggiani, 1992); *Chilocorus bipustulatus* Linnaeus (Peleg, 1983) and *Stethorus punctum* (Biddinger and Hull, 1995). The use of pyriproxyfen on southern African citrus is suspected to have led to the decline of *C. nigritus* populations on clumps of the giant bamboo *Dendrocalamus giganteus* Munro, which is used by the beetle as alternative habitat, around citrus orchards (Hattingh and Tate, 1995). This has serious economic implications, especially with reference to natural enemy control of pests in cirus orchards.

The activity of predators in cultivated agroecosystems may be considered to be far more effective in the control of economically damaging species than chemical means (Pyle *et al.*, 1981). Maintenance of coccinellid populations in citrus orchards would thus contribute to minimal pesticide use, with significant economic implications (Bedford, 1968; Hattingh, 1995).

Chilocorus nigritus is one of the most economically important coccinellids in the Swaziland lowveld, where IGRs are also recommended for use. This study was carried out to identify growth stages of *C. nigritus* susceptible to IGRs recommended for use on citrus in Swaziland. Adult fecundity, larval development and ovicidal activity would be observed in laboratory and field experiments.

MATERIALS AND METHODS

Chemicals used Commercially used formulations of three insect growth regulators were tested. These were, 1) buprofezin (Applaud, Nihon Nohyaku Co., Japan) 30g/100l 25% WP, 2) pyriproxyfen (Nemesis, Agrihold) 30ml/100l plus 300ml mineral oil EC and 3) teflubenzuron (Nomolt, Celamerck GmbH, Germany) 20ml/100l SC. All three chemicals are recommended for use against various citrus pests in Swaziland citrus orchards. Distilled water was used as a control.

Rearing of host scale *Aspidiotus nerii* and *Chilocorus nigritus* The Oleander scale, *Aspidiotus nerii* Bouche was used as a substitute prey for the beetle *Chilocorus nigritus*, collected from bamboo stands at Inyoni Yami Swaziland Irrigation Scheme (Tunzini). The Oleander scale was reared on potatoes *Solanum tuberosum* L. and butternuts *Curcubita moschcata* Turnhalle.

The vegetables were prepared by washing in 2.5% sodium hypochlorite solution then dipped in 0.2% solution of benomyl fungicide (Erichsen *et al.*, 1991). After drying at room temperature for at least 24 hours, they were infested with the scale. Potatoes were used for the stock culture from which the butternuts were infested. Butternuts infested with mature scale were then placed in wooden boxes used for rearing *C. nigritus*. The wooden-framed boxes measured 500mm x 500mm x 800mm, had netted sides, hard masonite bottoms and glass tops. Polyester fibre pads were provided as oviposition sites for the beetles. Water was provided daily by spraying inside the boxes with a microsyringe.Oviposition pads pf polyester fibre were removed three times a week and placed in hatcheries, which were cylindrical insect cages with clear sides and a netted top (380mm high and 200mm wide).

Newly-emerged adults were transferred into the rearing boxes. Any rotting butternuts were removed immediately. Prior to each experiment, *C. nigritus* adults were removed from the rearing boxes and sexed according to the guidelines of Samways and Tate (1984). Immature stages were also selected according to the life stage required for each experiment. Both adult and immature stages were used for the laboratory and field experiments.

Laboratory experiment The laboratory experiments were carried out to identify the IGR-susceptible stage and investigate the developmental effects of the IGRs on the development of *C. nigritus*. Larvae and adults of *C. nigritus* were exposed to IGRs in one of two ways:

(i) *C. nigritus* were fed prey treated with IGRs - Butternuts covered with various stages of *A. nerii* were dipped into either buprofezin, pyriproxyfen, teflubenzuron or the control for 10 seconds then air-dried for at least three hours. The life stage to be tested was then placed on the butternuts after which the butternuts were placed in netted cages measuring 200mm x 200mm x 150mm. Larvae were fed on the treated prey for three days or until moulted, whichever occurred first, then transferred to feed on untreated prey. Observations were made daily. Fecundity of adults over a 24-hour period was assessed at two and seven days after treatment.

(ii) *C. nigritus* were sprayed with IGRs - Specimens were placed in empty netted cages and sprayed with one of the IGRs and then air-dried for at least three hours. The specimens were then transferred onto untreated butternuts infested with scale. Observations were made daily. Adult fecundity over a 24-hour period was assessed at two and seven days after treatment.

Each treatment was replicated three times, with each replicate having at least nine larvae, depending on the availability of the life stage. Five pairs (male and female) of adults per replicate were used in experiments requiring the use of adults.

Field experiment The experiments were carried at the three commercial estates, i.e., Tambuti estate, Tambankulu estate, Inyoni Yami Swaziland Irrigation Scheme (Tunzini) whenever the required IGR was being sprayed. Five pairs of adults were placed inside a 200mm x 200mm x 150mm wooden framed, nylon mesh-covered cage with a butternut. Prior to the spraying of each orchard, four transect lines were measured from the edges of the orchard to be sprayed along the four cardinal points. Along each transect line, five intervals based on the log base two scale were measured, i.e., 2m, 4m, 8m, 16m and 32m.

Two cages were then placed on citrus trees at each interval, although those at 2m and 4m were usually placed on tall weedy plants since they were usually between the space separating orchards. Within the experimental orchard, four cages with adults were randomly placed. In addition, three cages with egg pads and two cages with various stages of *C. nigritus* on butternuts were placed within the experimental orchard. Larvae were also placed at the 2m and 32m intervals.

A full cover spray of buprofezin (0.4kg/2000l plus 3.5l/2000l Sunspray) was carried out at Inyoni Yami Swaziland Irrigation Schemes using J8 spray guns. Pyriproxyfen (600ml/2000l) plus oil 6l/2000l) was sprayed at Tambuti Estate using an eagle sprayer also at full cover. Spraying was carried out in the morning with no wind to minimise drift. Wind conditions were strictly monitored and spraying was postponed if conditions were unsuitable.

The cages were left in the field for 5-6 hours after spraying then taken to the laboratory where they were separated according to the respective intervals. The IGRs were sprayed at different times so that insects exposed to different treatments were never in the same room at the same time. Controls were placed in an unsprayed orchard and then taken to a separate laboratory.
Observations of adult and larval mortality, oviposition and larval development were made on a daily basis, and recording made at 24 hours, 48 hours and one week after spraying.

Statistical analyses The proportion of larvae moulting, dying or pupating was arcsin transformed, while adult oviposition was log (x+1) transformed before analysis using the SPSS statistical package.

RESULTS

Laboratory experiment

(i) *C. nigritus* fed prey treated with IGRs - After 24 hours there were no significant differences in larval mortality either due to the IGR ingested (p = 0.101) or life stage treated (p = 0.194). At 48 hours, larval mortality was significantly higher in those that had ingested buprofezin-treated prey (p = 0.005) and this was more pronounced after a week where mortality due to the same IGR was highly significant compared to the others (p = 0.000). All of the first-instar larvae which had ingested buprofezin-treated scale died during their first moult. The first moult was initiated, with either the old head or body cuticle cracking open, but the new larva was unable to emerge through the old cuticle and eventually died. Mortality of first-instar larvae which had ingested any of the IGRs was significantly higher than that of fourth-instar larvae one week after treatment (Tukey HSD, p = 0.05). Pyriproxyfen had the least effect on larvae after one week (Table 5) with mortality observed only in the first-instar larvae at this time. Larvae which had ingested teflubenzuron-treated scale had relatively low mortality and none of the fourth-instar larvae had died one week after treatment.

Despite the survival and successful moults of the larvae, all larvae which had ingested IGR treated scale did not pupate in all the treatments. These were significantly different from the control ($p = 0.000$) where 94.444± 7.857% of the larvae pupated. In the control group, fourth-instar larvae had a higher rate of successful pupation compared to the younger life stage (Tukey HSD, $p = 0.05$) (Table 5).

Table 5. Percent mortality (±1SD) and pupation (±1SD)of *C. nigritus* larvae fed scale treated with the various IGRs.

IGR used	Life stage treated	% Mortality at indicated days after treatment		% Pupating
		2	7	
Buprofezin	1st	5.135±4.806^{a}	100.00±0.000^{ac}	0.000±0.000^{b}
	2nd/3rd	17.362±23.230^{a}	60.556±34.170^{a}	0.000±0.000^{b}
	4th	10.556±12.946^{a}	2.222±3.849^{ad}	0.000±0.000^{b}
Pyriproxyfen	1st	0.000±0.000^{b}	6.667±11.547^{bc}	0.000±0.000^{b}
	2nd/3rd	0.000±0.000^{b}	0.00±0.000^{b}	0.000±0.000^{b}
	4th	0.000±0.000^{b}	0.00±0.000^{bd}	0.000±0.000^{b}
Teflubenzuron	1st	18.519±6.415^{ab}	18.519±6.415^{bc}	0.000±0.000^{b}
	2nd/3rd	0.000±0.000^{ab}	13.670 ±7.595^{b}	0.000±0.000^{b}
	4th	0.000±0.000^{ab}	0.000±0.000^{bd}	0.000±0.000^{b}
Control	1st	0.000±0.000^{b}	0.000±0.000^{b}	88.888±9.62^{a}
	2nd/3rd	0.000±0.000^{b}	0.000±0.000^{b}	97.22±3.932^{a}
	4th	0.000±0.000^{b}	0.000±0.000^{b}	100.0±0.000^{a}

Figures followed by the same letter (IGR,instar) not significantly different within same column (p=0.05)

A two-way ANOVA indicated a significant interaction between the IGR used for treatment and the life stage exposed ($p = 0.000$). This may be seen in Table 5 where first-instar larvae were observed to have higher mortality when exposed to the IGRs compared to fourth-instar larvae

(Tukey HSD, p = 0.05). There was also a significant interaction between pupation and IGR use (p = 0.000).

(ii) *C. nigritus* larvae sprayed with IGRs - When larvae were sprayed with IGRs and then transferred to uncontaminated scale, there was no significant difference in mortality after 24 hours according to the IGR used (p = 0.059). There was however, a significant difference according to the stadium treated (p = 0.019), with first-instar larvae experiencing significantly higher mortality (Tukey HSD, p = 0.05). After 48 hours, there was no significant difference according to life stage treated (p = 0.071) while there was a difference according to the IGR sprayed (p = 0.004) (Table 6). Further analysis indicated that buprofezin-sprayed larvae had higher mortality compared to the teflubenzuron-sprayed group and the control. After 1 week, this difference in mortality was highly significant (p = 0.001) with buprofezin-sprayed groups experiencing the highest mortality (Tukey HSD, p = 0.05). Differences in mortality relative to the life stages treated were significant among the three treatment groups a week after treatment.

Differences in development of larvae up to pupation was not affected by the life stages treated (p = 0.960) but were significant when comparing the IGR used for spraying (p = 0.000) (Table 6). The control group had the highest proportion of larvae pupating (94.444 ±7.857%). The buprofezin- treated group had the lowest proportion pupating (7.755 ± 13.927%) compared to the pyriproxyfen (44.075 ±17.813%) and teflubenzuron (65.766 ± 14.992%) groups (Tukey HSD, p = 0.05).

Table 6. Percent mortality (±1SD) and pupation (±1SD) of *C. nigritus* larvae after being sprayed with the respective IGRs.

IGR used	Life stage treated	% Mortality at indicated days after treatment		% Pupating
		2	7	
Buprofezin	1st	28.571±14.286^{a}	61.905±35.952^{a}	14.426±24.744^{c}
	2nd/3rd	25.181±7.035^{ac}	82.043±5.362^{ad}	8.978±2.682^{c}
	4th	2.381±4.124^{ad}	7.937±8.362^{ae}	0.000±0.000 ^{c}
Pyriproxyfen	1st	0.000±0.000^{a}	5.556±9.623^{b}	38.889±25.459^{b}
	2nd/3rd	29.167±29.463^{ac}	50.001±58.926^{bd}	41.667±0.000^{b}
	4th	5.159±4.507^{ad}	5.159±4.507^{be}	50.3968±14.103^{b}
Teflubenzuron	1st	5.556±9.623^{b}	11.111±9.623^{c}	67.413±17.797^{b}
	2nd/3rd	7.778±8.389^{bc}	18.333±21.279^{cd}	63.519±23.807^{b}
	4th	0.000±0.000^{bd}	0.000±0.000^{ce}	62.696±11.252^{b}
Control	1st	0.000±0.0.000^{b}	0.000±0.000^{c}	88.888±9.622^{a}
	2nd/3rd	0.000±0.000^{b}	0.000±0.000^{c}	97.22±3.912^{a}
	4th	0.000±0.000^{b}	0.000±0.000^{c}	100.00±0.00^{a}

Figures followed by the same letter (IGR,instar) not significantly different within same column (p=0.05)

Pupae resulting from larvae treated with buprofezin and teflubenzuron appeared normal but no adults emerged during the period of observation. Pupae developing from larvae treated with pyriproxyfen usually illustrated some deformities whereby the wings would spread out of the pupal case and none of these deformed pupae produced any adults. There was no significant interaction in mortality of larvae due to the life stage treated and IGR used (p = 0.051) seven days after treatment. Pupation success of the various life stages treated was also not affected by an interaction between the IGR used and life stage treated (p = 0.850).

In both experiments, there was no adult mortality observed during the period of observation, indicating that the IGRs had no impact on adult mortality. Seven days after treatment there was no significant difference in oviposition success over a 24-hour period according to IGR treatment, whether the adults were exposed through ingestion of treated scale (X^2= 5.574, p = 0.134) or sprayed (X^2= 2.979, p = 0.084). Oviposition was higher seven days after treatment in those exposed through ingestion of treated scale (X^2=4.888, p = 0.027). When the adults were sprayed, there was no significant difference in oviposition 48 hours and one week after treatment (X^2= 4.786, p = 0.188) (Table 7).

Table 7. Eggs deposited per female (±1SD) after the respective treatment over a 24-hour period.

IGR used	Adults fed treated scale		Adults only sprayed	
	2 days	7 days	2 days	7 days
Buprofezin	$0.556+0.337^a$	$2.639+1.228^a$	$1.611+1.434^a$	$2.694+0.376^a$
Pyriproxyfen	$2.833+0.583^a$	$3.306+0.918^a$	$2.417+2.315^a$	$4.625+1.458^a$
Teflubenzuron	$0.556+0.586^a$	$3.610+0.649^a$	$1.445+1.932^a$	$3.222+0.488^a$
Control	$0.917+0.301^a$	$1.111+0.337^a$	$0.917+0.301^a$	$1.111+0.337^a$

Figures followed by the same letter not significantly different within same column (p=0.05)

Field experiment None of the IGRs were directly toxic to adult *C. nigritus*. Death of adults was usually due to drowning as a result of exposure to the high volume of liquid during spraying. This was observed in individuals at the centre of the experimental orchard or those placed in close proximity to the orchard edges, e.g. at 2m. The viability of eggs produced by the experimental female *C. nigritus* could not be investigated due to the lack of equipment. Only one incubation chamber was available and the eggs would undoubtedly have been contaminated within the confined space.

There were no significant differences in oviposition according to the direction of transect whether the cages at the centre of the orchard were included ($p = 0.888$) or excluded ($p = 0.821$). There was however a significant difference in oviposition depending on the distance from the orchard edge ($p = 0.002$) (Table 8). When the cages at the centre of the orchard were included, there was a significant difference in oviposition success according to the interval at which the cages were placed ($p = 0.005$). Further investigations indicated that exposure at 2m resulted in a significantly lower oviposition from those at 16m and 32m (Tukey HSD, $p = 0.05$). When the cages at the centre of the orchard were excluded, oviposition at 2m was still significantly lower than that at 8m, 16m and 32m (Tukey HSD, $p = 0.05$).

There were significant differences in the oviposition rate when comparing IGRs. Oviposition in the control groups was significantly different from the buprofezin and pyriproxyfen treated groups ($p = 0.000$).There was no significant interaction between the IGR sprayed and the distance from the orchard, whether the central group was included ($p = 0.349$) or excluded ($p = 0.288$).

One week after treatment, the direction of transect still had no significant effect on subsequent oviposition, whether the centre was included ($p = 0.359$) or excluded ($p = 0.261$). Females at 2m had a significantly lower oviposition than those at 8m, 16, and 32m whether the central group was included ($p= 0.009$) or excluded ($p = 0.005$) (Table 8). There was a weakly significant difference in oviposition due to IGR treatment ($p = 0.05$).

There was no interaction between the IGR used and the distance from the orchard sprayed whether the cages at the orchard centre were included ($p = 0.608$) or excluded ($p = 0.740$). There was also no significant difference in oviposition two days and seven days after treatment ($p = 0.163$). In addition there was no interaction between the IGR used and duration of period after treatment ($p = 0.159$). Of the eggs exposed to buprofezin and pyriproxyfen, none hatched, while 80.089 ± 3.201% of the control group hatched. Eggs exposed to IGRs turned from their normal golden brown colour to black. Hatching success of experimental eggs was thus significantly different ($p = 0.000$).

Due to limitations in availability, larvae were placed only at the 1) centre of the orchard, 2) 2m and 3) 32m. Development of larvae up to pupation was significantly different between the IGRs, whereby the control group was significantly different from the treated groups ($p = 0.003$) (Table 9). A higher proportion of larvae exposed at 32m pupated compared to those at 2m and centre ($p =0.036$). 57.895% of larvae exposed to buprofezin developed into seemingly normal pupae but all failed to produce any adults. 15.789% formed deformed pupae and only 26.316% emerged as adults. In larvae treated with pyriproxyfen, the pupae were also deformed and never produced any adults during the period of observation. Other fourth-instar larvae never pupated, i.e., they persisted as larvae beyond the period of observation. Deformities in pupae were observed as the emergence of wings through the pupal case. The wings were smaller than normal.

Table 8. Eggs oviposited per female (±1SD) after exposure to IGRs during spraying . Eggs laid over a 24-hour period.

IGR used	Distance from orchard edge	Eggs per female at times indicated	
		2 days	7 days
Buprofezin	2m	0.417±0.083^{bc}	0.000±0.000^{ac}
	4m	0.667±0.609^{b}	1.125±1.235^{a}
	8m	0.875±1.103^{b}	1.208±1.462^{ad}
	16m	2.333±1.179^{bd}	1.958±1.572^{ad}
	32m	2.167±1.056^{bd}	2.583±1.853^{ad}
Pyriproxyfen	2m	0.000±0.000^{bc}	0.500±1.001^{ac}
	4m	0.875±1.117^{b}	3.208±2.175^{a}
	8m	1.708±1.250^{b}	3.125±2.303^{ad}
	16m	1.667±1.421^{bd}	4.208±3.568^{ad}
	32m	1.125±0.370^{bd}	2.875±2.299^{ad}
Control	2m	2.125±1.505^{a}	1.792±0.917^{a}
	4m	2.083±1.351^{a}	2.167±0.491^{a}
	8m	3.833±1.581^{a}	3.833±0.694^{a}
	16m	4.042±1.468^{a}	3.042±0.712^{a}
	32m	2.833±0.861^{a}	2.500±0.943^{a}

Figures followed by the same letter(IGR, distance) not significantly different within same column (p=0.05)

Table 9. Average % pupation (±1SD) of larvae exposed to IGRs during spraying.

Distance from orchard edge	% larval pupation after exposure to IGRs		
	Buprofezin	Pyriproxyfen	Control
0m/centre	7.407±12.828 ^{a}	6.110±6.734 ^{a}	81.814±12.850 ^{a}
2m	0.000±0.000 ^{a}	12.500±12.501 ^{a}	90.000±0.000 ^{a}
32m	53.125±27.717 ^{b}	46.875±15.729^{b}	90.000±7.352^{b}

Figures followed by the same letter not significantly different within same column (p=0.05)

DISCUSSION

Chitin synthesis actively occurs during larval development (Boneβ, 1983; Ishaaya, 1990) and as such, IGRs would be expected to have an impact on larval stages, with adults seldom suffering mortality due to these chemicals. This was observed in this study, whereby no adult mortality due to IGR activity was observed. Effects on adults were thus investigated in terms of their fecundity after exposure to IGR.

Despite the initial siginificant differences in fecundity of exposed females compared to the control after two days, there was only a slight non-significant difference after seven days. This therefore indicated that the IGRs did not have a significant impact on the overall fecundity of the exposed females, as also observed by Hattingh and Tate (1995) for the same species, Peleg (1983) for *C. bipustulatus* and Ascher *et al.* (1986) for *Carpophilus hemipterus* (Linnaeus).

The viability of the eggs oviposited could not be determined due to various constraints. Various studies, however, have indicated that egg viability is severely compromised after exposure of the female to IGRs. Egg hatch in *C. bipustulatus* females exposed to methoprene, diflubenzuron and fenoxycarb was completely inhibited, with similar results obtained when *C. hemipterus* was exposed to diflubenzuron, chlorfluazuron, XRD-473 and teflubenzuron (Peleg, 1983; Ascher *et al.*, 1986). Although egg viability was restored when the females were transferred to a clean environment, this would still have a detrimental effect on the predator populations in a field environment. The predators would fail to have a sufficiently consistent population increase so as to control prey (pest) populations effectively.

Although there were significant differences in fecundity due to distance from the orchard edges, this was unlikely to be due to IGR activity. The females at the centre of the orchard and at 2m from the edge were drenched during spraying. The slight decline in egg production may have been due to the disruption of suitable environmental conditions experienced during spraying. Mating and oviposition would thus be delayed while the individual recovered.

All the IGRs tested had detrimental effects on *C. nigritus* larvae to varying degrees. Since larval development involves moulting and associated chitin synthesis, the effects of these chemicals would thus be expected to be most pronounced during these developmental stages. As seen from the results, buprofezin activity was apparent at the end of the life stage treated, with the younger stages, i.e. first-instar larvae being the most susceptible. Fenoxycarb was also observed to have a more detrimental effect on earlier-instar larvae than mid-instar larvae of *Stethorus punctum* (LeConte) (Biddinger and Hull, 1995).

Buprofezin acts by the inhibition of chitin synthesis as well as disturbance of hormonal balance (Nagata, 1986; Kobayashi *et al.*, 1989; Konno, 1990). Such activity results in the inhibition of ecdysis, and mortality is the result of the larvae's inability to emerge from the old cuticle, as observed in this study. Development of larvae in the pyriproxyfen and teflubenzuron treatments was not significantly different from the control in both laboratory and field experiments. Teflubenzuron, methoprene and fenoxycarb also did not affect larval development in *Rodolia* *cardinalis* and *C. bipustulatus*, even when the larvae were fed treated scale (Peleg, 1983; Loia and Viggiani, 1992).

When larvae were fed IGR-treated scale, none of them pupated despite having experienced all the relevant moults, as in the pyriproxyfen and teflubenzuron treatments. In addition to the high retention of IGRs in larval tissue and lower detoxification, late-instar larvae and pupae are extremely sensitive to IGR activity, so the presence of these in the insect's body, even in minute quantities, would still have the effect of inhibiting further development during the late developmental stages (Staal, 1975; Ishaaya, 1990). Peleg (1983) also observed that despite *C. bipustulatus* having had normal larval moults and reaching the fourth stadium, all the larvae had extension of the fourth-instar larval period by 2-3 weeks before dying. None of these pupated after having fed on IGR-treated scale. *Coccinella septempuncata* larvae also failed to complete development after exposure to diofenolan. Inability of coccinellid larvae to complete development was reported as a typical IGR effect (Sechser, 1994).

IGRs act as stomach poisons and would thus have maximum activity when ingested, i.e. via contaminated prey for predators, rather than when the predators were exposed by contact, as confirmed by the higher mortality and failure to pupate in the group that had fed on IGR-treated scale compared to those which were only sprayed with the insect growth regulators. This is also possible in the orchard environment, where the less-mobile larvae would tend to feed on prey (pest) contaminated by IGRs sprayed within the orchard. Invariably, the predator populations would be affected by IGRs at the end of each larval stage during moulting and inhibition of pupation. This would result in the decline of predator populations due to the lack of progeny replacement and production of a reproductive generation (Kramer *et al.*, 1981).

IGRs have the most detrimental effect during the initial spray periods when the predators are in direct contact with the chemicals. Subsequent to this, the predators are able to continue with development, including the restoration of egg viability if in an uncontaminated environment. This was observed in various studies where the transfer of predators to an uncontaminated environment resulted in the recovery of specific developmental processes (Hattingh and Tate, 1995; Peleg, 1983). The larvae temporarily exposed in the field and laboratory illustrated this in their ability to develop up to the formation of pupae.Movement of adults to uncontaminated habitats is also possible in the field environment, where *C. nigritus* is known to inhabit bamboo stands where scales are available as alternative prey (Samways, 1984). Movement between the orchards and uncontaminated environments would however, be maximised if there is no preference for either scale species occupying the different habitat types and the predators are able to readily switch between the two prey species. If *C. nigritus* prefers scale species in the orchards then the predator would be continually exposed to contaminated prey resulting in the decline of the predator population.

All three IGRs had strong ovicidal activity so that egg hatch was completely inhibited in all the eggs exposed. According to Masner *et al.* (1987), the ovicidal activity of IGRs is through the inhibition of embryonic development in young eggs, as used in this case. Ovicidal effects may also be through the female whereby the IGRs are incorporated into the eggs through the ovaries, so the eggs laid by treated females also do not hatch and if they hatch, emerging larvae do not survive the early stadia (Staal, 1975; Ascher *et al.*, 1986; Yasui *et al.*, 1987; Ishaaya and Horowitz, 1992).

Results indicate that the IGR effects in the field were still aparent at 32m, which was the furthest distance assessed in this study. Larvae exposed at this distance had significantly lower pupation rates, 53% (buprofezin) and 47% (pyriproxyfen) , copmared to the controls (90%). The effects of these chemicals were further compounded by the formation of abnormal pupae and lower percentage of adult emergence.

Although adults were not affected by the IGRs per se, their reproductive success was adversely affected as a result of their laying non-viable eggs after exposure to IGRs (Ascher, *et al.*, 1986; Peleg, 1983, Hattingh and Tate, 1995). This would imply a decline in the population's reproductive potential. In addition to IGR effects on larval development and ovicidal activity, this would ultimately have a negative impact on *C. nigritus* populations. Lower predator populations would be unable to effectively control pest populations, which tend to recover much more quickly from adverse conditions. Even when scale populations are at low levels, predator presence is also necessary for a regulatory effect on the scale pests (Samways, 1984).

The persistence of these chemicals in the field also needs to be taken into account. Hattingh and Tate (1995) observed that pyriproxyfen residues still had adverse effects on female *C. nigritus* even 131 days after spraying. Despite having a shorter residual activity (Hattingh and Tate, 1995), the initial devastating effects of buprofezin observed in this study would still have a negative impact on predator population levels and contribute to the predator's inefficiency in controlling scale pests. IGR persistence needs serious consideration since these chemicals are sprayed in spring and early summer when predator populations are building up. Such an increase is likely to be hampered by the presence of IGRs, even if in residual form. In the field environment, buprofezin was the least detrimental since larvae exposed to it were able to develop up to the reproductive adult stage while those exposed to pyriproxyfen failed to do so. Although teflubenzuron was not tested here in the field, the laboratory experiments on this IGR illustrated it to be the least detrimental of the three compounds tested.

The spraying of IGRs in citrus coincides with periods of predator population build-up (spring, early summer). While predator populations increase, there are likely to be the immature larval stages developing. From the results obtained, larval and egg stages suffer the most detrimental effects from IGRs, especially the early instars. Development of these stages to effect coccinellid population increases in the orchards is thus likely to be impeded so that the rate of predator activity in the orchards is likely to be reduced. This is especially true if the residual activity of these IGRs is also taken into consideration. If IGR spraying cannot be avoided in orchards with high coccinellid activity, it may be advisable to avoid coincidence of spraying with periods of maximum larval and egg development. On a practical level, this implies the need for preventative action, i.e. encourage natural control and keep IGR use to a minimum, if at all.

C. nigritus is a valuable predator in southern Africa and sustainable population levels within the citrus agroecosystem are of considerable importance. Careful monitoring of IGR activity and their effects on economically valuable predators like the coccinellid *C. nigritus,* thus remains to be an essential undertaking for the agrochemical and agricultural industry.

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CHAPTER 5

THE ACTIVITY OF INSECT GROWTH REGULATORS ON A NON-TARGET SPECIES, *BOMBYX MORI* (LEPIDOPTERA : BOMBYCIDAE) IN THE FIELD AND LABORATORY.

ABSTRACT

Three insect growth regulators (IGRs) buprofezin, pyriproxyfen and teflubenzuron used against citrus pests,were tested in the laboratory and in the field on the silkworm, *Bombyx mori*. All IGRs were tested using the recommended dosage. Moulting success and survival after moulting were assessed for each treatment group. Laboratory experiments indicated that all three IGRs caused mortality around the moulting period or afterwards. Mortality was due to failure to complete moulting or occurred immediately after moulting. None of the experimental groups were able to complete larval development when larvae ingested leaves treated with any of the IGRs. The fifth instar was the most susceptible stage, failing to pupate in all treatments. 6.67% of 2nd-, 33.33% of 3rd- and 20% of 4th-instar larvae which had ingested pyriproxyfen treated leaves, and 13.33% of 4th-instar larvae which ingested buprofezin-treated leaves produced supernumerary larvae. In the field experiments, there was minimal spray drift from a knapsack sprayer. *Bombyx mori* larvae exposed to buprofezin from a knapsack sprayer were able to complete development, with adult emergence not significantly different from that of the control. Pyriproxyfen and teflubenzuron arrested larval development, with none pupating, unlike the buprofezin treatment and control. The use of IGRs in citrus may pose a threat to wild entomofauna and non-target species, resulting in possible disruption of integrated pest management programmes.

in the orders Homoptera (Lampson and Morse, 1992), Lepidoptera (Ismail and Wright, 1991) and Coleoptera (Ascher *et al.*, 1986; Eisa and Ammar, 1992). Triflumuron, another chitin- synthesis inhibitor, arrests instars of insects in at least four orders, i.e Diptera, Lepidoptera, Coleoptera and Hymenoptera, while larvae of some homopteran insects, e.g. *Psylla* and thysanopteran insects e.g. *Thrips* also have growth inhibition (Hammann and Sirrenberg, 1980; Blumberg *et al.*, 1985).

Pest outbreaks have been associated with the frequent application of synthetic insecticides, such as *Icerya purchasi* Maskell after regular application of IGRs in Israel (Mendel and Blumberg, 1991). While IGRs have been heralded as highly compatible with IPM practises in commercial agroecosystems, Hattingh and Tate (1995) and Hattingh (1996) indicate that some IGRs used in Southern Africa may not be as specific as promoted, and thus could be detrimental to the pest/predator balance.

The introduction of pyriproxyfen in the 1991/92 season led to widespread population outbreaks of the Australian bug *Icerya purchasi*, especially in orchards adjacent to those sprayed with IGRs. Populations of its natural enemy, *Rodolia cardinalis* (Mulsant) were unable colonise infested orchards and keep the pest at economically-acceptable levels. In addition, pyriproxyfen in particular, was implicated in the elevation of a secondary pest to primary pest status, i.e. the citrus mealybug, *Planococcus citri* (Risso) (Hattingh, 1996). Introduction of diofenolan against red scale, *Aonidiella aurantii* (Maskell) also led to mealybug outbreaks during the 1994/95 season. In addition, media reports suggested that the use of IGRs, particularly diofenolan, may have led to the decline of *Bombyx mori* (Linnaeus) cultures in local schools (Grout, 1998).These silkworms, growing to'giant specimens', had been fed mulberry leaves from trees growing in unsprayed areas within the vicinity of target orchards. The mulberry leaves could have been contaminated by spray drift settling on the mulberry trees. Diofenolan was subsequently withdrawn from the market, after only one season of use by farmers.

Several studies have been carried to investigate the activity of IGRs on non-target, especially beneficial, species. Investigations by Peleg (1983), Loia and Viggiani (1992) and Hattingh and Tate (1995) indicated that beneficial coccinellids were adversely affected by pyriproxyfen, teflubenzuron and buprofezin. *Aphytis* sp., parasitoids of red scale, were adversely affected by pyriproxyfen (Peleg, 1988), while other beneficial insects , such as *Typhlodromus* sp., were also adversely affected by triflumuron and fenoxycarb (Mansour et al, 1993). These studies suggest that IGRs could have some undesirable effects (Hattingh, 1996), and while these effects may be less than those of broad spectrum insecticides (Grenier and Grenier, 1993), further investigations still need to be carried out to ascertain their selectivity.

The present study investigates the toxicity and activity of buprofezin, pyriproxyfen and teflubenzuron on a non-target species, *Bombyx mori* in Swaziland. All three IGRs are presently recommended for use by the Swaziland citrus industry. Evaluations are both in the field and in the laboratory. Drift effects are also evaluated in the field.

MATERIALS AND METHODS

Insect growth regulators

Three insect growth regulators were tested against the silkworm, *Bombyx mori*. The IGRs, buprofezin ('Applaud', Nihon Nohyaku Co., Japan) 30g/100l 25% WP; pyriproxyfen ('Nemesis', Agrihold)30ml/100l plus 300ml oil EC, and teflubenzuron ('Nomolt', Celarmerck GmbH, Germany) 20ml/100/ SC, were prepared at the recommended dosages used on Swaziland citrus in the lowveld. Distilled water only was used for controls.

Rearing of *Bombyx mori*

Bombyx mori eggs were incubated at 26±2°C, and after five days newly-emerged larvae were checked daily and removed if present.These young larvae were kept in glass troughs at room temperature (25±2°C) and fed mulberry leaves. Fresh leaves were provided daily, with the simultaneous removal of waste material. The various instars were removed from the culture immediately after moulting so that same-aged instars were cultured together. They were transferred to petri-dishes in preparation for experimental work. Prepupae in both experimental and control trials were placed in paper cocoon nests to facilitate pupation. For practical reasons only the larvae and not the adults were tested, since they are phytophagous and thus most likely to be exposed to contaminated vegetation.

Field experiment

Three plots, each consisting of nine citrus seedlings (about 1m height) were planted in a field in the lowveld, and each plot was sprayed with one of the IGRs used.To determine the possible drift effects from the sprayed plots, four transect lines running along the four cardinal directions (north, east, west and south) were laid out from all four outer edges of each plot. Each transect was marked at intervals based on the log base 2 scale, i.e. 2m, 4m, 8m, 16m and 32m and additional seedlings were planted at each mark. A 200mm x 150mm x 150mm wooden-framed, nylon mesh-covered cage was placed at each interval. An uncovered petri-dish containing six silkworms was placed in each cage. Six additional cages were placed randomly on seedlings within each plot.

As would be observed in the commercial citrus estates, spraying was carried out only calm days. In each plot, only the nine central seedlings (making up a plot) were sprayed. Spraying was carried with a Cooper Pegler (CP3) Classic knapsack sprayer, using the cone-spray nozzle at high pressure (> 137.896KPa). Each tree was sprayed until run-off. The cages were left in the field for between 3.5 to 4.0 hours then removed due to excessive heat and ant activity, since these had proved to be detrimental to the silkworms during trials. The silkworms were taken to the laboratory where their growth and development were monitored. Special attention was paid to their mortality, ability to moult/pupate and adult emergence. The condition of the specimen and feeding habits were also noted. Monitoring was carried out until the larvae completed development (pupation and adult emergence) or died. All specimens were fed fresh, untreated mulberry leaves on a daily basis, sometimes twice a day, depending on the feeding rate and growth stage. Each experimental group had 156 silkworms and was replicated twice, except the control which had one replicate due to reasons explained in the discussion.

Laboratory experiment

Laboratory assays investigated the toxicity and developmental effects of the IGRs when ingested by various instars of *B. mori*. Mulberry leaves were treated by dipping, with agitation, into prepared IGR solutions for 10 seconds then air dried for 15 minutes. Controls were dipped in distilled water. Each instar was tested for susceptibility, whereby newly-moulted larvae (less than 24 hours old) were used for each instar. The experimental larvae were kept on a treated diet for five days or until they moulted, whichever was less, and subsequently fed on untreated leaves. Each IGR trial was replicated three times, with seven larvae per replicate in the first- and second-instar trials and five larvae in the latter instars. Mouthparts (mandibles) of specimens fed on pyriproxyfen and controls were carefully dissected and mounted on depression slides then observed under a microscope.

Statistical analyses

The proportion of larvae moulting successfully at each instar were noted for each of the IGRs and control. The proportions were then transformed using the arcsin transformation and analysed by ANOVA and multiple range tests.

RESULTS

Field experiment

Moulting success - In the buprofezin trial, the proportion of larvae successfully moulting was not affected either by distance from the plot edge ($p = 0.346$) or by the direction of transect ($p = 0.6187$). Similar results were obtained for the pyriproxyfen trial, where distance ($p = 0.782$) and direction (p = 0.266) had no effect on the moulting success. While direction had no significant effect on moulting success in the teflubenzuron trial (p = 0.923), specimens placed in cages at the various distances showed significantly different proportions moulting success (p = 0.001). Specimens placed at 2m from the edge, showed a significantly smaller proportion successfully developing into the next instar. At 2m, larvae were observed trying to moult but had varying levels of success. While some failed to remove the old body cuticle, thus remaining enclosed within, others managed to get the head cuticle off but died, having failed to emerge through the old body cuticle. This was true for all four transects at 2m around the teflubenzuron sprayed plot. Control specimens did not have significantly different proportions moulting, either with regard to distance from plot edge (p = 0.438) or to direction (p 0.418). Results suggest that there was an absence of the effect of drift from the buprofezin and pyriproxyfen application,while the teflubenzuron trial showed minimal drift up to 2m from the sprayed plot edge.

When the proportion of larvae moulting successfully for the three IGRs (in transects only) were compared to the control, the success in moulting into the next instar at the various distances was not significantly different (p = 0.172). However, when the specimens within the sprayed plots were also included, there was a significant difference in moulting success (p = 0.021). The larvae which were sprayed directly, (i.e. within plots) had a significantly lower proportion moulting successfully compared to larvae placed at 16m and 32m away from the plot's edge (Tukey HSD, p = 0.05).

Moulting success was significantly affected by the type of IGR sprayed (Table 10). When only specimens in the transects were examined (within plots excluded), IGR treatments were significantly different ($p = 0.000$). Larvae exposed to pyriproxyfen and teflubenzuron had a lower proportion developing into the next instar, while larvae exposed to buprofezin were not significantly different from the control (Tukey HSD, $p = 0.05$).

Observations of pyriproxyfen- and teflubenzuron-exposed larvae indicated that the IGRs produced different morphogenetic effects. Individuals exposed to pyriproxyfen usually died either due to their inability to complete moulting, only managing to get the head cuticle off or they were unable to feed after moulting. This therefore reduced the number of larvae developing into subsequent instars and resulted in no larvae pupating in this group. *B. mori* larvae treated with teflubenzuron also had incomplete moults. In addition to this, moulting failure was manifested by the exudation of body fluids (haemolymph) through the posterior or 'bleeding', whereby body fluids would be exuded through lesions on the body surface and points of weakness, e.g. between segments. Larvae experiencing this subsequently died. Other larvae moulted successfully but failed to spin cocoons after the fifth instar, so that no larvae in the teflubenzuron trial managed to pupate.

Inclusion of larvae exposed within the plots also indicated that IGR treatment significantly affected moulting success ($p = 0.000$). Examination of specimens only within the plots, i.e sprayed directly, also showed a significant difference in proportion moulting successfully depending on the IGR sprayed ($p = 0.031$) (Table 10). Teflubenzuron-treated larvae had a significantly lower proportion of successful moults compared to buprofezin-treated and control specimens.

Table 10.The proportion of successful moults (means ± 1 S.D.) at each of the intervals along the transect lines. Direction of transect not separated because not significant different from each other. See results section.

IGR treatment	PROPORTION OF SUCCESSFUL MOULTS Distance along transect					
	In plot	2m	4m	8m	16m	32m
Buprofezin	0.967a (±0.058)	0.95a (±0.100)	0.90 a (±0.200)	1.0a (±0)	0.95 a (±0.1)	1.0 a (±0)
Pyriproxyfen	0.55 b (±0.526)	0.875 b (±0.159)	0.833 b (±0.236)	0.667 b (±0.272)	0.917 b (±0. 096)	0.750 b (±0. 215)
Teflubenzuron	0.167c (±0.192)	0.208 c (±0.250)	0.75 b (±0.215)	0.75 b (±0.289)	0.958 b (±0.084)	0.958 b (±0.084)
Control	0.967 a (±0.058)	1.0 a (±0.000)	1.0 a (±0.000)	0.95 a (±0.100)	1.0 a (±0.000)	1.0 a (±0.000)

F = 12.0537, p = 0.000 (90, 3) with treated groups in centre included. Figures followed by the same letter not significantly different within columns (P=0.05).

Pupation and adult emergence - As indicated above, no larvae exposed to pyriproxyfen and teflubenzuron were able to pupate. Those that reached the fifth instar were unable to spin cocoons and thus died after prolonged periods of starvation. Comparisons were thus made between buprofezin-treated and control larvae. Larvae in the control trial had a significantly higher proportion managing to pupate, 0.75 ± 0.2 compared to 0.55 ± 0.27 ($p = 0.009$) in the buprofezin trial. However, 38% ± 21% and 48% ± 23% adult eclosion was observed in the buprofezin and control groups ($p = 0.190$). This indicated that a higher proportion of the buprofezin-treated pupae emerged while in the control trial, a slightly lower proportion emerged relative to the proportion that pupated.

Laboratory experiment

Successful moults - The proportion of successful moults was significantly different between the IGRs tested and control specimen (p = 0.000) (Table 11). Control larvae successfully moulted, while larvae treated with the IGRs had varying success and survival rates. Teflubenzuron-treated larvae had no success in moulting (and pupation) at all life stages, indicating high activity of this compound against *B. mori*. Sixteen percent of larvae which had fed on teflubenzuron-treated leaves started 'bleeding' at points of weakness, i.e through abdominal legs and intersegmental folds, and subsequently died, as observed in the field experiment. This treatment differed significantly from both buprofezin- and pyriproxyfen-ingesting larvae (Tukey HSD, p = 0.05).

Moulting success varied significantly between life stages (p =0.000). First and fifth instar larvae were the most susceptible stages, with none (buprofezin and teflubenzuron) or a very low proportion of only 0.143 (pyriproxyfen) of first instar larvae moulting into the subsequent life stage and no fifth instar larvae pupating (Tukey HSD, p =0.05). There was also a significant interaction between the IGR used and the life stage fed (p = 0.000). This suggested that the IGRs affected the moulting success of the various life stages differently. First and fifth instar larvae ingesting buprofezin-treated leaves had no moulting / pupation success, while pyriproxyfen seemed to have a significant impact on the fifth instar larvae. Second and third instar larvae treated with buprofezin or pyriproxyfen had a slightly higher proportion moulting successfully

Table 11. Proportion of successful moults per instar fed (mean ± 1 S.D.).

IGR applied to leaves	PROPORTION OF SUCCESSFUL MOULTS Life stage fed				
	1st	2nd	3rd	4th	5-Pupating
Buprofezin	0 b (±0.000)	1.0 b (±0.000)	0.933 b (±0.115)	0.133*b (±0.231)	0 b (±0.000)
Pyriproxyfen	0.143 b (±0.142)	1.0*b (±0.000)	0.867*b (±0.115)	0.667*b (±0.115)	0 b (±0.000)
Teflubenzuron	0 b (±0.000)	0 c (±0.000)	0 c (±0.000)	0 c (±0.000)	0 c (±0.000)
Control	1.0 a (±0.000)	1.0 a (±0.000)	1.0 a (±0.000)	1.0 a (±0.000)	1.0 a (±0.000)

Figures followed by the same letter not significantly different within columns (p = 0.05)
* = developed supernumerary instar

Table 12. The mortality of larvae (mean ± 1 S.D.) after successful moulting and period of larval development from 1st to 5th instar.

IGR used on leaves	PROPORTION MORTALITY AFTER MOULTING Life stage				Development period (days) 1st to 5th instar
	1st	2nd	3rd	4th	
Buprofezin	1.0* a (±0.000)	0.800 a (±0.000)	0.800 a (±0.000)	0.800 a (±0.346)	27.714 a (±4.716)
Pyriproxyfen	1.0 a (±0.000)	0.933 a (±0.115)	0.400 a (±0.200)	0.522 a (±0.115)	27.692 b (±3.066)
Teflubenzuron	1.0* a (±0.000)	1.0* a (±0.000)	1.0*a (±0.000)	1.0* a (±0.000)	not complete development
Control	0 b (±0.000)	0 b (±0.000)	0 b (±0.000)	0 b (±0.000)	20.833 c (±1.948)

* = unable to moult, mortality experienced around moulting period. Figures followed by the same letter not significantly different within columns (p = 0.05)

compared to the other instars (p = 0.0547). Teflubenzuron had a devastating impact on all stages, with no moulting success observed at any stage.

Survival after moult - Mortality was observed around the moulting period or immediately afterwards. There was a significant difference in the proportion of larvae dying depending on the IGR which was ingested (p = 0.000). This ranged from 0% mortality in controls to 100% mortality in the teflubenzuron-ingesting specimens. The proportion of larvae dying in both buprofezin and pyriproxyfen groups were significantly lower than those in the teflubenzuron group (Tukey HSD, p =0.05).

Ingestion of leaves treated with any of the three IGRs by first instars resulted in none of them surviving to the second life stage (Table 12). Buprofezin- and teflubenzuron-ingesting first instar larvae died around the moulting period, with individuals being unable to shed the old cuticle. Larvae ingesting buprofezin-treated leaves were observed to have very taut bodies with an enlarged fluid-filled cephalothoracic region. Teflubenzuron-treated first instar larvae exuded body fluids, and if moulting was initiated, the larvae were usually unable to complete the moult. Individuals were thus unable to move nor feed and died within 48 hours of having initiated the moult. An average of 14.29% of pyriproxyfen-ingesting first-instar larvae moulted, but they all died after having moulted into seemingly normal second instar larvae, while the rest died during partial moults.

A proportion of second and third instar larvae ingesting leaves treated with buprofezin or pyriproxyfen were able to successfully moult into third and fourth instars respectively (Table 11) but a significant proportion subsequently died without further moulting. Buprofezin-ingesting larvae showed mortality characteristics similar to first instar larvae or simply stopped feeding, making no attempt to feed on the leaves provided. The cause of death of successfully-moulted individuals ingesting pyriproxyfen was unknown. 22.86% of all instars which had ingested leaves treated with pyriproxyfen were unable to feed after moulting. These seemingly normal larvae had difficulties in feeding. Unlike the buprofezin non-feeders, the larvae would attempt to feed but failed to chew off any leaf material, as observed in a proportion of larvae in the semi-field experiment. As a result, these larvae probably died of starvation. This was observed in larvae fed at the 2nd, 3rd and 4th instars, with the majority unable to feed after the 4th instar moult (75% of non-feeders). On examination of the mandibles, the failure of the newly-moulted larvae to feed was not due to the diffences in mandibular structure since these were apparently not different from those of control larvae. However, muscles articulating the movement of the mandibles had excessive cell growth. The fibrils constituting the adductor and abductor muscles were clearly enlarged. Tracheoles supplying these muscles were also enlarged and clearly visible. In control specimens, the muscles were translucent so that the muscular fibres and tracheoles were not visible.

In the two groups which ingested either buprofezin- or pyriproxyfen-treated leaves, 13.3% and 66.7% of fourth instar larvae respectively succeeded in developing into the fifth instar. Larvae however, stopped feeding and did not spin cocoons. Larvae shrank and died without pupating.

Supernumerary instars were also observed in 6.67%, 33.33% and 20% of larvae which had been fed pyriproxyfen-treated leaves at the 2nd, 3rd and 4th instars respectively. In the buprofezin trial, a small proportion of larvae in the 4th instar only (13.33%) had a fifth moult. All supernumerary larvae had only one additional, sixth instar, which appeared larger than the previous one. The supernumeraries were however, unable to pupate.

The proportion of larvae reaching the fifth instar was significantly lower in the larvae fed leaves treated with either buprofezin or pyriproxyfen compared to the control ($p = 0.000$). The completion of larval development was also affected by the stage at which the IGR treated leaves were provided ($p = 0.003$). A significantly lower proportion reached the fifth instar when younger stages were exposed, compared to the provision of treated leaves at a later stage of development, as observed in the pyriproxyfen-treated group.

Only larvae in the control group were able to pupate. In all the experimental larvae, none was able to pupate, despite reaching the fifth instar in the pyriproxyfen and buprofezin groups. As a result, a comparison of the full developmental period could not be carried out. Longevity was compared between larvae that managed to survive to the fifth instar and a significant difference in the number of developmental days of the larvae was observed between buprofezin, pyriproxyfen and control larvae ($p = 0.000$) (Table 12). The control group had the shortest developmental period of 20.83 ± 1.95 days while buprofezin and pyriproxyfen groups had significantly longer developmental days of 27.71 ± 4.72 days and 27.69 ± 3.07 days respectively. The mean developmentalperiod also differed significantly between buprofezin and pyriproxyfen groups (Tukey HSD, $p = 0.05$).

DISCUSSION

From the results obtained, all three IGRs tested did not cause any direct mortality due to direct toxicity in both the field and laboratory experiments. Mortality occurred in a manner consistent with IGR activity, whereby the effect of the chemicals was the disruption of normal larval development (Boneβ, 1983; Friedell and McDowell, 1985; Schaeffer and Mulligan, 1991). This manifested itself in mortality during moulting, when larvae failed to emerge or after moulting when the newly-formed cuticle could not withstand haemolymph pressure and internal muscular traction (Cohen, 1970).

Comparison of the experimental plots and transect lines indicated that the larvae at 16m and 32m had a significantly higher proportion of larvae moulting successfully. This was probably due to some drift of the chemicals during spraying up to 2m in all directions. Drift in a particular wind direction would be the result of wind blowing in a certain direction, so that specimens within the transect in that direction would be affected differently from the transect in the opposing or adjacent directions. This was not the case in this experiment, since there was no significant difference in moulting success due to the direction of the transects. The calm conditions experienced during the spray days could also have minimised spray drift. Exclusion of the central plots, (i.e. comparison of larvae in the transect lines) resulted in no significant difference between larvae at the various distances and direction. This suggested that only minimal drift may have occurred during spraying. However, it should be borne in mind that only a knapsack sprayer was used and this equipment sprays at a much lower pressure compared to equipment used in commercial estates.

In the field experiment, buprofezin was the least detrimental of all the IGRs tested on *B. mori*. The silkworms were able to complete their development up to the adult stage, where the proportion of adults emerging was still not significantly different from the control. This suggests that in a field situation, *B. mori* may suffer little effect from this compound even when exposed directly, as in the central plots.

Both pyriproxyfen and teflubenzuron suppressed development of the exposed larvae to a certain extent, despite having exposed the larvae only during the treatment period. IGRs are known to be effective even at extremely low levels (Novak, 1975; Grenier and Grenier, 1993) and their effect probably persisted through the developmental stages. Low-level activity was also evident in the second replicate of controls which, after their accidental introduction into the experimental room, also failed to pupate.

Morphogenetic effects observed as a result of exposure to the IGRs also differed depending on the type of IGR sprayed or fed. Silkworms treated with buprofezin had unsuccessful attempts at moulting due to their inability to break through the old cuticle and failing to emerge. Moulting requires the exertion of pressure using the haemolymph so the insect can emerge through the ruptured, old cuticle (Wigglesworth, 1970). The enlargement of the cephalothoracic region could have resulted from the accumulation of haemolymph which had probably escaped through the improperly formed new cuticle beneath (Cohen 1987). The larvae would thus fail to generate enough pressure to emerge through the old cuticle, so remaining and dying inside. Some larvae exposed to buprofezin managed to moult but died before the next moult. Buprofezin is a chitin synthesis inhibitor and acts by severely inhibiting the deposition of the new cuticle through inhibition of chitin formation. Since chitin is a major, supporting component of cuticle, its absence results in a fragile and malformed cuticle (Wigglesworth, 1970; Ishaaya, 1990). Moult inhibitors, like buprofezin, thus compromise the elasticity and firmness of the cuticle and render it ineffective. This impaired cuticle would then be unable to support the affected larva, resulting in its death, despite having moulted successfully.

Buprofezin inhibits moulting by suppressing chitin formation in a way similar to benzoylphenylurea compounds (Cohen, 1987; Kobayashi *et al.*, 1989; Ishaaya *et al.*, 1989), a class to which teflubenzuron belongs. This may explain the similarity in the morphogenetic features of non-moulting larvae treated with either of the two IGRs. Teflubenzuron, a moult inhibitor, caused cuticular damage. Both field- and laboratory-treated larvae experienced incomplete moults. In addition to the above abnormalities, the teflubenzuron-treated groups also experienced 'bleeding' during their development, whereby the haemolymph would escape through the body. This probably reduced the internal pressure, rendering the larvae incapable of completing the moult. The body lesions, through which the haemolymph escaped, were as a result of the new cuticle's inability to withstand the internal pressure and contraction of intersegmental muscles in the abdomen since it lacked the vital chitin component (Wigglesworth, 1970; Cohen, 1987).

Mortality caused by pyriproxyfen was as a result of incomplete moulting or starvation. In both field- and laboratory-treated larvae, death during moults resulted when larvae were only able to moult off either the head or body cuticle. Moulting was therefore disturbed due to the presence of this compound. Muscles articulating the movement of the mandibles had excessive cell growth, leading to an enlargement of the total volume within the head region. Juvenile hormone analogs are known to have an effect on histogenesis in insects, producing excessive tissue proliferation (Novak, 1975). According to Staal (1975), additional metamorphic effects of juvenile hormone analogs are manifested through the impairment of behaviour, feeding and sensory functions. Proliferation of tissue around the mandibular muscles may be due to the induction of such impaired growth by a similar mechanism by the pyriproxyfen, a juvenile hormone analog (JHA). The resulting effect being the inability of the larvae to articulate their mandibles and failing to chew off any leaf material.

Of the three IGRs tested, pyriproxyfen also resulted in the production of a high proportion of supernumerary instars. Pyriproxyfen, as a JHA, encourages the persistence of juvenile or larval characteristics in the treated larvae (Wigglesworth, 1970), preventing development into the pupal stage. This was also observed in *Spodoptera litura* (Fabricius) (Lepidoptera) and *Blattella germanica* (Linnaeus) (Dictyoptera) when exposed to pyriproxyfen, and in *Galleria mellonella* (Linnaeus) and *Heliothis virescens* (Fabricius) (Lepidoptera) exposed to fenoxycarb, also a JHA with a similar mode of action to pyriproxyfen (Masner *et al.*, 1987; Koehler and Patterson, 1991; Grenier and Grenier, 1993).

The inability to pupate was also due to the effects of IGR exposure, whereby the exposed larvae simply had prolonged larval instars with no cocoon spinning. This indicated the high susceptibility of the fifth instar to the presence of the IGRs compared to the earlier instars. The last larval instar and pupal stage are known to be the stages most sensitive to IGR exposure, and earlier treatments have an effect through the persistence of the compound in some form within the larvae (Staal, 1975). This may therefore explain the complete failure of all the laboratory larvae in pupating. This was also observed in Italy and France, where *B. mori* exposed to pesticides with juvenilising actions failed to pupate (Grenier and Grenier, 1993). IGRs are highly effective even at extremely low doses, e.g. 8 x 10^{-5}ng/l and the picogram range (10^{-12}) (Ascher and Eliyahu, 1988; Grenier and Grenier, 1993). This could explain why even the second control replicate also failed to pupate after the introduction of pyriproxyfen- and teflubenzuron-treated groups.

The results from this study indicate that the IGRs tested have varying detrimental effects on a non-target species, despite literature reporting this group of pesticides as being highly specific. Even though no large-scale field experiment was carried out, the observation still provide a basis for caution, since IGRs are used extensively in the citrus industry and may pose a threat to wild entomofauna which may inadvertently be exposed during spraying, as observed in Mpumalanga (South Africa). The broad range of activity of IGRs may be their limitation in that they have a wide range of target species, even in different orders.This means that even non-target species are likely to be inadvertently affected.

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CHAPTER 6

GENERAL DISCUSSION*

The study investigated citrus management activities on non-target species, especially the economically important predatory family Coccinellidae. Citrus orchards are anthropogenic habitat patches formed by fragmentation and alteration of the natural landscape matrix. Once established, management practises need to be carried out to sustain the orchard system and minimise pest effects. Pesticide applications are the most widely used form of management in such systems. The study was thus carried out to investigate the distribution of the coccinellids within the agricultural landscape and ascertain the effects, if any, of insect growth regulators, a relatively new class of pesticides. This group of insecticides was specifically chosen as there were several unpublished reports from the field that there were severe impacts on non-target insects, even a considerable distance from the orchards.

Factors contributing to coccinellid distribution in the agricultural land mosaic

Tambuti estate, where this aspect of research was carried out, had a variety of habitats, i.e. orchards, pine wind breaks, natural veld and river borders. The intensively managed orchards had the highest coccinellid densities and species richness compared to the unmanaged river borders and natural veld. Economically important species at this estate, such as *Rodolia* spp. were

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* Detailed discussions were given at the end of each chapter. This discussion specifically addresses the interconnectedness between the chapters.

restricted to this orchard habitat and were not observed in the natural habitat. The restriction of these species to orchards was attributed to the presence of high prey densities.

Coccinellids are not efficient in prey location from a distance and their restriction to a single crop system may maximise their chances of prey encounter (Andow and Risch, 1985). While olfactory and tactile cues may contribute to prey location by coccinellids at the small scale level, an important environmental cue for favourable habitat location at the landscape level seems to be prominent features, e.g. silhouettes of trees and citrus leaf shape, which coccinellids associate with with profitable prey searches, as observed for *C. nigritus* (Hattingh and Samways, 1995). In citrus estates, the orchards provide suitable cues at both the small scale and landscape levels and this is likely to increase the chances of predators invading this habitat. The tendency for economically important species to be restricted to the orchards has favourable implications for pest control. Since the predators have a pattern of distribution close to that of the pest species, natural control is maximised, in the absence of any adverse conditions.

This landscape was also observed to have a few multihabitat species such as *Cheilomenes lunata, Scymnus* spp. and *Nephus* spp. Occurrence of these multihabitat species indicates the movement of coccinellids between the various types of habitat patches. Existence of these ecological flows between habitat patches is important as a linking process. Also, the persistence of these flows ensures the maintenance of the ecosystem's ecological processes, contributing to continued habitat availability for the coccinellids (Hobbs, 1993; Wiens, 1995).

The relationship between coccinellid distribution and their prey (host plants) was apparent for the species collected. Predatory species such as *Cheilomens lunata* and *Rodolia cardinalis* showed very close relationship with prey distribution, while the phytophagous species such as *Epilachna* spp. were restricted to their host plants. The other environmental variables measured, i.e. temperature, insolation and relative humidity apparently did not influence coccinellid distribution in the land mosaic studied. Their effects, however, were species specific. For example, certain species, e.g. *Nephus* spp. and *C. lunata* were almost always found at well insolated sites, while the tree dwelling species, e.g. *Ortalea ochracea* and *R. cardinalis* were always in cool areas with low insolation and low temperatures.

Insect growth regulator activity on non-target species

Effects on coccinellid populations - Economically important species showed limited inclination to move, as was shown in orchards under different pesticide treatment regimes. Orchards under an IPM programme, and where no IGRs were used, had significantly higher population densities of beneficial species compared to those where IGRs were used or those that had a conventional pesticide programme. While this was true for economically important species, assessment of the general (overall) coccinellid populations indicated no such significant differences. This was attributed to the fact that while the beneficial species were confined to the orchards, the other coccinellid species, i.e. those not recognised as having significant economic importance, freely moved in and out of the orchards. Differences between the various treatments were thus seen only when the less dispersive economically important species were analysed. Both the natural habitats and orchards proved to be suitable for the multihabitat species as observed by the presence of larvae and pupae on vegetation in these habitats (Hodek, 1967). These habitats are likely to serve as sources of coccinellid population sources after decimation of their populations in orchards, with the result that even the non-economically important species could effect some measure of control.

In the intensively-managed orchard habitats, the resident species, including all the economically important species, were vulnerable to exposure. The time of IGR application was also important. In early spring, predator populations increase. This period coincides with IGR spraying, and as seen from both field (chapter 3) and laboratory (chapter 4) experiments, these compounds have adverse effects on coccinellids.

The use of non-selective pesticides is one of the practises with the greatest adverse effect on local populations of beneficial coccinellids (Obrycki and Kring, 1998). Of the treatment regimes assessed, IPM seemed to be the most appropriate approach for encouragement of natural enemy populations. Such programmes emphasise the use of selective pesticides after careful monitoring of pest populations, with minimal effects on beneficial species. The success of IPM, however, lies in the understanding of the ecological interactions between insects and crops, which in turn, leads to an understanding of the agroecosystem structure and dynamics (Kogan 1998).

Specific IGR effects on the ladybird, Chilocorus nigritus and the moth, Bombyx mori - While field populations in the estates illustrated lower coccinellid densities in IGR treated orchards compared to those under IPM (with no IGRs), further field and laboratory experiments on non-target species were needed to provide a clear indication of the specific effects of these chemicals. Mortality of

Chilocorus nigritus from IGR activity was highest for the larval stages. IGRs disrupt larval development by inhibiting active chitin synthesis (Chapter 1). Inhibition of larval development was also apparent in the other experimental non-target species, *Bombyx mori*.

All stages of *C. nigritus* exposed to IGRs were vulnerable to these compounds. Reduction of the reproductive success of the adults, via the production of non-viable eggs, is likely to ultimately result in reduced predator populations in treated areas. This would have a negative impact on predator activity necessary for narural pest control. All IGRs tested had ovicidal activity on *C. nigritus* larvae. This again would reduce the effectiveness of this predator.

While some larvae were able to develop from early to late instars after exposure to IGRs, they were still unable to complete their life-cycle. This was observed either as failure to pupate (having long-instar periods) or through the formation of abnormal pupae and the failure of adults to emerge. The retention of IGRs in the organism's tissues, combined with their low detoxification rates (Staal, 1975), means that these compounds still have some effect long after the coccinellids have been exposed. Being stomach poisons, these compounds also achieve maximum activity when ingested, as observed in the experiments here. Spraying the IGRs against the target pest species, e.g. red scale on which *C. nigritus* feeds, results in inadvertent exposure of the coccinellids to the IGRs. In turn, IGR effects are likely to be most detrimental during the initial spraying period, when the coccinellids are exposed directly during spraying and indirectly through the consumption of contaminated prey. The long residual activity reported for these compounds is also of concern, since they would still have negative impacts on predator populations even a few months after spraying.

IGR effects observed at 32m outside the orchards have important implications for the occurrence of drift from orchards into adjacent areas. This is especially important for all the estates assessed. All these estates have river borders, which maintain relatively high coccinellid species richness and which could be readily and inadvertently impoverished. Furthermore, there could also be contamination of the rivers and impact on the aquatic diversity. The impact of the IGRs against *B. mori* suggests that these compounds may have even more far-reaching effects on non-target species.

IGRs had a clear impact on both *B. mori* and *C. nigritus* larvae. Most larvae exposed to buprofezin could not emerge from the old cuticle. In addition, teflubenzuron activity resulted in cuticular damage such as 'bleeding'. Pyriproxyfen activity in exposed larvae resulted in incomplete moults, in failure to feed in newly moulted larvae (in *B. mori*) and in the production of supernumerary larvae. Last instars of both species were affected to varying degrees as seen by their inability to pupate after exposure to IGRs, mainly pyriproxyfen and teflubenzuron.

In field experiments, buprofezin appeared to be the least detrimental of the three IGRs tested. Larvae of both species were able to develop up to the adult stage. The fecundity of these adults after removal from the contaminated environment, however, still remains untested.

Recommendations for preservation of sustainable coccinellid populations in land mosaics

Predaceous coccinellids have been linked to natural control more than any other taxa of predatory organisms (Obrycki and Kring, 1998). Their sustained presence within the ecosystem is thus of great importance in the agricultural land mosaic.

Their utilisation of the various habitat patches within the agricultural mosaics, which was evident in the field situation, emphasises the need for the preservation or promotion of biological diversity within the landscape. This would ensure the survival of both the beneficial species, restricted to the orchards, and the multihabitat species, which utilise a variety of habitats. Such diversity is defined in terms of vegetational variety and heterogeneity of their spatial arrangement (Samways, 1989; Burel and Baudry, 1995). The results have illustrated that particular landscape elements are essential for certain species. Preservation of the landscape's biological fauna would undoubtedly ensure conservation of the habitat patches within and around the agricultural landscape (Samways, 1989).

In addition to habitat conservation for these coccinellids, it is important to conserve their ecological roles within the ecosystem. Insects are known to have a diversity of functional roles in many aspects of an ecosystem. This means that elimination of a species may have unforeseen repercussions throughout that ecosystem. Conversely, the conservation of ecosystem function is also of great importance towards species preservation, as the species need a functioning ecosystem to persist (Hobbs, 1993; Samways, 1993; New, 1995).

Conservation of the landscape's spatial heterogeneity in isolation would, however, be fruitless if predators were to continue to be decimated in the orchards from inappropriate pest control, e.g. through the use of non-selective chemicals. The spring application of IGRs is one such factor which would impact on coccinellid population persistence.This was seen in the IGR-treated orchard at Tunzini (chapter 3), which initially had the highest populations of beneficial species, but at the end of the season these were ultimately the lowest. Due to the widespread use of IGRs on citrus in southern Africa, the use of an additional natural enemy to supplement coccinellid activity may have to be an option. In Israel, the parasitoid *Cryptochaetum iceryae* (Williston) was successfully introduced to assist *R. cardinalis* in the control of the Australian bug, *Icerya purchasi* (Mendel *et al.*, 1992). The importance of alternative habitats was also highlighted in this introduction, because the parasitoid was introduced onto a non-crop, broom plant for establishment before it freely invaded the orchards. Parasitoids also seem to be less susceptible to IGR activity, and so may play an important role in promoting natural control of pests in citrus orchards. Increased efficiency of natural enemy control would ultimately minimise the need for pesticide use. Southern Africa also has a variety of parasitoid species, e.g. *Aphytis* spp. and *Coccidoxenoides peregrinus*, which could provide supplementary control for pest species like *Aonidiella aurantii* and *Planococcus citri* (which are partially controlled by coccinellid predators) when coccinellid populations are low after use of non-selective pesticides.

Currently IPM emphasises the biological interactions between insects, natural enemies and non-crop plants (Andow, 1991). It is important to encourage coccinellid pest control capabilities through the preservation of heterogenous habitats and utilisation of selective pesticides, where necessary. The role of the natural control agents could facilitate a reduction in pests and ultimately, a reduction in pesticide utilisation if the natural enemies are able to flourish. The survival of coccinellid populations and their flow between natural and managed habitats may be promoted by the conservation of surrounding natural habitat around estates so as to maintain ecosystem function, not in a natural sense but the sense that economically injurious species are prevented from reaching damaging levels. This would ensure habitat preservation and retention of high species diversity in the agricultural land mosaic.

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CONCLUSIONS

- Orchards had the highest coccinellid densities and species richness, followed by the river borders. Natural veld habitats had the lowest coccinellid densities.

- Differences between the various treatment regimes are only apparent when populations of economically important species, rather than the whole coccinellid assemblage, are considered. Economically important species are principally restricted to orchards, while those whose economic value is unknown tend to be multihabitat species.

- Those orchards under an integrated pest management (IPM) programme, where no insect growth regulators (IGRs) were used, had the highest population densities and species richness of economically important coccinellid species, compared to those under a conventional pesticide programme or where IGRs were used.

- Distribution patterns of coccinellids are largely determined by prey presence / absence, while temperature, ground insolation and relative humidity had non-significant effects. Habitat choice was thus largely determined by prey presence. Highly dispersive species thus occupied a variety of habitats while the less mobile species were restricted to particulat habitats.

- In the laboratory, the IGRs tested had pernicious effects on two non-target species (the moth, *Bombyx mori* (Linnaeus) and the ladybird, *Chilocorus nigritus* (Fabricius), affecting larval and pupal development in both species. There was also ovicidal activity in the case of the ladybird, which compromises its overall reproductive success.

- Spring applications of IGRs coincide with coccinellid population increase, which leads to a decrease in natural control of pests.

- To maintain populations of coccinellids, it is best avoid the use of IGRs.

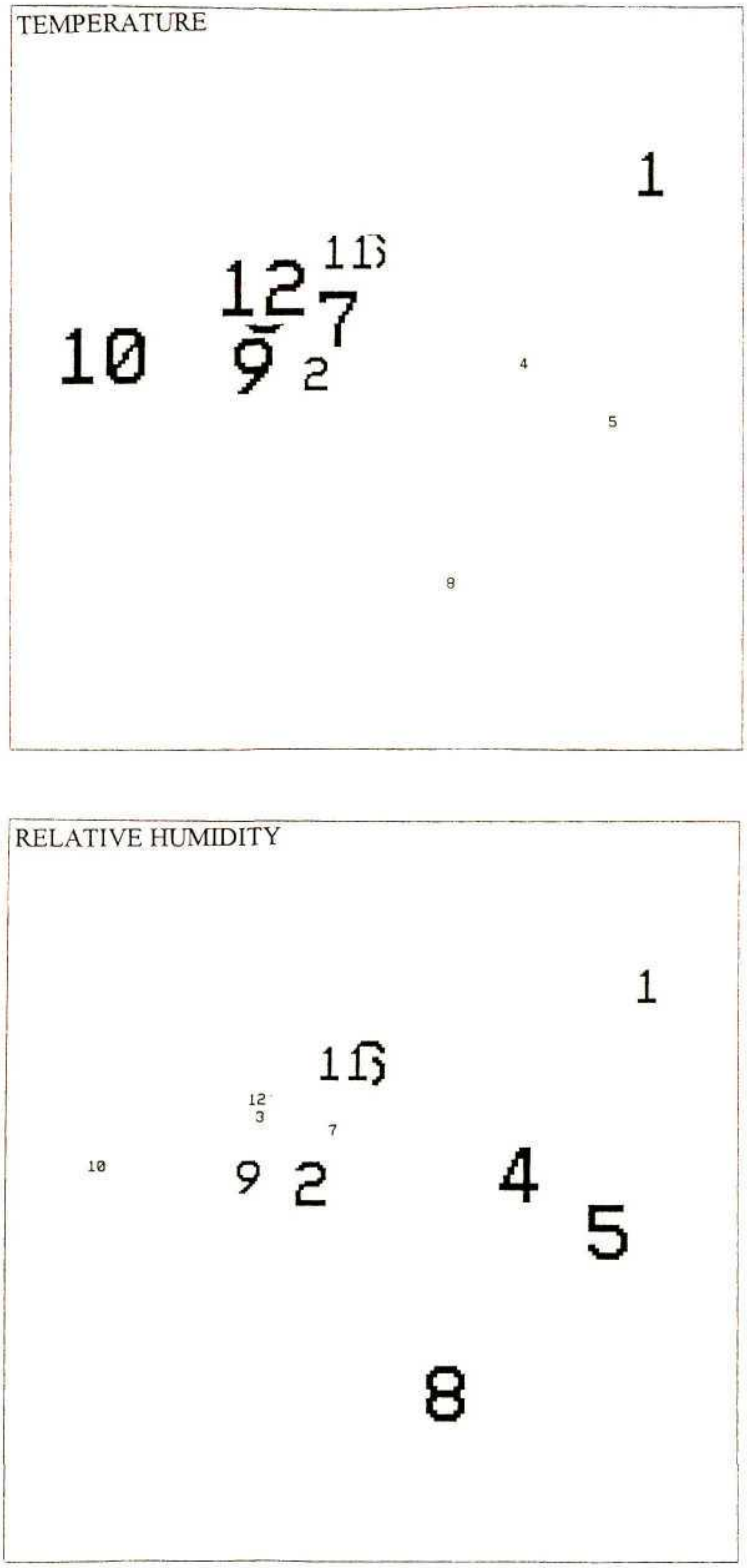
- Accidental drift during orchard spraying reduces non-target coccinellid populations in adjacent areas (orchards and natural habitats).

- Buprofezin is the least detrimental of the IGRs tested in the field, enabling larvae of both the experimental non-target species (*B. mori* and *C. nigritus*) to reach the reproductive adult stage after exposure.

- Presence of natural vegetation / veld around the estates contributes towards faunal diversity and preservation of beneficial coccinellid populations.

APPENDIX 1

NMDS ordination plots of the sites based on environmental variables indicated. 1=orchards, 2=100m east, 3=100m north, 4=100m south, 5=100m west, 6=200m east, 7=200m north, 8=200m south, 9=300m east, 10=300m north, 11=400m east, 12=400m north. (Stress = 0.08)



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