COMPARATIVE FEEDING AND FORAGING BEHAVIOUR OF THE BIOCONTROL AGENTS *CHILOCORUS* SPP. (COCCINELLIDAE)

BY

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PREFACE

This study was conducted in the department of Zoology and Entomology, University of Natal, Pietermaritzburg, from February 1988 to December 1991, under the supervision of Professor Michael J. Samways.

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 has been duly acknowledged in the text.

Vaughan Hattingh

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ABSTRACT

This study focuses on the effective biocontrol agent *Chilocorus nigritus* (Fabricius). Its behaviour and ecology were compared with other *Chilocorus* spp. where appropriate, to elucidate why this species is such an effective biocontrol agent, and how to improve methodology for its deployment as a natural enemy. An artificial diet for *C. nigritus* was developed, but was still sub-optimal. *Asterolecanium miliaris* (Boisduval) was a suitable prey for all life stages of *C. nigritus* and the adults of *Chilocorus bipustulatus* (Linnaeus) and *Chilocorus infernalis* Mulsant, but was inadequate for larvae of the last two species. Adult weight, measured at one day after adult eclosion, was an appropriate indicator of the effects of larval treatment on their development and on the fitness of subsequent adults. There was no improvement in culture vigour due to a behavioural response of individuals within one generation to fluctuating as opposed to constant temperature. Starvation for between 10h and 24h was appropriate for standardisation of hunger. Measuring feeding rate at a range of static temperatures did not reflect differences in the climatic adaptations of six *Chilocorus* spp., but mortality rates at increasingly high temperatures were useful. *Chilocorus* spp. showed little ability to choose between prey species. Prey substitutions adversely affected adults and larvae. Introduction of adults was the most effective method for field establishment. Giant bamboo *Dendrocalamus giganteus* Munro was a valuable site for field releases of *C. nigritus*, but less useful for *C.*

bipustulatus and *C. infernalis*. Counter to assumptions on which interference models have been based, no significant intraspecific interference, reducing predatory efficiency, was observed. Visually prominent features on the horizon and a specific leaf shape, were attractive to foraging *C. nigritus*. The location of prey patches by adults was facilitated by prey odour, but not so for larvae. Adults detected individual prey olfactorily and visually over short distances, but physical contact was required for detection by larvae. Prey location by larvae and adults was facilitated by alterations in movement patterns in response to prey consumption. Differences in prey detection and the effects of prey substitutions, between the life stages, were related to field behaviour. The relevance to biological control, of responses to rearing conditions and feeding and foraging behaviour, was investigated.

CHAPTER 1

INTRODUCTION

Background

The purpose of this study was to investigate the feeding and foraging behaviour of an effective biocontrol agent, in such a way that an academic contribution be made to our understanding of this behaviour, with practical implications for its use in biocontrol. The study focused on *Chilocorus nigritus* (Fabricius). A number of comparisons with other *Chilocorus* spp. of varied biocontrol status, were made where appropriate, to provide ecological and applied perspectives.

C. nigritus is a highly successful biocontrol agent of scale insects (Hemiptera: Diaspididae) on a number of agricultural crops in numerous countries. Originating from the Indian sub-continent, it has become widely distributed to include several Pacific and Indian Ocean Islands, East Africa, southern Africa, Brazil and West Africa (Vesey-FitzGerald, 1941, 1953; Chazeau *et al.*, 1974; Greathead & Pope, 1977; Georgala, 1979; Samways, 1984, 1989). This expansion is the result of planned introductions, redistribution through trade routes and natural colonization (Samways, 1984). Georgala (1979) first reported *C. nigritus* in South Africa, where its stronghold has become the Eastern Transvaal (Samways, 1984). Within South Africa, its distribution has been increased through natural colonisation and introductions of laboratory reared material,

to include all sub-tropical regions in the eastern part of the country (Samways, 1989). The distribution of the species in South Africa is limited by climate and extensive introductions into climatically unsuitable areas have not led to establishment (Samways, 1989).

C. nigritus attacks numerous scale pests on different crops and accepts a wide range of prey species (Samways, 1984). *C. nigritus* effectively controls scales on coconut palms in the Seychelles, Mauritius and New Hebrides (Vesey-FitzGerald, 1941, 1953; Moutia and Maimet, 1946; Chazeau, 1981). It provides economic control of red scale *Aonidiella aurantii* (Maskell) (Hemiptera: Diaspididae) on citrus in southern Africa, where its field behaviour compliments the role played by parasitoids in controlling this pest (Samways, 1984, 1988). *C. nigritus* has also been reported to have considerable impact on *A. aurantii* populations in India (Woglum, 1913).

C. nigritus formed the focal point of this study in view of: a) its economic value, b) the availability of information on its biology and field behaviour, c) the relative ease with which it can be cultured, d) the availability of field material to periodically revitalise laboratory cultures, e) the opportunity for comparison with other *Chilocorus* spp. The following species were used to a varying extent in comparisons with *C. nigritus*. Voucher specimens of the *Chilocorus* spp. are housed in the insect collection of the South African Museum, Cape Town, South Africa.

Chilocorus bipustulatus (Linnaeus) originates from the greater Mediterranean region and is a valuable natural enemy of numerous diaspidid scales (Smith, 1915; Nadel & Biron, 1964; Huffaker & Doutt, 1965; Rosen & Gerson, 1965; Gordon, 1985). It plays an important role in controlling citrus pests, particularly *A. aurantii*, in Israel (Nadel & Biron, 1964; Rosen & Gerson, 1965). It has established in California, where it preys upon diaspidid scales on olive trees (Huffaker & Doutt, 1965). *C. bipustulatus* was introduced into South Africa on two occasions (E.C.G. Bedford, pers. comm.) as a potential biocontrol agent of scale insects on citrus in the hotter, drier regions where *C. nigritus* is not effective, but has yet to become established.

Chilocorus cacti (Linnaeus) originating from southern North America, Central America and northern South America (Gordon, 1985; Hattingh & Samways, unpublished), was introduced into South Africa as a natural enemy of *A. aurantii* on citrus (E.C.G. Bedford, pers. comm.). This species established in the Western Cape, but does not provide economic control of scale outbreaks.

In Pakistan, *Chilocorus infernalis* Mulsant attacks a number of diaspidid scales (Ahmad & Ghani, 1966). It was imported into South Africa from the highlands of northern Pakistan (Samways, 1986b), against *A. aurantii* on citrus in the colder and higher altitude regions. Field establishment has not been achieved.

Chilocorus distigma (Klug) is widely distributed across Africa from the southern tip of the continent, to just South of the tropic of Cancer (Hattingh, 1991). This species was considered to be of some value in controlling *A. aurantii* on citrus in southern Africa in the past (Bedford, 1968), but is no longer important. *Chilocorus simoni* Sicard is a southern African species occurring on *Protea* spp., but is of no economic value as a natural enemy of scale insects on citrus in this region.

The feeding and foraging behaviour of Coccinellidae has been extensively studied (e.g. Hodek, 1973; Iablokoff-Khnzorian, 1982). However, numerous aspects which are relevant to understanding and managing their field performance, as well as more fundamental aspects of their behaviour, remain unclear.

Knowledge of the feeding behaviour of natural enemies is particularly valuable because it has direct bearing on how they affect the pest population. For example, the ability of *C. nigritus* to attack mature female *A. aurantii*, overcomes the problem of inverse density-dependence of *Aphytis* spp. parasitoids at high pest population densities (Samways, 1985; Samways & Wilson, 1988). This makes predation by *C. nigritus* at high pest population densities particularly important. This suggests that there is a difference in intraspecific interference behaviour between the parasitoids and predators, which makes effective high density predation possible, since the effectiveness of parasitoids in such situations is reduced by extensive interference (Hassell & Varley, 1969; Hassell *et*

al., 1976; Free *et al.*, 1977).

Foraging behaviour of natural enemies also reflects directly on their control effectiveness. This behaviour in coccinellids has been studied on the level of individual prey only, although it has had some implications for the location of prey patches (e.g. Carter & Dixon, 1982). There are aspects of this behaviour which remain unclear, such as the ability to detect individual prey over short distances. The effectiveness of *C. nigritus* in the field in southern Africa has been closely linked to their ability to shuttle between habitats (Samways, 1984, Hattingh & Samways, 1991). Although the location of habitats by coccinellids for aggregation and diapause has received some attention (Hagen, 1962) no reports of foraging behaviour at this level could be found. The location of habitats, prey patches, and individual prey items during foraging, was investigated in this study.

A start has been made in studying the behaviour of *C. nigritus* in the field and laboratory, which reflects upon their biocontrol value. Aspects of biology and feeding behaviour have been related to their field performance (Samways, 1984, 1986a, 1988; Samways & Wilson, 1988). Handling techniques which facilitate insectary rearing have been developed (Samways & Mapp, 1985; Samways & Tate, 1984, 1986). Samways (1989) proposed a method of climatic matching to improve the predictiveness of classical biological control introductions, using *C. nigritus* as a model.

There has however, been little quantitative laboratory work on their feeding behaviour. The effects of insectary procedures on the beetles reared, have not been evaluated. Techniques for improving the success rate of field introductions and their predictiveness, are urgently needed.

Rearing

Oleander scale *Aspidiotus nerii* Bouché (Hemiptera: Diaspididae) is a useful food source for maintaining laboratory culture of *Chilocorus* spp. Samways & Tate (1986) described methods for insectary culturing of *C. nigritus* using *A. nerii* on potatoes *Solanum tuberosum* Linnaeus and butternuts *Cucurbita moschata* cv. Waltham. However, maintenance of a large insectary culture of predators on natural prey has serious drawbacks. Such a system is labour intensive in that it requires the synchronous maintenance of two cultures. There is the ever-present threat of contamination of the prey culture by parasitoids, thus requiring strict quarantine. The vegetable hosts are susceptible to decay by pathogenic micro-organisms and seasonal availability presents a problem. Management of large cultures of scale insects, also present serious health hazards to insectary workers. This makes the development of a suitable artificial diet of considerable importance.

The vigour of coccinellid cultures is know to deteriorate after rearing for several successive generations (Hodek, 1973). Measurements often used in monitoring the vigour of insectary

cultures (Chambers, 1977), were used to periodically assess cultures which were maintained for more than five generations (*C. nigritus*, *C. bipustulatus* and *C. infernalis*). This provided physiological and behaviourial data, which were assessed for relevance to biocontrol effectiveness.

The quality of insects produced by insectary rearing may be extensively affected by handling procedures (Chambers, 1977). Rearing at fluctuating temperatures has produced fitter populations than rearing at constant temperatures (Hagstrum & Hagstrum, 1970; Hodek, 1973; Scriber & Slansky, 1981). It was unclear if this was due to selection or a behaviourial response to these conditions. If behaviour were responsible, then the exposure of one generation of insectary cultured insects to such conditions prior to field release, would be beneficial. This would make the need for maintenance of fluctuating conditions throughout the rearing process unnecessary.

Monitoring feeding rate is valuable in reflecting the direct effects of predators on the prey population. This measurement is also valuable as an indicator of other aspects of the predator-prey relationship, such as: a) foraging efficiency, b) predator activity, c) biotic and abiotic influences, d) in certain cases, prey suitability. It is necessary to standardise the hunger level of individuals used in such trials. There may be extensive variation in the hunger of predators on removal from rearing cages with plentiful available prey. This would result from inclusion of

individuals which had not fed immediately prior to removal, because of participation in activities such as cleaning copulation and oviposition. Standardisation of hunger by starving for a fixed period prior to conducting feeding rate trials, would reduce this variation. Nakamuta (1987) found the prey searching activity of the coccinellid *Coccinella septempunctata bruckii* Mulsant, was equivalent after 4h to 24h of starvation. Because of the extensive use of feeding rate as an indicator in this study, an appropriate starvation period for *C. nigritus* was determined.

The size of adult female insects is proportional to fecundity and longevity (Beddington, *et al.*, 1976; Slansky & Rodriguez, 1987). Sub-optimal nutrition and unfavourable environmental conditions during the larval stage, result in smaller subsequent adults (Hodek, 1973). Measurement of adult weight as an indicator of size, provides a convenient means of evaluating the effects of larval treatments, particularly on subsequent adult fitness. To exclude the effects of rearing conditions on the adults after eclosion, from the effects of larval treatments, weight must be measured shortly after eclosion. This presents a problem for interpretation of such measurements when the effect on adult fitness is also important, because of subsequent improvement in adult condition. The value of measuring adult weight at one day after eclosion was investigated.

Biocontrol improvement

The growing awareness of the environmental dangers associated with biocontrol introductions (Samways, 1988; Howarth, 1991), emphasizes the importance of improving the predictiveness of biocontrol introductions. Samways (1989) proposed the use of Walter & Leith's (1967) system of zonobiome classification based on climate, for matching areas of origin with suitable target areas for introduction of biocontrol agents. The distributional limits and geographical range over which *C. nigritus* is effective, suggest that temperature is a critical factor in determining climate suitability for this species. The value of measuring feeding rates at a range of static temperatures, in reflecting climatic adaptations, was evaluated in the laboratory using six *Chilocorus* spp.

Biocontrol agents are often reared on a prey species which is convenient for laboratory culturing, while the target pest in the field is a different species. *C. nigritus* is routinely reared on *A. nerii* and released on to *A. aurantii* and *Asterolecanium miliaris* (Boisduval) on giant bamboo *Dendrocalamus giganteus* Munro in the field, with *A. aurantii* on citrus as the target pest. The effects of such prey substitutions on these biocontrol agents had not previously been quantified and was thus investigated.

C. nigritus, *C. bipustulatus* and *C. infernalis* have been field released in whichever manner has been most convenient. These

different practices had not been comparatively evaluated. The most effective method for field establishment was determined in this study.

Feeding and foraging behaviour affecting biocontrol

A. miliaris on *D. giganteus* is important for the biocontrol success of *C. nigritus* in southern Africa (Samways, 1984; Hattingh & Samways, 1991). The nutritional adequacy of *A. miliaris* for *C. bipustulatus* and *C. infernalis* was unknown. If this alternative prey were unsuitable for these two predators, it would reflect on their potential as biocontrol agents of *A. aurantii* on citrus in this region. *A. miliaris* was evaluated as prey for these three species.

Intraspecific interference between parasitoids affects their foraging efficiency extensively (Rogers & Hassell, 1974; Beddington, 1975). In general, predators were assumed to behave in the same way, although there was little experimental verification. This interference behaviour has been modelled extensively (Hassell & Varley, 1969; Rogers & Hassell, 1974; Hassell *et al.*, 1976) and is important for foraging theories. Initial laboratory observations suggested that *C. nigritus*, *C. bipustulatus* and *C. infernalis* did not behave in this manner. Intraspecific interferences during feeding in these species was quantified and the implications for modelling explored.

Although the foraging behaviour of parasitoid biocontrol agents

has been well studied (van Alphen & Vet, 1986) there are important aspects of this behaviour by predatory biocontrol agents, which have not been addressed. Foraging behaviour of coccinellids on the level of individual prey, and to a lesser extent prey patches, has received attention (Carter & Dixon, 1982; 1984; Nakamuta, 1985; Podoler & Henen, 1986). On the level of habitat and to a large extent prey patch location, coccinellid foraging behaviour has not been studied in detail. The position of *C. nigritus* with regard to this behaviour was investigated.

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CHAPTER 2

Evaluation of artificial diets and two species of natural prey as laboratory food for *Chilocorus* spp. (Coleoptera: Coccinellidae)

ABSTRACT

Laboratory rearing of large numbers of *Chilocorus nigritus*, an economically valuable biocontrol agent of diaspidid scale insects, on natural prey is logistically difficult. An artificial diet is required to facilitate rearing for further research and would make commercial production of larger numbers viable. Promising diets were screened and the most successful, used as a base for modification. Additives used in developing artificial diets for other entomophagous insects, were tested for *C. nigritus*. Two suitable diets were obtained, one for adults and one for larvae. They were still sub-optimal and not adequate as the sole food source for rearing consecutive generations. They are valuable as a substitute food for insectary rearing during shortage of natural prey. Oleander scale *Aspidiotus nerii* Bouché and *Asterolecanium miliaris* (Boisduval) were evaluated as natural prey for *C. nigritus* and two other potential biocontrol agents in southern Africa, *C. bipustulatus* (Linnaeus) and *C. infernalis* Mulsant. *A. nerii* and *A. miliaris* were suitable for all life stages of *C. nigritus* and adults of *C. bipustulatus* and *C. infernalis*. *A.*

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miliaris was sub-optimal for larvae of *C. bipustulatus* and *C. infernalis*.

INTRODUCTION

C. nigritus (Fabricius) has been utilised by biocontrol specialists in numerous countries to control scale insect pests on several crops (Samways, 1984). It has been particularly valuable in economically controlling red scale, *Aonidiella aurantii* (Maskell) (Hemiptera: Diaspididae) on citrus in southern Africa (Samways, 1984, 1988).

Insectary rearing is required for experimental work aimed at improving utilisation of this beneficial species. The means to readily rear large quantities in an insectary are a pre-requisite for making commercial production possible. Rearing of entomophagous insects in general is logistically complicated (Waage *et al.*, 1985). Culture contamination, seasonal availability of food substrate for prey and seasonal population cycles, make maintenance of a continuous supply of adequate quantities of natural prey particularly difficult. Development of an artificial diet is essential for efficient rearing of *C. nigritus*.

No reports of an artificial diet suitable for rearing *C. nigritus* could be found in the literature. Smirnoff (1958) reared *C. bipustulatus* (Linnaeus) on an artificial diet suitable for numerous other coccinellids. Okada *et al.* (1972)

developed a valuable diet for the aphidophagous coccinellid *Harmonia axyridis* Pallas, based on macerated brood of honeybee drones. M.G. Hill (pers. comm.) had partial success with rearing *C. bipustulatus* and *C. cacti* (Linnaeus) on a brood-based diet. Brood-based diets have been widely used in artificial diets for coccinellids (Matsuka *et al.*, 1972; Niijima, 1979; Matsuka *et al.*, 1982). However, in the majority of cases, when rearing entomophagous insects on artificial diets, adult size is below average, larval survival is poor and fecundity suppressed.

The promising diets mentioned above were fed to *C. nigritus* and the most successful used as a base for improvement. Diet additives which have been valuable in developing artificial diets for other entomophagous insects, were selected from the literature. The base diet was altered and tested in steps, using *C. nigritus*.

An important prey for survival of *C. nigritus* in the field in southern Africa, is *Asterolecanium miliaris* (Boisduval) (Hemiptera: Diaspididae) on giant bamboo *Dendrocalamus giganteus* Munro (Samways, 1984; Hattingh & Samways, 1991). *Aspidiotus nerii* Bouché (Hemiptera: Diaspididae) is a valuable insectary prey for *C. nigritus* (Samways & Tate, 1986) *C. bipustulatus* and *C. infernalis* Mulsant are potential biocontrol agents of diaspidid scales on citrus in southern Africa. *A. miliaris* and *A. nerii* were comparatively evaluated as laboratory food for *C. nigritus*, *C. bipustulatus* and *C. infernalis*.

MATERIALS AND METHODS

Artificial diets

Trials were conducted under controlled environmental conditions of 25-26°C, 60-70% RH and 14L:10D photoperiod. Arenas were half-petri dishes, 100mm in diameter, 10mm deep, with white filter paper floors. The arenas were each covered with fine nylon gauze clamped around the petri dishes with elastic bands. Each arena contained a glass vial, 30mm long, 8mm in diameter, containing water with a wick of cotton wool. Polyester fibre egg pads, 20mm x 20mm x 5mm were replaced every 7d.

Beetles were reared on *A. nerii* prior to feeding on the diets. Adults were between 2 and 4 weeks old and first instar larvae were less than 2d old. One pair of adults was placed in each arena, five replicates per diet, and ten larvae per arena, four arenas per diet.

The freeze-dried diets B,C, and E to U, were stored in air-tight glass vials in a refrigerator at 5°C. The diet was powdered and sprinkled around the arenas. The highly hygroscopic diets became moist and soft within an hour in the high-humidity environment. Food and filter-paper floors were replaced every 7d.

Composition of artificial diets tested is given in Table 1. *C. nigritus* were reared on diet A, diet B (M.G. Hill, pers.

comm.), diet C and diet D (Smirnoff, 1958). The most successful of these, diet B, was then modified by adding potential phagostimulants, producing diets E to I. The two most successful of these were modified by adding vitamin and mineral mixes to one, producing diets J to M, and a series of miscellaneous supplements to the other, diets N to T. The most successful components were incorporated into diet U.

Initial screening

C. nigritus individuals were reared on *A. nerii* and on *A. miliaris*, and aspects of their biology measured for comparison with individuals reared on the artificial diets. A trial was conducted in which no food was provided but water was available, and another without food or water. Water was provided in vials with all other diets.

Diet A was presented as thin streaks on an inverted watch glass. The brood and royal jelly in diet B, were blended together, the wheatgerm, yeast and vitamin C, were combined, ground to a fine powder, thoroughly mixed with the wet constituents and the mixture freeze dried. The agar powder in diet C was mixed with hot water, cooled to 45°C and the other constituents, mixed as in diet A, added. Diet C and D were

Table 1. Composition of diets tested, percentages are proportions of the wet weights of the complete diets for each component

Component	Proportion of diet (%)																				
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U
honeybee brood		87.4	65.4		83.5	83.5	83.5	86.1	82.1	59.6	82.0	73.8	81.0	83.4	78.5	78.5	78.5	82.5	82.5	82.5	54.8
wheatgerm		8.8	6.6		8.4	8.4	8.4	8.6	8.3		8.3	7.4	8.1	8.4	8.4	8.4	8.4	8.4	8.4	8.4	8.3
brewers yeast		0.2	0.2	0.4	0.2	0.2	0.2	0.2	0.2	0.1	0.2	0.2	02.	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
ascorbic acid		1.8	1.4		1.7	1.7	1.7	1.8	1.7	1.2	1.7	1.5	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7
royal jelly		1.8	1.4	3.5	1.7	1.7	1.7	1.8	1.7	1.2	1.7	1.5	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7	3.0
agar powder			0.8	1																	
additional water	50		24.2	76.7																	
honey	50			4.6	4.5				4.5	3.3	4.5	4.1	4.4								4.5
oleander scale				1.5				1.5	1.5	1.1	1.5	1.3	1.5								3.0
sucrose				12.3		4.5															4.0
glucose							4.5							4.5	4.5	4.5	4.5	4.5	4.5	4.5	9.0
wheatgerm oil										33.5											0.5
vitamin E											0.1										
supplement 1 multi-vitamin & mineral												10.2									

Table 1. Continued

Component	Proportion of diet (%)																			
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T
supplement 2 (multi-vitamin)													1.4							
cholesterol														0.1						
casein															5.0					
amino acid concentrate																5.0				
dessicated liver																	5.0			
supplement 3																		1.0		
supplement 4																			1.0	
pollen																				5.0
fructose																				

allowed to set in plastic drinking straws, 6mm in diameter, split down the length and 3mm lengths of the diets were removed from the straws and placed in the arenas.

First alterations

A number of potential phagostimulants were added to diet B. In diet E (Table 1), the honey, royal jelly and brood were blended together and the dry constituents added. The sucrose and glucose of diets F and G respectively, were mixed with the dry constituents. The pulverised Oleander scale and Oleander scale + honey in diets H and I respectively were added to the wet constituents before mixing with the dry components.

Second alterations

Vitamin and mineral mixes were added to diet I. The wheatgerm oil and vitamin E in diets J and K respectively, were added to the wet components before mixing with the dry matter. The vitamin-mixture powders in diets L and M (Table 2) were added to the dry constituents.

Third alterations

Miscellaneous supplements were added to diet G. The cholesterol in diet N, was dissolved in 500mg ether and mixed with the wet components before adding the dry parts. The ether was evaporated off prior to freeze drying. An analysis of the

Table 2. Percentage composition of supplements 1 and 2 and amino acid concentrate in diets L, M and P respectively, proportion of wet weight of diet before freeze drying

Component	% of diet	Component	% of diet	Component	% of diet
		Diet L			
vitamin A	0.16	vitamin E	0.54	lecithin	0.12
vitamin D	<0.01	biotin	<0.01	I	<0.01
thiamine	0.39	Ca ascorbate	1.23	Fe	0.09
riboflavin	0.25	choline	0.39	Mg	0.15
nicotinamide	0.59	inositol	0.20	Cu	<0.01
pantothenic acid	0.39	benzoic acid	0.12	Zn	0.17
		glutamic acid	0.12	Mn	0.07
pyridoxine	0.25			K	0.34
vitamin B_{12}	<0.01	folic acid	<0.01	Ca	0.25
		Diet M			
vitamin B_1	<0.01	vitamin C	0.16	folic acid	<0.01
vitamin B_2	<0.01	nicotinamide	0.04	biotin	<0.01
vitamin B_6	<0.01	pantothenic acid	0.02	valeric acid	0.09
vitamin B_{12}	<0.01				
		Diet P			
phenylalanine	0.33	lysine	0.46	glycine	0.25
leucine	0.69	valine	0.46	aspartic acid	0.63
isoleucine	0.06	histidine	0.18		
threonine	0.21	arginine	0.20	serine	0.23
methionine	0.05	proline	0.28	glutamic acid	0.59
		alanine	0.42		

amino acid concentrate in diet P is given in Table 2.

Supplement 3 in diet R consisted of the following components at concentrations of less than 0.01% of wet weight of complete diet, except where percentage composition appears in parentheses: vitamin A, D, E (0.01%), C, B_1, B_2, B_6, B_{12}, nicotinamide (0,02%), biotin, folic acid, pantothenic acid, choline (0,02%), inositol (0,02%) ginseng (0,05%), lecithin, aspartic acid, lysine, arginine, leucine, isoleucine, phenylalanine, threonine, valine, glycine, proline, tryptophan, histidine, methionine, serine, tyrosine, alanine, cystine, glycerophosphates, Na, K, Mn, I, P, Ca, Mg, Zn, Se, S, Cu, Fe.

Supplement 4 in diet S, was similar to supplement 3 in diet R, but also contained mixed fatty acids, mixed sterols and bovine-organ and -gland extract. The pollen in diet T was removed from storage cells in a hive of bees foraging primarily on *Eucalyptus grandis* Hill ex Maiden. The most successful additives used in the study were added in combination to diet B to make diet U.

Natural prey

Time from egg hatch to adult eclosion for *C. bipustulatus*, *C. infernalis* and *C. nigritus* were determined when reared on *A. miliaris* on internode sections of bamboo, or on *A. nerii* on butternuts *Cucurbita moschata* cv. Waltham. The percentage survival of immature stages was recorded and the weights of

adults were measured one day after eclosion.

The availability of artificial substrates for the oviposition of *C. bipustulatus* and *C. nigritus*, made it possible to measure their fecundity. Individual pairs of *C. bipustulatus* were enclosed in rectangular cardboard arenas 106mm x 30mm and 30mm high, closed with fine nylon gauze clamped around the arenas with elastic bands. These arenas were attached to the surface of scale bearing bamboo and 20mm x 20mm egg pads of frayed linen provided. *C. nigritus* pairs were enclosed in circular plastic collars, 34mm in diameter and 10mm high, covered with fine nylon gauze clamped around the collars with elastic bands. These were attached to the surfaces of scale bearing butternuts and 10mm x 20mm x 5mm egg pads of polyester fibre provided. Once a week for three weeks, the arenas were moved to new positions to avoid prey depletion and the egg pads replaced.

RESULTS

The mean longevity of *C. nigritus* without food or water was 5.7d and without food, but with water provided, was 9d. Females survived for 7.2d and males for 4.4d without food and water. With water provided, females survived for 10.6d and males for 7.2d.

Measurements of survival, reproduction and development of immature stages, fed on the artificial diets, natural prey, or without food, are given in Table 3 and the diet modifications

Table 3. Survival, reproduction, fecundity (eggs/pair/7days), weights and development rate of *C. nigritus* fed on artificial diets, *A. nerii*, *A. miliaris*, or starved with or without water

Diet	% adult survival	reproducing pairs (%)	fecundity	% immature survival	adult weight (mg)	development time (days)
no food & no water	0	0	0	0	-	-
no food, + water	0	0	0	0	-	-
A. nerii	90	100	32.2	84	6.6	23
A. miliaris	90	90	31.5	85	6.8	25
A	70	0	0	0	-	-
B	90	80	2.2	22	4.7	29
C	100	20	0.4	0	-	-
D	90	40	1.7	0	-	-
E	90	60	3.3	57	4.6	28
F	90	60	2.4	62	4.7	28
G	100	80	6.5	55	5.0	28
H	90	80	1.5	53	4.8	28

Table 3. Continued

Diet	% adult survival	reproducing pairs (%)	fecundity	% immature survival	adult weight (mg)	development time (days)
I	100	100	3.7	42	5.0	28
J	0	0	0	0	-	-
K	100	60	3.8	44	4.6	28
L	20	0	0	0	-	-
M	90	100	1.5	26	5.2	-
N	100	60	0.9	9	3.5	-
O	100	80	2.3	8	4.0	-
P	90	40	0.4	10	3.2	-
Q	100	60	2.6	3	3.9	-
R	100	50	4.5	33	3.9	-
S	80	20	0.4	19	3.3	-
T	100	80	1.3	20	4.6	-
U	100	80	2.4	46	5.6	28

evaluated in Table 4.

Of diets A, B, C and D screened initially, diet B gave the best results (Table 4). Diets G and I were the most successful phagostimulatory modifications to diet B. Diets K and M were the most useful of the diets based on diet I with vitamin and mineral additives, but were not superior to diet I itself. Diets O, R and T were the most successful modifications of diet G, but were still not superior to diet G. Diet U, which incorporated the most useful additives used in this study, was superior to diet B and similar in value to diets G & I. Diet U produced the heaviest adults and the rate of egg laying was highest on diet G.

Weights of adult *C. bipustulatus* and *C. infernalis* from larvae reared on *A. miliaris* were significantly lower than weights when reared on *A. nerii* (Table 5). Weights of *C. nigritus* were not significantly different when reared on these two prey species. Duration of immature development of the three *Chilocorus* spp. was not significantly different when reared on these prey species. The rate of oviposition by *C. bipustulatus* and *C. nigritus*, did not differ significantly on the two prey species.

Table 4. Comparisons between artificial diets tested, average counts for each measurement ranked and the average rank for each diet calculated

Measurements	Ranks of measurements									
	Initial diets				1st modifications					
	A	B	C	D	E	F	G	H	I	B
Adult survival	4	2.5	1	2.5	4.5	4.5	1.5	4.5	1.5	4.5
no. pairs reproducing	4	1	3	2	5.5	5.5	3	3	1	3
Oviposition	4	1	3	2	3	4	1	6	2	5
Immature survival	3	1	3	3	2	1	3	4	5	6
Adult weight	3	1	3	3	6	4.5	1.5	3	1.5	4.5
Development rate	3	1	3	3	3	3	3	3	3	6
Average	3.0^{a}	1.3^{b}	2.7^{a}	2.6^{a}	4.0^{ab}	3.8^{ab}	2.2^{a}	3.9^{ab}	2.3^{a}	4.8^{b}

	2nd modification					
	J	K	L	M	I	B
Adult survival	5.5	4.5	5.5	1.5	1.5	3
no. pairs reproducing	5.5	4	5.5	1.5	1.5	3
Oviposition	5.5	1	5.5	4	2	3
Immature survival	5.5	1	5.5	1	2	3
Adult weight	5.5	4	5.5	1	2	3
Development rate	5.5	2	5.5	2	2	4
Average	5.5^{c}	2.7^{ab}	5.5^{c}	2.2^{ab}	1.8^{a}	3.3^{b}

Table 4. Continued

Measurements	Ranks of measurements							
	3rd modification							
	N	O	P	Q	R	S	T	G
Adult survival	3.5	3.5	7	3.5	3.5	8	3.5	3.5
no. pairs reproducing	4.5	2	6.5	4.5	6.5	8	2	2
Oviposition	6	4	7.5	3	2	7.5	5	1
Immature survival	6	7	5	8	2	4	3	1
Adult weight	6	3	8	4.5	4.5	7	2	1
Average	5.2^{bc}	3.9^{ab}	6.8^{c}	4.6^{bc}	3.7^{ab}	6.9^{c}	3.1^{ab}	3.1^{ab}

	Most successful diets			
	B	G	I	U
Adult survival	3	2	2	2
no. pairs reproducing	3	3	1	3
Oviposition	4	1	2	3
Immature survival	4	1	3	2
Adult weight	4	2.5	2.5	1
Development rate	4	2	2	2
Average	3.7^{a}	1.9^{b}	2.1^{b}	2.2^{b}

Absence of a common letter in the superscript indicates a significant difference, Friedman ANOVA, followed by a nonparametric multiple comparison (Siegel & Castellan

Table 5. Percentage survival of immature stages, weights of adults within 24h of eclosion and time from egg hatch to eclosion, for *C. bipustulatus*, *C. infernalis* and *C. nigritus*, reared on *A. nerii* and *A. miliaris*

Predator & prey spp.	% immature survival	Adult weight mean ± 1SE (*n*)	Development time (days) mean ± 1SE (*n*)	Eggs/pair/day mean ± 1SE (n)
C. bipustulatus				
A. nerii	69	6.7^{a} ± 0.2 (31)	29.0^{a} ± 0.4 (33)	6.2^{a} ± 0.6 (12)
A. miliaris	73	4.4^{b} ± 0.1 (29)	28.7^{a} ± 0.3 (29)	6.0^{a} ± 0.9 (12)
C. infernalis				
A. nerii	76	11.3^{a} ± 0.3 (36)	26.5^{a} ± 0.4 (37)	-
A. miliaris	60	5.7^{b} ± 0.2 (15)	25.0^{a} ± 0.5 (15)	-
C. nigritus				
A. nerii	78	6.6^{a} ± 0.1 (37)	22.3^{a} ± 0.3 (42)	4.6^{a} ± 0.4 (15)
A. miliaris	85	6.8^{a} ± 0.2 (28)	23.6^{a} ± 0.4 (34)	4.5^{a} ± 0.8 (15)

Absence of a common letter in the superscript indicates a significant difference, α=0.01, Mann-Whitney U-test, each *Chilocorus* sp. tested seperately.

DISCUSSION

Artificial diets for entomophagous insects are often sub-optimal regarding fecundity, adult size and growth rate (Waage *et al.*, 1985). This study produced two promising diets for *C. nigritus*, but with similar shortcomings. Diet U was the most successful larval diet, producing heavier subsequent adults than any of the other artificial diets, although these were still 15-18% lighter than when reared on natural prey. Immature survival to adult eclosion on diet U was 46% compared with 84% on *A. nerii*. The highest rate of egg laying was obtained on diet G, but was still only 20% of the rate when fed on *A. nerii*. These diets were not suitable for rearing consecutive generations because of the reduced size of adults.

These diets are still sub-optimal, but can be valuable as supplements to natural prey. At times of shortage of prey, diet G can be used to maintain the adult population. In this study there was 100% survival of adults on this diet for 60 days. Although fecundity was only 1/5 of the level on natural prey, this diet will at least ensure that the culture does not decline in size. This diet may be particularly useful when small quantities of prey are still available for larval rearing.

Fecundity and longevity of insects is reduced in below average sized adults (Beddington *et al.*, 1976; Slansky & Rodriguez, 1987). It is therefore important to reserve limited supplies

of nutritionally adequate prey for larval rearing, to avoid reducing fitness of individuals in the population through production of below average sized adults.

A. miliaris and *A. nerii* are both adequate food sources for rearing *C. nigritus* larvae. *A. miliaris* is however, a sub-optimal diet for larvae of *C. bipustulatus* and *C. infernalis*. This may have important implications for the survival of *C. bipustulatus* and *C. infernalis* in the field in southern Africa, and may in part explain the lack of success achieved with establishment of these species.

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CHAPTER 3

Physiological and behavioural characteristics in the laboratory of *Chilocorus* spp. (Coleoptera: Coccinellidae) relative to their effectiveness in the field as biocontrol agents

ABSTRACT

Chilocorus bipustulatus (Linnaeus), *C. infernalis* Mulsant and *C. nigritus* (Fabricius) were cultured and a quality monitoring programme undertaken to identify possible changes in culture vigour associated with prolonged laboratory rearing. Measuring adult weight at one day after eclosion was valuable in reflecting effects of larval treatments on adult fitness. There was no improvement in culture vigour due to behavioural responses of individuals within one generation to fluctuating as opposed to constant temperature. Feeding rates of *C. nigritus* adults and larvae were significantly lower during the dark phase than during the light phase. Starvation of *C. nigritus* adults for between 10h and 24h, resulted in similar subsequent feeding rates. In addition to the above three species, *C. cacti* (Linnaeus), *C. distigma* (Klug) and *C. simoni* Sicard were also laboratory reared. The extensive differences in the natural climatic adaptations of these six species, were not reflected in measurements of their feeding rates at a range of constant temperatures. However, mortality rates at

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increasingly high temperatures did show differences, in accordance with their distributions relative to climate. Measurement of these biological attributes in the laboratory, did not improve predictiveness of their biocontrol value.

INTRODUCTION

Economic background

Chilocorus nigritus (Fabricius), originating from the Indian sub-continent (Samways, 1989), is an economically valuable biocontrol agent in numerous countries including southern Africa, where it effectively controls red scale *Aonidiella aurantii* (Maskell) (Hemiptera: Diaspididae) on citrus (Samways, 1984, 1986a).

In contrast, *C. cacti* (Linnaeus) was introduced into South Africa in 1966 from Texas, United States (DeBach & Rosen, 1976), where it is useful in controlling various scale insects on citrus (Thomas, 1964). It became established in the SW Cape Province of South Africa, but failed to control scale outbreaks.

C. bipustulatus (Linnaeus) is a Mediterranean species, effective in controlling various diaspidid scale insects in several countries (Nadel & Biron, 1964; Huffaker & Doutt, 1965; Rosen & Gerson, 1965; Gordon, 1985). It was first imported into South Africa in 1963 as a potential biocontrol agent of

diaspidid scale insects on citrus and again in 1987 (ECG Bedford, pers. comm.), but failed to establish. *C. infernalis* (Mulsant) was imported from the northern highlands of Pakistan (Samways, 1986b) but also failed to establish in southern Africa.

Field collections of *Chilocorus* spp., indigenous to South Africa, were obtained for insectary culturing. *C. distigma* (Klug) was collected on giant bamboo *Dendrocalamus giganteus* Munro, feeding on the diaspidid scale *Asterolecanium miliaris* (Boisduval). *C. simoni* Sicard was collected on *Protea* spp., feeding on an unidentified scale insect. Laboratory cultures of these two species were established and maintained on Oleander scale *Aspidiotus nerii* Bouché (Hemiptera: Diaspididae).

C. angolensis Crotch, feeding on soft green scale *Pulvinaria aethiopica* (De Lotto) (Hemiptera: Coccidae) on citrus, was field collected, but could not be cultured on *A. nerii*. *C. solitus* Weise was collected on *Aloe* spp., but could not be maintained on *A. nerii* (Hattingh, 1991). The following rare species, were collected in insufficient numbers to establish cultures; *C. calvus* Weise, *C. cruentus* (Gorham), *C. quadriguttatus* Weise, *C. reinecki* Weise, *C. sexguttata* Weise, *C. wahlbergi* Mulsant.

None of the local species are of major economic importance in biocontrol of scale insects on citrus in southern Africa,

although *C. wahlbergi* feeds on *A. aurantii* in the orchards and *C. angolensis* on *P. aethiopica*. *C. distigma* was considered to be of some importance in controlling *A. aurantii* on citrus in earlier years (Bedford, 1968), but is now thought to be of minimal value.

Quality monitoring

We used the following measurements which are commonly used in monitoring the quality of laboratory cultures: adult weight, egg laying rate, immature-developmental rate and survival rate of immature stages (Moore *et al.*, 1985). Additionally I determined feeding rate, as this has a direct bearing on field performance of a biocontrol agent.

Adult age-weight and sex-weight relationships

The effect of larval treatments on subsequent adult fitness of *C. nigritus*, *C. bipustulatus* and *C. infernalis*, was evaluated by measuring adult weight. The change in weight with time after adult eclosion was quantified to determine the necessity of taking weight measurements at a fixed time after eclosion. Furthermore, to determine the validity of measuring adult weight shortly after eclosion in evaluation of larval treatments, it was necessary to quantify weight increase of adults which were lighter at eclosion due to deficient larval nutrition. The sex-weight relationship was also determined.

Constant versus fluctuating temperature

Fluctuating laboratory temperatures produce fitter populations than rearing at constant temperatures (Hagstrum & Hagstrum, 1970; Hodek, 1973; Scriber & Slansky, 1981). It was unclear from these studies if improved performance was a behavioural response of individuals to fluctuating temperatures, or if the improvement was due to selection for fitter individuals. This study determined whether there was an increase during one generation of *C. nigritus*, in the rate of egg laying, egg development tempo, percentage egg hatch, adult weight and adult feeding rate, in response to fluctuating temperatures.

Feeding rate: photophase and starvation

Feeding rate was used extensively in studies of the feeding and foraging behaviour of *Chilocorus* spp. (Hattingh & Samways, 1990, unpublished). Nakamuta (1987) cautioned that endogenous diurnal rhythms in feeding activity may predominate over hunger. The need for considering photophase during feeding rate measurements with *Chilocorus* spp. was determined. During feeding rate experiments, hunger was standardised by starving for fixed periods of time prior to measurement. The effects of various starvation periods on subsequent feeding rates were quantified.

Temperature-feeding rate relationship

Citrus is grown in southern Africa over a broad range of climatic regions. Climate has limited the distribution of *C. nigritus* in this region (Samways, 1989). *C. bipustulatus* and *C. infernalis*, were imported and targeted at areas in southern Africa where *C. nigritus* is not successful. In southern Africa, *C. cacti* is limited to the SW Cape Province, in spite of widespread attempts at introduction (E.C.G. Bedford, pers. comm.; DeBach & Rosen, 1976). In contrast, *C. distigma*, which is only of limited biocontrol value, is found throughout southern Africa where citrus is grown. It has further been recorded from most of Africa South of the Tropic of Cancer (Hattingh, 1991). *C. simoni* has been collected in the Drakensberg mountains and Transvaal Highveld, where there are large temperature fluctuations, both seasonally and diurnally.

The value of feeding rates and survival at various constant temperatures, in reflecting climatic adaptation and distribution of these species was investigated.

MATERIALS AND METHODS

Sex determination

C. nigritus could easily be sexed according to the method described by Samways & Tate (1984). Similarly, *C. infernalis* males had an invagination in the fifth and sixth abdominal

sternites, which was not present in any of the females identified during copulation. No easily recognisable differences between the sexes of *C. bipustulatus* could be found. However, when attached to sticky tape by their elytra and held upside down, they everted their genitalia, making sex determination possible.

Quality monitoring

Beetles were reared under controlled conditions of 25-26°C, 50-70% RH and a 14L:10D photoperiod. They were fed *A. nerii* cultured on butternuts *Cucurbita moschata* cv. Waltham. Free water was provided in the rearing cages by spraying with an atomizer once to three times per week. The rearing cages were 0.5m x 0.4m wooden floored, 0.42m high wooden framed, with sides covered in fine gauze and with glass lids.

Butternuts with *A. nerii* were removed from the adult rearing cages after one to two weeks. These had eggs laid beneath the scale coverings and attached to the surfaces of scales and vegetables. Newly hatched larvae were removed daily for development studies. The time from hatching to adult eclosion was determined and the percentage survival of the immature stages recorded. Adult beetles of both sexes, over 14d old, were weighed.

Suitable synthetic material for the oviposition of *C. bipustulatus* and *C. nigritus* was available. *C. bipustulatus*

lays eggs between strands of frayed linen (Nadel & Biron, 1964; Hattingh & Samways, 1991) and *C. nigritus* in pads of polyester fibre wadding (Samways & Tate, 1986; Hattingh & Samways, 1991). This made it possible to determine the percentage egg hatch and pre-oviposition period for these two species. Egg pads were removed from the rearing cages and were kept separate from larvae and adults. Hatching larvae were removed twice daily to avoid egg cannibalism.

The pre-oviposition periods were determined by sexing on eclosion and keeping pairs separately with egg pads until oviposition commenced.

The ease with which the number of eggs laid on synthetic egg pads by *C. bipustulatus* and *C. nigritus* could be determined, made it possible also to quantify the fecundity of these species. Pairs of adults, older than 14d, were each provided with an egg pad, which was replaced every 2d to 6d and the eggs counted.

The feeding rates of *C. bipustulatus*, *C. infernalis* and *C. nigritus* were determined at regular intervals during rearing. The numbers of female *A. nerii* consumed per individual per unit time were measured. Circular plastic collars, 10mm in height and 35mm in diameter, were attached to the surfaces of scale bearing butternuts. The scales were of approximately equal densities and were equal aged (one week before crawler production commenced) mature females. Beetles were enclosed

individually in the arenas with fine gauze over the tops, and clamped around the sides with elastic bands. Individual adults and fourth instar larvae were allowed to feed for 4h without prior starving. The number of scales eaten was determined from feeding damage. The feeding-rate data given here, were the standards determined at initial establishment of the laboratory cultures and were used thereafter as a reference for quality assessment.

Adult age-weight and sex-weight relationships

Larvae of *C. bipustulatus*, *C. infernalis* and *C. nigritus* were reared on *A. nerii* on butternuts in a cage separate from the adult culture. On eclosion adults were removed daily and weighed at regular intervals thereafter. To determine the ability of undernourished adults to recover once fed on an adequate diet, larvae were taken from rearing trials on sub-optimal synthetic diets. These adults were then fed *A. nerii* and weighed at regular intervals.

C. bipustulatus, *C. infernalis* and *C. nigritus* maintained on *A. nerii*, were sexed and weighed.

Constant versus fluctuating temperature

The effects of constant and fluctuating temperatures during rearing of *C. nigritus* were determined. A constant temperature of 25-26°C, with 50-70% RH and a 14L:10D photoperiod was

maintained. In the treatment, the temperature fluctuated gradually between 15°C at 02H00 and 28°C at 13H00 and RH fluctuated between 70% and 35% at 02H00 and 13H00 respectively.

Cages were wooden floored 0.2m x 0.2m and wooden framed, 0.2m high, covered in fine gauze netting. Each cage contained one butternut encrusted in *A. nerii* of several generations and mixed ages. Adult beetles for the trial were selected from the stock culture reared on *A. nerii* on butternuts at a constant 25-26°C. These beetles were four to six weeks old to ensure that they were sexually mature. They were weighed, and three pairs enclosed per cage, 10 cages per treatment.

The adult beetles were allowed two weeks to acclimate to the new environment before measurements began. One egg pad 10mm x 40mm x 200mm was wrapped around the butternut in each cage. These were replaced after three days and another pad placed in each arena for a further three days. For the three pairs in each cage, the total number of eggs laid over these 6d was determined. This was converted to the number of eggs laid per pair per day, and the counts at constant and fluctuating temperatures compared with a Mann-Whitney U-test. The time taken for these eggs to hatch and the percentages hatching recorded, pooled for each group of three pairs, and compared with Mann-Whitney U-tests.

The feeding rates of the adults were measured at three weeks after introduction into the two environments . This was done

as previously described in quality monitoring. Six beetles were enclosed per arena and the number of scale eaten in each arena divided by six to give a measurement of the number of scales eaten per individual, 10 replicates per environment. The feeding rates were measured during the first, middle and last 4h of the light phase. This was necessary since the average temperature in the fluctuating environment during the first and last 4h of the light phase, were significantly lower than in the constant temperature environment. These feeding rates in the two environments, were then compared with Mann-Whitney U-tests.

The weights of each group of six beetles were measured four weeks after introduction to these environments and compared with weights at commencement of the trial (Mann-Whitney U-tests).

Feeding rate: photophase and starvation

The expected reduction in feeding rates at night, of fourth instar and adult *C. nigritus* were measured as described in quality monitoring. Measurements were taken during the middle of the light and dark phases without starving prior to evaluation and compared with Mann-Whitney U-tests.

The effect of starving for 0h, 4h, 10h, 24h and 48h on the feeding rates of individual *C. nigritus* adults on *A. nerii*, was evaluated as previously described. A Kruskal Wallis ANOVA was

performed followed by a nonparametric multiple comparison between treatments (Siegel & Castellan, 1989).

Temperature-feeding rate relationship

The feeding rates of adult *C. bipustulatus*, *C. cacti*, *C. distigma*, *C. infernalis*, *C. nigritus* and *C. simoni* were determined at constant temperatures and relative humidities of 3°C and 90%, 10°C and 80%, 17°C and 53%, 24°C and 50%, 31°C and 48%, 38°C and 35%, and 41°C and 35%. Fifteen to 20 beetles per species were taken from the stock cultures maintained on *A. nerii* at a constant 25-26°C, 50-70% RH, exposed to the experimental temperature and fed *A. nerii*. Twenty four hours after commencement of exposure, the feeding rates were determined as described in quality monitoring. However, the scale insects were not mature as in the previous trials, but were three week old males and females. Survival after 48h of exposure to these temperatures and after 14h, 21h, 41h and 48h of exposure to 41°C, were recorded. Different individuals were used for each temperature trial.

RESULTS

Quality monitoring

A comparison of adult weight, larval development time and percentage survival of immature stages for the six *Chilocorus* spp. is given in Table 1.

Mean egg hatch for *C. bipustulatus* was 69% ± 5.8% (±1SE), *n*=8 egg pads, *n*=90 eggs. The mean hatch rate of *C. nigritus* was 74% ± 2.6% (±1SE), *n*=7 egg pads, *n*=677 eggs, Mann-Whitney U-test, *P*>0.05.

The first observation, during evaluation of the pre-oviposition period of *C. bipustulatus*, was made at 5d after adult eclosion and oviposition had already commenced in seven of the nine pairs tested. The remaining two pairs started ovipositing 13d and 15d after eclosion. The mean pre-oviposition period for *C. nigritus* was 14d ± 0.5d (±1SE), *n*=6 pairs.

A mean of 3.8 eggs ± 0.9 (±1SE), *n*=9 pairs, were recovered per pair of *C. bipustulatus* per day, over 25d. The mean recovery rate for *C. nigritus* was 2.8 eggs per pair per day, ± 0.4 (±1SE), *n*=17 pairs, recorded over 11d, Mann-Whitney U-test, *P*>0.05.

The mean numbers of mature female *A. nerii* consumed per adult beetle in 4h, were 2.4^{a} ± 0.4 (±1SE), *n*=20 for *C. bipustulatus*, 2.8^{ab} ± 0.4 (±1SE), *n*=49 for *C. nigritus* and 3.8^{b} ± 0.4 (±1SE), *n*=20 for *C. infernalis* (Kruskal Wallis ANOVA, followed by a nonparametric multiple comparison (Siegel & Castellan, 1989), α=0.05). Male *C. infernalis* adults consumed 3.4 scale insects ± 0.5 (±1SE), *n*=10 and females 4.1 ± 0.6 (±1SE), *n*=10, Mann-Whitney U-test, *P*>0.05. Male *C. nigritus* adults consumed 2.0

Table 1. Weights of adults older than 14d, times from egg hatch to adult eclosion, mean ± 1SE (*n*), and percentage survival of immature stages for six *Chilocorus* spp.

Species	Adult weight mg	Development time days	Percentage survival
C. bipustulatus	9.5^{a} ± 0.2 (24)	29.0^{c} ± 0.4 (33)	69
C. cacti	20.0^{c} ± 0.4 (52)	30.6^{c} ± 0.7 (21)	71
C. distigma	37.2^{d} ± 1.0 (28)	33.2^{d} ± 0.4 (40)	70
C. infernalis	15.5^{b} ± 0.4 (21)	26.2^{b} ± 0.4 (37)	76
C. nigritus	8.5^{a} ± 0.2 (40)	21.9^{a} ± 0.3 (39)	78
C. simoni	17.6^{bc} ± 0.6 (37)	>40 (10)	-

Kruskal Wallis ANOVA, followed by a nonparametric multiple comparison (Siegel & Castellan, 1989), α=0.05, absence of a common letter in the superscript indicates a significant difference.

scales ± 0.6 (±1SE), n=20 and females 3.5 ± 0.7 (±1SE), n=19, Mann-Whitney U-test, P<0.05. Fourth instar larvae consumed 2.6[a] scales per larva per 4h ± 0.5 (±1SE), n=10 for *C. bipustulatus*, 1.2[b] ± 0.2 (±1SE), n=24 for *C. nigritus* and 4.6[c] ± 0.7 (±1SE), n=9 for *C. infernalis* (Kruskal Wallis ANOVA, followed by a nonparametric multiple comparison (Siegel & Castellan, 1989), α=0.05).

Age-weight and sex-weight relationship

Weight increased for approximately 6d, 11d and 9d after eclosion, and levelled off at approximately 37%, 15% and 38% heavier than 1d after eclosion for *C. bipustulatus*, *C. infernalis* and *C. nigritus* respectively (Fig. 1).

C. nigritus adults which were undernourished at eclosion due to a deficient larval diet, were significantly lighter than 1d old adults from larvae reared on an adequate diet, Mann-Whitney U-test, P<0.05. Weight increase of these undersized adults lasted longer than for the adults from adequately fed larvae (Fig. 1). Their weights levelled off after approximately 23d, at 59% heavier than 1d after eclosion. These stable end weights were still significantly lower than those of adults from well-fed larvae, Mann-Whitney U-test, P<0.05.

The average weight at 1d after eclosion of *C. bipustulatus* males was 6.0mg ± 0.3 (±1SE), n=9 and females was 6.5mg ± 0.5 (±1SE), n=10, Mann-Whitney U-test, P>0.05. One day old *C.*

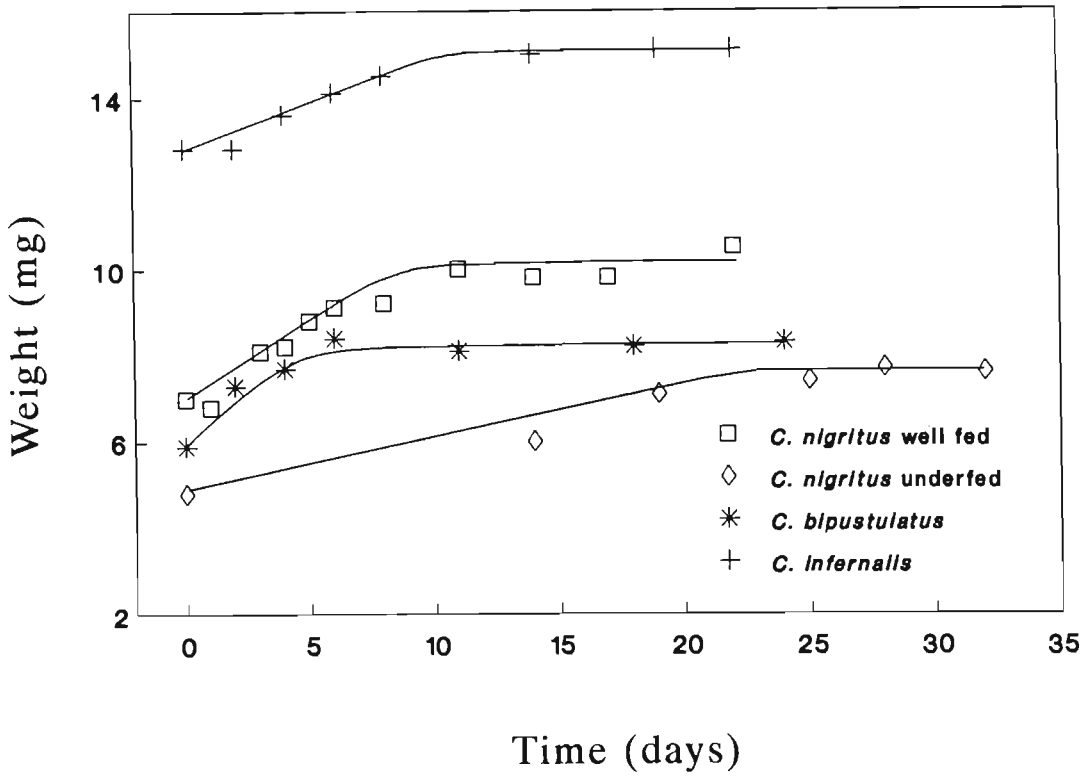

Fig. 1. Mean adult weights against time after eclosion for *C. bipustulatus*, *C. infernalis* and *C. nigritus* from well-fed larvae, and *C. nigritus* from larvae reared on a sub-optimal synthetic diet.

infernalis males weighed 12.4mg ± 0.6 (±1SE), *n*=8 and females 13.6mg ± 0.5 (±1SE), *n*=8, Mann-Whitney U-test, *P*>0.05. However, 22d old *C. infernalis* males weighed 13.7mg ± 0.6 (±1SE), *n*=8 and females 16.3mg ± 0.5 (±1SE), *n*=8, Mann-Whitney U-test, *P*<0.01. At 1d after eclosion *C. nigritus* males weighed 6.3mg ± 0.1 (±1SE), *n*=68 and females 6.6mg ± 0.1 (±1SE), *n*=67, Mann-Whitney U-test, *P*<0.05.

Constant versus fluctuating temperature

A comparison between the vigour of *C. nigritus* cultures maintained at constant and at fluctuating temperatures, is made in Table 2. Only three adults died during the trial, making a comparison of adult longevity under these conditions impossible without lengthier observation. Comparison of larval development times was abandoned due to a major breakdown of the environmental control equipment.

Feeding rate: photophase and starvation

The average number of mature female *A. nerii* eaten per adult *C. nigritus* in 4h was 1.4 ± 0.4 (±1SE), *n*=20 in the light phase and 0.4 ± 0.2 (±1SE), *n*=20 in the dark phase, Mann-Whitney U-test, *P*<0.05. The feeding rate of the fourth instar larvae in the light phase was 1.8 ± 0.1 (±1SE), *n*=11 and in the dark phase was 1.0 ± 0.3 (±1SE), *n*=11, Mann-Whitney U-test, *P*<0.05.

The feeding rate of *C. nigritus* adults after various periods

Table 2. Comparisons between vigour of *C. nigritus* cultures maintained at constant and fluctuating temperatures, using rate of egg laying, percentage egg hatch, adult survival, adult weight and adult feeding rate

Measurement	Mean ± 1SE (*n*)	
	Constant temperature	fluctuating temperature
eggs / pair / day	4.3^a ± 0.3 (10)	2.2^b ± 0.3 (10)
% egg hatch	74^a ± 6 (10)	71^a ± 9 (10)
% survival of adults over 40d	98 (60)	97 (10)
adult weight at start of trial (mg)	8.2^a ± 0.2 (10)	8.1^a ± 0.1 (10)
adult weight after 40d exposure to treatment	8.8^a ± 0.2 (10)	8.4^a ± 0.1 (10)
A. nerii eaten per beetle in first 4h of light phase	1.1^a ± 0.1 (10)	0.6^b ± 0.1 (10)
A. nerii eaten in middle 4h of light phase	1.5^a ± 0.2 (10)	1.5^a ± 0.2 (10)
A. nerii eaten in last 4h of light phase	1.4^a ± 0.2 (10)	0.7^b ± 0.1 (10)

Absence of a common superscript indicates a significant difference, seperate comparisons for each row, Mann-Whitney U-test, α=0.05.

of starvation are represented in Fig. 2.

Temperature-feeding rate relationship

The mean numbers of three week old male and female *A. nerii* eaten in 4h are plotted against temperature in Fig. 3. The survival rates of the six species at various constant temperatures is given in Table 3. The species could be ranked in order of increasing tolerance of high temperatures: *C. infernalis* < *C. simoni* < *C. nigritus* < *C. bipustulatus* and *C. distigma* < *C. cacti*.

DISCUSSION

Biocontrol predictiveness

Differences in measurements of biological parameters of these predators, as used here for quality assessment, do not accurately reflect differences in their value as biocontrol agents in the field. This cautions against predicting differences in field performance based only on small differences in laboratory performance. Nevertheless, accurate biogeographical climatic matching can be valuable in streamlining biocontrol projects (Samways, 1989).

The growing concern for environmental repercussions of introducing alien insects, even if considered to be beneficial (Howarth, 1983, 1991; Samways, 1988), emphasises the urgent

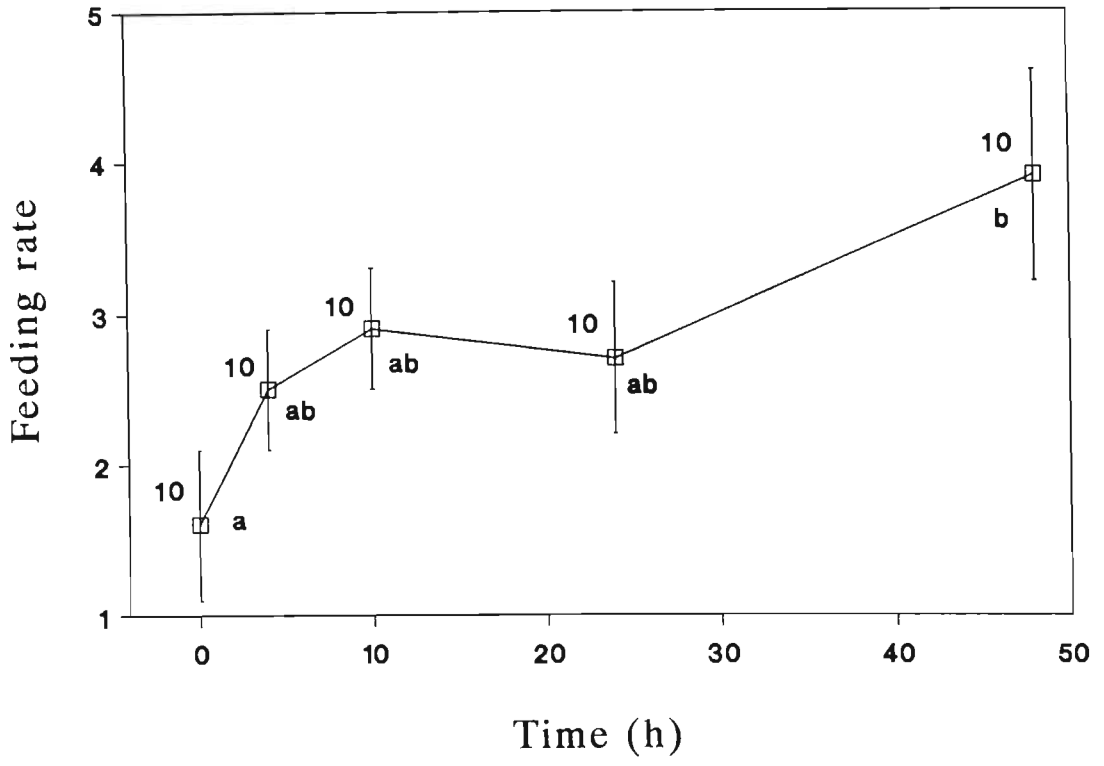

Fig. 2. Mean, ± 1SE, number of mature female *A. nerii* eaten per *C. nigritus* adult in 4h, after various periods of starvation, *n* left of SE bars; absence of a common letter in labels right of SE bars indicates a significant difference, Kruskal Wallis ANOVA, followed by a nonparametric multiple comparison.

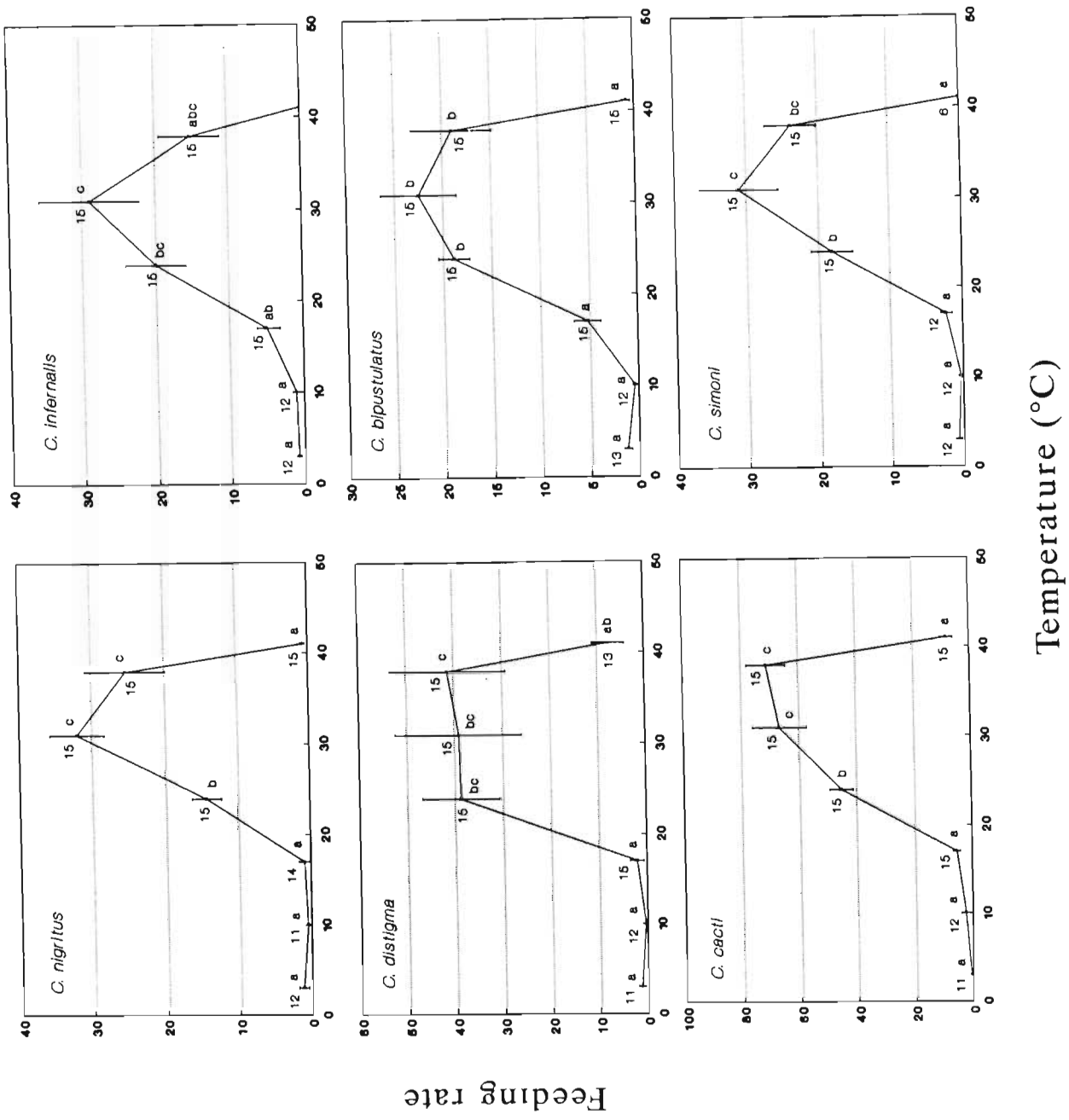

Fig. 3. Mean number of three-week old male and female *A. nerii* eaten per adult *Chilocorus* sp. in 4h at various constant temperatures, *n* left of SE bars, absence of a common letter in label right of SE bars indicates a significant difference, Kruskal Wallis ANOVA, followed by a nonparametric multiple comparison.

Table 3. Percentage of the population surviving 48h of exposure to a range of constant temperatures and survival after various periods of exposure to 41°C, for *C. bipustulatus*, *C. cacti*, *C. distigma*, *C. infernalis*, *C. nigritus* and *C. simoni*

Species	Percentage surviving 48h at						
	3°C	10°C	17°C	24°C	31°C	38°C	41°C
C. bipustulatus	85	94	100	100	100	100	0
C. cacti	100	95	95	95	100	100	0
C. distigma	100	100	100	100	100	100	0
C. infernalis	85	100	100	100	95	84	0
C. nigritus	100	88	100	95	95	95	0
C. simoni	100	93	94	100	100	88	0

	Percentage surviving at 41°C after exposure for			
	14h	21h	41h	48h
C. bipustulatus	91	62	5	0
C. cacti	100	95	50	0
C. distigma	100	57	0	0
C. infernalis	0	0	0	0
C. nigritus	90	5	0	0
C. simoni	43	7	0	0

need for further research on methods to reduce introductions of biocontrol agents which are not going to provide adequate control of pest insects.

Importance of parasitism

Compared with the highly successful biocontrol agent *C. nigritus*, the less economically important *C. cacti*, had a significantly higher feeding rate over a broad range of temperatures (Fig. 3), and could survive at higher temperatures. There is a good match between the climate in the region of origin of this species and parts of southern Africa. However, field performance in southern Africa is poor. This may be attributed to extensive parasitism of *C. cacti* in South Africa by *Oencyrtus sinis* Prinsloo (Hymenoptera: Encyrtidae), whereas no parasitism of *C. nigritus* has been reported in this region. The comparatively high level of performance of *C. cacti* in the laboratory however, indicates that this species may be a valuable biocontrol agent in other regions where they would not be exposed to such parasitism.

Biogeographical background

C. distigma is widely distributed through Africa, South of the Tropic of Cancer (Hattingh, 1991), encompassing Walter & Leith's (1967) zonobiomes 1, 2, 3, 4 and 5, which are briefly equatorial-humid, tropical, hot- and arid-subtropical, Mediterranean and warm-temperate humid regions respectively.

C. bipustulatus is indigenous to the greater Mediterranean region, but has become established in parts of California (Rosen & Gerson, 1965; Huffaker & Doutt, 1965; Gordon, 1985). The Mediterranean climate is classified as primarily zonobiome 4, with regions being zonobiome 3. *C. infernalis* distribution is centred in the highlands of northern India and Pakistan, being in zonobiomes 2 and 10, which are tropical with summer rainfall and mountainous.

C. nigritus is indigenous to the Indian sub-continent which is classified as zonobiome 2, and has become established in many other countries with zonobiome 2 climates (Samways, 1989). In southern Africa, it's distribution is restricted to the more humid low altitude areas (Samways, 1989).

C. simoni is a southern African species occurring in zonobiome 2, including higher altitude regions with low humidities and frost in winter and hot dry conditions in summer, unlike *C. nigritus*.

C. cacti is indigenous to the far southern USA, Central America and northern South America (Gordon, 1985). It's distribution is centred in zonobiomes 1 and 2, but also encompasses zonobiomes 3, 5, 7 (arid, with a cold season) and 10.

Comparing climatic adaptations

In spite of differences in climatic adaptations, there were no obvious differences in the temperatures at which the feeding rates of these species reached a peak.

Survival times at excessively high temperatures were valuable in directly comparing the temperature adaptations of these species (Table 1). The order in which the species tested, first showed extensive mortality at increasing temperatures, reflected differences in the climatic regions covered by their distributions. *C. infernalis* was the first species to die, followed by *C. simoni*, then *C. nigritus*, then *C. bipustulatus* and *C. distigma* at approximately the same time and finally *C. cacti*.

Adult weight

Fecundity of insects is widely accepted to be proportional to adult size (Beddington *et al.*, 1976; Slansky & Rodriguez, 1987) and the track width across which a foraging coccinellid detects individual prey by contact may be determined by measuring the foreleg span (Carter & Dixon, 1982). This makes measurement of adult size by means of weight a valuable measure of adult fitness.

Our results indicated that measurement at one day after eclosion is indicative. Newly-emerged adults which were

lighter due to a deficiency in larval diet, showed a greater subsequent increase in weight on an adequate diet than adults from well-fed larvae. However, the end weights attained by the originally undernourished insects, were still significantly lower than those of adults from larvae reared on a suitable diet. This avoids lengthy rearing and evaluation of the adults to determine the effects of larval treatments on subsequent adults.

Feeding rates

Nakamuta (1987) reported that the feeding rate of the coccinellid *Coccinella septempunctata bruckii* Mulsant was constant after 4h to 24h of starvation. Fig. 2 indicates a similar trend in *C. nigritus*. This provides guidelines for the duration of starving to standardise hunger prior to conducting feeding rate trials.

Adults and larvae fed significantly slower during the dark phase than the light phase, which is relevant to performing feeding rate trials in the laboratory. This reduction in feeding rate at night could in part be attributed to a reduction in their ability to locate prey by sight.

As coccinellids need to come very close to, or make contact with prey, for detection of individual prey items, a reduction in speed of movement during foraging will reduce the rate of prey encounters. The possibility of a temperature-induced

reduction in feeding rate at night was avoided by conducting the trials under controlled temperature conditions. The reduced feeding rate may however, be an adaptation to avoid reduced energy efficiency during foraging at the lower night temperatures encountered in the field. Adult beetles were observed in the laboratory rearing cages to go into a semi-torpid state at night, forming small aggregations of two to ten individuals.

Constant and fluctuating temperatures

This study indicated that the basis for improvement in culture vigour on exposure to fluctuating temperatures, is not a behavioural response of individuals, as there was no improvement within one generation. These observations coupled with reports of such improvement (Hagstrum & Hagstrum, 1970; Hodek, 1973; Scriber & Slansky, 1981), support the hypothesis that there might be strong selection pressures under different temperature regimes. The details of such rearing conditions, may well affect the ability of the biocontrol agents to establish on release into a particular climate.

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CHAPTER 4

Prey choice and substitution in two *Chilocorus* spp. (Coleoptera: Coccinellidae)

ABSTRACT

Choice between prey species by *Chilocorus nigritus* (Fabricius) and *C. bipustulatus* (Linnaeus) adults and larvae was determined. They showed virtually no discrimination between the prey types tested, and this was not affected by the type of prey previously eaten. The deleterious effects of a prey substitution, during larval development and adult maintenance, were investigated using *C. nigritus* with supplementary work on *C. bipustulatus*. These diet changes significantly retarded larval development rate, and subsequent adults were smaller than control individuals. Prey substitutions in the adult diet, suppressed oviposition for several days and feeding rate was reduced at one day after substitution. Larvae were more sensitive than adults to such diet changes. This was not a case of classical unsuitability of the new prey, as the fecundity and feeding rate returned to the same levels as before the substitution, after a few days of exposure to the new prey. Furthermore, both prey types were suitable for larval development of *C. nigritus* when they fed on one exclusively, but unsuitable when substitutions were made. The effects of prey substitutions may possibly be attributed to the

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presence, in the new prey, of plant toxins, which the predators are initially not physiologically capable of dealing with in large quantities. These results present difficulties for the concepts of monophagy and polyphagy, being less well defined than normally thought. In view of coccinellid foraging behaviour and larval habitat selection by adults, the temporary reduction in fitness following a diet change is considered to be adaptive.

INTRODUCTION

Chilocorus nigritus (Fabricius) and *Chilocorus bipustulatus* (Linnaeus) are well known as effective biocontrol agents of many scale insects (Nadel & Biron, 1964; Rosen & Gerson, 1965; Samways, 1984). These species have also been valuable in experimental investigation of the feeding and foraging behaviour of insect predators (Podoler & Henen, 1986; Samways & Wilson, 1988; Hattingh & Samways, 1990).

Chilocorus spp. were maintained in the insectary on Oleander scale *Aspidiotus nerii* Bouché (Hemiptera: Diaspididae) on butternuts *Cucurbita moschata* cv. Waltham and potatoes *Solanum tuberosum* Linnaeus, because of the ease with which these prey items can be cultured (Samways & Tate, 1986). Following an unexpected interruption in the supply of this prey, the scale insect *Asterolecanium miliaris* (Boisduval) (Hemiptera: Diaspididae), which is plentiful on giant bamboo *Dendrocalamus giganteus* Munro, and is a valuable alternative prey for *C.*

nigritus in the field in southern Africa (Samways, 1984), was used as a substitute. A temporary, unquantified drop in the rate of oviposition was observed.

Debilitating effects have been observed in other coccinellids following a change in their diet from suitable to less suitable prey (Hodek, 1973; Iablokoff-Khnzorian, 1982; Hagen, 1987). However, there is a dearth of information on the effects of alternating between suitable prey types. Samways & Wilson (1988) reported that a change from *A. nerii* to red scale *Aonidiella aurantii* (Maskell) (Hemiptera: Diaspididae), results in a drop in feeding rate of *C. nigritus* larvae, however the adult feeding rate was reported to increase. These responses are relevant to our understanding of their prey range, and could influence foraging theories.

Many field introductions of *C. bipustulatus* to achieve establishment, and *C. nigritus* to increase their distribution and local population levels in southern Africa, have been made in areas with suitable climates. These insectary reared specimens are however released onto *A. aurantii* in citrus orchards or *A. miliaris* on giant bamboo. To date however, there has not been a quantitative assessment of deleterious effects of prey substitution during larval development and adult maintenance. This paper evaluates through experimental manipulation in the laboratory, the significance of prey substitution.

Ability to choose and to alternate between prey types has an important bearing on the outcome of a biocontrol project using predatory insects. When one prey type becomes scarce, it may be important that the presence of another suitable local prey type will not only be suppressed by the agent, but may also be instrumental in stimulating the predator to stay in the area and not disperse. This phenomenon provides predator insurance against a possible resurgence in the first pest's numbers.

Another important consideration of prey substitution and subsequent suitability is in the insectary rearing of a predator. Often the target pest is not easy to rear, even though it may be notorious in the field. An alternative insectary prey may therefore determine whether the predator can be successfully maintained for later field release. The problem then is that such a secondary prey may or may not predispose the predator to switching to the target prey when released in the field.

Such prey substitution is intimately bound up with prey choice. Yet the ability of coccinellids to choose between prey types with different levels of suitability is not entirely clear. Earlier studies illustrate both the ability and inability to choose. Blackman (1967) reported that *Adalia bipunctata* (Linnaeus), cannot distinguish between the toxic vetch aphid *Megoura viciae* Bucht and the non-toxic pea aphid *Acyrthosiphon pisum* (Harris). In contrast, Dixon (1958) noted that as soon as *Adalia decempunctata* (Linnaeus) penetrates the body wall of

the mealy plum-aphid *Hyalopterus pruni* (Geoffroy), this prey is rejected. Thereafter, palpal contact is enough to reject this prey (Dixon, 1958). This study investigates the position of these *Chilocorus* spp. in this range of abilities.

MATERIALS AND METHODS

Beetle rearing

The experiments were conducted in the insectary at 25-26°C and 50-70% RH, with a 14L:10D photoperiod. The beetles were maintained on a biparental strain of *A. nerii* on butternuts and potatoes (Samways & Tate, 1986). *A. miliaris* on internode sections of giant bamboo were field collected as alternative prey.

Prey choice

Prey choice was evaluated by presenting *C. nigritus* and *C. bipustulatus* adults with *A. nerii* and purple scale *Chrysomphalus aonidum* (Linnaeus) on butternuts, *A. miliaris* on short sections of bamboo stem, and *A. aurantii* on oranges *Citrus sinensis* cv Valencia. The surface area of the different food substrates was the same, as were the densities of the various scale insects. The arena had a wooden floor 0.5m x 0.4m covered with clean brown paper with 12 equal-sized blocks drawn on it. The sides were also wood, with windows 0.24m x 0.24m, and 0.34m x 0.24m, covered with fine nylon netting. The

lid was a 5mm-thick glass plate resting on soft foam around the upper lip of the box, 0.42m above the floor. Symmetrically positioned overhead lighting was provided.

The four prey types were each randomly allocated three blocks on the floor of the arena. Twelve adults of each beetle species, reared on *A. nerii* and not starved prior to the trial, were released on a cardboard platform, 0.12m x 0.12m, suspended in the centre of the arena. Two-hourly counts were made of the beetles on the various prey types. A trial run showed that after 24h, the total number of beetles found on the food substrates no longer increased significantly. Thereafter, samples were taken at 24h after release. The various prey types were randomly rearranged, and different individual predators were used for each of the six replicates. For each beetle species the counts on the three examples of each prey type, were summed and analysed by Friedman two-way ANOVA.

A similar trial was conducted with fourth-instar larvae of the two species. The butternuts, oranges and bamboo half-rings, were placed side by side so that larvae could move between them without having to cross an open space on the floor of the arena. Two larvae of each species, reared on *A. nerii*, were placed on each prey substrate with a soft paint brush.

The effect of the prey type previously eaten by adults and fourth-instar larvae of *C. nigritus,* on their choice between *A. nerii* and *A. miliaris* was determined. Potatoes were used

that were infested with *A. nerii* and short sections of bamboo stem infested with *A. miliaris*, with approximately equal densities of scale insects and surface areas. One section of bamboo stem and one potato were used per replicate, and were placed in contact with each other in the centre of the arena. Controls were non-infested potatoes and bamboo sections, and beetles which had been reared on *A. miliaris*. The arenas had brown paper covered wooden floors 0.2m x 0.2m. The sides were 0.2m high, and roof consisted of a wooden frame covered in fine nylon netting.

Two beetle cultures were used, the one maintained on *A. nerii* and the other on *A. miliaris*. Three adults or fourth-instar larvae were placed on each food substrate in separate trials. A trial run was performed, using individuals from both feeding histories, in which it was found that after 24h the ratio of predators on the two prey types no longer changed significantly. Thereafter, the number of predators on the surface of the potato or bamboo were recorded 24h after being presented with the choice. There were six or seven replicates per developmental stage and feeding history category, and there were six control replicates with uninfested potatoes and bamboo. Counts were compared with permutation tests for related samples.

Prey substitution

The effect of a prey substitution on the larval development

rate and subsequent adult weight, was investigated with *C. nigritus*. Larvae were selected at the end of the first instar from a culture maintained on *A. nerii*. As Treatment 1, these larvae were then reared on *A. miliaris*, and as a control, larvae of the same age were selected from a culture maintained on *A. miliaris* and were further reared on *A. miliaris*. Treatment 2 larvae, at the end of the first instar, were selected from the culture maintained on *A. miliaris* and then fed *A. nerii*. The control larvae were maintained on *A. nerii* and after similar handling, continued feeding on *A. nerii*. The times taken for the adults to emerge were measured, and weighed one day after eclosion. A Kruskal Wallis ANOVA was performed, $\alpha = 0.05$, followed by a nonparametric multiple comparison, $\alpha = 0.05$ (Siegel & Castellan, 1989).

The effect of a prey substitution on the feeding rates of *C. nigritus* and *C. bipustulatus* adults was evaluated. Adult beetles which had previously been maintained on *A. miliaris* were then fed *A. nerii*. For the control, individuals reared and maintained on *A. nerii* were used. The feeding rates of individuals over 4h were recorded at various intervals after the switch, n=9 to 11 individuals per species.

Consumption rate of *A. miliaris* could not easily be measured. The entire scale covering was often removed and the presence or absence of the soft body beneath the covering could not be determined without removal of the scale covering. For this reason a reciprocal transfer from *A. nerii* to *A. miliaris* was

not performed.

Circular plastic collars, 35mm in diameter and 10mm high, were attached with "Prestik" to the surface of potatoes infested with approximately equal densities of equal aged (one week before crawler emergence) *A. nerii* females. Individual beetles were enclosed in the arenas with nylon netting which was clamped around the arenas with elastic bands. After feeding on *A. nerii*, the beetles leave behind an empty scale covering from which the soft body has been removed through a slit in the scale covering. The numbers of scale insects eaten were recorded after removal of the predators. For each species, a Friedman ANOVA was performed, followed by a nonparametric multiple comparison between treatments and controls, $\alpha = 0.05$ (Siegel & Castellan, 1989).

The rate of egg laying by individual pairs was determined by enclosure in arenas, with egg pads and prey. Arenas for enclosing coccinellids on potatoes with *A. nerii*, were circular plastic collars, 10mm high and 35mm in diameter. On bamboo with *A. miliaris*, rectangular cardboard arenas were used, 106mm x 30mm x 30mm. The arenas were attached to the surfaces of scale bearing substrates, each enclosed with fine nylon gauze clamped around the sides with elastic bands. *C. nigritus* laid eggs in polyester fibre pads (Samways & Mapp, 1983) and *C. bipustulatus* between strands of frayed linen (Nadel & Biron, 1964).

C. bipustulatus and *C. nigritus* reared on *A. nerii*, were enclosed on potatoes bearing *A. nerii*, their fecundity monitored, and then transferred to *A. miliaris* on bamboo and their fecundity monitored. *C. nigritus* adults within 1d of eclosion from larvae reared on *A. nerii*, were transferred to *A. miliaris*. After 30d their fecundity on *A. miliaris* was measured, they were then transferred to *A. nerii* and fecundity monitored. Changes in fecundity were compared with a Friedman ANOVA, followed by a nonparametric multiple comparison, $\alpha=0.05$ (Siegel & Castellan, 1989).

RESULTS

Prey choice

The Friedman test indicated that there were significantly more *C. bipustulatus* adults and larvae on one or more of the four prey types than on the remaining prey types presented in the choice experiment (Table 1). However, a nonparametric multiple comparison did not indicate a significant difference between any of the prey groups. These conflicting results can be attributed to differences in the power of the tests. Counts of *C. nigritus* adults and larvae on the four prey types were not significantly different by Friedman's ANOVA.

The numbers of *C. nigritus* adults and larvae on uninfested butternuts and bamboo, between 1h and 24h after presentation with the choice, were not significantly different (Friedman

Table 1. Mean numbers of *C. bipustulatus* and *C. nigritus* adults and fourth instar larvae on four prey spp. at 24h after being presented with the choice.

Predator	Mean no. of individuals on prey spp.			
	A. miliaris	*A. nerii*	*C. aonidum*	*A. aurantii*
Adults				
C. bipustulatus	2.3[a] ± 0.6	2.2[a] ± 0.3	0.7[a] ± 0.2	1.8[a] ± 0.5
C. nigritus	1.8 ± 0.8	2.0 ± 0.4	2.0 ± 0.6	2.8 ± 0.8
Larvae				
C. bipustulatus	4.2[a] ± 1.0	7.8[a] ± 0.5	6.7[a] ± 0.8	4.2[a] ± 0.8
C. nigritus	4.5 ± 0.4	6.7 ± 0.8	6.0 ± 0.4	4.2 ± 0.9

Absence of a common superscript indicates a significant difference, Friedman ANOVA, followed by a nonparametric multiple comparison, (Siegel & Castellan, 1989), α=0.05.

ANOVA, $P>0.05$). There were more *C. nigritus* adults on *A. nerii* than on *A. miliaris* when presented with a choice between these two prey types and this was not effected by feeding history (Table 2). There was no significant difference in the numbers of fourth-instar larvae on the two prey types, and this was not affected by feeding history (Table 2).

Prey substitution

A substitution in prey type at the end of the first instar resulted in the time to adult eclosion being significantly longer than for those larvae reared continuously on only one prey type (Fig. 1). Also, the weights of adults one day after eclosion were significantly lower when a prey substitution had taken place during larval development than without a substitution (Fig. 1).

A substitution in prey type did not significantly affect the feeding rate of *C. bipustulatus* adults during the first four hours of exposure to the new prey (Fig. 2). However, the feeding rate one day after the diet change was significantly lower than that of the control (Fig. 2). Thereafter an increase in the feeding rate to a level similar to that of the control was observed. A similar trend was observed with the feeding rate of *C. nigritus* after exposure to a diet change (Fig. 2).

The rates of egg laying by *C. bipustulatus* declined shortly

Table 2. Numbers of *C. nigritus* adults and larvae encountered on *A. nerii* and *A. miliaris* at 24h after presentation with the choice, mean ± 1SE (*n*)

Predator	Prey sp. on which reared			
	A. nerii counts at 24h on		*A. miliaris* counts at 24h on	
	A. nerii	*A. miliaris*	*A. nerii*	*A. miliaris*
C. nigritus adults	$1.5^{a} \pm 0.5$ (6)	0^{b} (6)	$3.0^{a} \pm 0.6$ (6)	$0.7^{b} \pm 0.3$ (6)
C. nigritus larvae	$4.3^{a} \pm 0.4$ (6)	$1.5^{a} \pm 0.5$ (6)	$2.6^{a} \pm 0.3$ (5)	$3.0^{a} \pm 0.5$ (5)

Absence of a common superscript indicates a significant difference, Permutation test for related samples, α=0.05.

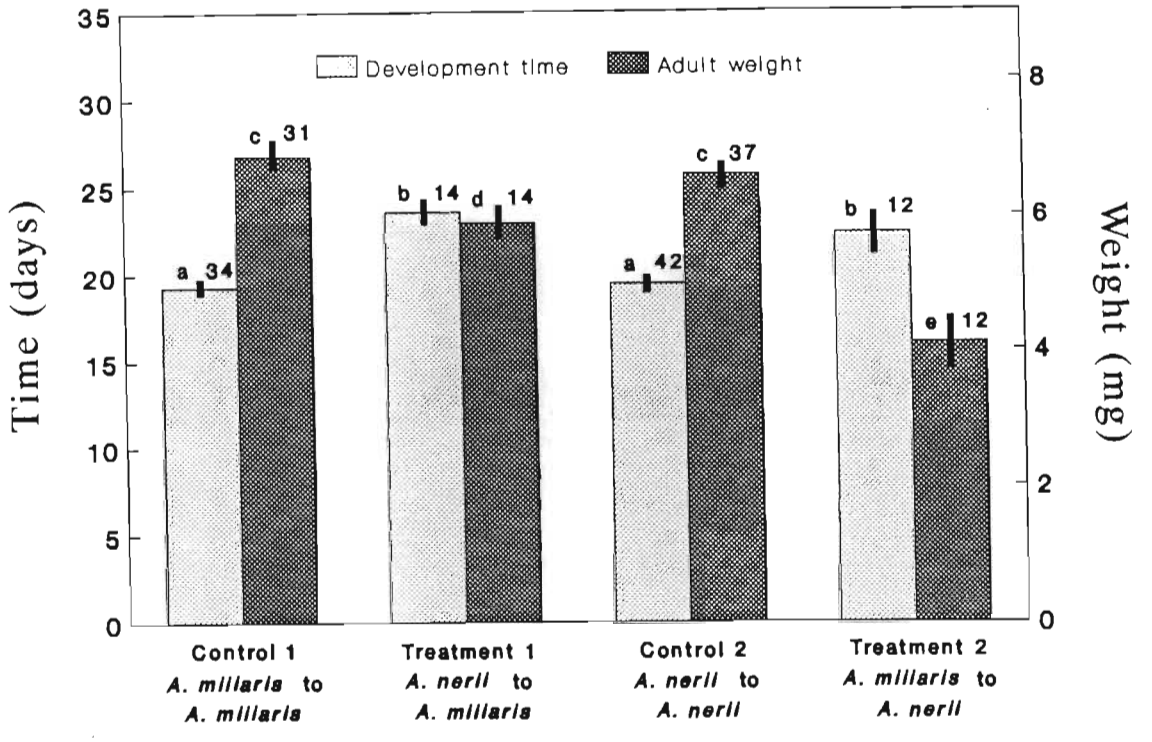

Fig. 1. Mean (± 1 SE), number of days from the end of the first larval instar to adult eclosion of *Chilocorus nigritus*, and mean weights of these adults, *n* to the right of SE bars. Control 1 reared continuously on *A. miliaris*, Treatment 1 transferred from *A. nerii* to *A. miliaris*, Control 2 reared on *A. nerii*, Treatment 2 transferred from *A. miliaris* to *A. nerii*. A common label indicates no significant difference, Kruskal Wallis ANOVA, followed by a nonparametric multiple comparison, α=0.05.

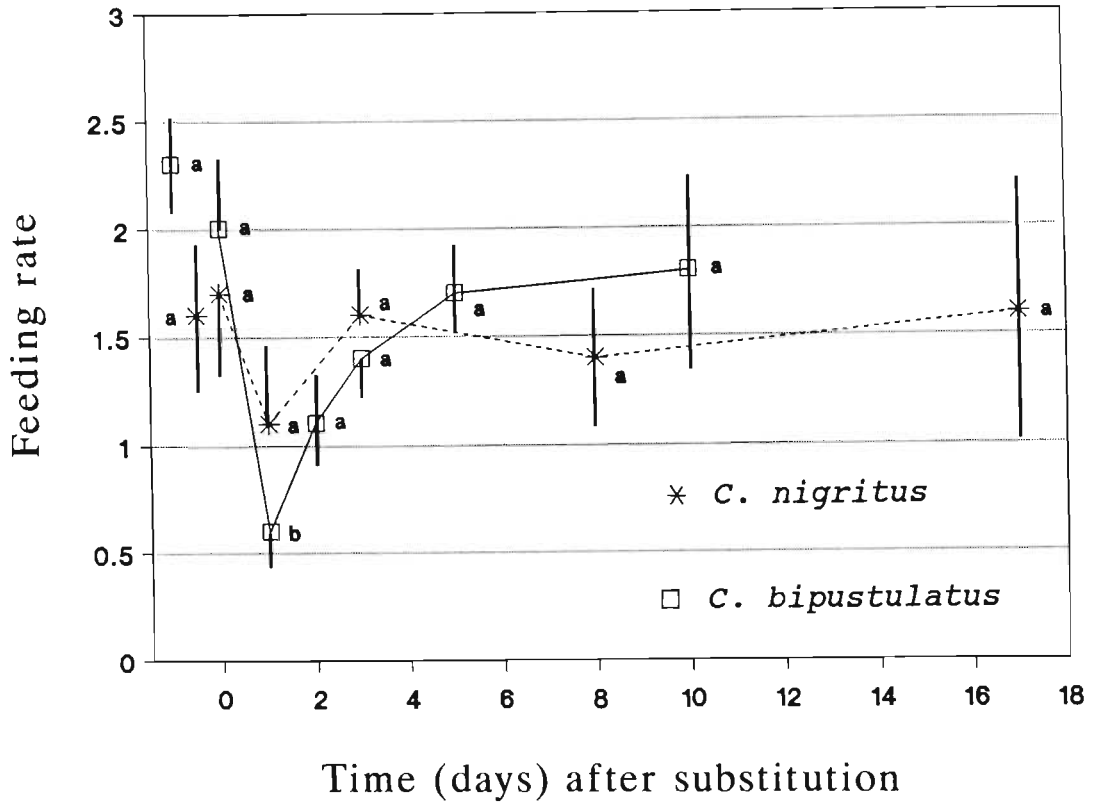

Fig. 2. Mean (± 1 SE) number of adult female *A. nerii* eaten per individual adult *C. bipustulatus* or *C. nigritus* in 4h, at various times after a change in diet from *A. miliaris*, *n*=9 to 11. Controls were reared and maintained on *A. nerii* exclusively. Common labels indicate no significant difference, Friedman ANOVA, followed by a nonparametric multiple comparison, α=0.05.

after a prey substitution and returned to a level equivalent to the rate prior to the substitution (Fig. 3). Following a transfer of *C. nigritus* from *A. nerii* to *A. miliaris*, the fecundity dropped for a few days before returning to the same level as before the transfer. There was no reduction in fecundity following the transfer of *C. nigritus* from *A. miliaris* to *A. nerii*.

DISCUSSION

These *Chilocorus* spp. demonstrated only limited ability to choose between prey species. In the case of *C. nigritus* adults showing a preference for one of two species provided in a choice experiment, the difference was only marginally significant. In these experiments, the prey types can be viewed as being in discrete patches with no species mixing. The beetles may be even less capable of choosing when prey species are inter-mixed. Blackman (1967) reported similar findings with *Adalia bipunctata* which could not avoid toxic *Aphis fabae* Scopoli and *Megoura viciae* when presented together with suitable prey. Also, slight preferences are not always for the more suitable prey and *Aphis fabae* and *Aphis sambuci* Linnaeus, although natural prey for *A. bipunctata* in the field, are less suitable in the laboratory than some other prey (Blackman, 1967). This cautions against using prey choice alone, to select suitable predator-prey relationships for biological control.

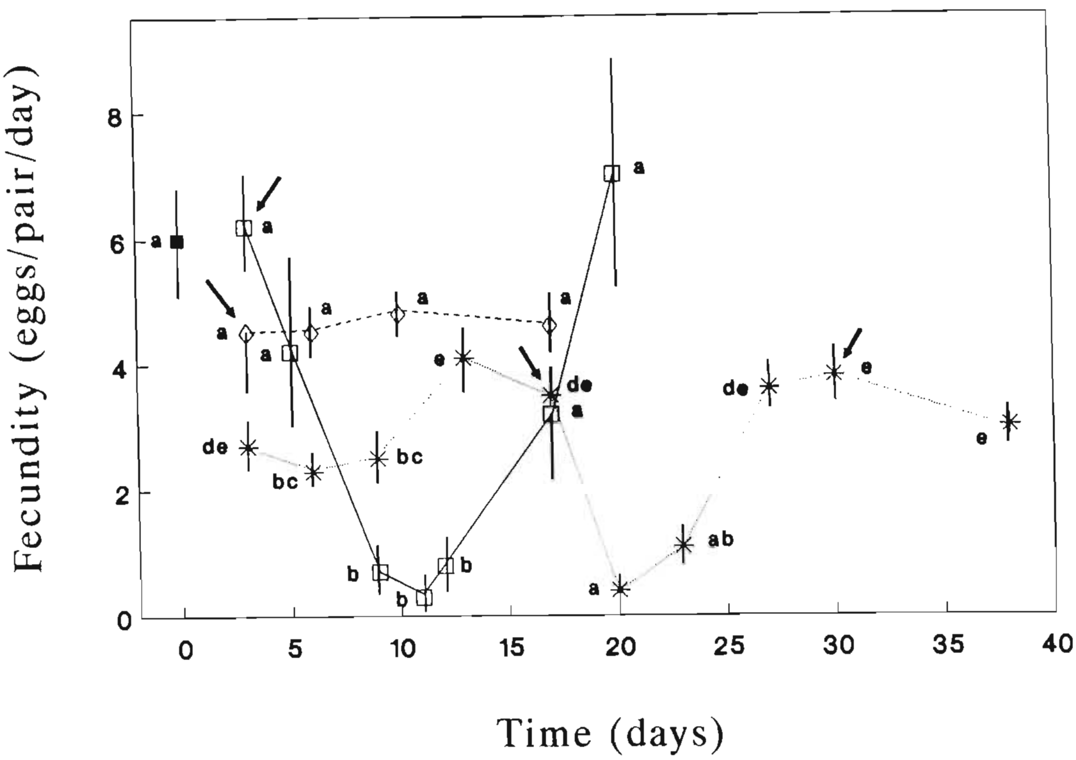

Fig. 3. Mean (± 1 SE) number of eggs laid per pair per day by *C. bipustulatus* and *C. nigritus*, before and after prey substitutions. The arrows indicate when the substitutions were made. *C. bipustulatus* were transferred from *A. nerii* to *A. miliaris* (□), *n*=6, and fecundity was also measured when fed on *A. miliaris* continuously (■), *n*=12. *C. nigritus* were transferred from *A. nerii* to *A. miliaris* (*) and vice versa (◇), *n*=15. Absence of a common letter in labels indicates a significant difference, Friedman ANOVA, followed by a nonparametric multiple comparison, ∝=0.05.

The reason why *C. nigritus* fecundity was not suppressed following a transfer from *A. miliaris* to *A. nerii* is not clear. It is possible that a particular prey substitution will adversely effect fecundity, but a substitution in the opposite direction may not have the same effect. Alternatively, having reared the larvae on *A. nerii* before transferring the adults to *A. miliaris* at 1d after eclosion may have predisposed the adults to making the transition from *A. miliaris* to *A. nerii*, without a consequent reduction in the rate of egg laying.

The deleterious effects of prey substitution during adult coccinellid feeding are of short duration. The effects of a change during larval development are more serious, resulting in slower development and reduced adult size. Since fecundity in insects is generally proportional to female size (Beddington *et al.*, 1976; Slansky & Rodriguez, 1987) this translates into reduced fitness. The larval life stage is the most vulnerable in the coccinellid life cycle, and therefore extending the duration of this life stage may be an obstacle to risk avoidance. In view of the serious effects of such diet alterations, and the lack of discrimination between prey types, a mechanism by which such situations are avoided can be expected, particularly in the case of larvae.

Since the adult is the dispersal life stage, they are likely to encounter unfamiliar prey in the new habitats following dispersal. In view of larval habitat selection being performed by adults via oviposition (Blackman, 1967; Hodek, 1973), this

response by adults to encountering a novel prey may be considered adaptive. Commencement of extensive search in the foraging of coccinellids is dependent on the time since the last prey was consumed (Carter & Dixon, 1982) and this search mode results in dispersal from the patch (Krebs *et al.*, 1974; Nakamuta, 1985). Podoler & Henen (1986) and Hattingh & Samways (unpublished) have found that these *Chilocorus* spp. forage in this way. On encountering a habitat where an unfamiliar prey type is encountered, the feeding rate of the adults would be suppressed. This reduced feeding rate would initiate the adoption of extensive foraging, resulting in dispersal from this habitat.

Simultaneously, fecundity would be suppressed and their progeny excluded from such a habitat, thus avoiding serious repercussions for the fitness of the next generation. Both *A. nerii* and *A. miliaris* are suitable for larval development of *C. nigritus* when fed exclusively on one of these species (Hattingh & Samways, unpublished). This study however, shows that substitution of one of these species for the other, makes such a diet unsuitable for the larvae. If adults were to disperse to a new habitat with an unfamiliar prey type, but one which is suitable for larvae if fed on exclusively, it would be disadvantageous to exclude their progeny from this habitat through cessation of oviposition. Indeed, the temporary nature of suppressed fecundity on encountering such a habitat, would avoid this. The extensive search by adults, induced by a reduction in feeding rate, would ensure sampling of a larger

area of the new habitat. Having primarily encountered only one prey type for several days, which would be suitable for larvae, egg laying would be resumed.

The duration of intensive area concentrated search by coccinellids following a prey encounter, increases with increasing hunger and this increases the time spent in a patch (Carter & Dixon, 1982). The hunger level of dispersing beetles on encountering a new habitat with mixed prey or unfamiliar prey could affect their decision whether to remain or to disperse from the habitat. Hungry individuals might well tolerate the adverse effects of the unfamiliar prey, by means of longer periods of intensive search following prey encounters, rather than starve. This would provide advantageous flexibility for the dispersal response to encountering a habitat with less suitable prey and would be dependent on the hunger level of the dispersing beetles.

These *Chilocorus* spp. did not actively avoid unfamiliar prey, and their feeding rate on first encountering unfamiliar prey was not reduced. This indicates that the reduced feeding rate one day after exposure to the new prey was not due to the absence of cues used to locate and recognise prey, the absence of a phagostimulant, or to the predators having greater difficulty in consuming the new prey.

The delayed and temporary deleterious effects of a change in diet probably have a physiological basis and are a result of

a different chemical composition of the new prey type. Hagen (1987) states that either the proportion of nutrients, or the possession of sequestered secondary plant metabolites, determines the suitability of the prey for the predator. A different proportion of nutrients may require more of certain enzymes or symbiotic microorganisms to metabolise. This could take some time to achieve and could explain the temporary reduction in feeding rate and consequently fecundity.

Numerous authors have attributed unsuitability of particular prey types to toxins taken up from the host plant (Hodek, 1973; Hagen, 1987, Moraes & McMurtry, 1987). The effects of feeding on prey which is unsuitable due to such toxins, are similar to those observed here (Hodek, 1973; Hagen, 1987). The temporary reduction in adult vigour on encountering such prey for the first time, could be a result of having to develop a mechanism to deal with such toxins. The high energy costs of maintaining detoxification enzymes make it advantageous for the animal to produce these only when required (Brattsten, 1979). The initial rapid feeding would provide the toxin required for induction of the necessary enzymes, and the ensuing period of reduced feeding would allow for production of sufficient enzyme to make consumption of large quantities of the new food possible. Induction can in cases be observed as rapidly as 30 minutes after exposure (Brattsten, 1979), but Terriere (1984) indicates that several hours is a more probable period, with maximal effect only evident after two or three days. This coincides with the duration of reduced feeding following

exposure to unfamiliar prey in the study reported here.

There are a number of possible reasons why larvae are less capable of tolerating these diet changes than adults. In the field, larvae can be expected to encounter such diet changes less often than the adults which are responsible for dispersal. Also, larval habitat selection is performed by adults, and a mechanism whereby larvae are excluded from such habitats by the adults probably exists, as proposed earlier. The energetic costs of maintaining mechanisms to cope with such prey changes may be high, thus increasing the duration of the vulnerable life stage. Further, the area-concentrated search adopted by these predators reduces dispersal from a site as long as the prey remain plentiful, thereby ensuring that the larvae remain in the suitable habitat selected by their parents. These may be the underlying reasons why larvae are less capable of tolerating diet changes.

These results present difficulties for definitions of terms such as monophagy and polyphagy. Scriber (1979) stated that classification of an organism as polyphagous, requires qualification on the basis of whether the term applies to the species, a population or an individual. These predators appear to be adapted to habitats in which predominantly monocultures of prey species occur. This appears to be a common feature of scale insect infestations in the field, although exceptions do occur (Hattingh & Samways, pers. obs.). Coccinellids may be theoretically polyphagous, searching out high-density patches

of preferred prey. In reality, the species may consist of populations with higher specificity, with the species as a whole having a far broader range of suitable prey.

It would be valuable to determine the duration of familiarity with the previous prey type, following a transfer to an unfamiliar prey species. This would relate to the success of these predators in controlling more than one prey species, following introduction into predominantly monoculture patches, or into mixtures of different prey species, after familiarisation. Also, how effectively can these natural enemies adjust to a diet of mixed prey types or will fitness continuously be suppressed?

These results caution against exposing larvae to such diet alterations during insectary rearing of coccinellid biocontrol agents for field introduction. Maintenance of insectary cultures on prey species other than the target species is acceptable provided that larvae are not released into the field. Furthermore, a period of two weeks for familiarisation of insectary adults with the target prey prior to release is advisable.

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CHAPTER 5

Determination of the most effective method for field establishment by biocontrol agents of the genus *Chilocorus* (Coleoptera : Coccinellidae)

ABSTRACT

The coccinellid biocontrol agent *Chilocorus nigritus* (Fabricius) can be established on the non-target scale *Asterolecanium miliaris* (Boisduval) on giant bamboo *Dendrocalamus giganteus* Munro. When these sites are adjacent to citrus orchards, the coccinellid readily moves across to reduce population levels of the target prey, red scale *Aonidiella aurantii* (Maskell)(Hemiptera: Diaspididae). *C. bipustulatus* (Linnaeus) and *C. infernalis* Mulsant are potential new biocontrol agents against *A. aurantii* in South Africa. Each life stage of *C. nigritus*, *C. bipustulatus* and *C. infernalis* has advantages and disadvantages for field introductions. This chapter investigates different methods of introduction of the different life stages for field establishment. A number of synthetic materials were tested as egg pads for *C. infernalis* without success. Introduction of eggs adhering to stiff paper cards was unsuccessful and impractical for these three species. Eggs, young larvae (first and second instars), older larvae (third and fourth instars) and adult *C. nigritus* were introduced into stands of bamboo.

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The adult was the most suitable life stage for establishment followed by the older larvae, then the younger larvae, with the least suitable being the egg stage. Adults of the three species were released onto scale-infested bamboo at two sites to evaluate the viability of such introductions and to compare their persistence at these sites. *C. nigritus* and *C. infernalis* persisted at one release site through the winter and the hottest period of the summer.

INTRODUCTION

Chilocorus nigritus (Fabricius) is an economically important biocontrol agent of red scale *Aonidiella aurantii* (Maskell) on citrus in southern Africa (Samways, 1984, 1986, 1988). It has also been utilised by biocontrol specialists in several countries against various other scales on several crops (Samways, 1984). This predator was first observed in southern Africa in the 1970's, possibly having entered the region through dispersal from Réunion, Aldabra or Madagascar (Samways, 1989). *C. nigritus* successfully colonised major portions of the eastern region of southern Africa. This increase in distribution has been aided by releases of insectary-reared beetles (Samways, 1989).

C. bipustulatus (Linnaeus), indigenous to the Mediterranean region (Smith, 1915), is a well known predator of various armoured and unarmoured scale insects (Smith, 1915; Huffaker & Doutt, 1965). *C. bipustulatus* is considered a valuable

biocontrol agent of *A. aurantii* on citrus in Israel (Nadel & Biron, 1964; Rosen & Gerson, 1965), and has become established in California where it preys on olive scale *Parlatoria oleae* (Colvée)(Hemiptera: Diaspididae) (Huffaker & Doutt, 1965; Gordon, 1985). This predator was imported into South Africa towards red scale control in hot and dry areas where *C. nigritus* has not been successful. The cold tolerant *C. infernalis* Mulsant from the foothills of the Himalayas, was also imported as a natural enemy of *A. aurantii* in the colder regions of southern Africa.

The citrus orchard is a relatively hostile environment for the introduction of coccinellids. Spring insecticide applications and year to year changes in prey population levels, make direct establishment in the orchard difficult. However, it is now well known that an important alternative prey for *C. nigritus* is the scale *Asterolecanium miliaris* (Boisduval) on giant bamboo *Dendrocalamus giganteus* Munro (Samways, 1984). Stands of this Asian plant support large populations of this scale insect throughout the year and are regularly seen along water courses in the eastern part of South Africa. This is also the country's major citrus producing area, and as the streams are of great importance for irrigation, many of the bamboo stands are close to the citrus orchards. *C. nigritus* regularly inhabits the bamboo stands and moves across to the citrus when *A. aurantii* levels begin to increase. This prey alternating behaviour has enormous economic benefits, and additionally, provides considerable security that the biocontrol oriented

pest management programme will be successful.

Although *A. miliaris* is a highly suitable alternative prey for *C. nigritus*, this does not appear to be so for the larvae of *C. bipustulatus* and *C. infernalis* (Hattingh & Samways, unpublished). This suggested that the use of bamboo for establishing and maintaining economically important populations of *C. bipustulatus* and *C. infernalis*, in climatic areas unsuitable for *C. nigritus*, was not likely to be successful.

In the insectary, females of the three species lay eggs beneath scale covers, and *C. nigritus* and *C. bipustulatus* will also readily oviposit on synthetic egg pads. Large quantities of *C. nigritus* eggs are easily obtained within a few days by draping 0.01 m^2 strips of polyester fibre wadding over vegetables infested with *Aspidiotus nerii* Bouché (Hemiptera: Diaspididae). *C. bipustulatus* lays eggs between the frayed strands along the edges of strips of linen (Nadel and Biron, 1964). These pads are convenient for transport and introduction into the field (Samways and Mapp, 1983). They are also useful to separate eggs and the subsequent young larvae from the adults in the insectary, thereby avoiding cannibalism. However, the ability of newly emerged larvae to escape from these pads was unknown. There appears to be no reports of a suitable artificial oviposition substrate for *C. infernalis*.

The use of egg pads was investigated as was the viability of using cardboard cards on which the eggs were glued. It was the

commercial distribution of glasshouse whitefly *Trialeurodes vaporariorum* Westwood 'pupae' (Hemiptera: Aleyrodidae), parasitised by *Encarsia formosa* Gahan (Hymenoptera: Aphelinidae), glued onto cards (Scopes & Pickford, 1985) that stimulated this latter approach.

All three species have been successfully insectary reared on *A. nerii*. This scale is extensively used because of the ease with which it can be cultured in the insectary (Samways & Tate, 1986) and its ready acceptance by the three beetle species. These insectary reared beetles have been released onto *A. aurantii* on citrus and *A. miliaris* scale on bamboo during field introductions. Samways & Wilson (1988) reported a reduction in feeding rate by *C. nigritus* larvae following a transfer from *A. nerii* to *A. aurantii* in the laboratory. The deleterious effects of a change in prey type have been observed for larvae and adults of all three species, and appear to be more acute among the larvae than the adults (Hattingh & Samways, unpublished). This may influence the suitability of the various life stages reared on *A. nerii*, for field releases onto *A. aurantii* or *A. miliaris*.

The introduction of eggs avoids the problem of a forced change in prey type. Many more eggs than adults can be introduced, with less insectary work required. However, the susceptibility of the eggs to the environmental extremes in the field is unknown. Also, the time required for larval development during which the coccinellids are particularly vulnerable, before

reproduction can begin, is a disadvantage.

The introduction of larvae requires less insectary work than adults, and therefore more individuals can be released. However, the larvae are difficult to work with, each must be individually transferred with a soft paint brush. Additionally, they are apparently more susceptible than adults to the deleterious effects of a change in diet (Hattingh & Samways, unpublished). Samways & Wilson (1988) showed that the younger *C. nigritus* larvae are incapable of eating the mature *A. nerii* and *A. aurantii* females on which fourth instar larvae and adults can feed.

In contrast, with adults, after mortality through the rearing process, only a limited number of individuals are available for release. Should these then disperse extensively on release, there is slim chance of establishment. Hattingh & Samways (1990) showed that the high density of beetles during transport and after intensive release, did not result in greatly increased dispersal owing to intraspecific interference. However, other factors such as the physical disturbance of handling, hunger levels, physical surroundings at the release site, and depletion of prey due to exploitation, are still possible causes of increased dispersal at the point of release.

The role of bamboo for field introductions of the three *Chilocorus* spp. was determined. The use of egg pads for insectary rearing and field introductions was investigated.

The various life stages of *C. nigritus* for field releases were evaluated.

MATERIALS AND METHODS

Egg pads

Materials were tested as egg pads for *C. infernalis* by placing 10 pads of each material in a rearing cage with approximately 200 adults of all ages. The pads were inspected for eggs daily on 10 consecutive days. Materials tested were polyester fibre wadding used for *C. nigritus*, frayed linen used for *C. bipustulatus*, double layered paper towelling, cotton wool, surgical gauze and open cell sponge.

Attempts were made to remove *C. nigritus* and *C. bipustulatus* eggs from the pads for attachment to cards with various adhesives. The pads were gently teased with a soft paint brush and the polyester fibre pads were stretched and compressed to release the eggs.

Experimental sites

The accuracy of judging the level of scale infestation on the bamboo stems by observation was quantified. Three sampling units were obtained from each of three of the release sites and two sampling units from an additional stand. Each sampling unit was a sliver of bamboo stem, and all were judged to be

equally infested with all life stages present. A cardboard sheet with a rectangular window 40mm x 10mm, was attached to the surface of each sampling unit. The number of live and dead mature *A. miliaris* were determined microscopically.

All life stages of the scale insects can be found on bamboo in the Pietermaritzburg area throughout the year provided extreme heat, cold or drought is not experienced. Qualitative observations were made of the scale population levels throughout the experimental period.

Five sites for evaluating the life stages of released *C. nigritus* were selected in parks and open plots in and around Pietermaritzburg, Natal, 29°35'S, 30°25'E. These sites were stands of bamboo with similar levels of *A. miliaris* infestation and of approximately equal proportions; being the density of the leaf canopy, the surface area covered ($95m^2$), the number of stems (120), and the height of the stems (15m). The persistence of adults of the three species at the release sites were compared at two localities. These were Site 5, used in the evaluation of life stages and Site 6, 800m west of Sites 2 and 3 and complying with the standards set for the other sites.

Evaluation of life stages

The arenas for the introduction of *C. nigritus* larvae and eggs were 2m sections of bamboo stems with similar levels of *A.*

miliaris infestation. Six stems per site were chosen, and care was taken to obtain similar distributions of the stems within each stand. The arena was enclosed by two barriers, each consisting of a double layer of "Bidim fibre" grade U 24 (used in the manufacture of ant bands to prevent access of ants to the canopy of citrus trees) wrapped around the stem. The lower barriers were 0.3m to 1m above the ground. All side branches and branches from other plants in contact with the arenas were cut away to prevent the larvae from escaping.

Adult *Chilocorus* spp. for release were transferred from the rearing cages well stocked with *A. nerii*. They were transported in brown paper bags 0.2m x 0.12m x 0.06m and released within one hour. The larvae were transported on the surface of *A. nerii*-infested butternuts *Cucurbita moschata* cv. Waltham, and transferred to the bamboo stems with a soft paint brush. Adults, larvae and eggs were introduced into the bamboo stand on 12 December 1988.

At Site 1, 100 *C. nigritus* eggs in polyester fibre pads were pinned to the stem in each of the six arenas. Site 2 was 2 km north of Site 1, and here 50 first and second instar *C. nigritus* larvae were released per arena. Site 3 was a separate stand of bamboo 5m from Site 2. Here 30 third and fourth instar *C. nigritus* larvae were released per arena. At Site 4, which was 5 km east of Sites 2 and 3, 120 *C. nigritus* adults were released from six brown paper bags. Each bag was opened carefully and pinned to the base of a bamboo stem in the centre

of the stand. Site 5 was 600 m from Site 4, and here 120 adult *C. nigritus* were gently brushed out of brown paper bags on to the foliage in the centre of the stand of bamboo.

The number of coccinellid larvae in the arenas at Sites 1, 2 and 3 were monitored weekly. For the first seven weeks the adult release sites 4 and 5 were sampled weekly and thereafter every two or three weeks for the next 42 weeks. Sites 4 and 5 were sampled by spending 20 min searching each stand. The stems were not climbed, and only those ladybirds positively identified were recorded. Recognition of *C. nigritus* from more than 2m was impossible since they could no longer be distinguished from the plentiful coccinellid *Exochomus flavipes* (Thunberg). None of these three *Chilocorus* spp. were present at the experimental sites prior to commencement of the trials, and there had been no previous reports of their occurrence in this area.

Species comparison

At Site 6, 50 adults of each species were released from brown paper bags in the centre of the stand on 10 April 1989. At Site 5, 100 adults of each species were released in the same way on the same day. The *C. nigritus* adults released at Site 5 were marked with a dot of white "Tippex" (typing correction fluid) on one elytron. This was done to distinguish the adults in this release from those already on the bamboo as a result of the previous release during evaluation of the life stages.

No apparent deleterious effects of such markings were observed in the insectary. These sites were sampled twice during the first two weeks, and then twice at two-weekly intervals, and thereafter monthly.

RESULTS

Egg pads

None of the materials tested was accepted by *C. infernalis*, as an oviposition substrate. *C. nigritus* and *C. bipustulatus* eggs could not be removed from the polyester fibre and frayed linen pads respectively, as the eggs were securely cemented to the fibres. Those eggs which did come free had damaged choria and soon became dehydrated.

C. nigritus and *C. bipustulatus* larvae hatching from eggs in pads did not have difficulty in escaping from the tangled fibres. No larvae were found trapped or dead in any of the egg pads used in insectary rearing.

Experimental sites

The mean number of live mature *A. miliaris* per 400mm^2 was 256 ± 42 (±1SE), n = 11, and the mean number of dead scales was 149 ± 43 (±1SE), n = 11. The small SE indicates that my judgement of the level of infestation was satisfactory.

Sites 1, 2, 3, 4 and 6 were on the banks of continuously flowing water courses and were therefore not exposed to drought. The temperatures during the experimental period were not extreme for this region. Regular qualitative observations showed that the scale population remained stable with all life stages present throughout the experimental period at Sites 1, 2, 3, 4 and 6. There was a slight increase in numbers of young scale in spring with a population peak in mid to late summer. At Site 5 the scale population was similar to the other sites for the duration of the life stage evaluation experiment. The plants at this site showed signs of water stress from 19 to 29 weeks after release in the species comparison experiment. This resulted in the loss of all the leaves from the plants and it became extremely difficult to find live scale insects.

Evaluation of life stages

At Site 1, the highest count of larvae from the 600 introduced eggs was one week after introduction, when 30 larvae were observed (Fig. 1). Only five of these larvae attained a size indicative of the fourth instar. None of these larvae pupated, and after three weeks no more live individuals were found.

The introduction of first and second instar larvae at Site 2 was more successful (Fig. 1). The mortality rate was still very high with only 9% of the larvae surviving the first three weeks to pupation. After four weeks, only two adults were found and thereafter no live individuals were recorded. SE

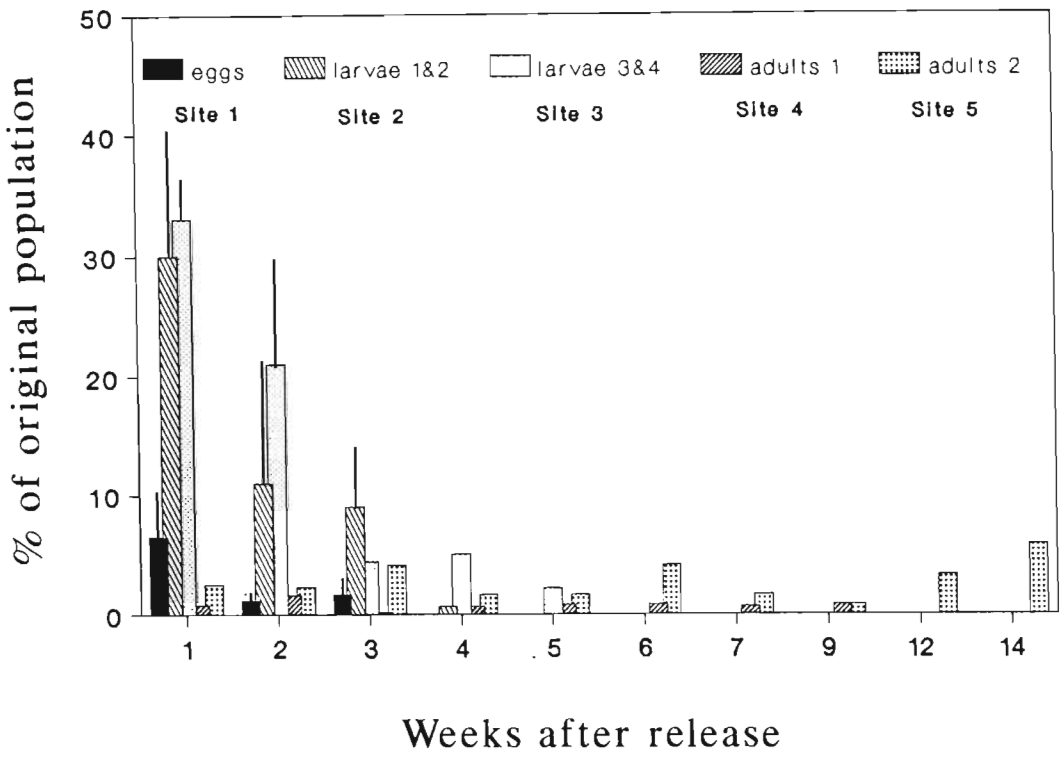

Fig 1. Mean percentages + 1SE and percentages of numbers released on 12 December 1988, observed during subsequent sampling at release sites, plotted against weeks after release, for various life stages of *Chilocorus nigritus,* six replicates for each immature life stage.

measurements (Fig. 1) were possible for larval counts only, since adults were not restricted to the arenas and thereby replication was lost. At Site 3, 21% of the released third and fourth instar larvae had pupated at the end of the second week. The maximum count of adults at Site 3 was 5% of the original number after four weeks. No individuals were recovered after six weeks.

Counts of zero to two *C. nigritus* adults were obtained at the adult release Site 4 for the first nine weeks, and thereafter none was observed (Fig. 1). The number of positive identifications of *C. nigritus* adults at Site 5 remained low at one to nine per sample. However, 49 weeks after release, adults and larvae were still present at this site. This colony had survived through the summer and the coldest part of winter.

Species comparison

The release of adults of the three species at Site 6 was not successful. One week after release, no *C. nigritus* or *C. infernalis* were found, and only one *C. bipustulatus* was sighted. Two weeks after release, one *C. bipustulatus* and one *C. infernalis* were found and thereafter no more specimens were observed.

The release of adults of the three species at Site 5 led to *C. bipustulatus* adults persisting at the site for two weeks (Fig. 2). Marked *C. nigritus* adults were present for four weeks,

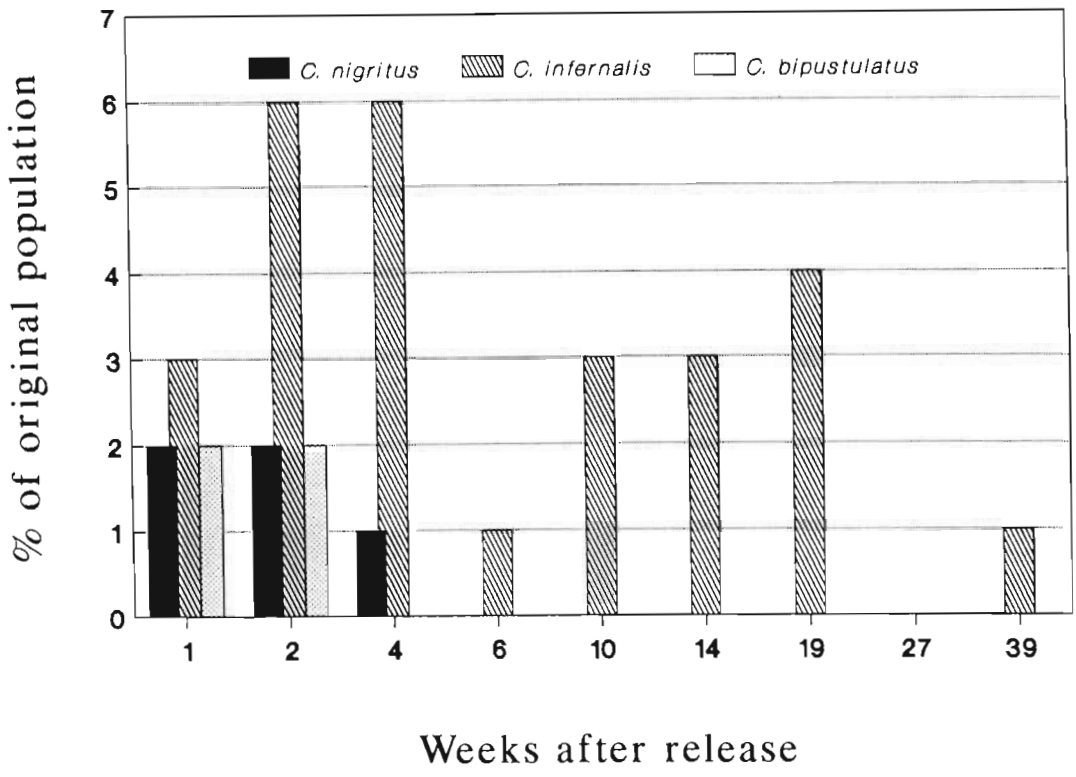

Fig. 2. Percentages of numbers of *Chilocorus bipustulatus*, *C. infernalis* and *C. nigritus* adults released at Site 5 on 10 April 1989, observed during subsequent sampling, plotted against weeks after release.

which could possibly have been long enough to produce a second generation. Unfortunately, individuals of the new generation could not be distinguished from those of the previous release and their offspring.

C. infernalis were present at Site 5 in every sample for the first 19 weeks (Fig. 2). However, there was a qualitatively observed gradual reduction with time in the size of adults observed. The mean weight of *C. infernalis* adults reared in the insectary on *A. nerii* and used for releases on bamboo was 12.1 mg ± 1.6 (±1SD), n = 40. The specimen located at the release site at 39 weeks after release was captured and weighed 6.9 mg. After 19 weeks a very dry period was experienced, the bamboo lost all leaves and live scale insects could not be found. In spite of this stressful period of 10 weeks, *C. infernalis* adults were still present 39 weeks after release. This population had therefore survived the whole winter and the severest part of the summer.

DISCUSSION

The low percentage recovery of first instar larvae from eggs and the poor survival of larvae, particularly the earlier instars, make eggs laid in pads unsuitable for field introductions. These pads nevertheless remain useful for harvesting eggs in the insectary. This is necessary to culture these *Chilocorus* spp. efficiently, as the rate of egg cannibalism by adults and larvae can be high (Hattingh,

unpublished). Adults are the most suitable life stage for field introductions. Introduction of *C. nigritus* adults onto bamboo to obtain establishment, before natural dispersal and colonisation of the citrus orchard, can be recommended. Field introduction of *C. bipustulatus* onto bamboo in the Natal midlands region is less likely to result in establishment than similar introductions of the other two species.

The prolonged survival of the *C. infernalis* colony on bamboo was surprising, since *A. miliaris* is considered a sub-optimal diet for the larval development of this coccinellid (Hattingh & Samways, unpublished). They found that *C. infernalis* larvae reared on *A. miliaris* in the laboratory, produced adults which were significantly smaller than those obtained with a suitable larval diet such as *A. nerii*. The gradual reduction with time in the size of *C. infernalis* adults observed in the stand of bamboo, was probably due to this dietary deficiency. In spite of an unsuitable prey type and the drought, *C. infernalis* survived through the severest part of the summer and winter. This suggests that *C. infernalis* is a good biocontrol candidate for survival in the Natal midlands region, but field introduction of this species onto bamboo is not advisable, making introduction directly into the citrus orchard necessary.

The variable results obtained with comparable releases in separate but very similar sites, demonstrates that introduction into an orchard without establishment does not indicate that an introduction into another nearby orchard will not be

successful. The only observed difference between Sites 4, 5 & 6 at which adults were released, were the vegetational surroundings. The most successful site, No.5, was in the middle of a lawn with the nearest shrub or tree 50m from the stand. At Sites 4 & 6 many large trees grew within 5m of the stands. The persistence of adults at Site 5 could be due to less dispersal from the release site. There was no large barrier of open space surrounding Sites 4 & 6, and more dispersal into the adjacent trees could be expected.

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CHAPTER 6

Absence of intraspecific interference during feeding by the predatory ladybirds *Chilocorus* spp.(Coleoptera: Coccinellidae)

ABSTRACT

The hypothesis was tested that intraspecific behavioural interference does not adversely affect the feeding behaviour of adults of three predatory coccinellid species, *Chilocorus nigritus* (Fabricius), *C. bipustulatus* (Linnaeus) and *C. infernalis* Mulsant, at densities found under field conditions. Feeding rates on mature oleander scale *Aspidiotus nerii* Bouché (Hemiptera: Diaspididae) were evaluated by two methods at various predator densities. Proportion of the population dispersing, was also measured for one of the species. Feeding rate did not decrease and dispersal did not increase with increasing predator density. No significant behavioural interference that might have reduced predatory efficiency was observed, counter to assumptions on which published interference models are based. Results here help to explain the relative importance of parasitoids and predators in the effective control of red scale *Aonidiella aurantii* (Maskell)(Hemiptera: Diaspididae). The results also provide guidelines for release of these biocontrol agents.

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INTRODUCTION

Mutual interference between insect parasitoids and predators in reducing their foraging efficiency has received much attention. The work has mostly modelled the interaction between parasitoids, with little experimental verification. Also, in earlier studies, predators were often assumed to behave in the same way as parasitoids in terms of intraspecific interference. In early population models (Lotka, 1925; Volterra, 1926; Nicholson & Bailey, 1935) searching efficiency (E) was assumed to be constant through fluctuations in parasitoid (P) and host density (N),

$$E = N_a/NP \qquad (1)$$

N_a is the total number of attacks per unit time and area. This assumption was shown to be invalid when Holling (1959) described efficiency as a decreasing function of host density based on his functional response expression. The assumption was also considered incorrect by Watt (1959) and Hassell & Varley (1969), who showed that efficiency declined with an increase in parasitoid density expressed as

$$E = QP^{-m} \qquad (2)$$

where Q is quest constant (level of efficiency of an individual parasitoid) and m is the interference constant. This reduction in efficiency was adopted in subsequent modelling of interference (Hassell, 1971a & b; Royama, 1971; Hassell & Rogers, 1972; Hassell & May, 1973; Rogers & Hassell, 1974; Beddington, 1975; Hassell, Lawton & Beddington, 1976; Free, Beddington & Lawton, 1977). Rogers & Hassell (1974)

attributed the reduced efficiency to temporary cessation of searching for hosts by a parasitoid following an encounter with another parasitoid. They described efficiency as

$$E = QP_s/P \qquad (3)$$

where Q is the searching capacity of a single parasitoid without interference, P is the total population size of parasitoids, and P_s is the number of parasitoids searching. This was expressed by Beddington (1975) as

$$E = a/(1 + at_hN + bt_wR) \qquad (4)$$

where a is the attack rate, t_h is the handling time, b is rate of encounters between parasitoids, t_w is the time wasted per encounter, and R=P-1. Hassell, Lawton & Beddington (1976) included in their explanation an increase in dispersal following an increase in parasitoid density.

Free *et al.* (1977), in turn, described these responses as behavioural interference and defined a further component called pseudo-interference. This they ascribed to a differential exploitation of hosts in areas of high density. Free *et al.* (1977) drew attention to the possibility of important differences between intraspecific parasitoid and predator interference relationships. Behavioural interference was also considered to be less important under natural conditions than previous work had suggested. Since the work of Free *et al.* (1977) attention has been focused on functional and numerical responses, leaving the question of the relevance of this work to predators unanswered.

In this study I investigated mutual interference in three coccinellid species: 1) *Chilocorus bipustulatus* (Linnaeus), the well-known biocontrol agent of problem scale insects (Hemiptera: Diaspididae) in Israel (Nadel & Biron, 1964), 2) *C. nigritus* (Fabricius), an effective predator of red scale *Aonidiella aurantii* (Maskell) (Hemiptera: Diaspididae) on citrus in certain climatic areas of southern Africa (Samways, 1984, 1986, 1989), and 3) *C. infernalis* Mulsant, which has been imported into South Africa from Pakistan as a potential biocontrol agent of *A. aurantii* in other climatic areas.

During routine laboratory rearing of *C. nigritus, C. bipustulatus* and *C. infernalis*, there was no indication of intraspecific behavioural interference. These observations prompted experimental evaluation of whether or not this interference occurred at densities similar to and greater than those found in the field.

MATERIALS AND METHODS

Predator culture

All three *Chilocorus* spp. were reared on Oleander scale *Aspidiotus nerii* Bouché (Hemiptera: Diaspididae) on potatoes *Solanum tuberosum* Linnaeus and butternuts *Cucurbita moschata* cv. Waltham at 26°C, 50-60% RH and a 14L:10D photoperiod. Experiments were under the same conditions, which were similar to optimal field conditions. Individuals were randomly selected

from those individuals on the surfaces of scale bearing vegetables in the rearing cages and transferred directly to experimental arenas. Thereby beetles on the sides of the rearing cages which often appeared to be in a semi-torpid state were avoided. Each insect was used only once.

Experiment I

The first experiment determined the feeding rate of *C. nigritus, C. bipustulatus* and *C. infernalis* adults. The prey were mature female *A. nerii* of equal age (approximately one week before crawler production commenced), reared on potatoes.

The feeding arenas were circular plastic collars, 35mm in diameter, 10mm high, attached to the surface of the potatoes with apparently inert 'Prestik'. The beetles were placed on to the surface of the potatoes and the arenas closed with fine nylon gauze clamped around the collars with elastic bands. The prey density per arena was sampled.

Coccinellids fed for 4h at densities of 1, 2, 4 or 8 beetles per arena, 6 to 20 replicates per density. Trials were run from the 7th to the 11th hour of the light phase. The trial was re-run with *C. nigritus* using 10 replicates per density. The number of prey eaten were counted using a dissecting microscope. With their sharp mandibles (Samways & Wilson, 1988), the ladybirds make an incision through the dorsal surface of the scale covering and remove the soft body, leaving

behind the empty scale covering. For each replicate, the total number of prey eaten was determined and divided by the number of predators in the arena. The mean of these figures for each species and density class was determined.

Experiment II

A limitation in design of Experiment I prompted a second experiment with adult *C. nigritus*. The small volume of the arena greatly restricted dispersal, and the search time was reduced by the very high prey density. The duration of the experiment may have been too short, further reducing the amount of available search time. If interference occurs primarily during searching, as suggested in the literature, these results would have underestimated the importance of interference. In the second experiment the arenas were made much larger, allowing relatively free dispersal. The duration of the experiment was increased, and the density of the prey was reduced. The total number of prey was not reduced, so avoiding any functional response due to a reduction in prey density.

The arenas in this second experiment were small cages in the shape of cubes with $0.04m^2$ sides. The floor was wooden, the sides and roof were fine nylon gauze attached to a wooden frame. The prey were mature female *A. nerii* (one week before crawler production commenced), at approximately equal densities on potatoes of approximately equal size. The number of prey

per potato was sampled. Each potato had a two-pronged wire fork on either side for handling, and rested on a collar to minimise scale damage. One potato was placed in each small cage and 1, 8 or 15 individuals placed on to the surface of the potato.

Ten replicates of each predator density were run over the last 10h of the light phase. These results were processed in the same way as the previous experiment. To determine dispersal, four counts at approximately 2h intervals were made of the number of coccinellids not on the potatoes. For each two hourly count, the sum of dispersing individuals in the 10 replicates for each density class, was divided by the total number of individuals per arena. The mean of these four values per class was then calculated.

RESULTS

Experiment I

The mean number of *A. nerii* per small circular arena was 179 ± 20 (±1SE), n=15. There was no significant difference between the number of prey eaten per predator in 4h, at the various densities, for any of the three species (Kruskal Wallis ANOVA, P>0.05) (Fig. 1). Thus the ratio N_a/P in equation (1) was constant, and since the prey density (N) was practically constant, efficiency (E) could be considered constant through the range of predator densities (P) tested.

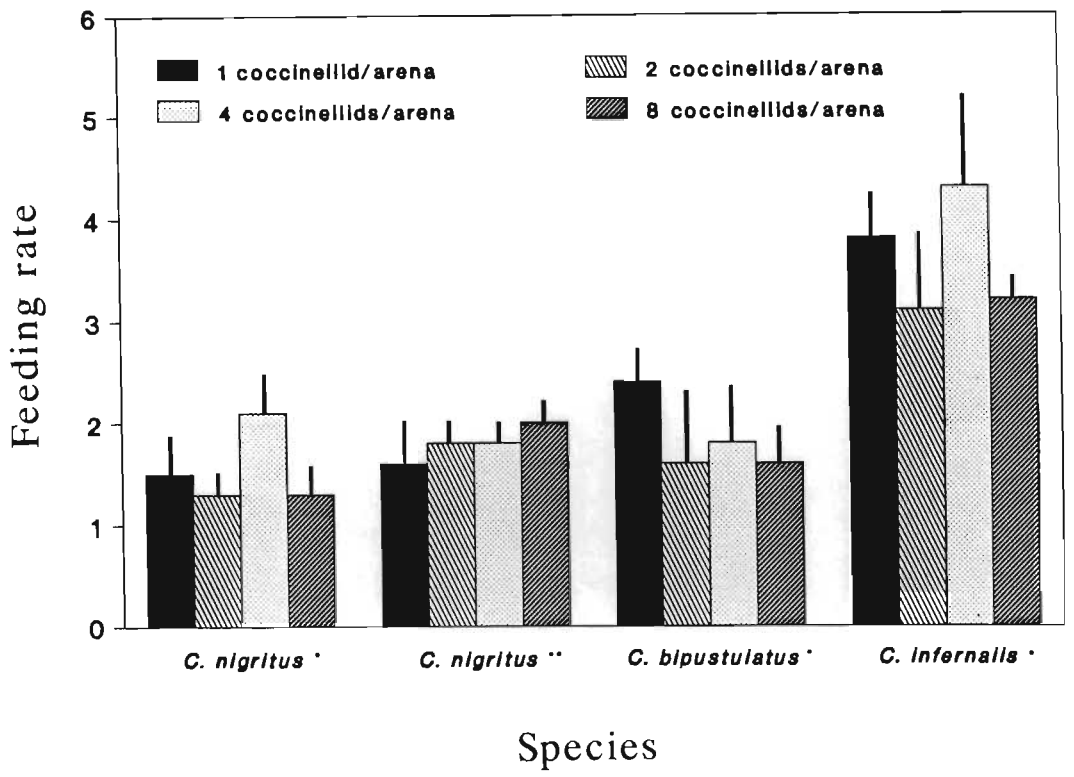

Fig. 1 Mean (+ 1SE) number of prey (Oleander scale, *Aspidiotus nerii*) consumed by *Chilocorus* spp. predators in 4h at various predator densities in circular arenas, * trial 1, ** trial 2.

Experiment II

The mean number of scale insects per potato sampled was 320 ± 21 (±1SE), n=10. There was no significant increase in dispersal with an increase in predator density (Table 1). Therefore, the ratio P_s/P in equation (3) was constant, making efficiency as expressed in this equation a constant at all predator densities. There were also no significant differences between the feeding rates at the various densities (Kruskal Wallis ANOVA, P>0.05) (Table 1). This can again be expressed as constant efficiency.

DISCUSSION

Congregations of *C. nigritus* adults on banyan trees *Ficus benghalensis* during periods of unfavourable climatic conditions, have been reported from Pakistan and India (Tirumala *et al.*, 1954; Ketkar, 1959; Ahmad, 1970). However, no feeding takes place during such aggregations because there are no suitable prey on these trees (Tirumala Rao *et al*, 1954; Ketkar, 1959). During several years of field observations of *C. nigritus* population levels on citrus in southern Africa, the highest density of feeding beetles encountered was two per leaf or four per orange, which, in some cases, were completely encrusted by red scale (Samways, pers. obs.). Interference may occur at predator densities higher than those used in my experiments. However, such extremely high densities would be of little relevance to their feeding under field conditions,

Table 1. Numbers of prey (*A. nerii*) consumed per *C. nigritus* in 10h at various *C. nigritus* densities in net cages, and proportions of predators dispersed from feeding sites

Predator density	Mean consumption rate ± 1SE (*n*)	Mean percentage dispersal (*n*)
1	4.7 ± 0.6 (10)	8 (4)
8	4.7 ± 0.5 (10)	10 (4)
15	4.9 ± 0.2 (10)	4 (4)

particularly in citrus orchards where they are utilised for biocontrol.

These results support Free *et al.*, (1977) in that behavioural interference is less important in predators than parasitoids. This difference may help to explain why predators are more important than parasitoids in the field control of *A. aurantii* at high population densities on citrus. Predators, particularly *C. nigritus*, become increasingly important at high scale densities (Samways, 1984), and the shift in relative importance of different biocontrol agents with increasing host/prey population levels, is integral to the efficient functioning of this complex of biocontrol agents (Samways, 1986, 1988).

Podoler & Henen (1986) have shown that *C. bipustulatus* adopts an area-concentrated foraging mode for a short period after consumption of a prey item. *C. nigritus* and *C. infernalis* show the same response to prey encounters, which concentrates the foraging predators in areas of high prey density. This behaviour has been considered general of insects searching for patchily distributed resources (Nakamuta, 1985). Such aggregation would be counteracted (through interference induced dispersal), or the effectiveness of the foraging mechanism reduced (through repeated temporary cessation of searching behaviour), by the existence of strong behavioural interference.

These results caution against lumping predator and parasitoid behaviours together as framed by interference models. These ladybirds show no interference behaviour akin to that for parasitoids: they do not disperse with increased crowding, and there is no temporary cessation of searching behaviour following an encounter with another predator. These results do not invalidate the models for predators, provided appropriate values are given to certain parameters: $m=0$, eq. (2) and $t_w=0$, eq. (4). However, criticism of early models, for assuming that efficiency is constant through fluctuations in predator density, is invalidated when applied to these predator-prey interactions.

The results here have a bearing on the use of these insects as biocontrol agents. The absence of severe intraspecific behavioural interference means that these insects can be reared, transported and released in the field at high densities without interference problems. Other biotic or even abiotic factors however, may be limiting, and require further research.

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CHAPTER 7

Location of habitat, prey patches and individual prey by the biocontrol agent *Chilocorus nigritus* (Coleoptera : Coccinellidae)

ABSTRACT

Field observations suggested that *Chilocorus nigritus* (Fabricius), a biocontrol agent of diaspidid scale insects, responds to cues associated with habitats to facilitate their location. Foraging behaviour at the level of habitat, prey patch and individual prey, was studied through laboratory experimentation. Vertically oriented geometric shapes were more attractive than the same shapes in a horizontal position. A simulated horizon with a tree line was preferred to a flat horizon. They were attracted to a two dimensional image of a tree. Leaf shape was recognised and the shape of a citrus leaf was preferred to compound bipinnate leaves and squares. These responses were associated with habitat selection for feeding and aggregation at aestivation sites. The location of prey patches was facilitated by prey odour for adults but not larvae. Response to the odour was not source oriented and did not effect movement patterns. Adults detected individual prey visually and olfactorily over short distances but physical contact with prey was required for detection by larvae. Prey location by adults and larvae was facilitated by alternating

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between intensive and extensive movement patterns in response to prey consumption and time since the last prey encounter. The differences in the ability of larvae and adults to detect individual prey and to locate prey patches, was associated with their roles in habitat selection in the field. These results provide guidelines for orchard management to maximise the biocontrol value of this species.

INTRODUCTION

Chilocorus nigritus (Fabricius) originating from the Indian sub-continent, is a biocontrol agent of scale insects (Hemiptera: Diaspididae) on several crops in numerous countries (Samways, 1984). It is particularly valuable in providing economic control of red scale *Aonidiella aurantii* (Maskell)(Hemiptera: Diaspididae) on citrus in southern Africa (Samways, 1984; 1986). In this region they shuttle between citrus orchards and giant bamboo *Dendrocalamus giganteus* Munro on which large numbers of the diaspidid scale *Asterolecanium miliaris* (Boisduval) are encountered (Samways, 1984; Hattingh & Samways, 1991). The success of *C. nigritus* in southern Africa has been linked to this behaviour (Hattingh & Samways, 1991).

Hassell and Southwood (1978) described three levels of predator foraging, these are single food items, patches or areas of aggregated food items and habitats. There is little information on coccinellid foraging at the level of habitat and

patch. Foraging for individual prey items has received some attention but aspects thereof remain unclear. There have been conflicting reports of coccinellids being able to detect individual prey visually and olfactorily over short distances (e.g. Allen *et al.* 1970; Stubbs, 1980; Obata, 1986) and detection only through physical contact (e.g. Fleschner, 1950; Kehat, 1968; Storch, 1976). A study of the foraging behaviour of one species of coccinellid, encompassing all three levels was required.

Predatory coccinellids maximise their encounter rate of patchily distributed prey, by adjusting movement patterns following prey encounters (Hassell & Southwood, 1978; Carter & Dixon, 1984). This behaviour is characterised by an increase in orthokinesis and a reduction in klinokinesis and scanning for a short period following prey consumption (Laing, 1937; Banks, 1957; Dixon, 1959; Chandler, 1969; Carter & Dixon, 1982, 1984; Nakamuta, 1985; Podoler & Henen, 1986). This movement has been called intensive foraging and reverts to extensive foraging after a period of unsuccessful search following the prey encounter (Carter & Dixon, 1982).

The effectiveness of shuttling by *C. nigritus* between alternative habitats in the field, suggests that habitat location is based on the recognition of cues associated with profitable habitats. Random dispersal and encounters of habitats, with retention based on prey encounters, does not explain their success.

This study, through laboratory experimentation, investigated the role played by visual cues in habitat location. The attractiveness of a two dimensional representation of a tree line as opposed to a flat horizon and the significance of vertically and horizontally oriented images was investigated. The response of *C. nigritus* to a two dimensional image of a tree and leaf shape was determined. The attractiveness of real leaves was also evaluated using simple ovate leaves (*Citrus sinensis* cv. Valencia) and compound bipinnate leaves (*Jacaranda mimosifolia* D. Don).

The role of sight and olfaction in the location of prey patches was investigated. Detection of individual prey items by *C. nigritus* over short distances and the appropriateness for these predators, of the foraging model based on intensive and extensive searching movements, was also investigated.

MATERIALS AND METHODS

A number of experiments were used to elucidate the sensory modalities involved in the different levels of foraging. The role of visual cues in the location of habitats was investigated in experiments a) to e), conducted in a flight chamber. Patch location was investigated using a further 5 experiments. The effect of prey consumption on orthokinesis and klinokinesis (movement patterns), which is relevant to the location of both prey patches and individual prey items, was determined. Finally the detection of individual prey items was

studied.

Habitat location

The walls and ceiling of a room with a 2.96m x 2.96m floor and 3.5m high walls, were covered in white, opaque paper. The temperature varied between 25°C and 30°C. A clear perspex cylindrical tunnel, 2.38m long with a 1.04m diameter and closed at both ends, was placed in the middle of the room, on a white platform 2.38m x 1.04m and 0.85m above the floor. Two flood lights and six incandescent desk lamps were positioned beneath the platform in such a way that the light intensity at both ends of the tunnel was 1000 lux.

A series of trials was conducted in which 60 *C. nigritus* adults per replicate, starved for 24h, were released in the centre of the perspex tunnel. Numbers of beetles in the terminal 0.5m at each end of the tunnel were periodically recorded for up to 135min after release. The tunnel was rotated through 180° between replicates to eliminate any bias towards either end of this apparatus. As a control, beetles were released in the tunnel without any images on the walls.

The attractiveness of a series of images painted on 2.01m wide and 1.21m high screens, with the base 0.85m above the floor, using chrome oxide green paint (GLS 013, "dekade paints"), were compared. Equivalent surface areas were covered in paint on both of the screens used in a particular comparison.

Eight parallel vertical stripes, 1.21m long and each 84mm broad, were painted on one screen. Five horizontal stripes, 2.01m long and 84mm broad on another. A horizon with a tree line was painted, with a solid base 2.01m wide, 0.31m high, from which various tree shapes protruded. Another screen had a solid base 2.01m x 0.49m without protruding tree shapes. The shape of a tree was also painted with a 0.15m broad and 0.34m high stem, on top of which the foliar portion was in the shape of a circular disk, 1.1m in diameter, with the lower fifth cut off. Twenty one leaves with the shape and size of a typical large citrus leaf were painted on a screen 1.67m high and 1.05m wide. On another screen of the same size, 21 solid squares 80mm x 80mm were painted.

The attractiveness of the following images against opposite walls at either end of the tunnel, were compared in experiments: a) vertical and horizontal stripes, b) the flat horizon and the horizon with a tree line, c) the shape of a tree and vertical stripes, d) paintings of citrus leaves and squares, e) real *C. sinensis* leaves and *J. mimosifolia* leaves.

The leaves were suspended with cotton threads against the walls at either end of the tunnel. Six citrus twigs with 75 leaves in total, were used per replicate and replaced daily. The total surface areas of *C. sinensis* and *J. mimosifolia* leaves used were equivalent.

Patch Location

Experiments were conducted with *C. nigritus* adults in the flight chamber without images on the walls, the perspex tunnel and platform.

Experiment 1: vision and olfaction

Four arenas were arranged in a square on top of a platform 0.8m x 0.8m and 0.85m above the floor. Arenas were wooden framed cages 0.2m x 0.2m x 0.2m covered in fine white netting with wooden floors. In each of the centrally positioned corners of the arenas, a butternut *Cucurbita moschata* cv. Waltham was placed. In the first trial these were uninfested and in the second, they were encrusted in *Aspidiotus nerii* Bouché (Hemiptera: Diaspididae) which is routinely used for rearing *C. nigritus* (Samways & Tate, 1986).

Ten *C. nigritus* adults per replicate starved for 24h, were placed in a glass petri dish, 25mm in diameter. These were placed on the floors of the arenas in the corners opposite the butternuts. Five minutes later the lids of the petri dishes were removed and the times taken for the first beetle to make contact with the butternuts in each arena recorded. The experiment was also conducted with fourth instar larvae, starved for 16h, five larvae per replicate. Times were compared with a Mann-Whitney U-test, $\alpha=0.05$.

Experiment 2 (choice): vision and olfaction

Four butternuts infested with *A. nerii* and four uninfested butternuts, were arranged on opposite sides of arenas with 0.5m x 0.4m floors and 0.4m high. Ten beetles per replicate, starved for 24h, were brushed out of petri dishes on to the floor covered in brown paper, between the two rows of butternuts. The times taken for the first beetles to encounter an uninfested or an infested butternut were recorded. Encounters with butternuts in less than 30s were disregarded to reduce the effect of chance encounters resulting from movement caused by the disturbance of handling, which was not associated with foraging. Measurements were compared with a Wilcoxon signed ranks test, $\alpha=0.05$.

Experiment 3: olfaction

In the one trial, an unprofitable site (no prey patch present) was provided in the presence of prey odour, but which did not emanate from the site itself. In the other trial, an unprofitable site was presented in the absence of prey odour. There were no visual differences between the sites in the two trials.

Experiment 3 was conducted with *C. nigritus* adults in a similar manner to experiment 1. The times taken to make contact with an uninfested butternut in the presence or absence of prey odours were compared. The arenas were placed on top of a wire

gauze box, 0.71m x 0.71m and 0.16m deep. Clean butternuts or *A. nerii* infested-butternuts were placed in the wire enclosure on which the arenas rested. Beetles were brushed out of petri dishes on to the arena floors in the corners opposite to the butternuts. Times in excess of 30s, for the first beetle to encounter a butternut, were compared with a one tailed Mann-Whitney U-test, $\alpha = 0.05$.

Experiment 4: olfaction and foraging paths

The foraging paths of *C. nigritus* adults in the presence or absence of *A. nerii* odour were compared. The arena in which the beetles searched was a circular perspex disk 0.05m in diameter, covered in white paper as a floor. A 50mm high PVC collar, supported a circular disk of clear perspex above the floor. The collar had six, equally-spaced, gauze covered, circular holes of 35mm in diameter. There were circular holes of 10mm in diameter in the floor and lid. Air was sucked through the hole in the floor and vented outside the experimental room. Beetles were inserted through the hole in the lid. The arena was placed on top of the wire box described above, which contained either clean butternuts or *A. nerii* infested butternuts.

Beetles were starved for 24h prior to being placed in the arena. Their movements were video taped and the paths followed later, from commencement of movement until contact was made with another individual, the perimeter of the arena or the hole

in the centre of the arena floor. The speed of movement and number of turns in excess of 20° was calculated.

Experiment 5: olfactometers

A Y-tube olfactometer was made of glass tubing with a 40mm diameter. The stem was 185mm long and the two branches, 47° apart, were each 110mm. Air was extracted at 15l min^{-1} from the base of the stem and vented outside the experimental room. Air was drawn through two glass chambers of 5l, each containing a 0.5l flask of water to balance humidity of the air, into each branch.

White NH_4Cl gas was drawn through one branch and its mixing with clean air from the other branch observed, to select an appropriate flow rate. The apparatus was surrounded by 0.75m-high walls of white polystyrene to eliminate visual aspects of the surroundings. Fluorescent and incandescent lighting was provided vertically above the apparatus and the temperature in the room was 25°C to 27°C. Forty *C. nigritus* adults starved for 24h, were inserted at the base of the stem and the numbers in the two branches periodically recorded.

A control was run where clean air was drawn through both branches. A trial was conducted in which one glass chamber contained only the flask with water and the other also contained three butternuts encrusted in *A. nerii*.

Another, olfactometer was constructed, consisting of a straight pipe of clear perspex, 53mm in diameter and 1m long. Each end was covered in gauze and inserted into a cardboard box 315mm x 230mm and 260mm high, through a hole in the side. The opposite side of each box had a 160mm x 10mm slit. Clean butternuts were placed in the one box and *A. nerii* infested butternuts in the other. Air was extracted from the centre of the tube at 0.4l min^{-1}. The apparatus was also surrounded by polystyrene walls.

Forty, *C. nigritus* adults, starved for 24h, were inserted at the centre of the tube. The numbers in the terminal 0.2m and 0.3m of each end were periodically recorded.

Movement patterns

The search patterns of *C. nigritus* adults and larvae on the upper surface of a large pumpkin *Cucurbita maxima* cv. Flat White Boer, with a circular boundary of polyester fibre padding, 250mm in diameter, were video taped. Recordings of extensive foraging after 24h of starvation, commenced 5min after introduction into the arena. Individuals were then each presented with a mature female *A. nerii* and the after feeding foraging patterns recorded. Paths were recorded until the foragers made contact with an obstacle or flew away. The arena was wiped with 90% alcohol between replicates.

The distances travelled per 10s were calculated. Distances

travelled/straight line distances between positions at 10s intervals, quantified tortuosity of the path (Nakamuta, 1985). Sizes of individual turns larger than 20° were measured. Long sweeping turns were divided into sections equivalent to 1.5 body lengths of a fourth instar larva, as the majority of the discrete turns were completed over a shorter distance.

Detection of individual prey

Arenas were plastic petri dishes, 87mm in diameter, 15mm deep. Each had a white filter paper floor and a 10mm diameter hole in the floor, 5mm from the perimeter, into which the mouth of a 40mm long glass vial fitted. Petri-dish arenas were placed on a raised wooden platform with holes through which the vials were inserted. Experiments were conducted in the flight chamber without images on the walls or the perspex tunnel and platform.

The prey site was positioned at the opposite side of the arena from the point of insertion of the vial, 5mm from the perimeter. One of the following was placed at the prey site of each arena: an intact mature female *A. nerii* (visual and olfactory aspects of prey present); a bees-wax imitation (only visual aspects); a drop of macerated *A. nerii* which was absorbed by the filter paper (only olfactory aspects); a very faint pencil mark (control).

*C. nigri*tus adults and fourth instar larvae were starved for

24h. Five individuals were placed in a vial with a polyester fibre plug in the neck and allowed 10min to settle after handling. The plug was removed with minimal disturbance and the vial inserted into the petri-dish arena. The time was recorded from emergence of the first individual into the petri dish, until contact was made with the prey site.

RESULTS

Habitat location

There were no significant differences between the numbers of beetles at either end of the perspex tunnel at 15min, 45min, 75min or 135min after introduction in the control (Table 1).

Experiment a)

There were significantly more beetles at the end of the tunnel facing the vertical stripes than at the opposite end facing the horizontal stripes at 45min and 75min after introduction. The differences at 15min and 105min were not significant (Table 1).

Experiment b)

There were significantly more beetles at the end facing the painting of a horizon with a tree line than at the opposite end facing a flat horizon at 15min, 45min, 75min, 105min and 135min after introduction (Table 1).

Table 1. Mean numbers of *C. nigritus* adults in the terminal 0.5m of either end of a cylindrical tunnel, at various times after introduction into the apparatus tunnel, with various visual images presented at opposite ends, against the walls of the experimental room

Images compared	Replicates *n*	Mean numbers of beetles at either end of the tunnel at various times after release				
		15min	45min	75min	105min	135min
control: no images; no images	10	4.6 ; 5.8	8.7 ; 10.3	10.8 ; 12.2		12.8 ; 14.4
vertical lines; horizontal lines	11	7.6 ; 5.8	14.3*; 10.8*	18.6*; 12.6*	19.5 ; 15.0	
horizon + tree line; flat horizon	10	11.2*; 6.7*	15.3*; 9.4*	19.1*; 10.8*	23.6*; 10.4*	24.4*; 11.4*
vertical lines; tree shape	12	7.3 ; 7.4	13.1 ; 12.2	15.6 ; 12.9	16.2 ; 10.8	17.2*; 13.5*
		30min	60min	90min	120min	
painted ovate leaves; squares	16	9.2*; 6.7*	13.4*; 9.1*	13.5 ; 11.8	13.6 ; 12.3	
real leaves: *C. sinensis*; *J. mimosifolia*	17	12.4 ; 9.8	15.5*; 10.7*	17.1 ; 13.6	15.2 ; 13.2	

* significantly different, α=0.05, permutation test for related samples for the first four comparisons, Wilcoxon signed ranks test for the last two comparisons.

Experiment c)

Counts were not significantly different at the end facing the vertical stripes and the end facing a painting of a tree at 15min, 45min, 75min and 105min. However, there were more beetles at the side with vertical stripes at 135min (Table 1).

Experiment d)

There were significantly more beetles at the end facing the painting of citrus leaves, than at the end facing the squares, at 30min and 60min. Counts at the two ends were not significantly different at 90min and 120min (Table 1).

Experiment e)

There were no significant differences in counts at the ends facing *C. sinensis* and *J. mimosifolia* leaves, at 30min, 90min and 120min after introduction. Counts at the *C. sinensis* side of the tunnel were significantly higher at 60min (Table 1).

Patch location

Experiments 1, 2 and 3

The times taken for adults to locate *A. nerii*-infested and clean butternuts, in the presence or absence of prey odour are given in Table 2. Infested butternuts were located more

Table 2. Time taken for first *C. nigritus* to make contact with a butternut, infested with *A. nerii* or uninfested, mean (s) ± 1SE (*n*)

Life stage	Experiment 1	
	uninfested butternut	infested butternut
Adults	351[a] ± 90 (15)	132[b] ± 30 (15)
Larvae	2132[a] ± 460 (20)	1727[a] ± 291 (20)
	Experiment 2: Choice	
	uninfested butternuts	infested butternuts
Adults	306[a] ± 84 (17)	246[a] ± 66 (17)
	Experiment 3	
	uninfested butternut + odour	uninfested butternut - odour
Adults	174[a] ± 36 (24)	348[b] ± 72 (24)

The absence of a common superscript indicates a significant difference, α=0.05, Mann-Whitney U-tests for experiments 1 & 3 and Wilcoxon signed ranks test for the choice experiment.

rapidly than uninfested butternuts when each was presented separately. Infested butternuts were not located more rapidly than uninfested butternuts in the choice experiment. Uninfested butternuts were located more rapidly in the presence of prey odour than in the absence of prey odour.

Experiment 4

There was no significant difference between the mean speed = 6.2mm s^{-1} ± 0.4 (±1SE), *n*=33 and mean 100 (number of turns/mm travelled) = 4.3 ± 0.3 (±1SE), *n*=33, in the presence of scale odour, and speed = 6.1mm s^{-1} ± 0.3 (±1SE), *n*=31 and 100 (turns mm^{-1}) = 3.8 ± 0.2 (±1SE), *n*=31 without prey odour. Mann-Whitney U-tests, $P>0.05$. When using movement paths from only the first 5min after release, there were also no significant differences: a) In the presence of scale odour, mean speed was 77mm s^{-1} ± 0.5 (±1SE), *n*=10 and mean 100 (turns mm^{-1}) was 3.3 ± 0.3 (±1SE), *n*=10; b) In the absence of prey odour, speed was 7.2mm s^{-1} ± 0.7 (±1SE), *n*=11 and 100 (turns mm^{-1}) was 3.2 ± 0.3 (±1SE), *n*=11.

Experiment 5: olfactometers

In the control, counts of beetles in each branch of the Y-tube were not significantly different at any of the times after introduction (Table 3). There were also no significant differences between counts in the two branches, when clean air was drawn through one, and air carrying *A. nerii* odour through

Table 3. Mean numbers of *C. nigritus* adults in the two branches of a Y-tube olfactometer at various times after introduction of 40 beetles per replicate into the apparatus, n=10

Time after Introduction (minutes)	Mean (±1 SE) numbers of beetles in the two arms of a Y-tube olfactometer			
	Control		Treatment	
	Branch A no prey odour	Branch B no prey odour	Branch A no prey odour	Branch B + prey odour
5	2.2 ± 0.5	2.5 ± 0.7	3.7 ± 0.5	2.8 ± 0.7
10	2.4 ± 0.6	2.9 ± 0.7	4.6 ± 0.7	2.8 ± 0.8
15	2.4 ± 0.7	2.9 ± 0.5	4.0 ± 0.8	2.5 ± 0.7
20	2.6 ± 0.5	2.7 ± 0.5	3.5 ± 0.8	2.6 ± 0.7
25	2.9 ± 0.4	2.8 ± 0.5	3.3 ± 0.8	2.6 ± 0.9
30	2.5 ± 0.5	2.8 ± 0.7	4.1 ± 0.6	2.7 ± 0.9
40	2.8 ± 0.5	3.0 ± 0.7	3.4 ± 0.6	2.9 ± 0.8
50	3.6 ± 0.8	4.0 ± 0.7	3.0 ± 0.7	3.7 ± 0.9
60	-	-	3.0 ± 0.9	3.4 ± 1.1
75	-	-	3.2 ± 0.7	2.7 ± 0.5
100	4.3 ± 0.8	4.1 ± 1.0	3.7 ± 0.7	2.5 ± 0.6
115	5.2 ± 1.0	5.1 ± 1.1	-	-
130	4.8 ± 0.9	5.6 ± 1.0	4.3 ± 0.7	2.8 ± 0.7
200	5.8 ± 0.6	5.4 ± 0.8	-	-

No significant differences between counts in the two branches at the different times after introduction in both contol and treatment, permutation test for related samples, α=0.05.

the other (Table 3).

In the control with the straight line olfactometer, there were no significant differences between the counts at the two ends of the tube (Table 4). No significant differences were found between counts at the end attached to the source of scale insect odour, and the opposite end without prey odour (Table 4).

Movement patterns

The search paths of *C. nigritus* adults and larvae are analysed in Table 5. The speed, tortuosity of search path and turns/distance travelled, for adults and larvae were significantly different for the first 60s after feeding, from the measurements before feeding. During the period 12min to 14min after feeding, these measurements had reverted back to levels similar to those prior to feeding.

Detection of individual prey

Times taken to locate an intact *A. nerii*, a wax imitation, macerated *A. nerii* and the control mark, are given in Table 6. Adults located the complete scale insect (vision and olfaction), the wax imitation (vision) and the site of absorbed macerated scale (olfaction) more rapidly than the control site. Larvae did not locate the complete scale insect more rapidly than the control site.

Table 4. Mean numbers (±1 SE) of *C. nigritus* adults in the terminal 0.2m and 0.3m at either end of an olfactometer, at various times after introduction of 40 beetles per replicate into the apparatus, n=20

Time after introduction (minutes)	Mean numbers at either end of the olfactometer in the terminal 0.2m		terminal 0.3 m	
	Side A no prey odour	Side B no prey odour	Side A no prey odour	Side B no prey odour
10	3.5 ± 0.5	3.6 ± 0.5	3.8 ± 0.5	3.7 ± 0.5
20	3.8 ± 0.5	3.5 ± 0.5	4.3 ± 0.5	3.9 ± 0.7
30	4.0 ± 0.7	3.7 ± 0.6	4.6 ± 0.6	4.2 ± 0.6
	Side A no prey odour	Side B + prey odour	Side A no prey odour	Side B + prey odour
10	4.2 ± 0.5	3.6 ± 0.5	4.5 ± 0.5	4.0 ± 0.6
20	4.7 ± 0.7	3.0 ± 0.5	4.9 ± 0.6	3.3 ± 0.5
30	4.1 ± 0.6	3.2 ± 0.5	4.5 ± 0.6	3.3 ± 0.5

No significant differences between counts at either end of the apparatus, at the different times after introduction, in both control and treatment, Wilcoxon signed ranks test, α=0.05.

Table 5. Analysis of *C. nigritus* search paths before and after feeding, speed, tortuosity factor (path distance/straight line distance for each 10s period) and turns/path distance

Measurement	Before feeding	First 60s after feeding	12 to 14 min after feeding
Adult			
speed (mm s^{-1})	$8.9^a \pm 0.8$ (6)	$4.3^b \pm 1.1$ (6)	$8.1^a \pm 1.1$ (4)
tortuosity factor	$1.21^a \pm 0.05$ (6)	$2.26^b \pm 0.31$ (6)	$1.37^a \pm 0.9$ (4)
100 (turns mm^{-1})	$4.1^a \pm 0.8$ (6)	$19.5^b \pm 2.3$ (6)	$7.3^a \pm 1.3$ (6)
Larvae			
speed (mm s^{-1})	$6.3^a \pm 0.2$ (5)	$3.5^b \pm 0.3$ (5)	$5.8^a \pm 1.0$ (4)
tortuosity factor	$1.42^a \pm 0.9$ (5)	$1.86^b \pm 0.2$ (5)	$1.50^a \pm 0.1$ (4)
100 (turns mm^{-1})	$3.6^a \pm 1.2$ (5)	$10.6^b \pm 1.7$ (5)	$7.3^a \pm 1.9$ (4)

Absence of a common superscript indicates a significant difference, Friedman ANOVA folowed by a nonparametric multiple comparison, α=0.05.

Table 6. Time taken for first *C. nigritus* adults and fourth instar larvae to locate the prey site, mean (s) ± 1SE (*n*)

Life stage	Prey site			
	A. nerii	Wax imitation	Macerated *A. nerii*	Control
Adults	217[a] ± 58 (20)	311[a] ± 75 (20)	326[a] ± 76 (19)	694[b] ± 117 (20)
Larvae	536[a] ± 112 (20)	-	-	633[a] ± 139 (20)

Absence of a common superscript indicates a significant difference, α=0.05, Kruskal Wallis ANOVA followed by a nonparametric multiple comparison for adults, and a Mann-Whitney U-test for larvae.

DISCUSSION

Aggregation of large numbers during unfavourable climatic conditions is a common feature among coccinellids (Hagen, 1962). Most aggregating species are attracted to prominent objects silhouetted on their horizon (Hagen, 1962). A characteristic of aggregation behaviour, is that they do not feed at these sites and enter a state of diapause (Hagen, 1962). Tirumala *et al.* (1954), Ketkar (1959) and Ahmad (1970) observed congregation of *C. nigritus*, mostly on the undersurfaces of leaves, but also on fruits and branches of banyan trees *Ficus benghalensis* in India and Pakistan. This behaviour was accompanied by a form of diapause during which they did not feed (Tirumala *et al.*, 1954).

In South Africa this species congregates on giant bamboo *D. giganteus* during the winter months, but they do not form tight groups (Samways, 1984). This behaviour in South Africa is also different in that they continue feeding and reproducing, although qualitative observations indicate that the reproductive rate is considerably lower.

Qualitative observations suggest that this seasonal rhythmicity persists to a degree, during rearing in the laboratory under controlled environmental conditions. Reduction in reproductive rate was observed from late summer to late winter of the southern hemisphere. In Pakistan and India congregation and diapause occurred during winter or summer depending on the

region (Tirumala *et al.*, 1954; Ketkar, 1959; Ahmad, 1970). The area from which the material which entered southern Africa, originated in Asia is unknown (Samways, 1984).

In South Africa, *C. nigritus* were also found congregating on the undersides of *Ficus sur* Forssk. leaves. This occurred on *F. sur* plants adjacent to clumps of *D. giganteus*. They formed small groups of two to five beetles, they were immobile, there was no prey on these plants, and no immature stages were found.

This study showed that during foraging, *C. nigritus* was optically attracted to prominently silhouetted features, such as a horizon with a tree line and individual trees. Vertically oriented parallel lines were also more attractive to these predators than horizontal lines. This explains their congregation on *D. giganteus* which are up to 20m tall and grow in dense stands, in which the vertical stems are visually prominent. This demonstrates the important role played by the visual aspects of the landscape in the location of habitats by these predators.

In this study, *C. nigritus* showed a preference for ovate leaves (characteristic of the plant species on which they are most often encountered), above compound bipinnate leaves. To eliminate the possibility of leaf size and subtle colour differences having effected this response, the attractiveness of paintings of leaves were compared with equal-sized squares. The leaf paintings were again preferred, indicating that it was

the shape of the leaf which was recognised.

Recognition of leaf shape may be an important cue in habitat location during foraging, as well as in locating congregation sites where they enter a form of diapause. This would explain the formation of small groups on *F. sur* leaves near to *D. giganteus* sites.

Most of the plants on which *C. nigritus* is regularly encountered, have leaves of similar shape, being ovate, elliptic or oblong, e.g. citrus, guava, mango, banyan, coffee and *Ficus* spp. This supports the hypothesis that leaf shape is an important visual cue for habitat location. Apparently contradictory evidence, is the abundance of *C. nigritus* on bamboo and coconut palms. However, the visually conspicuous nature of bamboo and coconut, because of their height, would facilitate their location.

The physical handling of the beetles during introduction into the perspex tunnel was probably disruptive. There may also have been a delay in the response of the beetles to the visual cues on initial exposure. Therefore the absence of a significant response during the first 15min of exposure, cannot be regarded as meaningful.

It was established that vertical lines were attractive to *C. nigritus*. The absence of a preference for vertical lines above the painting of a tree, indicated that the tree image was also

attractive. However, after 135min of unsuccessful foraging in the presence of these images, a preference for the vertical lines became evident. Similarly the preference for painted leaves above painted squares, *C. sinensis* leaves above *J. mimosifolia* leaves, and vertical lines above horizontal lines, was no longer evident after various periods of exposure without additional stimulus. This may be explained by habituation to these cues.

The preference for the vertical lines above the painting of a tree after prolonged exposure, may be indicative of a hierarchy in their responsiveness to cues. The vertical lines may be perceived as a longer-range cue than the tree. In the event of unsuccessful foraging, it would be advantageous to habituate to shorter-range cues more rapidly than to longer-range cues. The absence of a significant difference between the counts in the ends of the tunnel facing the vertical and horizontal lines at 105min after introduction, is only marginally insignificant, $P=0.055$. This indicates that this apparent anomaly, may not be real.

In experiment 1, adult *C. nigritus* located infested butternuts more rapidly than uninfested butternuts. This could have been in response to visual or olfactory stimulation. In experiment 2, infested butternuts were not located more rapidly than uninfested butternuts when presented with a choice. This suggested that response to the cue used to facilitate location of the patch, was not source oriented, which makes a visual cue

improbable. In experiment 3, uninfested butternuts were located more rapidly in the presence of prey odour, although not emanating from the butternut, than in the absence of prey odour. This indicated that patch location was facilitated by a response to olfactory stimulation, which was not source-oriented.

C. nigritus adults also did not select the odour-carrying branches of the olfactometers. Experiment 4 indicated that there were no differences, characteristic of alternations between intensive and extensive search, between movement patterns in the presence or absence of prey odour. A possible explanation is that prey odour stimulated the commencement of, and sustained, foraging behaviour, precluding other activities such as cleaning and resting.

The improved discovery rate in the presence of odour, could also have resulted from the triggering of sensitivity to a visual cue by olfactory stimulation. If this cue to which sensitivity was triggered, was not specific enough to discriminate between scale-infested and uninfested sites, it would explain their inability to select infested butternuts above clean butternuts in the choice experiment.

C. nigritus adults were capable of locating individual prey visually and olfactorily over short distances. Fourth instar larvae relied on making physical contact for detection of individual prey. Adults and larvae altered their movement

patterns from typically extensive search to intensive search following prey consumption. This reverted back to extensive search with time when subsequent prey were not encountered. This type of foraging maximises encounter rate of patchily distributed prey (Hassell & Southwood, 1978: Carter & Dixon, 1984).

Habitat selection for coccinellid larvae is performed by adults (Blackman, 1967; Hodek, 1973). Therefore, there is less likelihood of larvae having to locate prey in an unsuitable habitat than the adults. Furthermore, adults as the dispersal life stage, would greatly benefit from more effective mechanisms of locating prey. This may be the underlying reason for adults displaying superior ability to locate prey patches and individual prey.

There is still a great deal which remains to be discovered about the stimuli effecting habitat and patch location and selection. Possible interactions between visual and olfactory stimuli, and the role played by learning, should be investigated.

In view of the ability of these biocontrol agents to locate *D. giganteus* and shuttle between these sites and orchards, it would be valuable to promote planting of *D. giganteus* in close proximity to orchards. During winter when the scale insect population levels are low in the citrus orchards, and stringent chemical control is applied, such sites would serve as valuable

reservoirs of the biocontrol agents. There is, however, a conflict of interests here, since *D. giganteus* is an alien species in southern Africa. However, this plant is not an aggressive invader and is highly dependent on plentiful water. If plantings are limited to isolated water sources such as beside farm dams, and river-side plantings are avoided, the risks will be minimised.

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CHAPTER 8

DISCUSSION

This study has identified aspects of rearing, handling and field release of these *Chilocorus* spp., which can improve their biocontrol value, and the quality of laboratory cultures for experimental studies. Aspects of their feeding and foraging behaviour, which help to explain their field performance and indicate how their biocontrol value may be enhanced, were studied. Experimental investigation of their feeding and foraging behaviour, contributed towards the development of theories concerning predator-prey relationships.

Rearing and laboratory experimentation

The adverse effects of rearing larvae on an unsuitable prey species, or on a sub-optimal artificial diet, and substitutions of prey species, were reflected in significantly reduced weights of subsequent adults at one day after eclosion. This reduction in weight, persisted after the cessation of adult weight increase. It was concluded that measuring adult weight at 1d after eclosion, is a valuable indicator of the effect of larval treatments on larval development and the fitness of subsequent adults.

Rearing at fluctuating temperatures as opposed to constant temperatures, is associated with improvement in the quality of insects produced (Hagstrum & Hagstrum, 1970; Scriber & Slansky,

1981). This study established that improved vigour of cultures was not the result of a behavioural response by individuals to these conditions within one generation. This indicated that maintenance of fluctuating temperatures is necessary throughout the rearing process to obtain the benefits thereof. Exposure to such conditions, for one generation prior to field release, will not improve the vigour of these individuals.

The primary role for artificial diets at their present level of development, in the rearing of predaceous insects, is as a temporary substitute for natural prey (Waage *et al.*, 1985). This is also the case with the artificial diet developed for *C. nigritus*. The diet produced is however, of significant practical value despite the limitations of its suitability.

The comparison between the suitability of *Aspidiotus nerii* and *Asterolecanium miliaris* indicated that, in contrast to *A. nerii*, *A. miliaris* is not suitable for larval rearing of *C. bipustulatus* and *C. infernalis*. The availability in the field of large quantities of *A. miliaris* at extremely high population densities on *Dendrocalamus giganteus*, and the suitability of this prey species for all life stages of *C. nigritus*, have implications for insectary rearing. In short term studies, field harvesting of *D. giganteus* infested with *A. miliaris*, can be a valuable means of supporting a laboratory culture, thereby avoiding the complications associated with culturing of prey.

The deleterious effects of prey substitutions caution against

such practices during insectary rearing. The larval life stage in particular should be protected from such manipulation, because of the reduction in fitness of subsequent adults. Exposure of adults to such substitutions will adversely effect the reproductive rate of the culture.

Nakamuta (1987) found that endogenous circadian rhythmicity in the feeding activity of *Coccinella septempunctata bruckii* is dominant over hunger. This study identified a reduction in feeding rate of *C. nigritus* during the dark phase, demonstrating the need to consider this factor when conducting feeding behaviour experiments. An important consideration when conducting feeding rate trials is the standardisation of hunger levels. Nakamuta (1987) found that the feeding activity of *C. septempunctata bruckii* remained constant after between 4h and 24h of starvation. This study established that, prior to measuring feeding rates, a starvation period of between 10h and 24h was appropriate for *C. nigritus*.

Biocontrol improvement

Differences between the species in the basic biological data used for quality assessment, did not reflect differences in their biocontrol value. Also, measuring feeding rates at a range of static temperatures did not reflect differences in their climatic adaptations. However, survival rates after 48h of exposure to temperatures at the higher end of the range, were valuable in comparatively assessing the climatic

adaptations of the six *Chilocorus* spp. tested.

Species composition of diet was found to be an important consideration in insectary production of biocontrol agents for field release. Without the availability of a fully suitable artificial diet, rearing on a convenient natural prey species, which is often different from the target pest in the field, remains a necessity. However, these findings caution against the adverse effects of prey substitutions. Such diet changes during the larval stage, must be avoided to ensure production of high quality adults for field introduction. These results also have implications for the handling of adults prior to and shortly after release in the field, which will influence the effectiveness of such releases.

Adults were shown to be the preferred life stage for making field introductions of *Chilocorus* spp. Field introduction of immature life stages requires considerably less labour and eggs on synthetic pads are convenient to handle. However, the significantly lower survival rate of field released immatures, detracts from the value of this approach.

Feeding and foraging behaviour relevant to biocontrol

Prey suitability

The unsuitability of *A. miliaris* on *D. giganteus* for *C. bipustulatus* and *C. infernalis* larvae, indicates that field introduction of these species on to *D. giganteus* is not

advisable. The importance of *D. giganteus* as an alternative habitat to orchards for *C. nigritus* in southern Africa (Samways, 1984; Hattingh & Samways, 1991), and its unsuitability for *C. bipustulatus* and *C. infernalis*, may have extensive implications for the biocontrol potential of these two species in this region.

Scriber (1979) cautioned that describing an organism as polyphagous, requires qualification regarding its applicability to the species, a population or an individual. Hodek (1973) indicated that the prey ranges attributed to coccinellids are often broader than the range of species suitable for larval development. In numerous cases, an alternative prey may be acceptable for adult maintenance but not suitable for larval development (Hodek, 1973). This study established that *A. miliaris* was adequate for adults but unsuitable for larvae of *C. bipustulatus* and *C. infernalis*. The deleterious effects of alternating between prey species, and the existence of behavioural mechanisms to avoid such situations, indicated that individual populations of a *Chilocorus* sp., are considerably more specific than the species as a whole.

Coccinellids have been reported to lack ability to choose between available prey species, even when some of the choices represent unsuitable or even toxic prey (Blackman, 1967). This study demonstrated that *Chilocorus* spp. too, show little ability to choose between prey types. This cautions against the use of choice experiments in selecting suitable predator-

prey associations for biocontrol.

Intraspecific interference

The foraging efficiency of parasitoid biocontrol agents is reduced by intraspecific interference at high parasitoid population densities (Rogers & Hassell, 1974). In numerous models of this behaviour, it was assumed that predators behave in the same way. This study showed experimentally that this is not the case with these predators (Hattingh & Samways, 1990). These results provided guidelines for the application of interference models to predator-prey interactions.

The role of *C. nigritus* as a high density feeder is of particular biocontrol importance (Samways, 1985). The absence of intraspecific interference during feeding in *Chilocorus* spp., contributes towards an explanation of why they are more effective controllers of high population densities than parasitoids. This, together with the absence of inversely density-dependent feeding at high population densities, as in *Aphytis* spp. parasitoids (Samways, 1985), makes their biocontrol role complimentary to that of the parasitoid complex.

The absence of extensive intraspecific interference at high population densities, has implications for the rearing and release of these biocontrol agents. It makes rearing at very high densities without consequent creation of a stressful

environment, possible. It also indicates that these predators may be transported and field released at high densities, without resultant increase in extensive dispersal from the release site (Hattingh & Samways, 1991).

Location of habitats, prey patches and individual prey

The attractiveness of visually prominent features on the horizons of *C. nigritus* individuals was demonstrated. This was associated with foraging behaviour and the location of congregation sites during adverse conditions. This explains their success in shuttling between *D. giganteus* and citrus orchards in southern Africa, which has important implications for their biocontrol effectiveness (Samways, 1984; Hattingh & Samways, 1991). The existence of visual cues associated with individual trees and leaf shape, was also associated with habitat location for feeding and with behaviour associated with seasons of adverse climatic conditions.

Location of prey patches by adult *C. nigritus* was facilitated by the presence of prey odour, but the response to the odour was not source oriented. The mechanism of improved location was not conclusively identified. The classical intensive-extensive movement patterns associated with coccinellid foraging (Carter & Dixon, 1982) were evident in *C. nigritus*. This maximises the location of individual prey and facilitates foraging for patchily distributed prey (Hassell & Southwood, 1978). The uncertainty surrounding the ability of coccinellids

to detect individual prey over short distances, was clarified for *C. nigritus*. The location of individual prey by adults was facilitated by visual and olfactory detection over short distances.

C. nigritus larvae did not locate prey patches more effectively in the presence of prey odour than in its absence. The location of individual prey by larvae, was not improved by visual or olfactory detection over short distances. The effects of prey substitutions during the larval stage were also more biologically significant than with adults.

Differences between life stages

These findings reflect upon differences in the predator-prey relationships of larval and adult life stages. Larval habitat selection by coccinellids, is performed by adults through oviposition (Blackman, 1967; Hodek, 1973). This ensures that the larvae hatch in a suitable habitat with sufficient prey. Their intensive-extensive foraging behaviour and lack of intraspecific interference, ensure that they remain in that habitat as long as it remains profitable. Furthermore, this study has indicated that a mechanism exists whereby adults exclude their progeny from habitats in which they would be exposed to alternations between prey species. The selective pressure for developing more sophisticated means of locating prey, can thus be expected to be less intense for larvae than for adults. Furthermore, the capability to deal with changes

in diet, would be less important for larvae than for adults.

Further studies

The deleterious effects of prey substitutions may have far-reaching implications for theories concerning predator-prey interactions. Switching behaviour has been observed in insect parasitoids and predators in habitats with mixtures of prey species, whereby the foragers concentrate on the most abundant species (Murdoch, 1969). The optimality of this foraging behaviour is associated with the relative proportions of the different prey species in the habitat. Avoidance of prey substitutions may also be a significant selective force for development of such behaviour.

Another aspect of their foraging behaviour which requires investigation is the role played by learning, which is important in the foraging of parasitoids (Taylor, 1974; Wardle & Borden, 1985).

The development of the artificial diet for *C. nigritus* to a level of greater suitability, would be of great value. Much more work needs to be done on the higher levels of foraging behaviour. In particular, the possibility of interactions between olfactory and visual cues requires attention. The stimuli inducing dispersal from habitats, is also a promising direction for further research.

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