

Review

Factors affecting utility of *Chilocorus nigritus* (F.) (Coleoptera: Coccinellidae) as a biocontrol agent

D.J. Ponsonby*

Address: Ecology Research Group, Department of Geographical and Life Sciences, Canterbury Christ Church University, North Holmes Road, Canterbury, Kent. CT1 1QU, UK.

***Correspondence:** Email: David.ponsonby@canterbury.ac.uk

Received: 30 March 2009

Accepted: 25 June 2009

doi: 10.1079/PAVSNNR20094046

The electronic version of this article is the definitive one. It is located here: <http://www.cababstractsplus.org/cabreviews>

© CAB International 2009 (Online ISSN 1749-8848)

Abstract

Chilocorus nigritus (F.) has been one of the most successful coccidophagous coccinellids in the history of classical biological control. It is an effective predator of many species of Diaspididae, some Coccidae and some Asterolecaniidae, with an ability to colonize a relatively wide range of tropical and sub-tropical environments. It appears to have few natural enemies, a rapid numerical response and an excellent capacity to coexist in stable relationships with parasitoids. A great deal of literature relating to its distribution, biology, ecology, mass rearing and prey preferences exists, but there is much ambiguity and the beetle sometimes inexplicably fails to establish, even when conditions are apparently favourable. This review brings together the key research relating to factors that affect its utility in biocontrol programmes, including its use in indoor landscapes and temperate glasshouses. Data are collated and interpreted and areas where knowledge is lacking are identified. Recommendations are made for prioritizing further research and improving its use in biocontrol programmes.

Keywords: *Chilocorus nigritus*, Biological control, Prey relations, Glasshouses, Endosymbionts, *Hemisarcoptes*

Review Methodology: Literature for this review was gathered by searching the databases CAB Abstracts, ISI Web of Knowledge, Science Direct, and the search engine, SCIRUS, using the key words *Chilocorus*, *nigrita* and *nigritus*. Older literature was sourced using JSTOR and reference lists in articles discovered by the databases.

Introduction

Throughout the short history of classical biological control, the coccidophagous ladybird, *Chilocorus nigritus* (Fabricius, 1798) has featured prominently in a number of very successful programmes against mainly diaspidid pests (see Table 1 [1–29]). Consequently, it has become economically important in many parts of the world and two earlier reviews have examined its biology and economic value [19], and its ecology and biocontrol potential [30]. Fabricius originally named it *Coccinella nigrita* but Mulsant renamed it as *Chilocorus nigritus* in 1851 [19]. Technically, *nigrita* should have been retained as the specific epithet [22] and some workers continue to use this gender. Thus it has become embedded in the literature under both genders of the noun.

Members of the Diaspididae and Coccidae continue to be important pests of many crop species, often exhibiting extensive resistance to pesticides. Moreover, growing concerns over the effect of pesticides on biodiversity and public health have led to the introduction of restrictive legislation to control pesticide use and residues in food in many countries. These factors have led to an increased interest in biocontrol agents (BCAs) as part of integrated pest management (IPM) and organic crop production programmes. The strong success record of *C. nigritus* as a voracious, oligophagous BCA, linked to its considerable and expanding research literature demonstrates that it is extremely important as part of the natural enemy complex of a range of coccid prey in many parts of the world. This review examines the factors that affect its utility as a BCA, discusses the possible reasons for successful and

Table 1 Attempts at the use of *Chilocorus nigritus* as a biological control agent and records of natural control

Location and date	Mean temperature (maxima, minima) (°C)	Mean humidity (maxima, minima) (% RH)	Source of beetles	Introduction rates (where known)	Target pest species	Host plant	Native, introduced or invaded	Biocontrol type	Degree of success ¹	Reference
India, 1910	?	?	Native	Not applicable	<i>Aonidiella aurantii</i> Maskell	<i>Citrus</i> spp.	Native	Natural	S	[1]
California and Florida, 1910	37, 3	24, 91	India		<i>A. aurantii</i>	<i>Citrus</i> spp.	Introduced	Classical	F?	[1]
Sangi, Sulawesi, 1928–1933	34.7, 22.4	86, 67	Native	Not applicable	<i>Aspidiotus rigidus</i> Reyne	<i>Cocos nucifera</i> L.	Native	Natural	F	[2]
Western Sri Lanka, 1930s	34.5, 20.7	74, 59	Native	Not applicable	<i>Aspidiotus destructor</i> Signoret	<i>C. nucifera</i>	Native	Natural	S	[3]
Seychelles, December 1938	30, 24	74, 79	Coimbatore, India	400 adults	<i>Eucalymnatus tessellatus</i> (Signoret)	<i>C. nucifera</i>	Introduced	Classical	U	[4, 5]
					<i>Chrysomphalus aonidium</i> (L.)				C	
					<i>Ichnaspis longirostris</i> (Signoret)				C	
					<i>Pinnaspis buxi</i> (Bouché)				C	
Mauritius, May 1939	30.4, 16.6	81, 70	Sri Lanka	111 adults	<i>A. destructor</i>	<i>C. nucifera</i>	Introduced	Classical	C	[6]
					<i>Aulacaspis tegalensis</i> (Zehntner)	Sugar cane		Classical	P	[7]
Bermuda, 1947	27.2, 17.1	71, 81	Mauritius		<i>Carulaspis minima</i> (Signoret)	<i>Juniperus bermudiana</i> L.	Introduced	Classical	F	[8]
					<i>Lepidosaphes newsteadi</i> (Sulc)				F	
California, 1948	37, 3	91, 24	Guangdong province, China		<i>A. aurantii</i>	<i>Citrus</i> spp.	Introduced	Classical	F	[9]
Coimbatore, India, 1954	34.7, 19.2	25, 83	Native	Not applicable	<i>A. destructor</i>	<i>C. nucifera</i>	Native	IPM and Augmentative	S	[10]
					<i>A. aurantii</i>	<i>Citrus</i> spp.			S	
					<i>Parlatoria zizyphus</i> (Lucas)	<i>Citrus</i> spp.			S	
					<i>Coccus viridis</i> (Green)	<i>Coffea arabica</i> L.			U	
Agalega Islands, 1955	30.6, 22.5		Mauritius		Various scale insects	Various	Introduced	Classical	U	[11]
Chagos Archipelago, 1956, 1959	31, 24	83 (mean)	Mauritius	2000 adults (1956)	Various scale insects	Various	Introduced	Classical	U	[11]
Diego Garcia, October 1958	31, 24	83 (mean)	Mauritius		<i>A. destructor</i>	<i>C. nucifera</i>	Introduced	Classical	M	[12]
Hawaii (Oahu), October 1958	31.4, 18.6	81, 72	Chilaw, Sri Lanka	12 adults	<i>Aspidiotus</i> spp.	?	Introduced	Classical	F	[13, 14]
Ramisi, Kenya and Arusha Chini, Tanzania, 1968, 1969	32.8, 19.3 27, 16	85, 59 85, 35			<i>A. tegalensis</i>	Sugar cane	Invaded	Fortuitous	P	[15]

West Pakistan, 1970	38.7, 2.6	90, 19	Native	Not applicable	<i>A. destructor</i> <i>Aonidiella orientalis</i> (Newstead) <i>Pinnaspis strachani</i> (Cooley) <i>Diaspidiotus periniciosus</i> (Comstock)	? ? ? ?	Native	Natural	P P P P	[16]
Hawaii (Oahu), August 1971	31.4, 18.6	81, 72	Agana, Guam	35 adults	<i>A. destructor</i>	<i>C. nucifera</i>	Introduced	Classical	U	[14, 17]
Vanuatu, April 1977	30.5, 22.1	84 (mean)		Not applicable	<i>A. destructor</i>	<i>C. nucifera</i>	Invaded	Fortuitous	S	[18]
Messina, Northern Transvaal, April 1981	29.1, 7.2	?	South Africa	1000	<i>A. aurantii</i>	<i>Citrus</i> spp.	Introduced	Augmentative	S	[Anon in 19]
Nelspruit, South Africa, December to February 1983	29, 6	?	South Africa	20 000 eggs	<i>A. aurantii</i>	<i>Citrus sinensis</i> (L.) Cv Valencia.	Introduced	IPM Augmentative	F	[20]
Komatipoort, South Africa, December to March 1983	29, 5	?	South Africa	60 000 eggs	<i>A. aurantii</i>	<i>C. sinensis</i> Cv Valencia.	Introduced	IPM Augmentative	S	[20]
Zimbabwe, 1980s	28.9, 5.5	70, 30		Not applicable	<i>A. aurantii</i>	<i>Citrus</i> spp.	Invaded	Fortuitous	S	[Whittaker, in 19]
Oman, January, March and April 1985	32.4, 17.9	90, 50	Bangalore, India	240, 276 and 167 (total 683) adults	<i>A. destructor</i>	<i>C. nucifera</i>	Introduced	Classical	C	[21]
San Miguel de Tucuman, Argentina, 1986	33.9, 5.6	76, 54	Nelspruit, South Africa		?	?	Introduced	Classical	U	[22]
Rehovot, Israel, Feb 1987	30.6, 6.7	63.3 (mean)	Nelspruit, South Africa		?	?	Introduced	Classical	U	[22, 23]
Kachchh, India, January to December 1991	41.5, 11.8	81, 37	Native	Not applicable	<i>Parlatoria blanchardi</i> (Targioni-Tozzette)	<i>Pheonix dactylifera</i> L.	Native	Natural	S	[24]
Bangalore and Doddaballapur, India, July 1991 to January 1992	34.2, 15.1	45, 76	Native	Not applicable	<i>Drepanococcus chiton</i> (Green)	<i>Zyziphus mauritiana</i> L. and Guava	Native	Natural	M	[25]
UK, January to August 1993 Glasshouses at Royal Botanic Gardens Kew	27, 12	?	Rawalpindi, Pakistan	2990 eggs and 2548 adults (0.5 beetles m ⁻²)	<i>Acutaspis umbonifera</i> (Newstead), <i>P. buxi</i> , <i>Abgrallaspis cyanophylli</i> (Signoret), <i>Hemiberlesia lataniae</i> (Signoret), <i>Diaspis boiduvalii</i> (Signoret), <i>Coccus hesperidum</i> L., <i>Saissetia coffeae</i> (Walker)	Wide range of tropical and temperate species.	Introduced	Glasshouse, Augmentative and IPM	M	[26]
UK, February to July 1993 Plantasia, Swansea	?, 17	?	Rawalpindi, Pakistan	800 eggs and 325 adults (1.4 beetles m ⁻²)	<i>P. buxi</i> , <i>C. aonidium</i> (L.), <i>Hemiberlesia palmae</i> (Cockerell), <i>Ichnaspis longirostris</i> , <i>Saissetia coffeae</i> , <i>Bambusaspis bambusae</i> (Boisduval)	Wide range of tropical and temperate species.	Introduced	Glasshouse, inundative	S	[26]

Table 1 (Continued)

Location and date	Mean temperature (maxima, minima) (°C)	Mean humidity (maxima, minima) (% RH)	Source of beetles	Introduction rates (where known)	Target pest species	Host plant	Native, introduced or invaded	Biocontrol type	Degree of success ¹	Reference
Italy, 1994	25, 0.8	78, 62	South Africa		<i>Aonidiella citrina</i> (Coquillett)	<i>Citrus</i> spp.	Introduced	Classical	U	[27]
Bangalore, India, June 1994–March 1995	34.2, 15.1	45, 76	Native	Not applicable	<i>C. viridis</i>	<i>Citrus aurantifolia</i> Swingle	Native	Natural	S	[28]
Balçali, Turkey 2002–2004	33.9, 3.9	61, 49	Originally from Pakistan	2120 eggs and 2390 adults	<i>A. aurantii</i>	<i>Citrus</i> spp.	Introduced	Classical	M	[29]

¹C, completely successful, population levels permanently held beneath the economic threshold; S, substantially successful, populations held mostly below the economic threshold; P, partially successful, some control but less often below the economic threshold; M, minimal control, established (at least temporarily) but no major impact; U, outcome uncertain; F, failure to establish and/or control the target pest.

unsuccessful introductions, makes recommendations for future research and draws conclusions aimed at improving its use in biocontrol programmes.

Climatic Factors

Early records indicate the centre of origin of *C. nigritus* to be the Indian Sub-Continent [19, 22]. However, records prior to 1938 suggest that it was also indigenous to Sri Lanka, Sumatra, Vietnam and parts of China, its natural range being limited by the highlands and semi-desert of the North-West frontier state of Pakistan to the east and to the north and west by the Himalayas and the Indo-China Mountains [19, 22]. Shipments of the beetle from India to the Seychelles in 1938 [4, 5] followed by introductions to Mauritius in 1939 [7], the Chagos Archipelago in 1959 and Madagascar in the mid-1970s [15] paved the way for an apparently unaided colonization of the eastern seaboard of Africa, which was first noted in 1966 at Tanga in Tanzania, but which now stretches from Somalia to South Africa [22]. By 1988, it had become established, either by introduction or invasion, in South America, (north-east Brazil), Oman, Toga, Ghana, Java, the Solomon Islands, Reunion, New Caledonia, Hawaii, American Samoa, Guam, Tahiti, Oahu and Vanuatu [17–22]. The beetle now occurs within a range of 35° north to 29° south but is closely linked to a specific climate and vegetation type and does not occur at all locations within that range [29–31].

Samways [22] used the Zonobiome system as described by Walter [32, 33] to classify the climatic preferences of the beetle and found a very close match between successful colonization and the conditions found in Zonobiomes I and II. Where the species occurred in continental sites it favoured Zonobiome II, which consists of tropical, frost-free deciduous forest or savannah with heavy rains during the high summer period and a very dry cool season. Where it flourished in island habitats, the Zonobiome type was I or a merging of I and II, i.e., areas of equable tropical rainforest with evergreen vegetation where, if a dry season occurs, it tends to be relatively short. Altitude is also important in restricting the beetle's distribution as it does not occur above 2000 m in either zone and is not found above 800 m in South Africa or where frosts occur within Zonobiome II [22]. At first sight, its successful introduction into Oman in 1985 [21] appears to contradict these findings (Zonobiome III, subtropical desert). However, the introduction was made in the coastal plain of the Dhofar province, which receives up to 640 mm seasonal rainfall from June to October as part of the southwest monsoon, and remains humid near the sea [34]. The climate is thus similar to Zonobiome II. Areas where the beetle has been introduced but did not establish include California [1, 9], Florida [3], Bermuda [8], the Central-Northern Transvaal, Eastern Cape and Western Cape [19]. These areas have cool, wet winters

with hot, dry summers or warm temperate climates (Zonobiomes IV and V) with the exception of the Transvaal (Zonobiome II), which is subject to regular frosts and is generally at an altitude of about 1000 m. Attempts to introduce *C. nigrinus* into Israel [23] and Turkey [29] (Zonobiome IV) resulted in the beetles establishing in citrus orchards during the summer but being unable to survive the winters. An absence of reports of establishment in Argentina suggests that attempts to introduce it at San Miguel de Tucuman in 1986 [22] may not have been successful, even though the climate and vegetation match Zonobiome II.

Samways *et al.* [31] used an ecoclimatic computer modelling program to predict the distribution of *C. nigrinus* based on climate data from locations in Asia where it is indigenous. The presence of accurate data for the thermal requirements of *C. nigrinus* [35, 36] led to high levels of accuracy in predicting locations where successful establishment had already been accomplished. A detailed breakdown of potential sites where establishment of *C. nigrinus* is possible or likely is provided, indicating that this species could be established at many locations, mainly in a band between 27° north and 27° south of the equator [31].

In Pakistan and India, *C. nigrinus* has been found to congregate in groups of two to ten adults, totalling several hundred to one thousand on banyan trees (*Ficus bengalensis* L.), apparently to pass through periods of unfavourable conditions [10, 16, 37]. Under such conditions, lasting for periods of several months between September and July [16, 37], they did not feed and copulation was only observed to take place just before they dispersed with the onset of the Southwest Monsoon [10, 37]. Attempts to breed from the resting beetles failed, despite providing them with food under laboratory conditions [10]. Despite evidence of seasonal variations in predatory activity from a number of studies (e.g. [19, 28, 38]), this behaviour has not been reported from other locations. Beetles from Rawalpindi did not show any signs of obligate diapause in cultures [26], suggesting that such behaviour may be induced by local environmental conditions.

Temperature Relations

Accurate information relating to the thermal requirements of BCAs is essential for assessing the likelihood of successful introductions [31]. Four studies have assessed these criteria in *C. nigrinus* ecotypes from South Africa [35], Pakistan [36], India [39] and a Californian culture originating in Pakistan [29]. Summaries of the main findings are shown in Tables 2 and 3. Eggs and larvae were unable to complete development at constant temperatures of 18°C and developed only slowly at 20°C [29, 36]. Two studies on cultures originating in Pakistan and reared on *Abgrallaspis cyanophylli* (Signoret) [36] and *Aspidiotus nerii* (Bouché) [29] have independently found the lower

thermal threshold to be very similar, at 16.6 and 16.7°C, respectively. These two studies also found the thermal constant to be in close agreement, at 325 and 361 degree-days, respectively suggesting that values for these two parameters may be tentatively used to determine and/or compare a range of characteristics for *C. nigrinus*, including climate limitations, effects caused by varying prey type and abundance or host plant interactions. In the field, these parameters gave reliable predictions of developmental period while temperatures were in the favourable range [36]. A lack of developmental data at higher temperatures or a value for the upper thermal threshold for development prevented accurate estimations of developmental period during high temperature events [36]. A more recent study found that the net reproductive rate (R_0) was maximal at 26°C and that the maximum intrinsic rate of increase (r_{max}) peaked at 30°C [29]. Evidence from these and other studies suggest that the optimal temperatures, i.e. where oviposition rate, immature survival and adult longevity are all maximal, lie between 26 and 30°C (Tables 2 and 3). Glasshouse observations also suggest that in order to achieve relatively high first instar survival, mean temperatures need to be in excess of 24°C [36]. Two studies on cycling temperatures at regimes of 12 h/12 h at 14/30°C (mean=22°C) [36] and 12 h/12 h at 20/32°C (mean=26°C) [29] (Table 2) provided evidence that immature survival was either not severely compromised by cycling temperatures [36] or was marginally improved [29]. Lifetime fecundity was not significantly different to that of beetles at constant temperatures in either study and the r_{max} at 20/32°C was similar to that at a constant temperature of 30°C [29].

Longevity of males and females at varying temperatures from different studies are summarized in Table 2 and show that on average, males are longer-lived than females. Beetles lived longest at temperatures towards the lower end of the favourable range and at cycling temperatures.

Survival in *C. nigrinus* at the lower extremes of temperature is summarized in Table 3. One study [35] demonstrated that nearly all beetles could survive a 48 h period at temperatures ranging from 3°C to 17°C. A second study [26] examined the lower LT_{50} of adults in the context of being able to overwinter in temperate latitudes. The results show that at 0°C, *C. nigrinus* adults that had been acclimated at 20°C for a minimum of 7 days had an LT_{50} of 16.8 h (range 4–30 h). However, LT_{50} s at lower temperatures were dependent on the degree of acclimation the beetle received before exposure, since beetles drawn from cultures at 27°C without acclimation had an LT_{50} of only 2.9 h (range 0–5 h) [26]. A third study [39] examined the storage of mass-reared beetles for later release. Only 8.3% of adults survived for 21 days when held at 5°C and 16.7% survived when held at 10°C. However, beetles held at 15°C under storage conditions, had significantly higher survival rates after 42 days than those held at 20 or 29°C but were much less fecund and less long-lived after storage. Eggs that were 1–3 days old

Table 2 Effect of temperature on various characteristics of *C. nigritus* when fed on *A. nerii* (Bouché) [29] and *A. cyanophylli* (Signoret) [36]

Characteristic	Host insect	Category	Temperature (°C) (± 1)									
			18	20	22	24	26	28	30	34	14–30	20–32
Immature survival (%)	<i>A. nerii</i> (Bouché)	Egg	75	–	85	–	67	–	70	41	–	77
		L1	0	–	90	–	94	–	96	65	–	99
		Egg–Adult	N/A	–	66	–	62	–	63	23	–	83
	<i>A. cyanophylli</i> (Signoret)	Egg	88	91	88	81	89	88	95	–	–	–
		L1	0	33	49	71	77	61	61	–	–	–
		Egg–Adult	N/A	17	39	39	40	52	51	–	–	–
Adult longevity (days)	<i>A. nerii</i>	♂	–	–	279	–	218	–	113	29	–	199
		♀	–	–	172	–	185	–	90	17	–	187
		♂+♀	–	–	172	–	185	–	90	17	–	187
	<i>A. cyanophylli</i>	♂	–	147	105	101	102	–	65	–	130	–
		♀	–	88	115	123	102	–	77	–	106	–
		♂+♀	–	88	115	123	102	–	77	–	106	–
Life-time fecundity (total number of eggs)	<i>A. nerii</i>	0	–	360	–	432	–	283	0	–	378	
	<i>A. cyanophylli</i>	0	564	633	1361	1008	–	872	–	782	–	
Daily fecundity (eggs/day)	<i>A. nerii</i>	–	–	3.1	–	3.5	–	3.4	0	–	2.9	
	<i>A. cyanophylli</i>	–	2.9	2.9	6.3	5.9	–	7.8	–	4.8	–	
Adult size (mm)	<i>A. nerii</i>	♀ Length	–	–	3.58	–	3.48	–	3.44	3.39	–	3.63
		Width	–	–	3.27	–	3.23	–	3.20	3.08	–	3.35
		♂ Length	–	–	3.54	–	3.38	–	3.40	3.21	–	3.55
		Width	–	–	3.31	–	3.22	–	3.19	3.00	–	3.31
		♂	1.28	4.00	–	–	5.17	–	6.94	–	4.92	–
		♀	1.94	3.94	–	–	8.83	–	7.17	–	6.84	–
Adult feeding rate (adult scales/day)	<i>A. cyanophylli</i>	♂	1.28	4.00	–	–	5.17	–	6.94	–	4.92	–
		♀	1.94	3.94	–	–	8.83	–	7.17	–	6.84	–
Population parameters	<i>A. nerii</i>	R_0 ($\text{♀}/\text{♀}$)	–	–	115	–	130	–	82	–	–	125.14
		r_{\max} ($\text{♀}/\text{♀}/\text{day}$)	–	–	0.043	–	0.066	–	0.070	–	–	0.060
		T (days)	–	–	111	–	74	–	63	–	–	80.62

Table 3 Summary of key thermal and humidity parameters for *C. nigrinus*

Parameter (and reference)	Value	Comments
Lower threshold for development [29, 36]	16.6, 16.7°C	Both studies were on beetles of Pakistani origin
Upper threshold for development [29]	>34°C	Not yet determined
Thermal constant [29, 36]	325, 360.6	On <i>A. cyanophylli</i> and <i>A. nerii</i> respectively
Favourable temperature range [29, 36]	21–30°C	
Optimal range ¹ [29, 36, 39]	26–30°C	Maximal survival in 1st instar larvae at mean temperatures of 24°C or above
Lower LT ₅₀ (hours) [26]		
0°C	16.8 (4–30)	Acclimated at 20°C for 7 days
–2°C	2.8 (1–4)	
–4°C	1.8 (1–3)	
–6°C	0.6 (1–2)	
Lower critical limit [29, 36]	>18°C (larvae) ≥15°C (adults)	Eggs do not develop All beetles die within 12 days at 13°C
Upper critical limit [35]	>38 but <41	48 h of exposure
Storage conditions [39]	Eggs (<3 days) 15–20°C Adults 15–20°C	Can be stored for 10–15 days
Favourable humidity range [26, 29, 36]	33–80%	Ambiguous findings
Longevity (days) ² [29, 36]		
(% RH)	♂, ♀	No significant differences in life time fecundity or daily oviposition across the range
40	277, 162	
60	215, 185	
80	278, 220	
60–65	165, 132	
28–39	68, 70	

¹Maximal fecundity, survival, longevity and voracity.

²See Table 2 for longevity at different temperatures.

did not hatch when held at 5 or 10°C for a minimum of 5 days, but most eggs were still viable after being held for 15 days at 15 or 20°C [39]. These findings are in agreement with an earlier study in which most beetles that had been acclimated at 20°C and then held at 13°C did not eat and were unable to survive for 12 days, whereas those held at 15°C fed on small quantities of scale and all survived [40]. Feeding rates at temperatures of 3, 10 and 17°C were also shown to be very low or zero [35].

Temperatures at the upper extremes have been less well studied, particularly in terms of developmental rates. A constant temperature of 34°C only marginally decreased the developmental period in relation to that at 30°C [29], suggesting that it exceeds the optimum and is close to the upper thermal threshold for development. The latter has not yet been determined.

Hattingh and Samways [35] found that 88% of adults survived 48 h at 38°C but none survived a similar period at 41°C. Furthermore, 90% of beetles survived for 14 h at 41°C but only 5% survived for 21 h and none at all for 41 h, suggesting that the LT₅₀ at 41°C probably lies between 16 and 18 h.

Larval feeding rates in relation to varying temperature do not appear to have been studied but cultures of South African origin reared on *A. nerii* [35] and Pakistani origin reared on *A. cyanophylli* [40] indicated that adult feeding was very low at temperatures below 20°C but peaked at temperatures between 26 and 31°C. In the former study,

feeding continued at a lower rate at 38°C but did not occur at 41°C. Females ate more than males at 26 and 30°C and when temperatures cycled at 14 and 30°C but consumption was not significantly different between genders at the upper and lower temperatures [40] (Table 2). The two studies, however, found differing levels of adult food intakes at fluctuating temperatures. In common with earlier work on larval intake of other coccinellid species under such conditions [41, 42], Ponsonby and Copland [40] found adults significantly increased food intake by 40% during 12 days of a regime of 12 h/12 h at 14/30°C when compared with food intake during a similar period at a constant temperature of 26°C. In contrast, Hattingh and Samways [35] discovered that adults of a similar age ate 43% less under a gradually changing temperature regime of 15/28°C than those at a constant temperature of 25–26°C, but adult weights were not significantly different after 40 days of exposure. Adult intake under fluctuating temperatures therefore remains ambiguous but mean temperatures need to be above 20°C for *C. nigrinus* to be an effective predator.

Humidity Relations

Greathead and Pope [15] observed that *C. nigrinus* was better adapted to the dry savannahs of east Africa than the native *Chilocorus* species and its successful introduction

into the arid, subtropical desert region of Oman [21] supports this observation. As stated above, it thrives in island environments where there are wet, humid summers and warm, moderately humid winters, or in mainland regions where there are hot, humid summers with cooler, drier winters [19, 22, 31]. However, when the beetle was fed in the laboratory on *A. cyanophylli*, relative humidity (RH) in the order of 30% ($\pm 10\%$) significantly reduced egg and larval survival rates when compared with a controlled RH of 65% ($\pm 5\%$), but pupal survival, adult oviposition rates and egg viability were unaffected [26, 43]. The egg and pupal developmental period was significantly reduced by low humidity at most constant temperatures in the range of 20–30°C and cycling temperatures of 12 h/12 h at 14/30°C but larval development was not significantly altered. Adult longevity was significantly reduced by lower humidity [26] (Table 3).

When reared at 26°C on *A. nerii* in another laboratory study, all stages of *C. nigrinus* development were significantly reduced at 40% ($\pm 5\%$) RH, compared with 60% ($\pm 5\%$) RH and 80% ($\pm 5\%$) RH [29]. Immature death rates were higher and adult female longevity was reduced at 40% RH and 60% RH compared with 80% RH (Table 3). Male longevity and preoviposition period were not significantly altered at the upper and lower levels of humidity but were reduced at 60% RH. In common with Ponsonby and Copland [43], daily oviposition rate and total fecundity were not affected, but there was a stronger female bias in the secondary sex ratio at 40% RH [29]. Senal [29] calculated that the net reproductive rate (R_0) and the maximum intrinsic rate of increase (r_{max}) were 1.4 and 1.2 times higher (respectively) at 40% RH, than at 60% and 1.6 and 1.3 times higher than at 80%, leading to the conclusion that low humidity improves the use of this species in biocontrol programmes. However, personal experience with this beetle in heterogeneous glasshouse environments has shown it to perform well under conditions of high humidity and less well in arid conditions [26], suggesting that other factors such as temperature, prey species and abundance may be more important than humidity in this context.

Host-Prey Relations

It has become apparent from a substantial literature that the prey relations of predatory coccinellids are extremely complex. Numerous studies have shown that clear differences may be drawn between 'essential' or 'suitable' prey that allow larva to complete development with high survival rates and result in reproductively fit adults, and 'supplementary' or 'marginal' prey that allow survival but do not support development of immatures or oviposition in adults (e.g. [41, 42, 44]). Hodek and Honěk [42, p. 167] recommend that in most cases, only experimental evidence is 'adequately unequivocal' and it has become routine to collect data, not only on the developmental

period of the immatures but also on the reproductive performance of the F_1 adults. Using these criteria, Table 4 provides a summary of prey that have unequivocally been shown to be 'essential' prey as well as a list of 'supplementary prey' that have reliable records of being hosts to *C. nigrinus* but as yet, no experimental evidence of being 'essential'. Table 5 summarizes the key bionomic performance indicators of beetles fed on varying prey species.

The literature on host relations of *C. nigrinus* is extremely ambiguous and appears to suggest the presence of different ecotypes and/or biotypes. For example, first instar larvae of South African origin were capable only of feeding on first instar *A. nerii* [51], while larvae of a similar stage from Pakistan were able to feed on first and second instar *A. cyanophylli* [40]. More recently, newly hatched first instar *C. nigrinus* larvae of Pakistani origin were observed to penetrate the scale cover and take their first feed on adult female *A. nerii* (biparental strain) (Rena Boothe, personal communication, 2008). South African larvae were also unable to switch from feeding on *A. nerii* to *Aonidiella aurantii* (Maskell) or vice versa [51], and suffered longer developmental periods and lower adult weight when switched between *Bambusaspis mliaris* (Boiduval) and *A. nerii* (and vice versa) [52, 53]. However, larvae from Pakistan exhibited almost no ill effects when switching from *A. cyanophylli* to *Coccus hesperidum* L. and vice versa [54].

Senal [29] found no significant differences in pre-oviposition, oviposition and postoviposition period, lifetime fecundity, oviposition rate or adult longevity when beetles were reared on *A. aurantii* throughout when compared with those reared from egg hatch to pupation on *A. nerii* and then switched to *A. aurantii* as adults. However, they were much more fecund and long-lived when reared on *A. nerii* throughout. The same author calculated R_0 to be 3.7 times higher and r_{max} to be 1.4 times higher when reared on *A. nerii* compared with *A. aurantii*.

C. nigrinus has been recorded feeding on Coccidae in India [10, 28, 55, 56], Pakistan [38], the Seychelles [5] and the New Hebrides [18]. Beetles from Pakistan were recorded feeding on *C. hesperidum* and *Saissetia coffeae* (Walker) [54] but in South Africa, they have rarely been seen feeding on coccids and did not feed on *Protospulvinaria pyriformis* (Cockerell) on avocado even when confined with them [19].

Records of *C. nigrinus* feeding on other groups from the Hemiptera are common and Samways [19] lists accounts of 46 species of prey from eight different families. Reliable accounts record adults feeding on aphids [57] and pseudo-coccids [19] but Hill and Blackmore [58] concluded that an increase in density of *C. nigrinus* on populations of the margorodid, *Icerya seychellarum* Westwood in the Seychelles was most likely the result of them preying on populations of *Hemiberlesia lataniae* (Signoret) cohabiting with the margorodids. Most of the evidence in the

Table 4 Species that can be considered 'essential' and 'acceptable' prey for *C. nigrinus*

Essential prey	Reference	Supplementary prey ¹	Reference
Coccidae			
<i>Coccus hesperidum</i> Linnaeus	[54]	<i>Chloropulvinaria psidii</i> (Maskell)	[55]
<i>Coccus viridis</i> (Green)	[18, 28, 55]	<i>Coccus colemani</i> Kannan	[56]
<i>Megapulvinaria maxima</i> (Green)	[10]	<i>Drepanococcus chiton</i> (Green)	[25]
<i>Saissetia coffeae</i> (Walker)	[54]	<i>Drepanococcus cajani</i> (Maskell)	[10]
		<i>Eucalymnatus tessellatus</i> (Sign)	[5]
		<i>Parasaissetia nigra</i> (Nietner)	[10]
		<i>Pulvinaria polygonata</i> Cockerell	[38]
Asterolecanidae			
<i>Bambusaspis miliaris</i> (Boisduval)	[52, 60]	<i>Bambusaspis bambusae</i> (Boisduval)	[26]
Diaspididae			
<i>Abgrallaspis cyanophylli</i> (Signoret)	[36]	<i>Acutaspis umbonifera</i> (Newstead)	[54]
<i>Aonidiella aurantii</i> (Maskell)	[29, 71]	<i>Aonidiella citrina</i> (Cochquillet)	[27]
<i>Aonidiella orientalis</i> (Newstead)	[16]	<i>Aonidiella simplex</i> (Grandpré and Charmoi)	[7]
<i>Aonidomytilus albus</i> (Cockerell)	[71]	<i>Aulacaspis citri</i> Chen	[45]
<i>Aspidiotus destructor</i> Signoret	[71, 90]	<i>Aulacaspis vitis</i> (Green)	[56]
<i>Aspidiotus nerii</i> Bouché	[29, 76, 92]	<i>Chrysomphalus dictyospermi</i> (Morgan)	[7]
<i>Aulacaspis tegalensis</i> (Zehntner)	[15]	<i>Chrysomphalus diversicolor</i> (Green)	[7]
<i>Aulacaspis tubercularis</i> Newstead	[71]	<i>Diaspis bromeliae</i> (Kerner)	[7]
<i>Chrysomphalus aonidum</i> Linnaeus	[71]	<i>Diaspis echinocacti</i> (Bouché)	[46]
<i>Hemberlesia lataniae</i> (Signoret)	[71]	<i>Hemiberlesia palmae</i> (Cockerell)	[26]
<i>Lepidosaphes cornuta</i> Ramakrishna Ayyar	[71]	<i>Ischnaspis longirostris</i> (Signoret)	[5, 26, 54]
<i>Melanaspis glomerata</i> (Green)	[47, 71]	<i>Insulaspis gloverii</i> (Packard)	[48, 49]
<i>Parlatoria blanchardii</i> (Targioni Tozzetti)	[59]	<i>Parlatoria crypta</i> McKenzie	[38]
<i>Pinnaspis buxi</i> (Bouché)	[54]	<i>Parlatoria zizyphi</i> (Lucas)	[10]
<i>Quadraspidiotus perniciosus</i> (Comstock)	[71]	<i>Pinnaspis strachani</i> (Cooley)	[16]
		<i>Pseudaulacaspis pentagona</i> (Targioni-Tozzetti)	[7]
		<i>Selenaspis articulatus</i> (Morgan)	[18]

¹Prey species that have reliable records of having been fed upon but no experimental evidence yet found to show that they are 'essential prey'.

literature points to the likelihood that the preferred prey of *C. nigrinus* are diaspidids and asterolecaniids and some species of coccid, notably *Coccus viridis* (Green) and *C. hesperidum* (e.g. [10, 15, 19, 26, 30, 38, 54]). Particularly favoured diaspidid prey (i.e. where control of populations in field situations is reported to be complete or substantially complete) include *A. aurantii* [1, 10, 19, 20, 38, 52], *Chrysomphalus aonidum* L. [5, 26], *Ischnaspis longirostris* (Signoret) [5, 26, 54], *Parlatoria blanchardii* (Targioni Tozzetti) [24, 58], *Pinnaspis buxi* (Bouché) [4, 5, 54] and *Aspidiotus destructor* (Signoret) [5, 7, 18, 21, 38]. Asterolecaniids also appear to be particularly favoured as prey, especially when diaspidids are in seasonal decline [52, 60] and populations of *Bambusaspis bambusae* on *Dendrocalamus giganteus* supported large populations of immature *C. nigrinus* in the glasshouses of the Plantasia complex, Swansea, UK [26]. The beetle did not feed on *Aspidiotus rigidus* Reyne unless it was dead [2].

Prey species may not be as important as the prey population structure when utilizing *C. nigrinus* as a BCA [61]. Unlike the aphidophagous Coccinellidae (e.g. [42, 62]), very little research has been done on the effect of prey population structure on feeding or reproductive behaviour of coccidophagous coccinellids. Strong prey synchrony is an important prerequisite for successful

colonization of prey patches and evidence from research on aphidophagous species suggests that patch quality and, in particular, the age structure of the prey or the phenological age of the plant play an important role in stimulating oviposition behaviour [62]. This has led to the postulation of an 'egg window hypothesis', whereby females optimize their fitness by choosing oviposition sites that support a theoretical minimum density of suitable aphid hosts for their offspring [62, 63]. It is assumed that a theoretical minimum density of suitable prey required to suit the survival of the first instar larvae opens the window [62], while the relatively recent discovery of oviposition-detering pheromones produced by conspecific larvae acts to close the window [64, 65]. Age and experience of the adult beetles are also important factors in such a model [66]. When *C. nigrinus* adults, in the absence of conspecific larvae, were subjected to a reduction in population density of *A. cyanophylli* of heterogeneous age structure (overlapping generations of all prey stages from crawler to adult) at levels above those needed to sustain egg production in the females (i.e. from 207 insects/cm² to 87 and 37 insects/cm²), a significant but transient decline in egg production occurred [61]. When females were offered populations of *A. cyanophylli* with varying homogeneous age structures of 1st and 2nd instar

Table 5 Effect of prey species on various bionomical characteristics in *C. nigratus* according to different authors

Prey species and stage	Host plant	No. of scales eaten per day by larvae (L) and adults (A)	Developmental period (days egg to adult) (and temperature °C)	Accumulated heat (degree-days) ¹	Fecundity (total number of eggs)	Adult longevity (days)	Reference
<i>Megapulvinaria maxima</i> (Green) stage unknown	?	L(?) 8	27–30 (?)	?	'Very poor'	?	[10]
<i>Saissetia coffeae</i> (Walker) all stages	<i>Solanum tuberosum</i> L.	?	38–64 (24 ± 1)	283–477 ²	?	?	[54]
<i>Coccus hesperidum</i> L. all stages	<i>Cucurbita moschata</i> (Duchesne ex Lam.).	?	34 (26 ± 1)	321	?	?	[54]
<i>Bambusaspis miliaris</i> (Boisduval) stage not defined	<i>Dendrocalamus giganteus</i> Munro	?	30.6 ³ (25–26)	272	?	?	[60]
<i>Abgrallaspis cyanophylli</i> (Signoret) 2nd and 3rd instar females, puparial males	<i>S. tuberosum</i>	L3 33.5 L4 16.7 A 7.0	34 (26 ± 1)	320	1008	♂ 101 ♀ 123	[36, 40, 43]
<i>Aonidiella aurantii</i> (Maskell) 20–25 days old	<i>C. moschata</i> Pumpkin	? L? 31.8 A 62.3	29 (26 ± 1) 34.1 (27 ± 1.8)	272 355	– 81	73	[76] [71]
All stages	<i>C. moschata</i>		27.4 (26 ± 1)	258	194	♂ 128 ♀ 100	[29]
<i>Aonidiella orientalis</i> (Newstead) 'females'	?	L3 12 L4 27	36.7 (24 ± 2.2)	272	292	♂ 104 ♀ 123	[16]
<i>Aonidimytilus albus</i> (Cockerell) 20–25 days old	Pumpkin	L(?) 31.0 A 62.4	35.1 (27 ± 1.8)	365	57	73	[16]
<i>Aspidiotus destructor</i> Signoret 20–25 days old	Pumpkin	L(?) 38.0 A 68.3	33.4 (27 ± 1.8)	347	93	81	[71]
Prey stage not defined	<i>Cocos nucifera</i> L.	L1 7.4 L2 13.1 L3 19.7 L4 23.8 A 17.7	29.7 (29.5 ± 2.1)	383	121	67	[50]
<i>Aspidiotus nerii</i> (Signoret) adult females	<i>C. moschata</i>	L(3) 18 L(4) 36 A 22.8	29.3 ² (25–26)	261	?	?	[51, 60]
All stages	<i>S. tuberosum</i>		30.07 (26 ± 1)	283	483	♂ 277 ♀ 162	[29]

<i>Aulacaspis tegalensis</i> (Zehntner) 'all stages'	Sugar cane	?	37.1 (21)	?	370+	♂ 110 ♀ 210	[15]
<i>Aulacaspis tubercularis</i> Newstead 20–25 days old	Pumpkin	L(?) 30.0 A 61.4	34.1 (27 ± 1.8)	355	87	69	[71]
<i>Chrysomphalus aonidum</i> L. 20–25 days old	Pumpkin	L(?) 29.7 A 60.7	34.2 (27 ± 1.8)	356	72	70	[71]
<i>Hemberlesia lataniae</i> (Signoret) 20–25 days old	Pumpkin	L(?) 33.6 A 67.6	35.1 (27 ± 1.8)	365	79	70	[71]
	Pumpkin	L1 8.1 L2 13.1 L3 18.8 L4 24.4 A 17.2	28.3 (29.5 ± 2.1)	365	102	64	[50]
<i>Lepidosaphes cornutus</i> (Ramakrishna Ayyar) 20–25 days old	Pumpkin	L(?) 30.0 A 65.6	34.3 (27 ± 1.8)	357	71	83	[71]
<i>Melanaspis glomerata</i> (Green) 20–25 days old	Sugar cane	L(?) 32.0 A 63.0	32.9 (27 ± 1.8)	342	79	75	[71]
Prey stage not defined	Sugar cane	L1 13.0 L2 17.5 L3 21.5 L4 27.3 A 17.2	26.1 (29.5 ± 2.1)	337	136	70	[50]
<i>Parlatoria blanchardii</i> (Targioni Tozzetti) adults	<i>Phoenix dactylifera</i> L.	L(1) 8.3 L(3) 34.8 L(4) 55.6 A 20.9	29.9 (27.3–2.5)	?	151	♂ 50 ♀ 76	[59]
<i>Pinnaspis buxi</i> (Bouché) all stages	<i>Ctenanthe oppenheimiana</i> (E. Morren)	–	36.5 (24)	308	?	?	[54]
<i>Pinnaspis strachani</i> (Cooley) 'females'	?	L(3) 19 L(4) 41 A 32	?(24 ± 2.2)	?	?	?	[16]
<i>Quadraspidiotus perniciosus</i> (Comstock) 20–25 days old	Pumpkin	L(?) 34.1 A 71.4	32.3 (27 ± 1.8)	336	86	77	[71]

¹Based on a lower thermal threshold of 16.6°C [36].

²Development delayed because of a shortage of prey [43].

³Data provided for egg hatch to adult eclosion only, figure based on an estimated egg development period of 7 days [36].

and gravid females (males of similar age present in each case), egg production declined permanently and significantly compared with those kept on heterogeneous populations with overlapping generations [61]. The authors concluded that, provided sufficient food is available for adult beetles to be satiated, prey population structure is more important than host density for stimulating oviposition and that, *C. nigritus* were more likely to successfully colonize well-established crops containing populations of scale insects with complex age structures than recently established ones with a single generation of colonizers. However, a similar, more recent study using a biparental strain of *A. nerii* as prey (i.e. with both males and females present) revealed that a homogeneous population of newly moulted third instar females stimulated more egg production than a heterogeneous one with overlapping generations (Rena Boothe, personal communication, 2009). Such ambiguity prevents the possibility of generalizations when making recommendations or predictions for successful pest control and indicates the need for more detailed research on prey population effects.

Vesey-Fitzgerald [5] described *C. nigritus* as showing 'great aptitude for finding its prey' and studies using flight chambers, olfactometry and search arenas demonstrated that *C. nigritus* adults were able to respond to visual and/or olfactory and, possibly, gustatory cues from their prey and the host plants [67–69]. These findings offer the potential for manipulating beetle populations using behaviour-modifying semiochemicals, but no recent work was found in literature searches on *C. nigritus* in this context.

Unlike parasitoids, which temporarily cease searching on contact with another parasitoid, *C. nigritus* adults were unaffected by contact with conspecifics and showed no tendency to disperse with increasing density of feeding beetles [70]. These observations, coupled to the high voracity of the larvae and adults (e.g. [40, 71]), its capacity to coexist in stable relationships with parasitoids (feeding on parasitized scale but not reducing host:parasitoid ratios) [20] and its ability to cope with all stages of prey [20, 40, 51, 70], help to explain the exceptional capability of this ladybird species to quickly reduce high population densities of scale insects.

Host Plant Effects

Accounts of the tritrophic interactions between coccinellids, their prey and their host plants are scarce but those that exist highlight an urgent need to elucidate such effects. For example, the margorodid, *Icerya purchasi* is toxic to *Rodolia cardinalis* when the host plants are *Spartium*, *Genista* or *Cocculus* species [41, 72], presumably because of the accumulation of toxic secondary metabolites within the prey. It is also well known that the topography of the plant surface can significantly affect larval and adult movement, the availability or vulnerability of oviposition sites, and the susceptibility of larvae to be

impaled on hooked trichomes [42]. Similarly, glandular trichomes may poison or trap insects and there is also evidence that host plant effects can alter the functional response [73]. Boothe *et al.* [74] recently examined the tritrophic effects of two biparental prey species, *A. cyanophylli* and *A. nerii*, on two plants commonly used to mass rear *C. nigritus*, butternut squash (*Cucurbita moschata* (Duchesne ex Lam.)) and potato tubers (*Solanum tuberosum* L.). They found that the beetles took almost 6 days longer to develop when fed *A. cyanophylli* on *S. tuberosum* than when fed the same prey on *C. moschata* but the numbers of beetles surviving on both prey species on the squashes were significantly lower, indicating that *S. tuberosum* is the more desirable host plant for mass rearing *C. nigritus*.

Major crop plants associated with successful introductions of *C. nigritus* are listed in Table 1. However, Samways [19] provides a relatively comprehensive account of the plant species on which the beetle has been observed feeding and notes that it apparently shows a weak preference for some plants. For example, in citrus orchards in South Africa, it usually occurs first in young grapefruit orchards rather than Valencia oranges. Ghani and Muzaffar [38] also provide an account of prey/host plant interactions, indicating that in Pakistan, *C. nigritus* occurs mainly as a predator of Diaspididae but with some evidence of a host plant bias. The need for further study of tritrophic interactions is indicated for key prey and host plant species, including plant nutritional and water status, the presence or absence of plant secondary metabolites and specific cultivar effects relating to pest resistance.

Natural Enemies and Pathogens

One of the key elements attributed to the success of *C. nigritus* as a BCA is an apparent lack of its own effective natural enemy complex. Very few reports exist of any noticeable level of predation or parasitoid activity against the beetle. Only one record of 'very low' parasitoid activity by a *Homalotylus* sp. attacking 4th instar larvae has been reported [55], while Hill and Blackmore [58] found that ants defended coccids against *C. nigritus*, such that their activity on ant-attended bushes was significantly reduced when compared with those bushes where ants were excluded. We have found adults dead in spider webs in glasshouse situations but have never observed any other predator or parasitoid activity.

It is known that coccinellids in general suffer from infection by four fungal genera and two gut-dwelling sporozoan groups, Gregarinida and Microsporidia [41, 75, 76]. No records have been found of pathogens having affected this species, possibly because of a lack of research aimed at this phenomenon. We have often observed impaction of the hindgut in beetles that die prematurely, suggesting a possible pathogenic cause. The impact of

pathogens on *C. nigritus* is unclear and intensive study is indicated.

Endosymbionts

Studies have shown that, in common with many invertebrate species, coccinellids harbour cytoplasmically inherited bacteria, which predominantly inhabit the oocytes and selectively kill male embryos [75, 77, 78]. Infected populations typically exhibit strong female-biased sex ratios. Several taxa of vertically transmitted, obligate, intracellular bacteria have now been implicated in this process, including *Rickettsia*, *Wolbachia*, *Spiroplasma*, *Cardinium* and Flavobacteria. A recent study used Polymerase Chain Reaction (PCR) to screen large samples from 21 species of UK and European Coccinellidae and found that 11 beetle species were infected, some with multiple symbiont species [79]. There appears to be some evolutionary advantage for wild populations, since Majerus [75] reported that 7% of an *Adalia bipunctata* population from the Cambridge area of the UK were infected with a *Rickettsia* species and that 87% of the offspring of infected females carried the male killers. From this he calculated that each infected female must produce 25% more surviving female offspring in order to prevent the extinction of the bacteria. The same author summarized three types of benefit accruing from the infection: (1) the avoidance of in-breeding; (2) 'resource allocation' either directly in the form of non-viable eggs for the emerging female larvae to eat, or indirectly by reducing competition for scarce food and (3) a reduction in sibling cannibalism of female eggs.

Several authors have reported female-biased secondary sex ratios on *C. nigritus* [29, 36, 80–83] ranging from 2.85 [83] to 1.13 [29] females per male. A recent PCR assay on *C. nigritus*, originally from Pakistan, revealed the presence of *Wolbachia pipientis* Hertig Clade A [83]. More recently, a wider assay by the same author and co-workers discovered the presence of *Wolbachia* (80%) and *Rickettsia* (70%) in the same biotype; *Wolbachia* (50%) and *Rickettsia* (60%) in a commercially reared strain of South African origin; *Rickettsia* (80%) in field-collected beetles of Indian origin; and *Wolbachia* (60%), *Rickettsia* (50%) and *Spiroplasma ixodetis* Tully *et al.* (10%) in field-collected beetles from South Africa (Joseph Burman and Jennifer Teague, personal communication, 2009). Many of the beetles had multiple infections.

There has been much speculation over the evolutionary advantages or disadvantages to harbouring male-killing bacteria (e.g. [84]) but, as far as we are aware, no work has been done on the importance of such endosymbionts to biological control in the coccidiphagous Coccinellidae and only a little on other BCAs. There is evidence that the genetically closed populations so often used for the mass-rearing of insects for augmentative release in biological control programmes quickly accrue infection levels as high as 100%, even if the starting levels are very low [85]. In a recent review on the relevance of the bacterial species,

Wolbachia in biocontrol research, Floate *et al.* [86] reported that only 1% of peer-reviewed articles on this male-killing species were in biocontrol-specific journals. The impact of male killing in terms of commercial production (mass rearing), and on pest control efficacy remain completely untested in any ladybird species but it is known that infections can cause a 60% reduction in hatch rates [87]. Urgent research is indicated in order to examine the ecological and economical effects of these pathogens on the utility of *C. nigritus* as a BCA, particularly in relation to mass rearing (see further discussion below).

Mass Rearing

The ease with which beetles can be mass reared and the quality of such beetles are important considerations for its use in pest management programmes. Several authors have described mass-rearing techniques (Table 6) but the best survival rates and adult fecundity appear to be from beetles cultured on biparental and *A. nerii* reared on potato tubers (*S. tuberosum*) [19, 29, 76, 89]. The same scale species reared on *C. moschata* appears to be a good alternative, particularly when potatoes are in short supply. *Melanaspis glomerata* (Green) reared on sugar cane setts, *Quadraspidiotus perniciosus* (Comstock) on 'pumpkin' [71] and *A. cyanophylli* on potatoes [43] are also suitable alternatives. Optimal culture temperatures are 26–30°C with a 12 h/12 h photophase/scotophase and RH between 40 and 70%. When using butternuts, medium to high densities of *A. nerii* are needed because female beetles tend to disperse from imminently doomed squashes with very high scale densities [82]. Our experience of rearing beetles from Pakistan on *A. nerii* and *A. cyanophylli* on potatoes is that highest levels of beetle reproduction occur when there are high densities of overlapping generations of scale that include all stages of development [54].

Although males live longer on average (Tables 2 and 3), our experience of beetles in insectaries has shown this to be skewed by a minority of very long-lived individuals [26]. This does not seem to be the case with females and thus ageing laboratory and mass-rearing cultures tend to be dominated by males. Samways and Tate [80] also found this to be the case in their cultures and recommended the introduction of younger females at regular intervals. Female-biased secondary sex ratios have been recorded among young adults in wild populations and cultures but the latter changed from a ratio of 1:1.27 males to females at 3 days in the insectary, to 1:0.42 by 2–6 months [80]. Reduced female longevity may be linked to infection by male-killing endosymbionts as described above and research is indicated to confirm this and determine if there is an advantage to producing endosymbiont-free cultures for biocontrol purposes.

Beetles of South African origin required flying space for maximal longevity and fecundity in the insectary [89] but beetles of Pakistani origin achieved maximum egg

Table 6 Accounts of mass rearing of *C. nigritus* using different host species

Host-prey insect	Host plant	Comments	Reference
<i>Abgrallaspis cyanophylli</i> (Signoret)	<i>Solanum tuberosum</i> L., <i>Cucurbita moschata</i> (Duchesne ex Lam.)	As above but immature survival rates not as high as with <i>A. nerii</i>	[36, 74]
<i>Aspidiotus destructor</i> Signoret	<i>Cucurbita maxima</i> (Duchesne) and <i>Cocos nucifera</i> L. leaves	Fully mature and ripe fruits with the stalk intact were infested as stock cultures and then fresh coconut leaves were infested by wrapping them around the fruit. Infested leaves cut into 12 cm lengths and fed fresh every 2 days	[88]
<i>Aspidiotus nerii</i> (Bouché)	<i>S. tuberosum</i> <i>C. moschata</i>	Both plant organs provide robust substrates. Higher infestation levels on potatoes and superior survival rates provide optimum rearing conditions	[19, 29, 74, 89]
<i>Aulacaspis tegalensis</i> (Zehntner)	Sugar cane	5 cm lengths changed every 2 days	[15]
<i>Aonidiella aurantii</i> Maskell	<i>C. moschata</i>	Slightly higher immature death rate, shorter lifespan of adults, lower fecundity compared with those reared on <i>A. nerii</i> on potatoes	[29]
<i>Coccus hesperidum</i> L.	<i>C. moschata</i> , <i>C. maxima</i> 'Blue Hubbard'	Good survival rates but larger quantities of scale required	[54]
<i>Megapulvinaria maxima</i> (Green)	?	Poor reproduction reported	[10]

production and longevity when they were reared in small, ventilated containers rather than large flight cages [26].

Efforts to formulate artificial diets have not yet yielded a suitable alternative to natural prey for mass production through successive generations, although Hattingh and Samways [60] describe a diet that can be used to support development in larvae and another that can be used for adult maintenance when prey are in short supply.

There is growing evidence that mixed diets are important for ladybird health [92]. In *C. nigritus*, supplemental semi-synthetic diets containing honey (50%), multivitamins (10%) and tocopheryl acetate (0.5%), when fed alongside *H. lataniae*, enhanced oviposition by 28% compared with those fed honey (50%) only [91]. The same study also describes the optimal substrate for females to oviposit into as 4×4 cm pads of absorbent cotton. However, Samways [19] found *C. nigritus* to lay about twice as many eggs in polyester fibres as on flannel, and that polyester was easier to handle and did not waterlog during periods of heavy rain or irrigation. Conversely, surgical gauzes were highly suitable as a substrate for oviposition and thus for augmentative releases in glasshouse environments [26]. Such ambiguity provides an indication that much has yet to be done to optimize the mass-rearing process of this predator and that differing biotypes/ecotypes may require different rearing conditions.

Use of *C. nigritus* in Pest Management Programmes

Assuming that a suitable climate, host plant and prey species are all present, several key elements affect the use

of this species in pest management programmes. Of particular importance are the timing, method and density of releases. In India, the beetles were mass-reared and released to good effect on coconut to control *A. destructor*, and on citrus to control *Parlatoria zizyphus* (Lucas) and *A. aurantii* after treatments with the (now defunct) non-persistent pesticide, hexaethyl tetraphosphate [10]. These authors recommended releasing the beetles late in the evening to prevent rapid dispersal, a problem that was also reported from South Africa following augmentative releases that often resulted in failure to achieve control of *A. aurantii* on citrus crops [19]. The problem was partially overcome by introducing eggs rather than adults [20, 92], although a later study [93] concluded that egg introductions were unsuitable for field release, the adult being the most suitable stage. Our experience has been that eggs can be useful for glasshouse introductions, particularly against species of Coccidae [26, 54].

As noted above, adult coccinellids in crop production, forestry and ornamental systems have a tendency to disperse, even on occasions when habitat and target pest populations have been apparently favourable for their reproductive success (e.g. [19, 42]). Male coccinellids have been found to respond to aggregates of conspecific females rather than their prey [94] and thus the key to successful colonization by ladybird beetles must lie in understanding what attracts the females into crops and ensuring that they are provided with the necessary cues to oviposit. More research is indicated for this issue but work to date suggests that visual, olfactory and gustatory cues from the host plant and/or the target prey are important in this respect [61, 67–69].

Table 7 Pesticide tolerance in *C. nigritus*

Active ingredient	Stage(s) tested	Outcome	Recommendations	Reference
Triazophos	Adults (field trials)	Mortality in adults for up to 4 days and up to 15 days in last instars	Apply at 428 g/l (AI) : 60 ml/100 litres dose twice (5 weeks apart) rather than 125 ml/100 litres	[48]
	Larvae and adults (glasshouse trials)	Evidence of some resistance evolution	Not enough resistance to warrant breeding a resistant strain	[97]
	Larvae and adults (laboratory trials)			
	Adults (susceptibility trials)			
Tartar emetic	Adults (field trials)	No residual effect	Suitable for IPM	[48]
Chlorpyrifos	Adults (field trials)	No residual effect	Suitable for IPM	[48]
Chlorpyrifos plus mineral oil	Adults (field trials)	No residual effect	Suitable for IPM	[48]
Mineral oil	Adults (field trials)	No residual effect	Suitable for IPM	[48]
Buprofezin	Eggs laid, egg viability	Reduced fecundity and egg hatch	Not suitable for IPM	[99]
		Residual effect (19 weeks)		
	Larvae	Increased larval mortality		[100]
	Adults	No effect on mortality		
Triflumuron	Eggs laid, egg viability	Reduced fecundity and egg hatch	Not suitable for IPM	[99]
		Residual effect (19 weeks)		
	Larvae	Increased larval mortality		[100]
	Adults	No effect on mortality		
Pyriproxyfen	Eggs laid, egg viability	Reduced fecundity and egg hatch	Not suitable for IPM	[99]
		Residual effect (19 weeks)		
	Larvae	Increased larval mortality		[100]
	Adults	No effect on mortality		

In spite of the fact that *C. nigritus* appears to have well-defined seasonal peaks of activity in its native range [10, 28, 37, 38, 95, 96], sometimes anecdotally [16] and sometimes causally linked to prey abundance [28], surprisingly few guidelines exist in the literature for the timing of releases during attempts at classical or augmentative biological control. In South Africa, releases of 1000 adults in April 1981 in the Northern Transvaal led to a 'remarkable' reduction in *A. aurantii* levels [19], while the introduction of 20 000 eggs on 25 citrus between December and February 1984 at Nelspruit resulted in zero establishment of *C. nigritus*, probably because of low prey density [20]. A similar, simultaneous introduction of 60 000 eggs at Komatipoort (December to March, 1984) resulted in good establishment of the beetle and control of the pest without the need for pesticides during 1985 and 1986 [20]. Where known, release dates have been included in Table 1 but despite considerable literature searches, no specific guidelines for the timing of augmentative releases in terms of target prey species, population density and structure, or crop growth stage have been found for any country where the beetle is native or has been established. The beetles do not overwinter in Mediterranean regions [29] or temperate glasshouse/indoor landscape environments [26] and thus are suitable only for augmentative biocontrol programmes during the summer months.

Of considerable interest in IPM programmes is the ability of *C. nigritus* to survive applications of pesticides. Table 7 summarizes specific work carried out with this species while Iperiti [98] provides a more comprehensive summary of toxicity effects of pesticides on European Coccinellids. Generally, these studies show that short-lived pesticides and longer-lived carbamates are suitable for IPM programmes including coccinellids, whereas persistent pesticides of all types, other than carbamates, are unsuitable. Insect growth regulators appear to be particularly disruptive over long periods [99, 100]. Research on the effects of recently approved pesticides is indicated.

***C. nigritus* as a Glasshouse BCA**

Only one account of trials on the efficacy of *C. nigritus* as a BCA in glasshouses has been found during literature searches [26] despite the fact that it has been available from biocontrol companies for this purpose in temperate regions since at least 1987 [101]. The beetle was introduced into the UK for this purpose in 1992 from the International Institute of Biological Control, Rawalpindi and released under licence in glasshouses during the summer of 1993, viz., the Royal Botanic Gardens, Kew (Palm House, Princess of Wales Conservatory (POWC) and the Temperate House) and the Plantasia Complex,

Swansea [26] (Table 1). Introductions were made both as eggs and adults against a range of armoured and soft scale species. At Kew, plants were monitored for scale insect infestations before and after introductions, while at Swansea, analysis relied on occasional observations. The beetle established during the period from July to September in the Palm House, July to October in the POWC (Tropical Wet zone) and from July to August in the Temperate House. Pest populations declined slightly during the sampling period, but it was not clear if this was the result of beetle activity or other factors. *C. nigrinus* failed to establish at all in the tropical arid zone of the POWC although this was probably because the practice of hosing cacti down to remove mealy bug infestations continued during the course of the trials. This practice was also used in the other houses and probably contributed to failure of the beetle to establish until late June when hosing was stopped for a trial period [26]. The introduction of eggs and adults at Plantasia in Swansea resulted in rapid establishment and substantial control of all scale insect species present. Such big differences in control success, despite similar temperature and humidity regimes and scale population levels, indicate that factors other than climate have a substantial impact on the utility of BCAs.

Temperature and humidity data from beetles of Pakistani origin suggest that in glasshouses and indoor landscapes in temperate regions, mean daily temperatures need to be at least 20°C [36, 43] but in excess of 22°C in order to maximize its efficiency [40]. It is also recommended that if eggs are introduced, temperatures should ideally be temporarily maintained at 24°C or above in order to reduce first instar mortality [36] and the practice of hosing down avoided during the period of establishment. In the UK, this would restrict the use of *C. nigrinus* from May to early October in cool temperate glasshouses and from March to late October for hothouse conditions [26]. Most commercial outlets for the beetle in temperate climates recommend releasing between 10 and 25 beetles or larvae per plant. Depending on the size of the plant, this may be an adequate introduction rate but glasshouse trials with heterogeneous plant and scale populations suggest minimum introduction rates of about 1.5 eggs and/or adults per square metre [26].

More research is needed to optimize the use of *C. nigrinus* in glasshouses.

Hemisarcoptes* and *C. nigrinus

Astigmatid mites from the genus *Hemisarcoptes* Lignières are important generalized obligate predators and/or ectoparasites of diaspidid scale insects [102]. They occur on all continents and offer significant potential as BCAs with rates of infestation of up to 75% having been recorded in the field on some scale species [103]. The mites are multivoltine with four mobile stages normally occurring (lava, protonymph, tritonymph and adult) but an

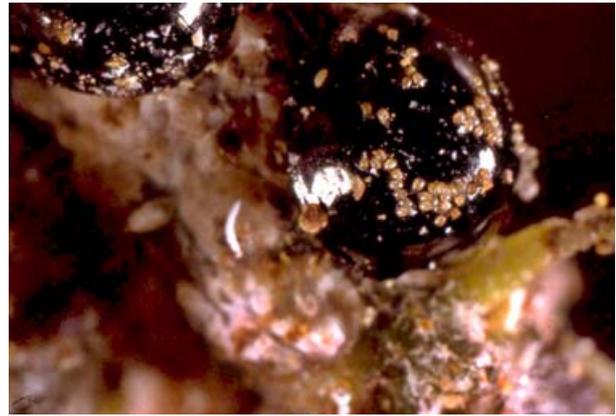


Figure 1 *Chilocorus nigrinus* adult with heavy infestation of *Hemisarcoptes* mites on the upper surface of the elytra

extra nymphal instar occurs in approximately 6% of individuals [102, 104]. This is a sclerotized entomophagous deutonymph (hypopus) that is environmentally resistant and specialized for a stenoxenic relationship with adult beetles of the genus *Chilocorus* [102, 104]. The beetles then act as a dispersal agent for the mite between scale insect patches (within-patch dispersal being effected by the other mite stages). Laboratory studies have shown that the deutonymphs of *Hemisarcoptes cooremani* (Thomas) attach themselves to the undersides of the elytra of adult *Chilocorus cacti* L. and maintain contact for periods lasting from 5 to 21 days, even if scale prey are plentiful [105]. The hypopi do not survive if no contact is made with the beetle and evidence from studies using radioactively labelled tritium demonstrate that they assimilate fluids from their host, probably through the discoidal suckers of the attachment organ, suggesting that the relationship is parasitic rather than phoretic [106]. However, a later study provided evidence that the beetles also obtain some fluids from the mite and the relationship is now considered to be mutualistic [107]. There are apparently no detrimental effects on the beetle other than when large numbers of hypopi make the beetle sluggish [102]. This 'two predator model' offers significant potential for biocontrol programmes but not all species of *Chilocorus* are suitable hosts for the mite because of subelytral setae that can prevent the attachment of the caudal sucker [108]. With one exception [26] no records have been found of this relationship occurring with *C. nigrinus*. Beetles obtained from Pakistan were highly suited as 'hosts' for an unidentified species of mite because they often became so densely populated that no space was left on the subelytral surface and the hypopi were forced to adhere in large numbers to the external surfaces of the elytra [26] (see Figure 1). Neither time nor funding allowed further study of the mite but it proved extremely effective as a control of *A. cyanophylli* on potato tubers, rendering a culture of the scale completely unsuitable for rearing the beetle when it was accidentally introduced

(personal observation). The lack of reports of *Hemisarcoptes* species preying on diaspidids in regions where *C. nigrinus* is native or endemic suggests that either they are being overlooked, or the relationship is relatively rare. Experiments to examine the bionomic impact and the relationship between *C. nigrinus* and *Hemisarcoptes* species are strongly indicated.

Conclusion

C. nigrinus has proved to be one of the most effective coccinellid BCAs of economically important soft and armoured scale insects, most likely because it has few natural enemies; is able to exploit every stage of the host prey; exhibits very limited negative responses to intra-specific competition; has an excellent capacity to coexist in stable relationships with parasitoids; is extremely voracious; lends itself well to mass rearing and has an outstanding propensity for a rapid numerical response. However, as with most BCAs, it sometimes fails to exert an effect on pest populations, even when conditions are apparently favourable. Its climatic requirements are now generally well defined but more research is urgently needed in order to elucidate the complex relationship that the beetle exhibits across trophic levels with its prey, the host plant and its endosymbionts. Few guidelines exist in the public domain for augmentative use in its native range (including conservation) and there is circumstantial evidence for the existence of differing ecotypes or biotypes, particularly in relation to abiotic requirements and interactions with prey species. These shortfalls in knowledge need to be explored and the implications for its utilization as a BCA determined. The population structure of the prey clearly affects the beetles' ability to colonize prey patches and research is needed to determine the cues used by the adult females to find prey patches and stimulate oviposition. Such research offers the potential for manipulation of beetle populations using 'push-pull' strategies. In particular, the relationship between *C. nigrinus* and *Hemisarcoptes* mites and other natural enemies of scale insects should be explored because it may have strong potential for suppressing pest populations. *C. nigrinus* also has excellent potential for use in IPM programmes but data relating to its integration with modern pesticides are limited and more research is indicated.

Acknowledgements

I wish to thank David Bedford from the Canterbury Christ Church University (CCCU) Library and Val McAtear from the Royal Entomological Society Library for their excellent service. I thank Samantha Peterson, Rena Boothe, Joe Burman, Jennie Teague and John Hills, from CCCU and Dr Mike Copland, Imperial College, for their respective inputs. I also thank Dr Derya Senal and Hakan Kumru for their help in interpreting Dr Senal's thesis.

References

1. Woglum RS. Report of a trip to India and the Orient in search of natural enemies of the citrus whitefly. Bulletin No. 120. Bureau of Entomology, United States Department of Agriculture, Washington, DC; 1913. p. 9–53.
2. Reyne A. Studies on a serious outbreak of *Aspidiotus destructor rigidus* on the coconut-palms of Sangi (North Celebes). Tijdschrift Voor Entomologie 1948;89:83–123.
3. Hutson JC. The coconut scale (*Aspidiotus destructor*). Tropical Agriculture in Ceylon 1933;80:254–6.
4. Vesey-Fitzgerald D. Progress on the control of coconut-feeding Coccidae on coconut palms in The Seychelles. Bulletin of Entomological Research 1941;32:405–13.
5. Vesey-Fitzgerald D. Review of the biological control of coccids on coconut palms in The Seychelles. Bulletin of Entomological Research 1953;44:405–13.
6. Moutia LA. The Sugar cane scale, *Aulacaspis tegalensis* Zehl. Bulletin of Entomological Research 1944;35:69–77.
7. Moutia LA, Mamet R. A review of 25 years of economic entomology in the island of Mauritius. Bulletin of Entomological Research 1946;36:439–72.
8. Bennett FD, Hughes IW. Biological control of insect pests in Bermuda. Bulletin of Entomological Research 1959; 50:423–36.
9. Smith HS, Flanders SE. Recent introductions of entomophagous insects into California. Journal of Economic Entomology 1949;42:995–6.
10. Tirumala Rao v, Leela David A, Mohan Rao KR. Attempts at the utilisation of *Chilocorus nigrinus* (F.) (Coleoptera: Coccinellidae) in the Madras State. Indian Journal of Entomology 1954;16:205–9.
11. Greathead DJ. A Review of Biological Control in the Ethiopian Region. Commonwealth Institute of Biological Control Technical Communication No. 5. Commonwealth Agricultural Bureaux, Farnham, UK; 1971. 162 p.
12. Oriens AJE. Report on a visit to Diego Garcia. Revue Agricole et Sucrière de L'île Maurice 1959;38:127–43.
13. Davis CJ. Recent introductions for biological control in Hawaii IV. Proceedings of the Hawaiian Entomological Society 1959;17:62–6.
14. Leeper JR. A Review of the Hawaiian Coccinellidae. Proceedings of the Hawaiian Entomological Society 1976;22:279–306.
15. Greathead DJ, Pope RD. Studies on the biology and taxonomy of some *Chilocorus* spp. (Coleoptera: Coccinellidae) preying on *Aulacaspis* spp. (Homoptera: Diaspididae) in East Africa, with the description of a new species. Bulletin of Entomological Research 1977;67: 259–70.
16. Ahmad R. Studies in West Pakistan on the biology of one nitidulid species and two coccinellid species (Coleoptera) that attack scale insect species (Homoptera: Coccoidea). Bulletin of Entomological Research 1970;60:5–16.
17. Davis CJ. Recent introductions for biological control in Hawaii XVII. Proceedings of the Hawaiian Entomological Society 1972;21:187–90.
18. Chazeau J. La lutte biologique contre la cochenille transparente du cocotier *Temnaspidiotus destructor*

18 Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources

- (Signoret) aux Nouvelles-Hébrides (Homoptera: Diaspididae). Cahiers O.R.S.T.O.M. Série Biologique 1981;44:11–22.
19. Samways MJ. Biology and economic value of the Scale Insect Predator *Chilocorus nigritus* (F.) (Coccinellidae). Biocontrol News and Information 1984;5:91–105.
20. Samways MJ. Combined effect of natural enemies (Hymenoptera: Aphelinidae and Coleoptera: Coccinellidae) with different niche breadths in reducing high populations of red scale *Aonidiella aurantii* (Maskell) (Homoptera: Diaspididae). Bulletin of Entomological Research 1986; 76:671–83.
21. Kinawy MM. Biological control of the coconut scale insect (*Aspidiotus destructor* Sign., Homoptera: Diaspididae) in the southern region of Oman (Dhofar). Tropical Pest Management 1991;37:387–9.
22. Samways MJ. Climate diagrams and biological control: an example from the aerogeography of the ladybird *Chilocorus nigritus* (Fabricius, 1798) (Insecta, Coleoptera, Coccinellidae). Journal of Biogeography 1989;16:345–51.
23. Argov Y, Rossler Y. Introduction of insects into Israel for the control of insect pests. Phytoparasitica 1988;16:303–15.
24. Muralidharan CM. Scale insects of the date palm (*Phoenix dactylifera*) and their natural enemies in the date groves of Kachchh. Gujarat Plant Protection Bulletin 1993;45:31–3.
25. Mani M. Studies on the natural enemies of the wax scale *Drepanococcus chiton* (Green) on ber and guva. Entomon 1995;20:55–8.
26. Ponsonby DJ. Biological control of glasshouse scale insects using the coccinellid predator, *Chilocorus nigritus* [PhD. thesis]. Wye College, University of London, London; 1995.
27. Longo S, Mazzeo G, Russo A, Siscaro G. *Aonidiella citrina* (Coquillet) nuovo parassita degli agrumi in Italia. Infamatore Fitopatologico 1994;44:19–25.
28. Mani M, Krishnamoorthy A. Suppression of the soft green scale *Coccus viridis* (Green) on acid lime in India. Advances in IPM for horticultural crops. In: Proceedings of the 1st National Symposium on Pest Management in Horticultural Crops: Environmental Implications and Thrusts, 15–17 October 1997, Bangalore, India. Association for the Advancement of Pest Management in Horticultural Ecosystems, Indian Institute of Horticultural Research, Bangalore; 1998. p 210–2.
29. Senal D. Avci böcek *Chilocorus nigritus* (Fabricius) (Coleoptera: Coccinellidae)'un bazı biyolojik ve ekolojik özellikleri ile doğaya adaptasyonu üzerinde araştırmalar [PhD thesis]. Department of Plant Protection, Institute of Natural and Applied Sciences, University of Çukurova, Turkey; 2006.
30. Omkar, Pervez A. Ecology and biocontrol potential of a scale-predator, *Chilocorus nigritus*. Biocontrol Science and Technology 2003;13:379–90.
31. Samways MJ, Osborn R, Hastings H, Hattingh V. Global climate change and accuracy of prediction of species' geographical ranges: establishment success of introduced ladybirds (Coccinellidae, *Chilocorus* spp.) worldwide. Journal of Biogeography 1999;26:795–812.
32. Walter H. Vegetation of the Earth in relation to Climate and Eco-physical Conditions. Springer-Verlag, New York; 1973.
33. Walter H. Vegetation of the Earth and Ecological Systems of the Geobiosphere. 3rd ed. Springer-Verlag, Berlin; 1984.
34. Anon. Climate, Plant and Animal Life in Oman. In Encyclopaedia Britannica, Deluxe Millenium Edition [on CD-ROM]. Focus Multimedia, Rugeley, UK; 2000.
35. Hattingh V, Samways MJ. Physiological and behavioural characteristics of *Chilocorus* spp. (Coleoptera: Coccinellidae) in the laboratory relative to effectiveness in the field as biocontrol agents. Journal of Economic Entomology 1994; 87:31–8.
36. Ponsonby DJ, Copland MJW. Effect of temperature on development and immature survival in the Scale Insect Predator, *Chilocorus nigritus* (F.) (Coleoptera: Coccinellidae). Biocontrol Science and Technology 1996;6:101–9.
37. Ketkar SM. Mass assemblage of the coccinellid beetle *Chilocorus nigritus* Fabr. on Banyan trees in Poona. Science and Culture 1959;25:273.
38. Ghani A, Muzaffar N. Relations between the Parasite–Predator Complex and the Host Plants of Scale Insects in Pakistan. Commonwealth Institute of Biological Control Miscellaneous Publication No. 5. Commonwealth Agricultural Bureaux, Farnham, UK; 1974. 92 p.
39. Pandian G, Murali Baskaran RK, Muthulakshmi S, Mahadevan NR. Thermal requirement of black beetle, *Chilocorus nigrita*. Indian Journal of Agricultural Sciences 2002;72:373–5.
40. Ponsonby DJ, Copland MJW. Maximum feeding potential of larvae and adults of the Scale Insect Predator, *Chilocorus nigritus* (F.) with a new method of estimating food intake. BioControl 2000;45:295–310.
41. Hodek I. Biology of the Coccinellidae. W. Junk, The Hague, The Netherlands; 1973.
42. Hodek I, Honěk A. Ecology of the Coccinellidae. Kluwer Academic Publishers, Dordrecht, The Netherlands; 1996.
43. Ponsonby DJ, Copland MJW. Environmental influences on fecundity, egg viability and egg cannibalism in the Scale Insect Predator, *Chilocorus nigritus* (F.) (Coleoptera: Coccinellidae). BioControl 1998;43:39–52.
44. Michaud JP. On the assessment of prey suitability in aphidophagous Coccinellidae. European Journal of Entomology 2005;102:385–90.
45. Lin Y, Peng YK, Chen SZ. Studies on the bionomics of *Aulacaspis citri* Chen and its control. Journal of Southwest Agricultural University 1997;19:442–6.
46. Lima IMM, Gama ES. Record of host plants (Cactaceae) and new dissemination strategy of *Diaspis echinocacti* (Bouché) (Homoptera: Diaspididae), prickly-pear-scale, in the States of Pernambuco and Alagoas, Brazil. Neotropical Entomology 2001;30:479–81.
47. Dorge SK, Dalaya VP, Prahdan AG. Studies on two predatory coccinellid beetles, *Pharoscyrnus horni* Weise and *Chilocorus nigritus* (F.), feeding on sugar cane scales *Aspidiotus glomeratus* G. Labdev. Journal of Science and Technology 1972;10B:138–41.
48. Bruwer IJ, Schoeman AS. Residual toxicity of four citrus insecticides in South Africa to the scale predator *Chilocorus nigritus* I (Coleoptera: Coccinellidae). Journal of Economic Entomology 1988;81:1178–80.
49. Bruwer IJ, Schoeman AS. Key factor analysis of two populations of the long mussel scale, *Inulaspis gloveri*

- (Packard) (Hemiptera: Diaspididae). Journal of the Entomological Society of South Africa 1990;53:101–5.
50. Baskaran RK, Suresh K. Comparative biology and predatory potential of black beetle, *Chilocorus nigrita* (Fab.) on three scale insects. Journal of Entomological Research 2006; 30:159–64.
 51. Samways MJ, Wilson SJ. Aspects of the feeding behaviour of *Chilocorus nigritus* (F.) (Coleoptera: Coccinellidae) relative to its effectiveness as a biocontrol agent. Journal of Applied Entomology 1988;106:177–82.
 52. Hattingh V, Samways MJ. A forced change in prey type during field introductions of coccidophagous biocontrol agents *Chilocorus* species (Coleoptera: Coccinellidae): is it an important consideration in achieving establishment. In: Polgár L, Chambers RJ, Dixon AFG, Hodek I, editors. Behaviour and Impact of Aphidophaga. Academic Publishing, The Hague, Netherlands; 1991. p. 143–8.
 53. Hattingh V, Samways MJ. Prey choice and substitution in *Chilocorus* spp. (Coleoptera: Coccinellidae). Bulletin of Entomological Research 1992;82:327–34.
 54. Ponsonby DJ, Copland MJW. Aspects of prey relations in the coccidophagous ladybird *Chilocorus nigritus* relevant to its use as a biological control agent of scale insects in temperate glasshouses. BioControl 2007;52:629–40.
 55. Puttarudrah M, Channabasavanna GP. Beneficial insects of Mysore – I. Indian Journal of Entomology 1953;15:87–96.
 56. Puttarudrah M, Channabasavanna GP. Beneficial insects of Mysore – II. Indian Journal of Entomology 1955;17:1–5.
 57. Omkar, Bind RB. Records of aphid natural enemies complex of Uttar Pradesh. IV. The coccinellids. Journal of Advanced Zoology 1995;16:67–71.
 58. Hill MG, Blackmore PJM. Interactions between the Coccid *Icerya seychellarum* on Aldabra Atoll. Oecologia 1980; 45:360–5.
 59. Muralidharan CM. Biology and feeding potential of black beetle (*Chilocorus nigritus*), a predator of the date palm scale (*Parlatoria blanchardii*). Indian Journal of Agricultural Sciences 1994;64:270–1.
 60. Hattingh V, Samways MJ. Evaluation of artificial diets and two species of natural prey as laboratory food for *Chilocorus* spp. Entomologia Experimentalis et Applicata 1993;69:13–20.
 61. Ponsonby DJ, Copland MJW. Influence of host density and population structure on egg production in the coccidophagous ladybird, *Chilocorus nigritus* F. (Coleoptera: Coccinellidae). Agricultural and Forest Entomology 2007;9:287–98.
 62. Dixon AFG. Insect Predator–Prey Dynamics: Ladybird Beetles and Biological Control. Cambridge University Press, Cambridge, UK; 2000.
 63. Evans EW. Searching and reproductive behaviour of female aphidophagous ladybirds (Coleoptera: Coccinellidae): a review. European Journal of Entomology 2003;100:1–10.
 64. Růžička Z. Recognition of oviposition-detering allomones by aphidophagous predators. European Journal of Entomology 1997;94:431–4.
 65. Růžička Z. Oviposition-detering effects of conspecific and heterospecific larval tracks on *Cheilomenes sexmaculata* (Coleoptera: Coccinellidae). European Journal of Entomology 2006;103:757–63.
 66. Fréchette B, Dixon AFG, Alazuet C, Hemptinne JL. Age and experience influence patch assessment for oviposition by an insect predator. Ecological Entomology 2004;29:578–93.
 67. Hattingh V, Samways MJ. Visual and olfactory location of biotopes, prey patches and individual prey by the ladybeetle, *Chilocorus nigritus*. Entomologia Experimentalis et Applicata 1995;75:87–98.
 68. Ponsonby DJ, Copland MJW. Olfactory responses by the scale insect predator *Chilocorus nigritus* (F.) (Coleoptera: Coccinellidae). Biocontrol Science and Technology 1995;5:83–93.
 69. Boothe RA, Ponsonby DJ. Searching behaviour in *Chilocorus nigritus* (F.) (Coleoptera: Coccinellidae). Communications in Agricultural and Applied Biological Sciences 2006;71:253–61.
 70. Hattingh V, Samways MJ. Absence of intraspecific interference during feeding by the predatory ladybirds *Chilocorus* spp. (Coleoptera: Coccinellidae). Ecological Entomology 1990;15:385–90.
 71. Jalali SK, Singh SP. Biotic potential of three coccinellid predators on various diaspine hosts. Journal of Biological Control 1989;3:20–3.
 72. Debach P, Rosen D. Biological Control by Natural Enemies. 2nd ed. Cambridge University Press, Cambridge, UK; 1991.
 73. Timms JE, Oliver TH, Straw NA, Leather SR. The effects of host plant on the coccinellid functional response: Is the conifer specialist *Aphidecta oblitterata* (L.) (Coleoptera: Coccinellidae) better adapted to spruce than the generalist *Adalia bipunctata* (L.) (Coleoptera: Coccinellidae)? Biological Control 2008;47:273–81.
 74. Boothe RA, Ponsonby DJ, Preziosi RF, Copland MJW. Tritrophic effects on survival and development in a coccidophagous coccinellid. Communications in Agricultural and Applied Biological Sciences 2008;73:383–93.
 75. Majerus MEN. Ladybirds. Harper Collins, London, UK; 1994.
 76. Drea JJ, Gordon RD. Predators: Coccinellidae. In: Rosen D, editor. World Crop Pests Volume 4B. Armoured Scale Insects: Their Biology, Natural Enemies and Control. Elsevier, Amsterdam, The Netherlands; 1990. p. 19–40.
 77. Hurst GDD, Majerus MEN, Walker LE. Cytoplasmic male killing elements in *Adalia bipunctata* (Linnaeus) (Coleoptera: Coccinellidae). Heredity 1992;69:84–91.
 78. Hurst GDD, Majerus MEN, Walker LE. The importance of cytoplasmic male killing elements in natural populations of the two-spot ladybird, *Adalia bipunctata* (Linnaeus) (Coleoptera: Coccinellidae). Biological Journal of the Linnean Society 1993;49:195–202.
 79. Weinert LA, Tinsley MC, Temperley M, Jiggins M. Are we understanding the diversity and incidence of insect bacterial symbionts? A case study in ladybird beetles. Biology Letters 2007;3:678–81.
 80. Samways MJ, Tate BA. Sexing of *Chilocorus nigritus* (F.) (Coccinellidae). Citrus and Subtropical Fruit Journal 1984;607:4–5.
 81. Henderson SA, Albrecht JSM. Abnormal and variable sex ratios in population samples of ladybirds. Biological Journal of the Linnean Society 1988;35:275–96.
 82. Erichsen C, Samways MJ, Hattingh V. Reaction of the ladybird *Chilocorus nigritus* (F.) (Coleoptera: Coccinellidae)

- to a doomed food resource. *Journal of Applied Entomology* 1991;112:493–8.
83. Burman JP. *Wolbachia* infection found in the important biological control agent *Chilocorus nigritus*: implications for current control systems [dissertation], Canterbury Christ Church University, Canterbury, UK; 2007.
 84. Majerus MEN. The impact of male-killing bacteria on the evolution of aphidophagous coccinellids. *European Journal of Entomology* 2006;103:1–7.
 85. Kyei-Poku GK, Floate KD, Benkel B, Goettel MS. Elimination of *Wolbachia* from *Urolepis rufipes* (Hymenoptera: Pteromalidae) with heat and antibiotic treatments: implications for host reproduction. *Biocontrol Science and Technology* 2003;13:341–54.
 86. Floate KD, Kyei-Poku GK, Coghlin PC. Overview and relevance of *Wolbachia* in biocontrol research. *Biocontrol Science and Technology* 2006;16:767–88.
 87. Majerus MEN, Hinrich J, Schulenburg GVD, Zakharov IA. Multiple causes of male-killing in a single sample of the two-spot ladybird, *Adalia bipunctata* (Coleoptera: Coccinellidae). *Heredity* 1994;84:605–9.
 88. Sadakathulla S. Technique of mass production of the predatory coccinellid, *Chilocorus nigritus* (Fabricius) on coconut scale, *Aspidiotus destructor* Sign. *Indian Coconut Journal* 1993;23:12–13.
 89. Samways MJ, Tate BA. Mass rearing of the scale predator *Chilocorus nigritus* (F.) (Coccinellidae). *Citrus and Subtropical Fruit Journal* 1986;630:9–14.
 90. Evans EW, Stevenson AT, Richards DR. Essential versus alternative foods of insect predators: benefits of a mixed diet. *Oecologia* 1999;121:107–21.
 91. Baskaran RK, Suresh K. Influence of semisynthetic diets and non-living substrata on fecundity of Black Beetle, *Chilocorus nigrita* (Fabricius) (Coleoptera: Coccinellidae). *Journal of Entomological Research* 2007;31:243–6.
 92. Samways MJ, Mapp J. A new method for the mass introduction of *Chilocorus nigritus* (F.) (Coccinellidae) into citrus orchards. *Citrus and Subtropical Fruit Journal* 1983;598:4–6.
 93. Hattingh V, Samways MJ. Determination of the most effective method for field establishment of biocontrol agents of the genus *Chilocorus* (Coleoptera: Coccinellidae). *Bulletin of Entomological Research* 1991;81:169–74.
 94. Hemptinne JL, Lognay G, Dixon AFG. Searching behaviour and mate recognition by males of the two-spot ladybird beetle, *Adalia bipunctata*. *Ecological Entomology* 1996;21:165–70.
 95. Muralidharan CM, Sodagar NN, Ramdevputra MV, Patel PK. Date palm scale (*Parlatoria blanchardii*), a new host for black beetle (*Chilocorus nigritus*). *Indian Journal of Agricultural Sciences* 1992;62:720–1.
 96. Mani M, Ganga Visalakshy PN, Krishnamoorthy A, Venugopalan R. Role of *Coccophagus* sp. in the suppression of the soft green scale *Coccus viridis* (Green) (Homoptera: Coccidae) on spota. *Biocontrol Science and Technology* 2008;18:721–5.
 97. Schoeman AS. Susceptibility of *Chilocorus nigritus* (F.) to triazophos (Coleoptera:Coccinellidae). *Journal of the Entomological Society of South Africa* 1987;50:371–4.
 98. Iperti G. Biodiversity of predaceous Coccinellidae in relation to bioindication and economic importance. *Agriculture, Ecosystems and Environment* 1999;74:323–42.
 99. Hattingh V, Tate B. Effects of field-weathered residues of insect growth regulators on some Coccinellidae (Coleoptera) of economic importance as biocontrol agents. *Bulletin of Entomological Research* 1995;85:489–93.
 100. Magagula CN, Samways MJ. Effects of growth regulators on *Chilocorus nigritus* (Fabricius) (Coleoptera: Coccinellidae), a non-target natural enemy of citrus red scale, *Aonidiella aurantii* (Maskell) (Homoptera: Diaspididae) in Southern Africa: evidence from laboratory field trials. *African Entomology* 2000;8:47–56.
 101. Steiner MY, Elliot DP. *Biological Pest Management for Interior Plantscapes*. 2nd ed. Alberta Environmental Centre, Vegreville, AB, Canada; 1987.
 102. Gerson U, O'Connor BM, Houck MA. Acari. In: Rosen D, editor. *World Crop Pests Volume 4B. Armoured Scale Insects: Their Biology, Natural Enemies and Control*. Elsevier, Amsterdam, The Netherlands; 1990. p. 77–97.
 103. Smirnoff WA. La cochenille du palmier dattier (*Parlatoria blanchardii* Targ.) en Afrique du Nord, comportement, importance économique, prédateurs et lutte biologique. *Entomophaga* 1957;2:1–98.
 104. Houck MA, O'Connor BM. Ontogeny and life-history of *Hemisarcoptes cooremani* (Acari, Hemisarcoptidae). *Annals of the Entomological Society of America* 1993;83:869–86.
 105. Houck MA. Adaptation and transition into parasitism from commensalisms: a phoretic model. In: Houck MA, editor. *Mites: Ecological and Evolutionary Analyses of Life History Patterns*. Chapman and Hall, New York; 1994. p. 252–81.
 106. Houck MA, Cohen AC. The potential role of phoresy in the evolution of parasitism: radiolabelling (tritium) evidence from an astigmatid mite. *Experimental and Applied Acarology* 1995;19:677–94.
 107. Holte AE, Houck MA, Collie NL. Potential role of parasitism in the evolution of mutualism in astigmatid mites: *Hemisarcoptes cooremani* as a model. *Experimental and Applied Acarology* 2001;25:97–107.
 108. Houck MA. Phoresy by *Hemisarcoptes* (Acari: Hemisarcoptidae) on *Chilocorus* (Coleoptera: Coccinellidae): influence of subelytral ultrastructure. *Experimental and Applied Acarology* 1999;23:97–118.