

Influence of host density and population structure on egg production in the coccidophagous ladybird, *Chilocorus nigritus* F. (Coleoptera: Coccinellidae)

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- Abstract**
- 1 Studies on aphidophagous coccinellids have indicated that patch quality, and, in particular, the age structure of the prey or the phenological age of the plant, may play an important role in stimulating oviposition behaviour. However, little is known about the egg-laying tactics of coccidophagous species.
 - 2 When restricted to a single, large colony of overlapping generations of their diaspid host *Abgrallaspis cyanophylli* (Signoret), adult females of the coccidophagous ladybird *Chilocorus nigritus* F. varied their egg production rate in a cyclic pattern that lasted for approximately 22 days. This information was used to generate hypotheses relating to eliciting cues for oviposition.
 - 3 Two experiments were carried out in the absence of conspecific larvae to assess: (i) whether changes in host density at levels above those needed to sustain egg production in the females affected egg output and (ii) the effect of restricting beetles to various homogeneous prey population structures on daily egg production.
 - 4 Changes in host density caused a significant but transient decline in egg production whereas a heterogeneous prey population elicited significantly higher levels of oviposition than homogeneous ones with similar host densities, irrespective of the growth stage of the prey.
 - 5 Beetles were fed to satiation throughout the experiments but the data obtained suggest that cues for eliciting oviposition operated in a density-dependent fashion. The results of the study are used to discuss the possibility that visual and chemical cues are quantitatively used by ovipositing beetles to assess patch suitability.

Keywords *Abgrallaspis cyanophylli*, *Chilocorus nigritus*, Coccinellidae, oviposition cues.

Introduction

Adult coccinellids are highly mobile (Evans, 2003) and have been notoriously difficult to establish in crop production, forestry and ornamental systems due to their tendency to disperse, even on occasions when habitat and target pest populations have been apparently favourable for their reproductive success (Samways, 1984; Frazer & Raworth, 1985;

Hodek & Honek, 1996). Male coccinellids are apparently more likely to respond to aggregates of conspecific females than their aphid hosts (Hemptinne *et al.*, 1996) and thus the key to successful colonization by coccinellids must lie in attracting females into a favourable habitat and providing them with the necessary cues to oviposit. Traditional models for ladybird reproduction predict that well-fed females lay more eggs and become less active, resulting in eggs being laid in the proximity of prey colonies (Dixon, 1959, 1970), but more recent studies on aphidophagous species have led to theories 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 (Dixon, 2000).

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Indeed, there is much evidence from field studies to support an 'egg window hypothesis' where females optimize their fitness by choosing oviposition sites that support a theoretical minimum density of suitable aphid hosts for their offspring (Dixon, 2000; Evans, 2003). Dixon (2000) suggests that the minimum density of aphids required to support the survival of the first-instar larvae might determine the opening of the egg window. Recent studies have shown that oviposition-detering pheromones from conspecific larvae (Růžička, 1997, 2006), age and experience (Fréchette *et al.*, 2004) are also important factors in such a model, and it is assumed that the adults' reaction to the larval tracks act to close the window (Dixon, 2000). All of these findings are consistent with the application of optimal foraging theory to oviposition behaviour as proposed by Mangel (1987). However, as described above, females often disperse from patches even when prey numbers are high, leading to recent suggestions that they may have an innate bet-hedging dispersal mechanism that is independent of local conditions (Evans, 2003). This may be the case with aphidophagous species where it is assumed that the ephemeral and unpredictable nature of the host causes female beetles to move rapidly between sites and habitats (Evans, 2003) but little is known about the egg-laying tactics of coccidophagous species (Dixon, 2000) whose prey tend to be more enduring. Furthermore, recent work has shown that aphid colonies were randomly distributed in space whereas coccids formed smaller, aggregated colonies (Borges *et al.*, 2006), suggesting that differing spatial, as well as temporal, patterns of prey distribution may select for differing host location and ovipositing behaviours in coccidophagous coccinellids. Thus, any information that might explain dispersal mechanisms or provide information as to the necessary cues required to retain ladybirds in a habitat and stimulate oviposition would be extremely valuable to conservation and pest management programmes.

Chilocorus nigritus (F.) is native to the Indian subcontinent and south-east Asia where it is an important component in the natural enemy complex attacking diaspid scale insect pests (Samways, 1984; Omkar & Pervez, 2003). It has also been used in classical biological control projects in numerous tropical and subtropical locations (Omkar & Pervez, 2003), as well as temperate glasshouses (Ponsonby, 1995). However, as with many coccinellid species, both native and non-native, not all such projects have been successful, even though the climate was deemed to be suitable (Bennett & Hughes, 1959; Davis, 1972). Thus, there is much interest in determining the ecological requirements of this and other coccidophagous species. During trials for an earlier study on age-specific fecundity in *C. nigritus* (Ponsonby & Copland, 1998), the daily egg production of females was observed to vary in a cyclical manner (unpublished data) that was clearly related to their food source, the biparental strain of the armoured scale insect *Abgrallaspis cyanophylli* (Signoret) (Hemiptera: Diaspididae), a tropicopolitan, multivoltine species, common throughout the same geographical range as *C. nigritus*. The present study began by carrying out post-hoc analysis of the daily egg production data from Ponsonby & Copland (1998), which established the presence of cyclic oviposition behaviour in the absence of conspecific larvae.

These findings were then used to generate hypotheses to further examine egg-laying behaviour in *C. nigritus*.

It is well known that limiting food supplies below a certain threshold reduces egg production (Dixon, 2000; Evans, 2003). Thus, two experiments were devised to test some aspects of ovipositing behaviour at prey densities in excess of those required to provide the daily requirement for sustained egg production. The first of these aimed: (i) to test whether cyclic oviposition behaviour was obligatory and (ii) to examine the effect of changes in host density on daily fecundity. The aim of the second experiment was to determine whether a particular age structure or host stage was required to elicit or maximize egg production.

Materials and methods

Post-hoc analysis was carried out on the daily fecundity data of single female *C. nigritus* paired with males of a similar age and supplied with a dense colony of seven to nine week old overlapping generations of *A. cyanophylli* reared on a potato tuber (*Solanum tuberosum* L.). Data were analysed from beetles maintained at constant temperatures of 24 °C, 26 °C and 30 °C and relative humidity (RH) in the range 61–63%. Insect cultures and methodology were similar to that described in Experiments 1 and 2 below (for full details, see Ponsonby & Copland, 1998). A 'cycle' was deemed to be the period that females were confined to a single *A. cyanophylli* culture. Because these were post-hoc analyses being used for hypothesis generation, this period was not standardized but arbitrarily terminated when beetles ceased ovipositing, at which point a new colony was provided. All eggs were removed daily. Because variations in cycle length occurred due to temperature effects (see below), data were standardized by calculating the proportion of eggs laid in each fifth of the cycle. These data were then tested using the Statview, version 5.0.1 analysis of variance (ANOVA) procedure (SAS Institute, 1998) after carrying out an arcsine transformation (Quinn & Keough, 2002). Means were further separated by using the Statview Tukey–Kramer test procedure (SAS Institute, 1998). Five cycles were analysed from each of ten randomly selected beetles. After examining tubers with scale colonies to assess whether sufficient quantities of all stages of prey remained for at least a further cycle, four beetles were left on their scale colonies after oviposition had ceased to determine whether egg production rates remained at zero, or increased again. Three of these were at 30 °C, and one at 26 °C.

Abgrallaspis cyanophylli cultures

The life cycle of this species when reared on potato tubers has been described by Ponsonby & Copland (2000a, b). Of relevance to the present study is that the developmental period (crawler to crawler) varied from approximately 50 days at 24 °C to 45 days at 30 °C (39 days at 26 °C) (Ponsonby & Copland, 2000a). Females are almost certainly ovoviviparous (Balachowski, 1939) and undergo three moults during their life cycle. They continued ovipositing for 3–4 months at 26 °C

(Ponsonby, 1995). Males underwent four moults, emerging as winged imagos 5–7 days before females began ovipositing (Ponsonby & Copland, 2000b). When reared on potato tubers at 27 °C in the present study, dense colonies of all stages of offspring from the first generation occurred after approximately 7–8 weeks and high densities were supported for 12–13 weeks on average (Ponsonby, 1995). Offspring from the second generation emerged after approximately 10–11 weeks.

Experiment 1: effect of host density on oviposition behaviour, egg cannibalism and egg viability

Adult beetles were obtained from the International Institute of Biological Control, Rawalpindi, Pakistan and reared on the diaspid *A. cyanophylli* cultured on potato tubers (*S. tuberosum* cv. 'Romano') at temperatures of 27 ± 1 °C, under an LD 12 : 12 h photoperiod and a RH of $55 \pm 10\%$. Adult beetles were allowed to oviposit into 100 × 100 mm eight-ply surgical gauze pads laid on top of the potato cultures for 1 week. The resulting eggs were reared in separate cages to adulthood and the emerging imagos were removed on a daily basis and placed under constant light at a temperature of 26 ± 1 °C for 28 days. Beetles of uniform age were then sexed according to the method of Samways & Tate (1984) and placed, one female and one male, into controlled humidity units (CHUs). CHUs consisted of two 1 lb (approximately 450 g) honey jars in tandem, one upright containing a saturated salt solution of ammonium nitrate to provide a constant humidity of $62 \pm 5\%$, the other inverted and containing the beetle imagos along with a small potato tuber (40–50 mm in diameter) infested with 8–9-week-old *A. cyanophylli* (two overlapping generations containing all instars). CHUs containing beetle pairs were incubated at 26 ± 1 °C in illuminated, cooled incubators. Illumination was via six 8-W fluorescent tubes providing a light intensity in the range of 15–25 W/m. All beetles were acclimated to these test conditions for 7 days prior to the experiment, thus providing sufficient time to standardize nutritional status amongst the beetles and ensure that females were ovipositing. Potato/scale colonies were changed for fresh ones every 2–3 days. All of the tuber colonies supplied at that stage were selected with a very high density of scale infestation, using total coverage of the tuber as a criterion. At the start of the experimental run, enough beetles were ovipositing for each of three levels of host density to be replicated six times. Each replicate was then randomly assigned to one of the three treatments. Levels of host density were determined by visually sorting tuber colonies of uniform age and size (40–50 mm in diameter), infested as described above, into three categories

using the criteria: Treatment 1; high prey density: those totally covered by insects (generally white in appearance with a yellow 'film' of crawlers clearly visible; Treatment 2; medium prey density: those with a generally grey appearance where a medium portion of the tuber was infested; and Treatment 3; low prey density: those with a generally brown appearance where relatively few insects were present. Prey densities for each of the treatments were quantified by randomly placing a 10 × 10 mm quadrat onto the surface of the tuber and counting the scale insects present. This process was repeated ten times on ten randomly selected tubers from each treatment and the mean density was calculated. Results are presented in Table 1. To standardize the nutritional status of the female ladybirds and to establish some indication of their natural variability, all replicates were supplied daily with fresh, high-density scale colonies for 9 days. After that period, the various treatment groups were provided daily with scale-infested tubers at the appropriate density for a further 14 days. Earlier experiments (Ponsonby & Copland, 2000b) had shown that, to reach satiation, there was a daily requirement of approximately nine adult female *A. cyanophylli* for ovipositing female imagos and five for males. Thus, beetles in the low-density treatment were provided with tubers upon which there was a minimum of 14 adult female scale insects (although all other immature stages were also present). All tubers were examined after removal for evidence of uneaten scale to confirm that beetles were fed to satiation throughout the experiment.

To assess oviposition behaviour, clean surgical gauze pads (as described above) were cut into quarters and placed inside the CHUs with the beetles to provide them with an ovipositing substrate. Gauze pads were removed daily throughout the experiment and the numbers of eggs were counted. At the same time, the CHU was examined for any eggs laid on the jar surface and the potato was searched for any eggs laid beneath dead scales, amongst the potato sprouts or on the surface of the potato tuber. These were counted and carefully removed from the CHU, a process that usually resulted in their destruction. In case eggs were missed by this process, discarded potato colonies were kept for a further 10 days and examined daily for larval emergence. Eggs laid on the gauze, and any that were successfully removed from the CHU, were incubated at 26 °C in small, ventilated, transparent plastic boxes (173 × 155 × 65 mm) under constant light conditions. Earlier work by Ponsonby (1995) had shown that varying humidity did not affect egg viability and thus eggs were reared at ambient humidity (approximately $30 \pm 10\%$). Egg viability (%) was estimated by counting the number of emerging larvae. Eggs were frequently cannibalized by the parents but

Table 1 Mean \pm SD host density (per cm²) and population structure of 9-week-old *Abgrallaspis cyanophylli* cultures on ten potato tubers selected for use as high, medium and low density treatments (means derived from ten counts on each potato)

	Crawlers	First- and second-instar nymphs	Adult females	Total
High density (Treatment 1)	7.9 \pm 3.2	168.5 \pm 43.9	30.1 \pm 14.0	206.5 \pm 35.4
Medium density (Treatment 2)	5.7 \pm 5.2	72.8 \pm 36.8	8.0 \pm 4.0	86.5 \pm 40.1
Low density (Treatment 3)	2.5 \pm 1.4	32.2 \pm 13.5	2.0 \pm 2.1	36.7 \pm 14.3

these could be detected by the presence of the chorion, which was never observed being fully eaten. Similarly, damaged eggs could be detected by a lack of turgidity and whereas both these and eaten eggs were added to the total of eggs laid, they were removed from the egg viability analysis.

Experiment 2: effect of population age structure on oviposition behaviour, egg cannibalism and egg viability

Abgrallaspis cyanophylli populations of uniform age and structure were inoculated on potato tubers of a uniform size (40–50 mm) by placing them on a mother culture, and reared under the conditions described for Experiment 1 above for 24 h. A strong light source was placed above them, encouraging large numbers of crawlers to migrate to, and settle on, the clean tubers. The freshly inoculated tubers were then removed and incubated in illuminated incubators under the same conditions as the mother culture. Such colonies were initiated on a weekly basis over several weeks to ensure that there was a constant supply of uniformly structured scale populations throughout the experiment.

Ovipositing pairs of *C. nigrinus* uniform age (one of each sex) were reared and acclimated in CHUs under constant light at 26 °C and 62% RH according to the method described for Experiment 1. Enough pairs were ovipositing at the end of the acclimation period to allow the experiment to be replicated five times at each of four levels of population structure. Each replicate was then randomly assigned to one of the four treatments: Treatment 1; control: 8–9-week-old colonies with overlapping generations and all developmental stages present; Treatment 2: settled first-instar nymphs only (males and females); Treatment 3: second-instar nymphs only (males and females); Treatment 4: mated, preovipositing adult females only (male scale covers were left on the colonies because most had enclosed by the start of the experiment). As with Experiment 1, the nutritional status of the females was standardized and their natural variability assessed during the first 10 days of the trial by providing all replicates with colonies as for Treatment 1. After that period, the various treatment groups were provided daily with tubers infested with the appropriate population structure for a further 10 days. To prevent restrictions in food intake and provide comparable host densities to Treatment 1, only colonies with very high densities of the various host stages were provided. Daily egg production, egg viability and egg cannibalism data were gathered as for Experiment 1. The sex of the adult beetles in all experiments was confirmed after trials by dissection and examination of the genitalia.

Statistical analysis

Daily egg production has been shown to vary with age in *C. nigrinus* (Ponsonby & Copland, 1998) and in aphidophagous coccinellids (Dixon & Agarwala, 2002). Therefore, statistical analysis for Experiment 1 was based on the null hypothesis that if there were no effects from the various levels of host density, the curves of daily egg production against time for each treatment would not be significantly different in shape.

A similar null hypothesis was formulated for the effects of the various population age structures in Experiment 2. The SAS 'Repeated Measures' procedure (SAS Institute, 1992) was used to test both hypotheses using multivariate analysis of variance and linear partitioning. Pre-determined orthogonal contrasts were used to test treatment means.

All other results were analysed using Statview v 5.0.1 (SAS Institute, 1998). Between-treatment egg viability data were analysed using the Kruskal–Wallis *k*-sample test on the medians carried out on data from both before and after changes were made. This ensured that any differences due to effects other than host density (Experiment 1) or population structure (Experiment 2) were detected before such changes were made (e.g. experimental error, infertile males or natural variability in the females). Within-treatment analyses (i.e. before and after changes to density or population structure) were carried out using Wilcoxon's signed rank test after first pairing the data. Statistical analyses of control treatments (Treatment 1 in both experiments) were carried out on the arcsine transformed data using ANOVA after first standardizing them by dividing the ovipositing period by five and presenting number of eggs in each period as a proportion of the total (as described above for post-hoc analysis of Ponsonby & Copland, 1998).

Results

Post-hoc analysis of data from Ponsonby & Copland (1998) revealed that egg production increased, reached a peak several days afterwards and then declined to zero, the latter in spite of an abundant supply of the host prey and the absence of conspecific larvae. Once a fresh tuber was provided, egg production again increased and the cycle was repeated. Increased activity by the captive beetles at the end of the egg-laying period was assumed to be indicative of attempts to disperse. Significant differences occurred between oviposition rates at different stages in the cycle ($F = 16.3$, d.f. = 4, 245, $P < 0.0001$; Fig. 1) but not between different individuals ($F = 0.002$, d.f. = 9, 240, $P > 0.99$) or different temperatures ($F = 0.01$, d.f. = 9, 240, $P = 0.99$). The duration of the cycle varied from a mean of 26 days at 24 °C, 22 days at 26 °C and 18 days at 30 °C (mean \pm SD = 22 ± 4.7 days; RH in the range 61–63%). Four individual beetles that were left on the colony after egg laying had ceased indicated that after a mean \pm SD of 5.4 ± 1.1 days, oviposition recommenced, peaked and then again declined, in a similar pattern to the initial bout of egg-laying (Fig. 2). In one such case, a third cycle of oviposition was observed (Fig. 2A).

Experiment 1: effect of host density on oviposition behaviour, egg cannibalism and egg viability

Multivariate analysis of variance revealed that daily egg production varied significantly with time ($F = 12.43$, d.f. = 22, 308, $P < 0.0001$) and with the various treatments against time ($F = 1.88$, d.f. = 44, 308, $P = 0.0011$). Orthogonal contrasts showed that oviposition rate was significantly different between Treatments 1 and 2 ($P = 0.02$ – 0.04) and Treatments

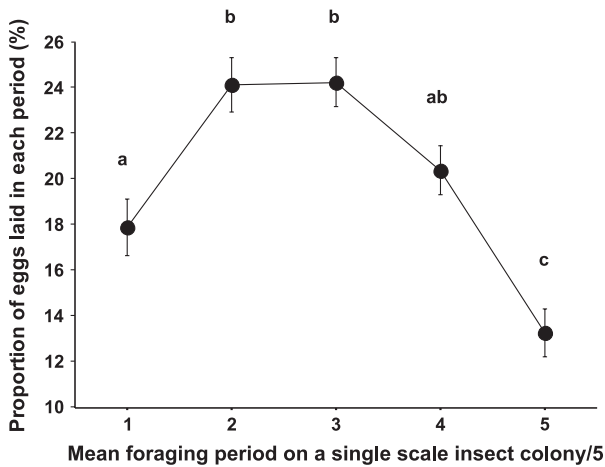


Figure 1 Standardized data (based on mean foraging period) of the mean proportion of eggs laid by *Chilocorus nigritus* females at 24–30 °C and RH in the range 61–63% when foraging on a single *Abgrallaspis cyanophylli* colony with all prey stages present in excess of requirements ($n = 10$ beetles, each completing five cycles). Range bars indicate the mean \pm SE. Mean \pm SD total foraging period = 22.0 ± 4.7 days. Means with different letters above error bars are significantly different at $P < 0.05$ (critical difference = 4.424, Tukey–Kramer test).

1 and 3 ($P = 0.001$ – 0.005), but not between treatments 2 and 3, from day 12 to day 16 (respectively) ($P > 0.05$ in all cases). On day 17, Treatment 1 was significantly different from Treatment 3 ($P = 0.02$). There were no significant differences at any other time ($P > 0.05$ in all cases) and all pairs were producing similar numbers of eggs at the end of the experimental run (Fig. 3). An ANOVA of the transformed standardized data for Treatment 1 revealed that there was no tendency towards the cyclic egg-laying behaviour observed in Fig. 1 ($F =$

1.94, d.f. = 4, 20, $P = 0.14$). Within-treatment analysis revealed no differences between individuals ($F = 0.22$, d.f. = 4, 20, $P = 0.92$). Between-treatment and within-treatment analyses of egg viability data detected no significant differences and the pooled data showed little change in egg viability from a median of 54% (interquartile range = 22%) during the first 9 days, to 55% (interquartile range = 24%) in the remaining period (Kruskal–Wallis k -sample test, $H = 4.1$, d.f. = 2, $P = 0.13$ before switching; $H = 0.79$, d.f. = 2, $P = 0.67$, after switching; Wilcoxon signed rank test, P -values in the range 0.14–0.92, $n = 6$). Egg cannibalism was not significantly affected by changes in host density and the pooled data revealed an overall median of 6% (interquartile range = 27%) (Kruskal–Wallis k -sample test, $H = 0.77$, d.f. = 2, $P = 0.96$ before switching; $H = 0.45$, d.f. = 2, $P = 0.80$ after switching; Wilcoxon signed rank test, $P = 0.14$ – 0.893 , $n = 6$).

Experiment 2: effect of population age structure on oviposition behaviour, egg cannibalism and egg viability

Multivariate analysis of variance revealed that oviposition rate differed significantly with time ($F = 7.23$, d.f. = 19, 304, $P < 0.0001$) and with the various treatments against time ($F = 2.42$, d.f. = 57, 304, $P < 0.0001$). Orthogonal contrasts showed that there was a significant difference in oviposition rate between Treatment 1 and Treatments 2, 3 and 4 on day 1 and day 12 and similarly, from day 14 to day 20 ($P < 0.05$ in all cases) (Fig. 4). In addition, Treatment 3 was significantly different from Treatments 2 and 4 on day 15 ($P = 0.02$ and 0.03 , respectively) but, at all other times, there were no other significant differences ($P > 0.05$ in all cases). At the end of the experimental run, beetles restricted to a single host stage (pooled data) were laying approximately 11 eggs per day fewer on average than those in Treatment 1. As in Experiment 1, analysis of variance on the

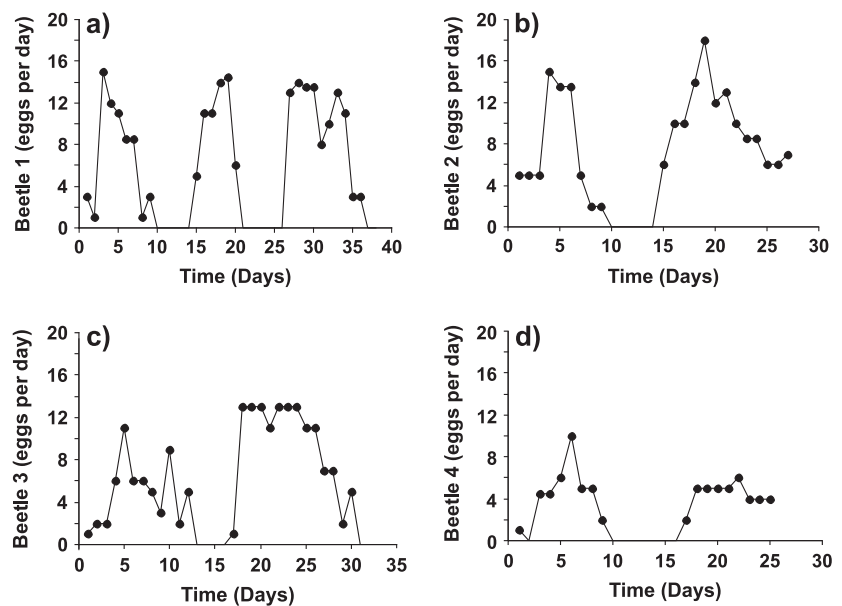


Figure 2 Daily egg production rates of individual female *Chilocorus nigritus* at 26–30 °C and RH in the range 61–63% when foraging on a single *Abgrallaspis cyanophylli* colony with all prey stages present in excess of requirements. (A–C) Maintained at 30 °C; (D) maintained at 26 °C.

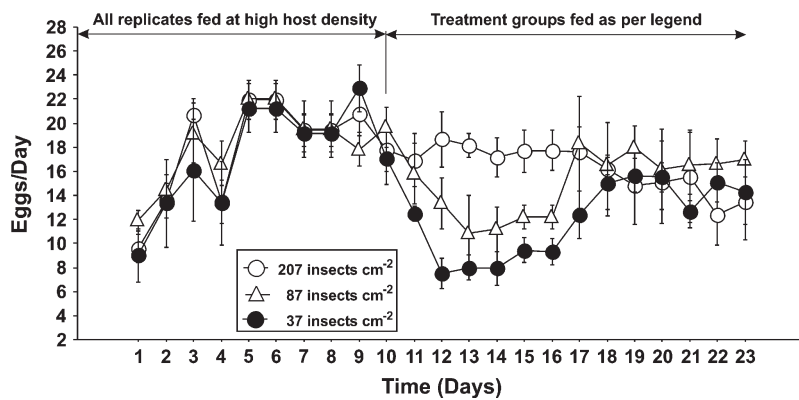


Figure 3 Effect of change in host density on daily fecundity in *Chilocorus nigritus* when fed on 9-week-old colonies of *Abgrallaspis cyanophylli* at 26 °C and 62% RH ($n = 6$ pairs at each level; range bars indicate the mean \pm SE).

transformed standardized data from Treatment 1 revealed no evidence of cyclic egg laying in the control group with mixed population structure ($F = 0.64$, d.f. = 4, 20, $P = 0.64$). Within-treatment analysis revealed no differences between individuals ($F = 0.002$, d.f. = 4, 20, $P > 0.99$).

Eggs laid during the first 2 days of the experiment were excluded from the analyses because of an incubator malfunction. Within-treatment analysis of the remaining data revealed that egg viability declined slightly in treatments 1 and 2 but increased in Treatments 3 and 4. However, only the latter result was significant (Wilcoxon signed rank test, $Z = -2.02$, $n = 5$, $P = 0.04$; Table 2). Between-treatment analysis revealed no significant differences (Kruskal–Wallis k -sample test, $H = 0.29$, d.f. = 3, $P = 0.96$ before switching; $H = 4.2$, d.f. = 3, $P = 0.24$ after switching; Wilcoxon signed rank test, $P = 0.13$ – 0.23 , $n = 5$). Egg cannibalism decreased from 33% to 2% when beetles were switched to second-instar prey (Treatment 3), narrowly missing significance (Wilcoxon signed rank test, $Z = -1.75$, $n = 5$, $P = 0.07$). There were no other significant differences between or within treatments (Kruskal–Wallis k -sample test, $H = 3.5$, d.f. = 3, $P = 0.32$ before switching; $H = 4.3$, d.f. = 3, $P = 0.23$ after switching; Wilcoxon signed rank test, $P = 0.07$ – 0.89 , $n = 5$;

Table 2) and the overall median level of 6% was comparable to that of Experiment 1.

Discussion

Post-hoc analyses of data from Ponsonby & Copland (1998) provided strong evidence of cyclic egg-laying behaviour when beetles were restricted to a single host colony (Fig. 1). Where beetles were left for longer periods on a single colony, cyclic oviposition behaviour was repeated after a short period (Fig. 2) but analysis of the standardized data did not reflect this where colonies were changed more frequently (Fig. 1). These findings were used to generate the following hypotheses, where cyclic egg-laying behaviour was induced by: (i) Selective feeding that caused temporary depletion below a critical level of a nutritional or gustatory cue (e.g. a particular host stage) or some other eliciting cue such as semiochemicals released by the prey or the host plant. Aphidophagous species are known to use such chemicals as cues for oviposition (Evans & Dixon, 1986). (ii) An innate ability to match the number of eggs laid to the availability of suitable host stages for the emerging beetle larvae. Several

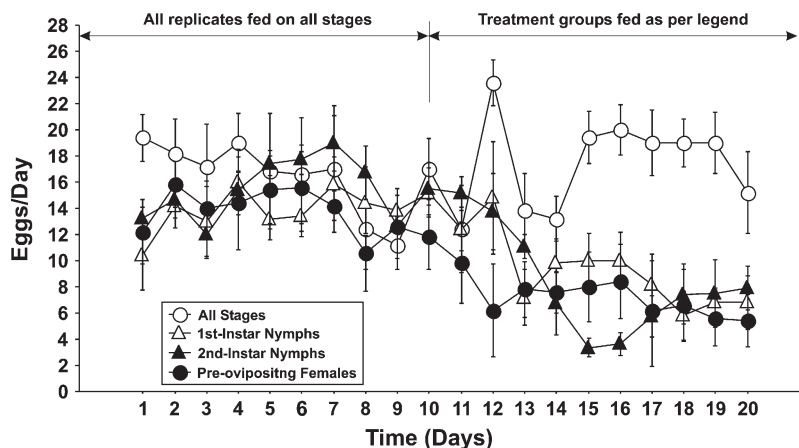


Figure 4 Effect of change in population structure on daily fecundity in *Chilocorus nigritus* when fed on colonies of *Abgrallaspis cyanophylli* at 26 °C and 62% RH ($n = 6$ pairs at each level; range bars indicate the mean \pm SE).

Table 2 Between-treatment and within-treatment effects of a change in host population structures on mean total egg production, median egg viability and median egg cannibalism in *Chilocorus nigritus* fed on *Abgrallaspis cyanophylli* at 26 °C and 62% RH (10 days on all host stages followed by 10 days on treatment stage; $n = 5$)

Host stage	No. of eggs laid (SD)	% Hatched (interquartile range)	% Eaten (interquartile range)
Treatment 1			
10 days on all stages	165.0 (57.6)	51 (24) ^{a,c}	10 (21) ^a
10 days on all stages	174.2 (26.4)	33 (32) ^{a,c}	24 (29) ^a
Treatment 2			
10 days on all stages	141.2 (33.4)	47 (34) ^{a,c}	4 (12) ^a
10 days on first-instar nymphs	91.0 (37.6)	42 (15) ^{a,c}	10 (16) ^a
Treatment 3			
10 days on all stages	138.6 (48.1)	39 (31) ^{a,c}	33 (30) ^a
10 days on second-instar nymphs	69.6 (41.3)	50 (35) ^{a,c}	2 (9) ^a
Treatment 4			
10 days on all stages	135.8 (37.8)	38 (19) ^a	7 (10) ^a
10 days on adult Females	71.4 (57.1)	49 (18) ^{b,c}	16 (16) ^a

For statistical analysis of No. of eggs laid, see Fig. 3 and text.

Medians within a column with the same superscript letter are not significantly different at $P = 0.05$ [Kruskal–Wallis k -sample test (between treatments) and Wilcoxon's signed rank test (within treatments)].

authors have proposed such a hypothesis (Banks, 1956; Dixon, 1970; Hodek, 1973), based on the model that limited food supplies will affect the ability of the females to mature eggs. In this instance, food supply was apparently not limited (Fig. 2), although selective feeding may have temporarily depleted suitable prey stages as in (i) above. (iii) A reaction to quality of the prey, possibly due to an accumulation of secondary metabolites from the plant, a depletion of available nutrients within the potato tuber or to changes in the age structure of the scale population. Erichsen *et al.* (1991) reported a strong tendency for *C. nigritus* females to avoid imminently doomed scale insect colonies on ageing fruit and squashes supporting very high host densities. However, in the present study, potato tubers supported high *A. cyanophylli* densities for 12–13 weeks on average and were not considered to be 'imminently doomed' during the period of the trials. Furthermore, the data presented in Fig. 2 do not support this hypothesis. (iv) An innate cycling of the rate of oviposition that could possibly be linked to a dispersal mechanism within the adult female as proposed by Frazer *et al.* (1981) and recently reviewed by Dixon (2000) and Evans (2003). In this context, a possible model might be a gradual decline in egg production on a given host colony leading to an increase in fat deposition (assuming food intake remains constant), therefore providing beetles with sufficient energy reserves to forage for new host colonies. (v) A negative feedback function controlling oviposition rate that was not linked to dispersal but to a need to recoup energy depleted during high levels of egg production. (vi) The absence of conspecific ladybird larvae suggests that their oviposition-detering pheromones do not act exclusively to close the egg window in this coccidophagous species, raising the strong possibility that the males or, more likely, the females, produce their own oviposition-detering signals.

Oviposition rates were very similar between treatment groups when all were held on high-density scale colonies

during the first phase of Experiment 1 (Fig. 3). Those beetles held on this treatment (Treatment 1) continued to produce a similar number of eggs per day with little overall variation. This provides very strong evidence that beetles base their decision to oviposit on cues that they receive from the host colony and argues robustly against an innate cycling or negative feedback mechanism linked to dispersal or energy depletion as postulated in hypotheses 4 and 5 above. Beetles exposed to a decrease in host density showed a temporary decline in the daily number of eggs produced. This was unlikely to be linked to a reduction in food intake because even the 'low' densities were sufficiently high to avoid the need for extensive searching by the beetles (Table 1). It is also likely that any effect linked to a reduced food intake or to a nutritional dependence on the relative abundance of a particular host stage would be more permanent. Thus, it is reasonably safe to conclude that, although they eventually adjusted to the new density, a change in density in itself was sufficient to elicit a transient negative response in ovipositing behaviour. The mechanism for this remains unclear but the data suggest that an acclimation process occurred that allowed the females to compensate for such a change.

No evidence of cyclic oviposition was observed in the control group with mixed population structure (Treatment 1) in Experiment 2. Similarly, mean oviposition rates were comparable between treatment groups when reared on mixed population scale colonies. The overall mean daily egg production was similar to the comparable treatment in Experiment 1. However, restricting ovipositing beetles to a single host stage caused a decline in egg laying that showed no sign of recovery by the end of the experiment. It was assumed that feeding beetles to satiation during the experimental runs would provide them with sufficient nutrition to maintain full egg-laying potential. However, independent assessment of egg development when preying on the various stages is indicated to confirm this. The strongest decline in

egg laying was elicited by restricting beetles to second-instar scales but this was a transitory response and by the end of the experiment, Treatments 2, 3 and 4 had remarkably similar mean oviposition rates compared with the control. First, it is possible that one of the four host stadia or stages not offered (i.e. active crawlers; male imagos; unmated, receptive females or parturient females) is required to elicit high rates of oviposition or, second, that a combination of stages is required for maximal egg production. Regarding the latter, diets including alternative prey have been shown to increase fecundity in aphidophagous ladybirds (Evans *et al.*, 1999; Soares *et al.*, 2004) but, whichever is the case, the question remains as to whether the stimulus is a nutritional requirement (i.e. that the consumption of the stages mentioned above stimulates the maturation of more beetle eggs), a nutritional cue (such that the oviposition reflex is triggered) or some other enabling mechanism that, when above a critical threshold, is independent of food intake (e.g. a semiochemical or visual cue). With regard to nutritional stimuli, most first-instar coccinellid larvae require first-instar (or relatively young) prey to survive (Dixon, 1970; Samways & Wilson, 1988; Majerus, 1994). This requires a high degree of prey synchrony, which has been observed amongst many coccidophagous species (Hagen, 1962), including *C. nigrinus* (Samways & Wilson, 1988). Previous studies have shown that a high degree of synchrony would be maintained in this species if females laid eggs in the presence of the unmated (but receptive), gravid or parturient female scale (Ponsonby & Copland, 1996, 2000b). First-instar *A. cyanophylli* would also be a suitable synchronous stage for beetles to oviposit onto provided eggs were laid early in their development (Ponsonby & Copland, 2000b). However, coccinellid larvae hatching on recently moulted second-instar *A. cyanophylli* would have to endure a minimum of 22 days before suitable prey could be found (Ponsonby & Copland, 2000b). Thus, unless they were to feed entirely on conspecific eggs, they would have no chance of survival. Indeed, lowest egg production from this experiment occurred when beetles fed on this stage (Treatment 3). Gravid female *A. cyanophylli* provided as the sole diet in Treatment 4 in the current study were within 3–4 days of parturition and therefore would have been full of developing embryos. However, this stage did not stimulate a high rate of oviposition, despite the fact that egg viability in beetles that fed on this group significantly increased after the diet change (Table 2).

Armoured scale insects do not excrete honeydew or other waste material and thus gustatory cues are likely to be limited to the scale cover or the insect body. However, *C. nigrinus* adults are known to use visual and olfactory cues to locate their prey (Hattingh & Samways, 1995; Ponsonby & Copland, 1995; Boothe & Ponsonby, 2006) whereas unmated, receptive females of several armoured scale insect species are known to release sex pheromones (Geiselmann, 1990; Einhorn *et al.*, 1998). Because this scale stage is consistent with a strong prey synchrony in the current predator/prey relationship, it is feasible that semiochemicals released by them (and possibly their host plants) act as cues for oviposition, perhaps in conjunction with visual cues (e.g. the presence of crawlers). The former has been shown to be

the case in aphidophagous ladybirds where the presence of aphid odours and honeydew elicited egg-laying behaviour (Evans & Dixon, 1986). Such cues could be linked directly to density-dependent responses as indicated by the results of Experiment 1 in which host density was manipulated because the concentration of semiochemicals in the boundary layer would be proportional to the number of scale insects present.

Unlike aphidophagous coccinellid species (Evans, 2003), oviposition by female *C. nigrinus* in the absence of their prey is extremely rare (Ponsonby, 1995), and thus it is evident that an eliciting cue is obligatory. Larval oviposition-detering pheromones have been reported in many coccinellid species, including the coccidophagous *Cryptolaemus montrouzieri* (Muls.) (Merlin *et al.*, 1996). Although this phenomenon has not been reported in *C. nigrinus*, it is unlikely that this species will be an exception but the results obtained in the present study suggest that other cues also affect oviposition behaviour.

Gustatory or visual contact in a heterogeneous population would result in a density dependent response (Experiment 1) only if the beetles were able to count and monitor suitable prey levels over time. This may be possible; but any oviposition cues are most likely to be linked to factors that elicit quantitative physiological changes or a combination of visual or gustatory factors for example. Nevertheless, this model cannot easily explain the cycling nature of the oviposition cue when insects are confined to a single host colony because the mean period of the egg-laying cycle does not match the generation period of the scale.

With the exception of Treatment 4 in Experiment 2 where beetles were fed only on gravid female scales, egg viability data showed a nonsignificant tendency to decline during the experiments, which is consistent with other age-specific studies (Ponsonby & Copland, 1998; Dixon & Agarwala, 2002). Egg cannibalism by adults in both of the current experiments remained largely unaffected by the various treatments and was similar to the study by Ponsonby & Copland (1998).

Laboratory studies are often poor indicators of field situations, and many other factors such as host plant/prey interactions (including their coevolutionary history), beetle age, interspecific competition and the presence of conspecifics are likely to play a major role in eliciting ovipositing behaviour. However, the present study suggests that, provided sufficient food is available for adult beetles to be satiated, prey population structure is more important than host density for stimulating oviposition in this coccidophagous coccinellid. Thus, *C. nigrinus* are 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 colonisers.

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