

Effect of spatial heterogeneity on the role of *Coccinella septempunctata* as an intra-guild predator of the aphid pathogen *Pandora neoaphidis*

H.E. Roy,^{a,b,*} P.G. Alderson,^b and J.K. Pell^a

^a Plant and Invertebrate Ecology Division, Rothamsted Research, Harpenden, Hertfordshire, AL5 2JQ, UK

^b Department of Agriculture and Horticulture, University of Nottingham, Sutton Bonington Campus, Loughborough, Leicestershire, LE15 5RD, UK

Received 25 April 2002; accepted 3 December 2002

Abstract

The foraging behavior of starved and non-starved adult and larval *Coccinella septempunctata* on groups of plants in the presence of *Pandora neoaphidis*-infected *Acyrtosiphon pisum*, uninfected aphids or a mixture of these two prey types was compared. In general results of these studies confirmed the results of previous work comparing foraging behavior on a smaller spatial scale in Petri dishes. However, behaviors were modified in response to spatial complexity, prey quality, and the host plant. Starved *C. septempunctata* adults and larvae fed for longer and consumed more aphids than non-starved coccinellids. Both larvae and adults fed on infected aphids and in some cases entirely consumed them. This was thought to be due to the ease of capture of infected (dead) aphids and the feeding stimuli provided by the presence of the host plant and, where there was a choice of prey, uninfected aphids in the environment. Both larvae and adults spent the majority of the time foraging in the upper regions of plants and visited more plants when they were not starved or when they were in the presence of less suitable, infected aphid prey.

© 2003 Elsevier Science (USA). All rights reserved.

Keywords: Biological control; Foraging behavior; Intra-guild predation; Spatial scale

1. Introduction

Coccinellids have been largely unsuccessful when used as single biological control agents of aphids, despite the adaptations which increase their foraging efficiency on patchily distributed and short-lived aphid colonies (Majerus, 1994). More recently, the potential of using multiple biological control species has received increasing attention (Dennis, 1991; Frazer et al., 1981; Roy and Pell, 2000). The effects of multiple natural enemies on a prey population could be synergistic, additive or non-additive (Ferguson and Stiling, 1996; Rosenheim et al., 1995). Alternatively, an increase in the number of natural enemy species could result in an increase of the

host if interference between the natural enemies was sufficient (Hochberg and Lawton, 1996).

Intra-guild predation is a dramatic example of interference between natural enemies, which could result in antagonism, and reduced mortality of the host. This process occurs if competing predators also engage in a trophic interaction (predation or parasitism) with one another (Polis and Holt, 1992), and is prevalent within many communities of biological control agents (Flexner et al., 1986; Goettel et al., 1990; Rosenheim et al., 1995; Roy and Pell, 2000). In behavioral studies in Petri dish arenas, larval and adult *Coccinella septempunctata* L. consumed aphids infected with the entomopathogenic fungus *Pandora neoaphidis* (Remaudière and Hennebert) Humber (Pell et al., 1997; Roy et al., 1998) and can, therefore, be considered as intra-guild predators of the pathogen. This interaction could have implications for the combined biological control potential of these aphid natural enemies. However, the foraging efficiency of *C. septempunctata* depends on many interacting factors

* Corresponding author. Present address: Environmental Science Research Center/Department of Life Sciences, Anglia Polytechnic University, East Road, Cambridge, CB1 1PT, UK. Fax: +01-223-352-979.

E-mail address: h.e.roy@anglia.ac.uk (H.E. Roy).

(aphid density, plant topography, and abiotic conditions) that define heterogeneity of habitat. Studies on the behavior of natural enemies within Petri dish arenas are valuable but they only allow a limited repertoire of behaviors to be observed. Some behaviors and interactions require examination on entire plants. Searching coccinellids, like many aphid species, are negatively geotactic and positively phototactic (Dixon, 1959; Fleschner, 1950; Obata, 1986). Many invertebrate predators of aphids, including coccinellids, search along the prominent contours of plants, for example the veins of leaves. Such behavior often leads a predator to the preferred feeding sites of its prey (Dixon, 1959; Wratten, 1973) although this is dependent on plant structure (Carter et al., 1984). Morphological features of plants such as waxy leaves, dense trichomes, or complex surfaces may reduce the searching efficiency of a predator (Grevstad and Klepetka, 1992). This paper describes experiments investigating the behavior of *C. septempunctata* larvae and adults on whole bean plants to determine the importance of spatial heterogeneity on the interactions between *C. septempunctata* and *E. neoaphidis*.

2. Materials and methods

2.1. Insect, fungus, and plant cultures

The aphid species, *Acyrtosiphon pisum* Harris (Hemiptera: Aphididae), used in these studies was maintained on three to four-week old dwarf broad bean plants (*Vicia faba* L. cultivar The Sutton) under controlled conditions of 18 °C and a 16 h photoperiod. The aphids were transferred to new plants every one to two weeks. Aphids were regularly harvested to feed a *C. septempunctata* colony and for culturing *E. neoaphidis*. *C. septempunctata* larvae were derived from eggs laid by adults collected in the field.

Isolate X4 (from the Rothamsted collection, original host = *A. pisum*) of *E. neoaphidis* was used in these studies. An in vivo culture of isolate X4 was maintained by regular passage through adult apterous *A. pisum* as described by Wilding (1973). Recently killed, infected aphids were dried and the resulting cadavers stored at 20% RH and 4 °C. For further aphid inoculation, cadavers were rehydrated at 10 °C and 100% RH. After 15 h the emerging fungus was sporulating profusely and could be used to infect aphids.

2.2. Effect of prey type (infected/uninfected aphids) and starvation on the behavior of fourth instar and adult *C. septempunctata* larvae

2.2.1. Whole plant arenas

The foraging behavior of fourth instar larvae and adult coccinellids on whole plants infested with infected

or uninfected aphids was assessed using a standardized experimental arena. Three bean plants (three weeks old) were transplanted into a 130 mm plant pot (containing moistened compost) in a triangular arrangement. The plants were arranged such that the leaves of adjacent plants overlapped. The stems of each plant were approximately 50 mm apart. Ten prey items were placed on each plant resulting in a total of 30 per pot. A single coccinellid was placed on the upper leaves of one of the three plants in each treatment and its behavior monitored.

2.2.2. Periods of starvation and prey treatments

The adult and larval coccinellids had either been allowed to feed immediately prior to the experiment or had been starved for the preceding 24 h. Individual coccinellids were placed in arenas with either: (i) all sporulating infected aphids, (ii) all live uninfected aphids, or (iii) a mixture of sporulating infected and live uninfected aphids (five of each per plant). The prey items (total of ten per plant) were placed on the top of the upper and mid leaves of the plants 15 min before the coccinellid was added to the arena to enable the live aphids to settle. The sporulating aphids were placed in similar positions to the uninfected aphids. Behavioral observations on coccinellid larvae were replicated six times for each prey treatment and starvation level. Behavioral observations on both male and female adult coccinellids were replicated seven times for each sex, prey treatment and starvation level.

2.2.3. Behavioral observations

Immediately after the coccinellid was added to the behavioral arena its behavior was recorded for 30 min or until it left the arena. It was only possible to assess continually the behavior of one coccinellid at a time and, therefore, the order in which treatments were recorded was fully randomized. Experiments were conducted throughout the day (between 10.00 and 18.00 h) in ambient laboratory conditions (23 ± 2 °C).

The behaviors were classified into seven categories: searching, feeding, resting, walking, cleaning, flying, and grazing (feeding on the plant). Searching was characterized by sinuous, slow tracks, interrupted by numerous stops, compared with walking which was a much more linear and faster movement (Ferran and Dixon, 1993). Larvae were often observed to contact an aphid with their mouthparts without any visible ingestion. This behavior was also categorized as searching. The total time spent on each behavior was calculated. The frequency of the feeding category was recorded and the number of entire aphids consumed was quantified. The number of partially consumed aphids was not quantified. The position of the coccinellid on the plant was recorded in addition to the behavioral observations. The plant was divided into three sections, upper, middle, and

lower, and the total time spent in each position, and also on the soil, was calculated. The number of plants visited throughout the observational period was also recorded.

2.2.4. Statistical analyses

Experiments involving male and female adult coccinellids were conducted in parallel. The experiments on larval coccinellids were conducted separately from the adult coccinellid experiments. Therefore, the analyses of adult behavior included sex as an additional factor and were done separately to those on larval behavior.

In all analyses involving behavioral categories or plant positions, zeros were adjusted for using the following formulae (Aitchison, 1986):

$$\delta(C + 1)(D - C)/D^2 \quad (\text{added to all zeros})$$

$$\delta C(C + 1)/D^2 \quad (\text{subtracted from all non-zero values})$$

where δ is the rounding error (0.5 s); C the number of zeros per replicate; and D is the number of behavioral categories.

The dominant behaviors of both larvae and adults (male and female) were feeding, searching, resting, and cleaning and, therefore, they alone were analyzed using a log ratio analysis (Pell et al., 1997). The log ratios of the time spent feeding vs. the time spent in all other dominant behaviors were compared for non-starved and starved coccinellids in the presence of the different prey treatments using ANOVA. The log ratios of the remaining behaviors (cleaning; resting and searching; resting) were analyzed using MANOVA. The total number of aphids entirely consumed was very low and did not warrant statistical analysis.

The analyses used to compare the time spent in each region of the plant were similar to those for the behavioral categories. The log ratios of upper vs. all other regions were analyzed using ANOVA. The log ratios of

the remaining regions of the plant (middle vs. lower and soil vs. lower) were analyzed using MANOVA.

The proportions of time spent in each of the three categories searching, resting, and cleaning were represented using barycentric triangles (Aitchison, 1986; Pell et al., 1997) in which a point equidistant from all vertices represents equal activity in all three behaviors. The time spent in the different regions of the plant (middle, lower, and soil) were also represented in this way.

The numbers of plants visited (either one, two, or three plants) by non-starved and starved adult coccinellids in the presence of different prey were interpreted as ordered categories and were analyzed using proportional odds regression via the generalized linear model facilities of Genstat 5 (Genstat 5 Committee, 1995). The numbers of plants visited by starved and non-starved larvae were also analyzed using proportional odds regression, although the numbers of plants visited by larvae in the choice treatments were excluded from the analysis because larvae only ever visited one plant.

The total time spent in the whole plant arenas (comprising different prey treatments) by non-starved and starved coccinellids was analyzed using ANOVA after a log transformation.

3. Results

3.1. Behavior of fourth instar *C. septempunctata* larvae

Starved larvae spent a significantly longer time feeding than non-starved larvae ($F_{1,30} = 24.03$; $p < 0.001$; Fig. 1), and there was a significant difference in the time spent feeding in the presence of different prey treatments ($F_{2,30} = 3.33$; $p < 0.05$; Fig. 1). There was no significant interaction between starvation and prey treatment in the time spent feeding ($F_{2,30} = 0.69$; $p > 0.05$).

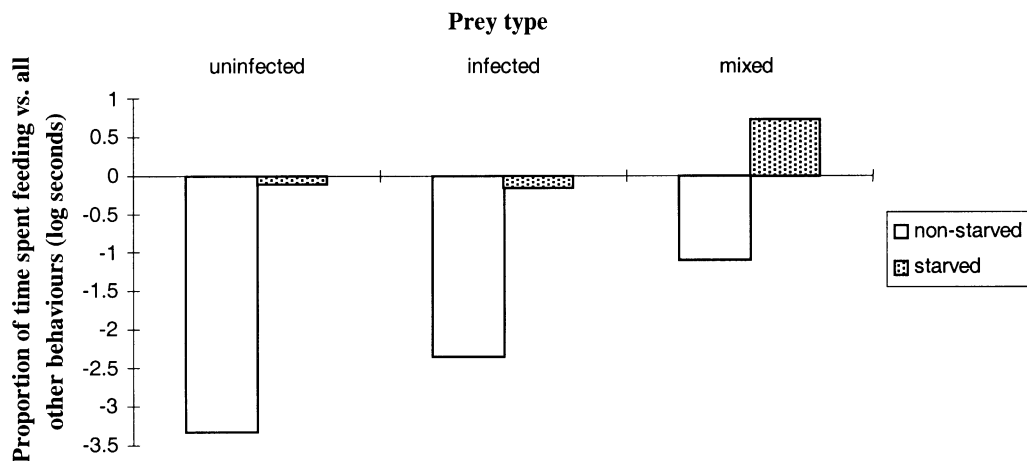


Fig. 1. Proportion of time spent feeding by non-starved and starved fourth instar coccinellid larvae foraging on bean plants infested with uninfected and infected *A. pisum* (SE=0.86).

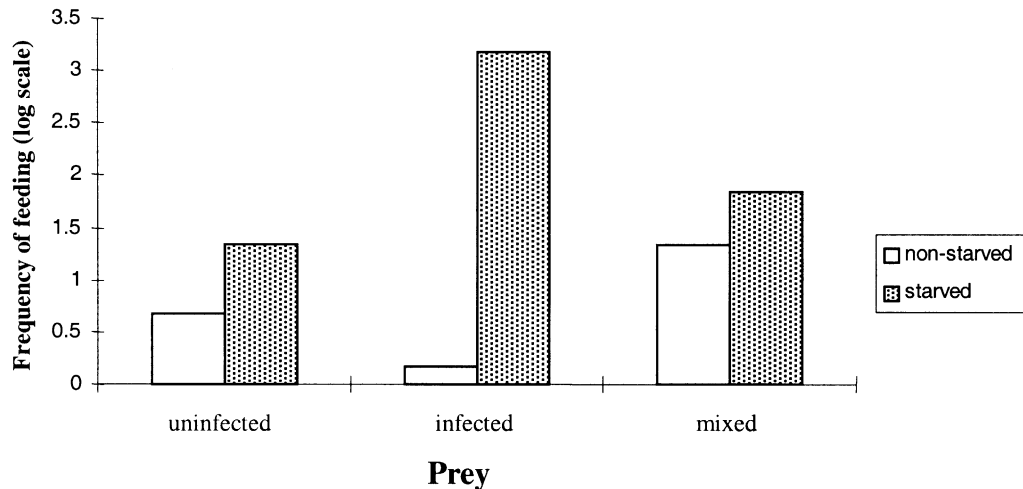


Fig. 2. Frequency of the behavior feeding by non-starved and starved fourth instar coccinellid larvae foraging on bean plants infested with uninfected and infected *A. pisum* (SE = 0.26).

There was a significant difference in the time spent feeding on infected compared to uninfected aphids by larvae presented with a mixture of both ($F_{1,10} = 6.98$; $p < 0.05$). Starved larvae spent more time feeding on uninfected (mean = 1403 s) than infected (mean = 64 s) aphids. In contrast, although non-starved larvae did not feed for long on either infected or uninfected aphids, they fed for longer on infected aphids (mean = 315 s) than uninfected aphids (mean = 147 s).

There were significantly more feeding bouts by starved larvae compared to non-starved larvae ($F_{1,30} = 14.69$; $p < 0.001$; Fig. 2). There was no significant difference in the frequency of feeding in the presence of different prey treatments ($F_{2,30} = 0.098$; $p > 0.05$), however, the interaction between starvation and prey treatment was significant ($F_{2,30} = 4.12$; $p < 0.05$; Fig. 2). The frequency of feeding by non-starved larvae in the presence of infected prey was significantly less than starved larvae. The number of bouts of feeding was similar for both non-starved and starved larvae in the presence of uninfected prey or a choice of prey.

The number of aphids consumed by non-starved and starved larvae was generally very low, however, starved and non-starved larvae presented with single prey treatments ate more uninfected aphids than infected aphids (sum of all replicates for starved larvae = 9 uninfected and 3 infected and for non-starved larvae = 1 uninfected and 0 infected). Starved larvae did not consume infected aphids when presented with a choice of infected and uninfected aphids but consumed a total of 6 uninfected aphids. In contrast non-starved larvae did consume a total of 1 infected aphid and 2 uninfected when presented with a choice.

There was no significant difference in the time spent searching, resting and cleaning by non-starved larvae compared to starved larvae ($F_{2,29} = 2.17$; $p > 0.05$; Fig. 3) or by larvae in the presence of different prey treatments

($F_{4,58} = 0.39$; $p > 0.05$). There was no significant interaction between starvation and prey treatment.

Larvae spent longer in the upper regions of the bean plant than any other region. The time spent in the upper region of the plant did not vary with starvation

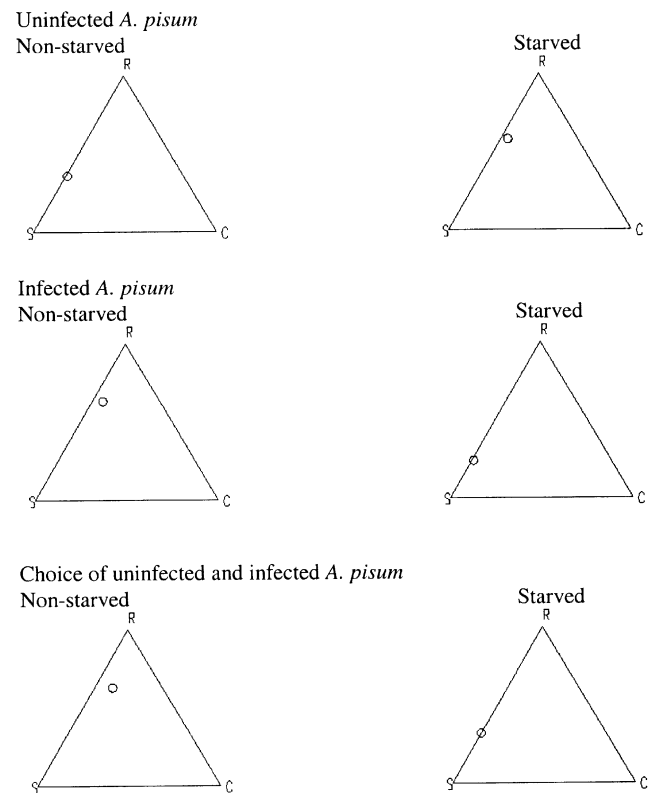


Fig. 3. Proportions of time spent on behaviors other than feeding by non-starved and starved fourth instar coccinellid larvae in the presence of uninfected and infected *A. pisum*. The time (mean) spent searching (S), resting (R), and cleaning (C) are represented within the triangles. A point on a vertex of a triangle represents 100% of time spent in that behavior. The time spent in these behaviors did not vary significantly between starved and non-starved larvae or in the presence of different prey types.

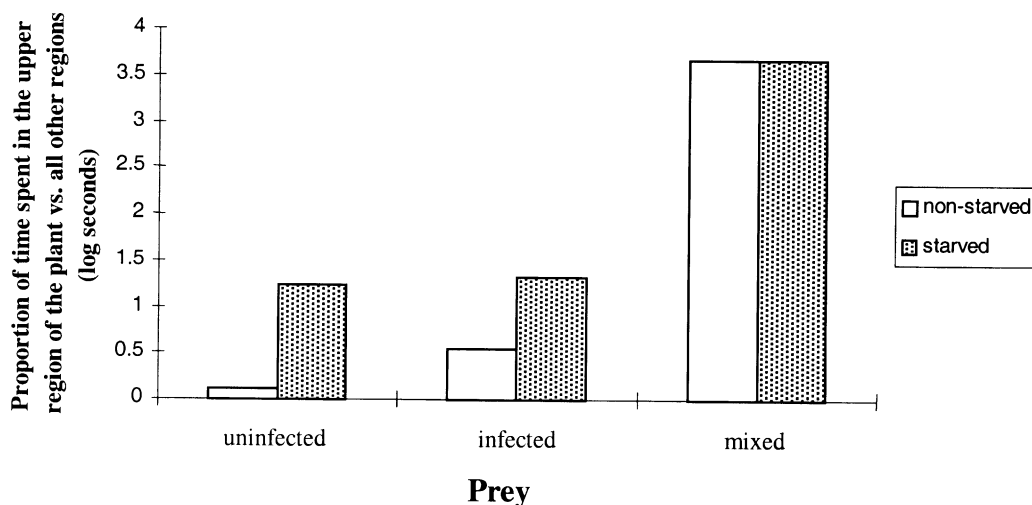


Fig. 4. Proportion of time spent in the upper region compared to the other regions of a bean plant by non-starved and starved fourth instar coccinellid larvae in the presence of uninfected and infected aphids (SE=0.70).

($F_{1,30} = 2.43$; $p > 0.05$) but did vary significantly with prey treatment ($F_{2,30} = 22.96$; $p < 0.001$; Fig. 4). There was no significant interaction between starvation and prey treatment ($F_{2,30} = 0.67$; $p > 0.05$).

Larvae spent longer in the upper region of the plant when presented with a choice of uninfected and infected aphids than when only one prey type was present (uninfected or infected). Indeed, larvae presented with a choice of prey foraged exclusively in the upper regions of the plant.

There was no significant difference in the time spent on the soil or in the middle or lower regions of the plant between non-starved and starved larvae ($F_{2,29} = 0.45$; $p > 0.05$). There was a significant difference in the time spent in these regions by larvae in the presence of different prey types ($F_{4,58} = 4.34$; $p < 0.01$), which could be attributed to the fact that larvae presented with a choice of prey foraged exclusively in the upper region of the plant. There was no significant interaction between starvation and prey treatment ($F_{4,58} = 1.18$; $p > 0.05$).

In the presence of a choice of prey, larvae spent the entire observation period on one plant. Larvae in the presence of either uninfected or infected aphids generally visited more than one plant, however, there was no significant difference in the number of plants visited by non-starved or starved larvae ($F_{1,5} = 1.35$; $p > 0.05$) or by larvae in the presence of either all uninfected or all infected aphids ($F_{1,5} = 0.10$; $p > 0.05$). There was no significant interaction between starvation and prey type ($F_{2,5} = 1.43$; $p > 0.05$).

3.2. Behavior of *C. septempunctata* adults

Starved adult coccinellids spent longer feeding than non-starved coccinellids ($F_{1,72} = 22.29$; $p < 0.001$; Fig. 5). There was no significant difference in the time

spent feeding on different prey types ($F_{2,72} = 1.24$; $p > 0.05$) or between males and females ($F_{1,72} = 3.50$; $p > 0.05$). However, there were significant interactions between starvation and prey treatment ($F_{2,72} = 3.71$; $p < 0.05$) and between starvation, prey treatment and the sex of the coccinellid ($F_{2,72} = 3.65$; $p < 0.05$). Non-starved and starved adult coccinellids spent similar periods of time feeding when presented with a choice of prey types, but, when presented with either uninfected or infected aphids alone, starved adults spent longer feeding than non-starved adults (Fig. 5).

There was no difference in the time spent feeding by non-starved male and female coccinellids but there was a significant difference between starved females and starved males in the time spent feeding. Starved females fed for longer in the presence of a choice of prey or all uninfected prey than in the presence of infected prey. Both starved male and female coccinellids spent similar periods of time feeding on infected aphids but starved females fed for longer when presented with uninfected aphids or a choice of prey than starved males (Fig. 5). Male and female coccinellids presented with a choice of uninfected or infected aphids spent similar periods of time (mean = 133 s) feeding on both prey types regardless of starvation ($F_{1,24} = 0.59$; $p > 0.05$) or sex ($F_{1,24} = 3.84$; $p > 0.05$). There was no significant interaction between starvation and sex ($F_{1,24} = 0.02$; $p > 0.05$).

The frequency of the behavior feeding was significantly lower for non-starved adult coccinellids (mean number of feeding bouts = 0.7; SE = 0.2) compared to starved adults (mean number of feeding bouts = 2.4; SE = 0.2) ($F_{1,72} = 18.35$; $p < 0.001$). There was no significant difference in the frequency of feeding by adults in the presence of different prey types ($F_{2,72} = 2.06$; $p > 0.05$) or by male and female coccinellids

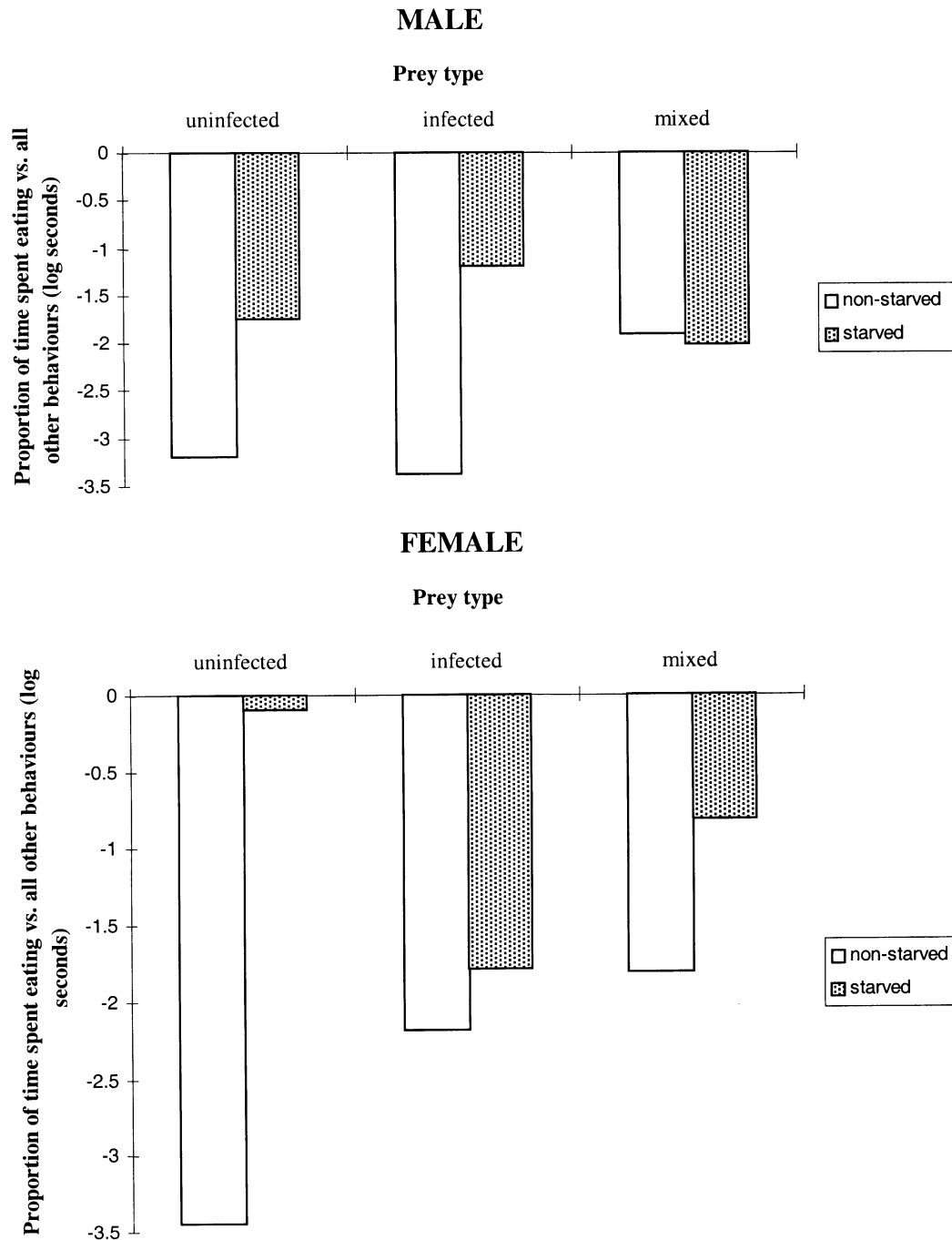


Fig. 5. Proportion of time spent feeding by non-starved and starved adult coccinellids foraging on bean plants infested with uninfected and infected aphids (SE = 0.72).

($F_{1,72} = 2.83$; $p > 0.05$) and there were no significant interactions between the factors.

Adult coccinellids consumed less prey when presented with only infected aphids as opposed to the other two prey types and, both non-starved and starved adults ate few infected aphids. However, whereas non-starved adults ate only a few aphids in all prey treatments (sum of all replicates = 3 uninfected and 4 infected), starved adults ate more aphids when in the presence of only

uninfected aphids (sum of all replicates = 17) or a choice of uninfected and infected aphids (sum of all replicates = 8 uninfected and 2 infected) than when presented with infected aphids (sum of all replicates = 3) alone.

Starved adults spent less time searching and resting than non-starved adults and more time cleaning than non-starved adults ($F_{2,71} = 5.15$; $p < 0.01$; Fig. 6), however, there was no significant difference between males and

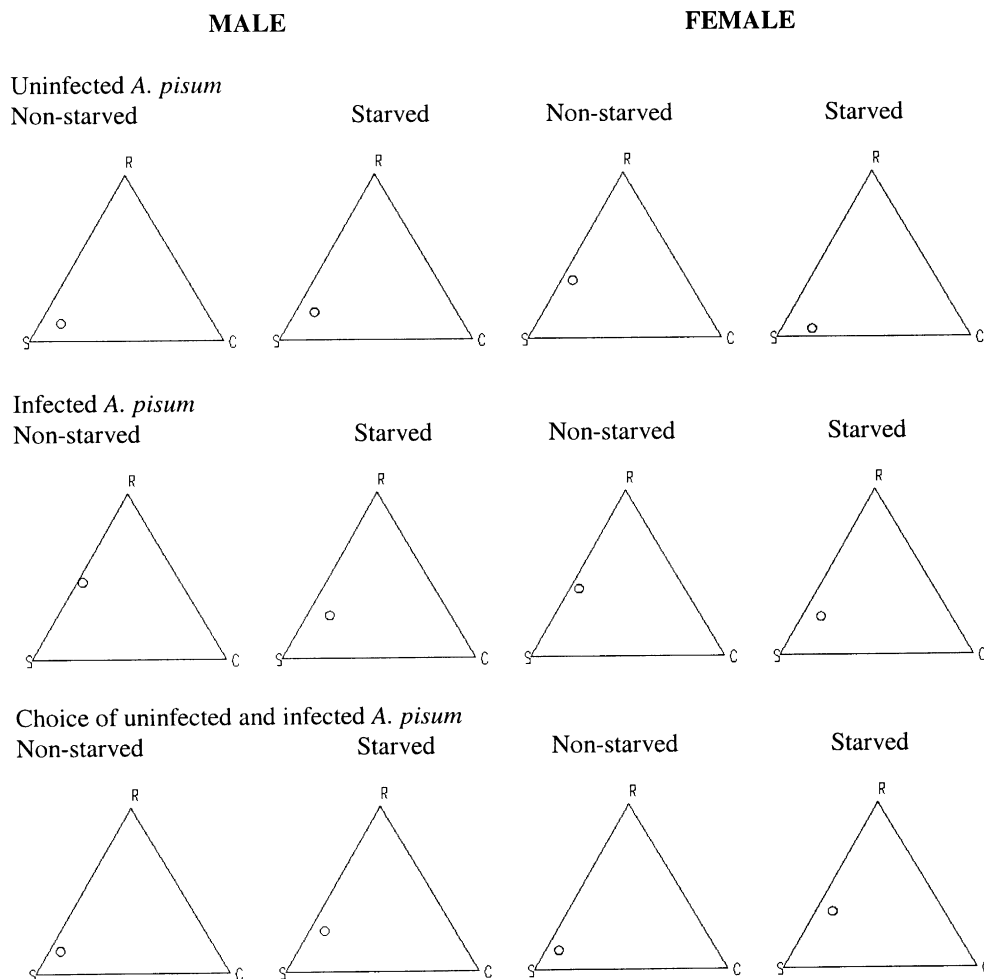


Fig. 6. Proportions of time spent on behaviors other than feeding by non-starved and starved adult coccinellids in the presence of uninfected and infected *A. pisum*. The time (mean) spent searching (S), resting (R), and cleaning (C) are represented within the triangles. A point on a vertex of a triangle represents 100% of time spent in that behavior. Starved adults spent significantly longer feeding than non-starved adults regardless of prey type or sex.

females ($F_{2,71} = 0.14$; $p > 0.05$) or between adults in the presence of different prey treatments ($F_{4,142} = 0.68$; $p > 0.05$). There were no significant interactions between starvation, prey treatment, and sex.

Adult coccinellids spent longer in the upper regions of the bean plant than any other region (Fig. 7). This time varied significantly with starvation ($F_{1,72} = 4.44$; $p < 0.05$) and prey treatment ($F_{2,72} = 3.48$; $p < 0.05$) but did not vary significantly between sexes ($F_{1,72} = 0.22$; $p > 0.05$). There was a significant interaction between starvation and prey treatment ($F_{2,72} = 4.09$; $p < 0.05$).

Starved adult coccinellids spent longer in the upper regions of the plant than non-starved adults. In addition, both non-starved and starved adults spent longer in the upper regions of plants infested with either all uninfected aphids or a choice of uninfected and infected aphids. Non-starved adults spent longer periods of time in the upper regions of plants infested with infected aphids when compared with starved adults.

There was no significant difference in the time spent on the soil or in the middle or lower regions of the plant between non-starved and starved adult coccinellids ($F_{2,71} = 0.99$; $p > 0.05$), by adults in the presence of different prey types ($F_{4,142} = 0.75$; $p > 0.05$) or by male or female coccinellids ($F_{2,71} = 0.05$; $p > 0.05$). There were no significant interactions.

There was a significant difference in the number of plants visited by non-starved and starved adults ($\chi^2_{1,11} = 4.80$; $p < 0.05$). Sixty percent of non-starved adults commonly visited all three plants during the study period compared to starved adults where the numbers visiting two (36%) or three plants (43%) was similar and lower. There was no significant difference in the number of plants visited by adults in the presence of different prey types ($F_{1,11} = 0.32$; $p > 0.05$) or by males or females ($F_{1,11} = 4.48$; $p > 0.05$). There were no significant interactions between any of the factors (starvation, prey treatment and sex).

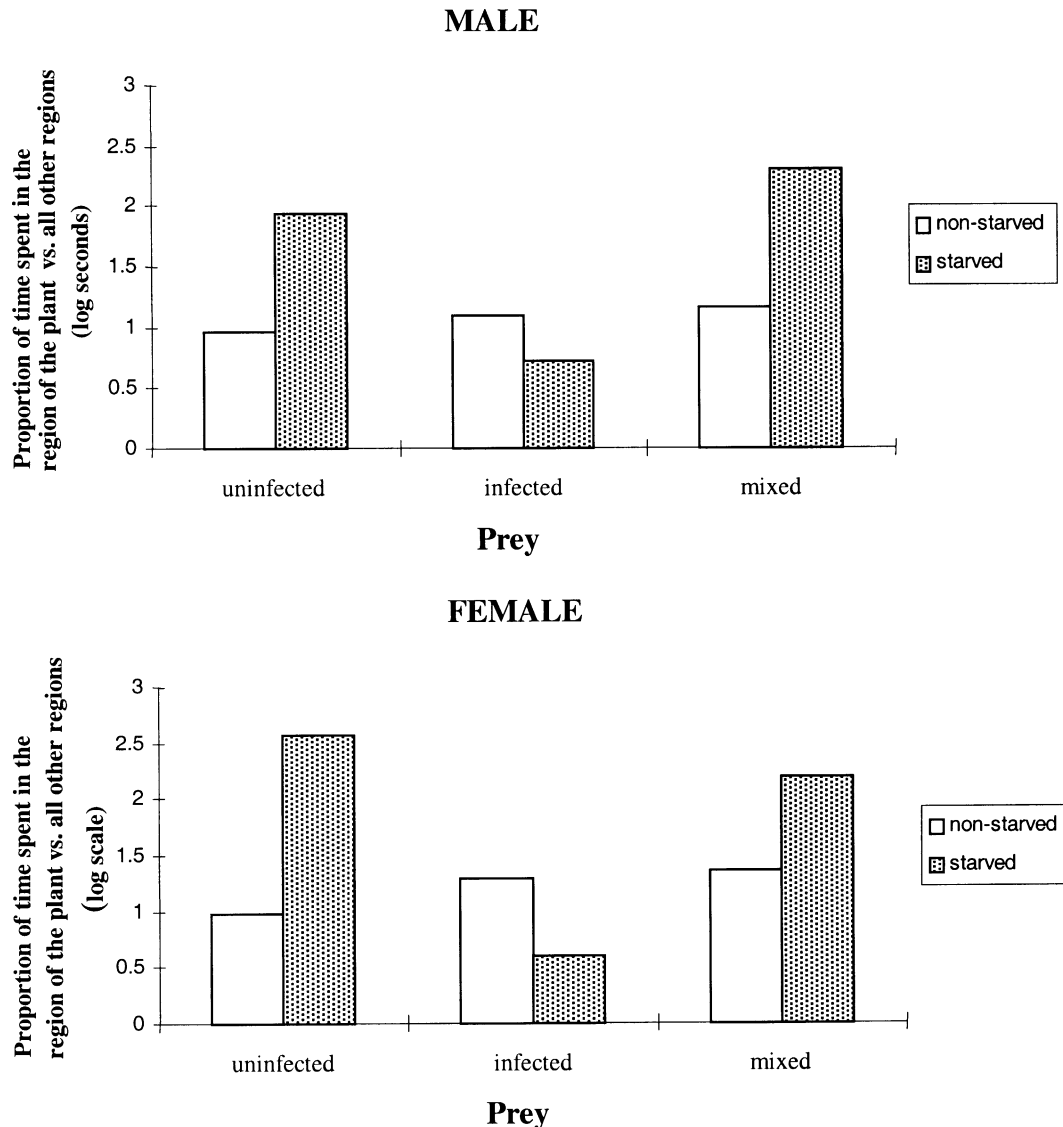


Fig. 7. Proportion of time spent in the upper region of a bean plant by non-starved and starved adult coccinellids in the presence of uninfected and infected *A. pisum* (SE = 0.67).

4. Discussion

Simple behavioral arenas, such as Petri dishes, provide environments in which preliminary observations on invertebrate behavior can be made. However, conclusions from such studies are limited by the context in which the observations have been made. The behavior of invertebrates is undoubtedly affected by the complexities of the multidimensional environment in which they forage (Chang, 1996; Sengonca and Frings, 1985).

The whole plant experiments described in this paper do largely support the results obtained from Petri dish studies (Pell et al., 1997; Roy et al., 1998). In general starved *C. septempunctata* larvae and adults fed for longer and consumed more aphids than non-starved coccinellids, and both larval and adult coccinellids

consumed infected *A. pisum* although more uninfected aphids were consumed. However, in contrast to the Petri dish studies, both adults and larvae foraging on whole plants consumed entire infected aphids. The importance of herbivore-induced host-plant odors in prey location and searching behavior by natural enemies has been demonstrated (Du et al., 1997; Ninkovic et al., 2001; Powell and Zhang, 1983; Powell et al., 1998; Vet and Dicke, 1992). The presence of such odors may encourage coccinellids to feed on less palatable prey (i.e., infected cadavers) due to the surrounding odors. The relative ease of capturing infected aphids compared to uninfected aphids on a bean plant could also contribute to explaining the increased incidence of feeding on infected aphids. Uninfected *A. pisum* can avoid capture by dislodging or walking away from the predator (Brodsky

and Barlow, 1986; McConnell and Kring, 1990) and in our study such aphid escape behaviors were frequently observed. In most of the Petri dish studies the uninfected aphids had been killed prior to the experiments, and even when live aphids were used, the confines of the Petri dish increased the incidence of encounter between predator and prey (Pell et al., 1997; Roy et al., 1998).

Starved female adult coccinellids did not consume any infected aphids when presented with these alone. Coccinellids can respond to both the quality as well as the abundance of their prey (Hemptinne et al., 1993). Aphid consumption is essential for the development of female ovaries and, thus, starved female coccinellids may have an increased motivation to feed on prey of a suitable quality (namely uninfected aphids). Honek (1985) demonstrated that the rate of aphid predation in females was higher than males, and that the activity of males was linked to the motivation to find a mate in addition to prey. Therefore, females may discriminate more than males between uninfected and infected aphids because of the importance of high quality prey consumption for ovary maturation. Interestingly, starved females did consume infected aphids when in the presence of both infected and uninfected aphids, although more uninfected aphids were eaten. Honeydew and aphid alarm pheromone are arrestant stimuli for coccinellids (Al Abassi et al., 2000; Carter and Dixon, 1984), therefore, in the presence of both uninfected and infected aphids, the female coccinellids may consume infected aphids as a consequence of the arrestant stimuli provided by the neighboring uninfected aphids. The consumption of infected aphids may have long-term effects on the fecundity and longevity of coccinellids, however this requires further investigation.

Infected aphids may be a less acceptable prey type for larvae because of the increased dependence of larvae, compared to adults, on extra-oral digestion (Hodek, 1973). Such digestion may be less effective on infected aphids. Although the foraging behavior of larvae can vary according to the prey previously consumed (Ettifouri and Ferran, 1993), starved larvae did not avoid infected aphids after initial encounters. Larvae require sufficient prey to complete their development and so, in the absence of uninfected aphids, infected aphids are consumed (Ferran and Dixon, 1993).

Both larvae and adults spent the majority of the observation period foraging in the upper regions of the bean plants. Searching coccinellids are known to be negatively geotactic and positively phototactic (Dixon, 1959; Fleschner, 1950; Obata, 1986), resulting in their predominance in the upper regions of plants, where aphids are generally situated.

Larvae presented with a choice of uninfected and infected prey generally foraged on only one of the three bean plants available and exclusively in the upper regions. In contrast, larvae on plants with single prey

types searched more extensively. Larvae can become conditioned to a particular aphid species (Ettifouri and Ferran, 1993) and switch from extensive to intensive search after encountering familiar prey, however on encountering new prey they continue to search extensively. The larvae used in these studies were fed uninfected *A. pisum* and *S. avenae* prior to the experiments and may have become conditioned to this prey type. Thus, larvae in the presence of only infected aphids may search more widely within a plant and between plants. Uninfected aphids exhibit escape behaviors in response to approaching predators (Dill et al., 1990). The greater area covered by larvae when searching in the presence of uninfected aphids may have been a response to the increased movement of the uninfected aphids. Although the movement of the aphids was not quantified in our study escape behaviors were frequently observed. The area covered by foraging larvae in the presence of both uninfected and infected prey larvae was less than when in the presence of either all uninfected or all infected aphids. Larvae on plants with a choice of prey spent considerable periods of time feeding which would account for the restrictive movement. Larvae in the presence of a choice of prey or all uninfected prey consumed similar numbers of entire aphids. However, larvae spent longer feeding when presented with a choice because infected aphids encountered were partially consumed before rejection. This appears to have the effect of concentrating search patterns of the larvae to a relatively small region of the arena.

Starved adults fed for a greater proportion of the study period than non-starved adults. Intensive search is more common after prey consumption (Ferran and Dixon, 1993) and this could explain the smaller area searched by starved adults compared to non-starved adults. The searching behavior of non-starved adults may be driven by other motivational responses than prey location for example, finding suitable oviposition sites or mates and this seems to result in them searching a greater area. Starved larvae have a greater motivation to feed than non-starved larvae (Ferran and Dixon, 1993) and this could explain their increased persistence in the arena. In addition, starvation causes larvae to adopt an intensive search mode (Ferran and Dixon, 1993) which would also restrict them to the arena. In contrast, non-starved larvae search more extensively and this searching mode may have resulted in the larvae leaving the arena.

In conclusion, larval and adult coccinellids are intra-guild predators of *E. neoaphidis*. Intra-guild predators are generally larger than their intra-guild prey, and omnivory is common amongst intra-guild predators (Polis and Strong, 1996), as is cannibalism (Polis et al., 1989). *C. septempunctata* principally preys upon a range of aphid species but has also been reported feeding on coccids, adelgids, mites, mildew, honeydew, pollen and nectar (Majerus, 1994), and many species of coccinellid,

including *C. septempunctata*, are highly cannibalistic (Agarwala and Dixon, 1992; Majerus, 1994). Therefore, it is not surprising that *C. septempunctata* is reported as an intra-guild predator both within this system and other invertebrate communities (Evans and England, 1996; Sengonca and Frings, 1985).

Experiments at the spatial scale of a Petri dish provide very simple environments in which to observe behavior and can be criticized for being unrealistic. However, they can be useful in identifying particular interactions that may be important at larger spatial scales and in more realistic environments. In the experiments described here we were able to demonstrate the importance of a more realistic environment in modifying behaviors initially observed in Petri dishes. Petri dish experiments demonstrated that although *C. septempunctata* had the potential as an intra-guild predator of *E. neoaphidis* it was unlikely to have a significant detrimental effect on *E. neoaphidis* populations. Under the more realistic conditions on small groups of plants the potential for intra-guild predation increased.

The consumption of infected aphids by coccinellids may reduce the pathogen population density and, thus, the probability of the pathogen becoming epizootic (Fuxa and Tanada, 1987). However, the presence of foraging coccinellids can result in an increase in the transmission of *E. neoaphidis* (Pell et al., 1997; Roy et al., 1998; Roy et al., 2001). Furthermore, coccinellids are still more likely to consume uninfected aphids than infected aphids. Field observations (Roy, 1997) on adult coccinellid behavior supported this hypothesis, indeed in the field *C. septempunctata* adults fed exclusively on living, presumably uninfected aphids when foraging in colonies of both infected and uninfected aphids. To determine the ultimate outcome of these interactions with respect to the aphid prey population and the populations of coccinellids and *E. neoaphidis* it will now be necessary to quantify populations at even larger spatial scales and over longer periods of time. These experiments will not only demonstrate whether intra-guild predation really has an influence on the population development of herbivores and their associated, interacting predators but also the real potential of natural enemies to be exploited for biological control purposes either as single species or as complexes.

Acknowledgments

HER was funded by the Lawes Trust and Anglia Polytechnic University and JKP by the Department for Environment, Food, and Rural Affairs, UK. Rothamsted Research receives grant-aided support from the Biotechnology and Biological Sciences Research Council of the UK.

References

- Agarwala, B.K., Dixon, A.F.G., 1992. Laboratory study of cannibalism and interspecific predation in ladybirds. *Ecological Entomology* 17, 303–309.
- Aitchison, J., 1986. *The Statistical Analysis of Compositional Data*. Chapman and Hall, London.
- Al Abassi, S., Birkett, M.A., Pettersson, J., Pickett, J.A., Wadhams, L.J., Woodcock, C.M., 2000. Response of the seven spot ladybird to an aphid alarm pheromone and an alarm pheromone inhibitor is mediated by paired olfactory cells. *Journal of Chemical Ecology* 26, 1765–1771.
- Brodsky, L.M., Barlow, C.A., 1986. Escape responses of the pea aphid *Acyrtosiphon pisum* Harris: influence of predator type and temperature. *Canadian Journal of Zoology* 64, 937–939.
- Carter, M.C., Dixon, A.F.G., 1984. Honeydew: an arrestant stimulus for coccinellids. *Ecological Entomology* 9, 383–387.
- Carter, M.C., Sutherland, D., Dixon, A.F.G., 1984. Plant structure and the searching efficiency of coccinellid larvae. *Oecologia* 63, 394–397.
- Chang, G.C., 1996. Comparison of single versus multiple species of generalist predators for biological control. *Environmental Entomology* 25, 207–212.
- Dennis, P., 1991. The temporal and spatial distribution of arthropod predators of the aphids *Rhopalosiphum padi* W. *Sitobion avenae* F. in cereals next to field-margin habitats. *Norwegian Journal of Agricultural Sciences* 5, 79–88.
- Dill, L.M., Fraser, A.F.G., Roitberg, B.D., 1990. The economics of escape behavior in the pea aphid, *Acyrtosiphon pisum*. *Oecologia* 83, 473–478.
- Dixon, A.F.G., 1959. An experimental study of the searching behavior of the predatory coccinellid beetle, *Adalia decempunctata* L. *Journal of Animal Ecology* 28, 259–281.
- Du, Y.-J., Poppy, G.M., Powell, W., Wadhams, L.J., 1997. Chemically mediated associative learning in the host foraging behavior of the aphid parasitoid *Aphidius ervi* (Hymenoptera: Braconidae). *Journal of Insect Behavior* 10, 509–522.
- Ettifouri, M., Ferran, A., 1993. Influence of larval rearing diet on the intensive searching behavior of *Harmonia axyridis* larvae. *Entomophaga* 38, 51–59.
- Evans, E.W., England, S., 1996. Indirect interactions in biological control of insects: pests and natural enemies in alfalfa. *Ecological Applications* 6, 920–930.
- Ferguson, K.I., Stiling, P., 1996. Non-additive effects of multiple natural enemies on aphid populations. *Oecologia* 108, 375–379.
- Ferran, A., Dixon, A.F.G., 1993. Foraging behavior of ladybird larvae. *European Journal of Entomology* 90, 383–402.
- Fleschner, C.A., 1950. Studies on searching capacity of the larvae of three predators of the citrus red mites. *Hilgardia* 20, 233–265.
- Flexner, J.L., Lighthart, B., Croft, B.A., 1986. The effects of microbial pesticides on non-target, beneficial arthropods. *Agricultural Ecosystems and Environment* 16, 203–254.
- Frazer, B.D., Gilbert, N., Nealis, V., Raworth, D.A., 1981. Control of aphid density by a complex of predators. *Canadian Entomologist* 113, 1035–1041.
- Fuxa, J.R., Tanada, Y., 1987. *Epizootiology of Insect Diseases*. Wiley-Interscience, New York.
- Genstat 5 Committee, 1995. *Genstat 5 Release 3 Reference Manual*. Clarendon Press, Oxford.
- Goettel, M.S., Poprawski, T.J., Vandenberg, J.D., Li, Z., Roberts, D.W., 1990. Safety to non-target invertebrates of fungal biocontrol agents. In: Laird, M., Lacey, L.A., Davidson, E.W. (Eds.), *Safety of Microbial Insecticides*. CRC Press, Boca Raton, FL, USA, pp. 209–231.
- Grevstad, F.S., Klepetka, B.W., 1992. The influence of plant architecture on the foraging efficiencies of a suite of ladybird beetles feeding on aphids. *Oecologia* 92, 399–404.

- Hemiptinae, J.-L., Dixon, A.F.G., Douchet, J.-L., Petersen, J.-E., 1993. Optimal foraging by hoverflies (Diptera: Syrphidae) and ladybirds (Coleoptera: Coccinellidae): Mechanisms. *European Journal of Entomology* 90, 451–455.
- Hochberg, M.E., Lawton, J.H., 1996. Competition between kingdoms. *Trends in Evolution and Ecology* 5, 367–371.
- Hodek, I., 1973. *Biology of Coccinellidae*. Dr. Wunk N.V., The Hague and Academia, Prague.
- Honek, A., 1985. Activity and predation of *Coccinella septempunctata* adults in the field (Col., Coccinellidae). *Zeitschrift für Angewandte Entomologie* 100, 399–409.
- McConnell, J.A., Kring, T.J., 1990. Predation and dislodgement of *Schizaphis graminum* (Hemiptera: Aphididae), by adult *Coccinella septempunctata* (Coleoptera: Coccinellidae). *Environmental Entomology* 19, 1798–1802.
- Majerus, M.E.N., 1994. *Ladybirds*. Harper Collins, London.
- Ninkovic, V., AlAbassi, S., Pettersson, J., 2001. The influence of aphid-induced plant volatiles on ladybird beetle searching behavior. *Biological Control* 21, 191–195.
- Obata, S., 1986. Mechanisms of prey finding in the aphidophagous ladybird beetle, *Harmonia axyridis* (Coleoptera: Coccinellidae). *Entomophaga* 31, 303–311.
- Pell, J.K., Pluke, R., Clark, S.J., Kenward, M.G., Alderson, P.G., 1997. Interactions between two aphid natural enemies, the entomopathogenic fungus *Pandora neoaphidis* Remaudière & Hennebert (Zygomycetes: Entomophthorales) and the predatory beetle *Coccinella septempunctata* L. (Coleoptera: Coccinellidae). *Journal of Invertebrate Pathology* 69, 261–268.
- Polis, G.A., Holt, R.D., 1992. Intra-guild predation: the dynamics of complex trophic interactions. *Trends in Ecology and Evolution* 7, 151–154.
- Polis, G.A., Strong, D.R., 1996. Food web complexity and community dynamics. *American Naturalist* 147, 813–846.
- Polis, G.A., Myers, C.A., Holt, R.D., 1989. The ecology and evolution of intra-guild predation: potential competitors that eat each other. *Annual Review of Ecological Systematics* 20, 297–330.
- Powell, W., Zhang, Z.-L., 1983. The reactions of two cereal aphid parasitoids, *Aphidius uzbekistanicus* and *A. ervi* to host aphids and their food plants. *Physiological Entomology* 8, 439–443.
- Powell, W., Pennacchio, F., Poppy, G.M., Tremblay, E., 1998. Strategies involved in the location of hosts by the parasitoid *Aphidius ervi* Haliday (Hymenoptera: Braconidae: Aphidiinae). *Biological Control* 11, 104–112.
- Rosenheim, J.A., Kaya, H.K., Ehler, L.E., Marois, J.J., Jaffee, B.A., 1995. Intra-guild predation among biological control agents: theory and evidence. *Biological Control* 5, 303–335.
- Roy, H.E., 1997. Interactions between aphid predators and the entomopathogenic fungus *Pandora neoaphidis*. Ph.D. Thesis, University of Nottingham.
- Roy, H.E., Pell, J.K., Clark, S.J., Alderson, P.G., 1998. Implications of predator foraging on aphid pathogen dynamics. *Journal of Invertebrate Pathology* 71, 236–247.
- Roy, H.E., Pell, J.K., 2000. Interactions between entomopathogenic fungi and other natural enemies: Implications for biological control. *Biocontrol Science and Technology* 10, 737–752.
- Roy, H.E., Pell, J.K., Alderson, P.G., 2001. Targeted dispersal of the aphid pathogenic fungus *Pandora neoaphidis* by the aphid predator *Coccinella septempunctata*. *Biocontrol Science and Technology* 11, 99–110.
- Sengonca, C., Frings, B., 1985. Interference and competitive behavior of the aphid predators, *Chrysoperla carnea* and *Coccinella septempunctata* in the laboratory. *Entomophaga* 30, 245–251.
- Vet, L.E.M., Dicke, M., 1992. Ecology of infochemical use by natural enemies in a tritrophic context. *Annual Review of Entomology* 37, 141–172.
- Wilding, N., 1973. The survival of *Entomophthora* spp. in mummified aphids at different temperatures and humidities. *Journal of Invertebrate Pathology* 21, 309–311.
- Wratten, S.D., 1973. The effectiveness of the coccinellid beetle *Adalia bipunctata* L. as a predator of the lime aphid *Eucallipterus tilliae* L. *Journal of Animal Ecology* 42, 785–802.