

# Interactions between Two Aphid Natural Enemies, the Entomopathogenic Fungus *Erynia neoaphidis* Remaudière & Hennebert (Zygomycetes: Entomophthorales) and the Predatory Beetle *Coccinella septempunctata* L. (Coleoptera: Coccinellidae)

J. K. Pell,\* R. Pluke,† S. J. Clark,‡ M. G. Kenward,‡ and P. G. Alderson†

\*Entomology & Nematology Department and †Statistics Department, IACR—Rothamsted, Harpenden, Hertfordshire, AL5 2JQ, United Kingdom; and ‡Department of Agriculture and Horticulture, University of Nottingham, Sutton Bonington Campus, Loughborough, Leicestershire LE12 5RD, United Kingdom

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Interactions between the pea aphid, *Acyrtosiphon pisum*, and two of its natural enemies, the ladybird *Coccinella septempunctata* and the fungus *Erynia neoaphidis*, were examined in the laboratory. Adult *C. septempunctata* starved for 48 or 24 hr or nonstarved were presented with aphid prey items at different stages of infection with *E. neoaphidis* and their feeding behaviors monitored. This demonstrated that, under laboratory conditions, *C. septempunctata* adults, particularly when starved for 48 hr, perceived *A. pisum* infected with *E. neoaphidis* as acceptable food sources. This is a potentially antagonistic interaction as inoculum necessary for further transmission and the development of epizootics would be removed from aphid populations. However, less time was spent feeding, except by 48-hr-starved individuals, on infected than on uninfected aphids and the number of “feeding” versus “ignore” mouthpart encounters was greater for uninfected than infected aphids, thus limiting the severity of this antagonism. In addition, under laboratory conditions, *C. septempunctata* adults were able to passively vector infective conidia of *E. neoaphidis* to susceptible aphids and initiate infection in 10–11% of the population. Ladybird foraging on *E. neoaphidis*-infected aphids and their ability to vector infective conidia to susceptible aphid populations is discussed in relation to the epizootiology of the fungus and its implications for the manipulation of natural enemies in integrated management strategies. © 1997 Academic Press

**KEY WORDS:** *Coccinella septempunctata*; feeding behavior; *Erynia neoaphidis*; vectoring; interaction.

## INTRODUCTION

Aphids are an important group of agricultural pests causing direct feeding damage to crops and also indi-

rect damage as vectors of plant virus diseases. They are attacked by a number of natural enemies including entomopathogenic fungi (mostly of the order Entomophthorales), various arthropod predators, and hymenopteran parasitoids. Many studies have examined the impact of these natural enemies independently of each other, demonstrating their potential, under certain conditions, to keep aphid populations below damaging levels (Wilding and Perry, 1980; Chambers *et al.*, 1986; Gutierrez *et al.*, 1990; Wratten and Powell, 1991; Wraight *et al.*, 1993). However, there have been fewer studies examining interspecific interactions between aphid natural enemies and the potential impact of these interactions on control potential. Previous studies have focused largely on fungus and parasitoid interactions (e.g., Vinson, 1976; Milner *et al.*, 1984; Powell *et al.*, 1986; Brobyn *et al.*, 1988; Poprawski *et al.*, 1992), with fungus and predator interactions limited to susceptibility tests against coccinellid beetles of entomopathogenic fungi under development for aphid biological control (Magalhaes *et al.*, 1988; James and Lighthart, 1994; Poprawski *et al.*, 1995). As natural enemies co-occur within a habitat, utilizing the same host insects, a greater understanding of the interactions between them is essential for their more effective manipulation and enhanced aphid control.

This paper describes experiments designed to examine interactions between a commonly occurring aphid pathogen in the UK, *Erynia neoaphidis* Remaudière & Hennebert (Zygomycetes: Entomophthorales), and one of the most common predatory coccinellid beetles, *Coccinella septempunctata* L. (Coleoptera: Coccinellidae). The feeding behavior of *C. septempunctata* on *E. neoaphidis*-infected *Acyrtosiphon pisum* Harris aphids and the potential of ladybirds to passively vector infective conidia to susceptible aphids were examined.

## MATERIALS AND METHODS

### *Insects*

A laboratory colony of the pea aphid, *A. pisum*, was maintained on dwarf bean plants (*Vicia faba*: cv. Sutton) in an insectary at 18°C and a 16-hr (16L:8D) photoperiod. These aphids were used both for the maintenance of the fungus *E. neoaphidis* (see below) and as prey for the coccinellid *C. septempunctata*.

The *C. septempunctata* colony was started using 100 adults collected from Thetford Forest (donated by Dr. M. Majerus, University of Cambridge, UK, in February, 1993). These adults were placed on *A. pisum*-infested bean plants in an insectary cage (0.5 × 0.5 × 1 m) at 21°C, 16-hr (16L:8D) photoperiod, and monitored daily for egg production. More aphids and new bean plants were provided as required. As egg batches were laid they were either removed to ventilated boxes, if laid on leaves, or protected behind ventilated Petri dish lids, if laid on the cage sides, to prevent cannibalism. On hatching, larvae were held individually in nylon mesh-covered tubes with aphids and a bean leaf. The leaf and aphids were replaced every other day to ensure a constant food supply until pupation of the coccinellids. After hardening of the cuticle, freshly emerged adults were placed in a clean insectary cage with aphid-infested bean plants to begin the cycle again, or were used in experiments.

### *Fungus*

*Erynia neoaphidis* (Strain ×4, Rothamsted Collection) was maintained in the laboratory by exposing healthy adult *A. pisum* for 3 to 5 hr to conidia discharged from infected aphids. The aphids were then incubated at 20°C in a 16-hr (16L:8D) photoperiod on bean plants within a lamp glass closed with polythene to ensure moist conditions suitable for infection of the aphids by the fungus. The polythene was replaced by nylon mesh after 24 hr, and aphids died 3 to 4 days after infection. Recently deceased aphids were then dried and stored at 4°C and 20% R.H. for future use (Wilding, 1973). Profuse sporulation occurred from these cadavers following rehydration and incubation at 100% R.H. and 10°C for 15 hr. Aphids were inoculated over a period of 3 days so that a range of individuals at different stages of infection were simultaneously available for experimentation.

### *Feeding Behavior Study*

Ladybirds at three levels of starvation were presented with adult aphids at different stages of *E. neoaphidis* infection as their food source. The three starvation levels were 48-hr-starved, 24-hr-starved, and nonstarved. Nonstarved ladybirds were kept in the

presence of aphid prey at all times until the start of the experiment. The six food types were as follows:

- (i) Five dead, uninfected aphids killed with CO<sub>2</sub>;
- (ii) Five live, uninfected aphids;
- (iii) Five dying, infected aphids which were straw-colored and swollen and, at this stage of infection, would have died on the same day at the end of the photoperiod;
- (iv) Five dead, infected aphids which had just died and turned brown, and were swollen and firm to the touch;
- (v) Five sporulating aphids which were off-white in color, fluffy in appearance due to external fungal growth, and were produced from cadavers moistened 14–18 hr before the experiment and held at 10°C in darkness to reach the stage of rapid conidia production during experimentation;
- (vi) No aphids present.

Each food type was presented separately as five aphids placed, in a pentagonal arrangement, on a damp filter paper within a 5-cm glass Petri dish. No food type represented a choice of prey items. Living aphids were also presented in this arrangement; all their subsequent movements were not inhibited in any way.

A single, 3- to 5-day-old adult ladybird was placed into each dish and its behavior was monitored. Activities were recorded as they occurred onto a single side of a D60 audiotape (approximately 30 min) using a Bush 3150 recorder. Recording of activity started immediately after the ladybird was placed into the Petri dish and continued until the tape cassette finished. The behavior was divided into six distinct modes: standing still, walking, searching, feeding, cleaning, and time after encounter. These modes are mostly self-explanatory, though a distinction between walking and searching was made: a ladybird was recorded as walking when it demonstrated "extensive search behaviour" as described by Dixon (1959), that is, it moved rapidly around the dish and only occasionally changed direction. Searching behavior, or "area-concentrated search behaviour," was characterized by rapid movement of the maxillary palps and an increase in the  $D_s/D_b$  ratio (mean ratio of length of actual search path to beeline distance during 15-sec intervals, as described by Bell and Kramer (1979)) and also by a decrease in the rate of forward movement. The time after encounter mode was the time between the ladybird encountering the food items and the onset of the resulting action. The number of aphid encounters that the ladybird made were also recorded and divided into mouthpart and non-mouthpart encounters which were then further categorized into the action which followed the encounter, i.e., (i) ignored response to aphid encounter, (ii) rejected response to aphid encounter, (iii) escape of the aphid, or

(iv) feeding response to aphid encounter. All recordings were done in a 21°C controlled environment room within the photoperiod phase between 08:00 and 17:00 when ladybirds were active. At least one replicate of each food type and level of starvation combination were completed during 1 day of the experiment, the whole procedure being repeated on further days to increase the replication. On each day of the experiment the order in which treatments were selected was random. There were a minimum of 6 and a maximum of 10 replicates for each aphid food type at each starvation level. Each ladybird was used only once.

The audiotape was then transcribed and the time spent on each activity and the number and type of encounters were recorded. All analyses were done using the statistical package Genstat 5 (Genstat 5 Committee, 1993). The raw data for analysis consisted of the times (seconds) spent by each ladybird in each of the six activity categories for the 18 treatments (six food types by three starvation regimes), as described earlier. Note that neither feeding nor encounters could occur for the control where no aphid food items were present. Also, after-encounter times were very short, so this category was analyzed separately. Four analyses were done:

(1) The proportion of time spent feeding out of the time spent in all activities except after-encounter time (Not Feeding) was compared among all treatments except the control (i.e., 15 combinations).

(2) The proportions of time spent in each of the nonfeeding activities (standing still, searching, cleaning, and walking) were compared among all 18 treatments.

The data [(1) and (2) above] are compositional, i.e., the times spent in each response category sum to the total response time for each ladybird (excluding the time after encounter). In (1) the data are bivariate ( $n = 2$ ) and in (2) they are multivariate ( $n = 4$ ). In each case, the data can be represented by  $n - 1$  variables, since, given  $n - 1$ , the  $n$ th is known. All the compositional information in the data is retained in  $n - 1$  variables. In each case a log-ratio analysis (Aitchison, 1986) was used. For case (1), the log (base 10) of the ratio of time spent not feeding to the time spent feeding was analyzed using analysis of variance (ANOVA), and in case (2) the log of the ratios of standing still to walking, searching to walking, and cleaning to walking were analyzed using multivariate analysis of variance (MANOVA). The choice of which category to use as the denominator in each case does not affect the test statistics, but potential numerical problems can be reduced by choosing a variable which has a reasonable range of data and does not include too many extreme times. Although the data were unbalanced, due to the different number of ladybirds tested per treatment, the order in which treatment terms were fitted made no

difference to the conclusions. The multivariate proportions of case (2) were represented using triangles or barycentric coordinate spaces (Aitchison, 1986) whose area depended on the proportion of time spent cleaning out of the time spent not feeding. The times spent walking, searching, and standing still as proportions of the time spent not cleaning (or feeding) were represented within the triangles. A point marked on the triangle at a vertex represents 100% activity in the single behavior represented by that vertex, while a point midway along the side opposite to that vertex represents 0% activity in that same behavior. A point equidistant from all three vertices represents equal activity in all three behaviors.

(3) The after-encounter times (logged base 10) after adding an offset of one in order to stabilize the variance) were compared among all treatments except control (i.e., 15 combinations), using regression analysis.

(4) Nonmouthpart encounters only resulted in "ignore" responses by ladybirds and were thus excluded from further analysis. Only "ignore" and "feed" responses were analyzed for the mouthpart encounters as they represented the majority of the responses (786 and 209 of the 1114 in total, respectively). These data were also analyzed using a log ratio analysis, where the log of the ratio feed/ignore was calculated for each ladybird, after adjusting for zeros when necessary (Aitchison, 1986), and treatments compared using ANOVA.

### Vectoring Study

This study was designed to determine whether ladybirds were able to passively vector *E. neoaphidis* infection.

One- to 6-day-old nonstarved ladybirds were inoculated for 2.5 hr with *E. neoaphidis* conidia discharged from 40 sporulating aphid cadavers in a 5-cm Petri dish. Ladybirds held under similar conditions but not inoculated with conidia were used as controls. Each ladybird was then transferred, with minimal handling, onto a 3-week-old bean plant infested with 25 1- to 3-day-old *A. pisum* adults. The plant was covered with either a 500-ml plastic beaker or a lamp glass closed with polythene to ensure moist conditions and the ladybird allowed to forage on or in the environment of the plant for 22 hr. Control plants were set up in the same way with aphids but no foraging ladybird. After removal of the ladybird, those aphids remaining on each plant were counted and transferred to clean bean plants within closed lamp glasses. Mortality due to fungal infection was monitored. A further 25 1- to 3-day-old *A. pisum* adults were then placed onto the original plants within closed lamp glasses and again the mortality due to fungal infection was monitored. The polythene covers on all lamp-glasses were replaced

with nylon mesh 72 hr after initiation of the experiments.

The proportions of original aphids on new plants and new aphids on original plants infected with *E. neoaphidis* were determined and compared. The median mortalities with time of the two aphid populations were compared using a nonparametric Mann-Whitney test.

**RESULTS**

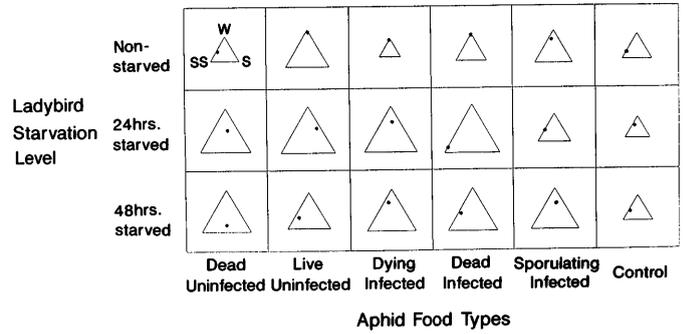
*Feeding Behavior Study*

*Time spent feeding (Table 1).* Significant differences were found among food types ( $F_{4,90} = 11.95, P < 0.001$ ) and between starvation regimes ( $F_{2,90} = 30.61, P < 0.001$ ), and an interaction between the two existed ( $F_{8,90} = 6.16, P < 0.001$ ).

Ladybirds spent a substantial proportion of their time feeding on all treatments after 48 hr starvation, whereas after only 24 hr starvation dead infected and sporulating aphids were not fed upon to any significant extent. If not starved, they generally only fed on dead uninfected aphids. Regardless of their starvation, they always fed on dead uninfected aphids, but to a lesser degree if they were less hungry.

*Time spent on other activities (Fig. 1).* Significant differences were found among food types ( $F_{15,290} = 4.58, P < 0.001$ ) and between starvation regimes ( $F_{6,210} = 7.16, P < 0.001$ ), and an interaction between the two existed ( $F_{30,309} = 3.05, P < 0.001$ ).

Ladybirds spent significantly more time cleaning in the presence of aphids (dead uninfected, live un-



**FIG. 1.** Proportions of the total time spent on activities other than feeding by nonstarved and 24- and 48-hr-starved ladybirds presented with different food types: dead uninfected, live uninfected, dying infected, dead infected, and sporulating infected aphids, and control (where no aphids were present). The area of the triangles is related to the proportion of time spent cleaning; the larger the triangle, the greater the proportion of time spent cleaning. The times spent walking (w), standing still (ss), and searching (s) as proportions of the time spent not cleaning are represented within the triangles. A point marked on the triangle at a vertex represents 100% activity in the single behavior represented by that vertex, while a point midway along the side opposite to that vertex represents 0% activity in that same behavior. A point equidistant from all three vertices represents equal activity in all three behaviors.

infected, dying infected, dead infected, and sporulating infected aphid food types), i.e., the triangles in Fig. 1 are generally larger for these food types than for the control. When aphids were present, more cleaning occurred in the starved ladybird treatments than in the nonstarved treatments. There was no difference in the time spent cleaning between infected and uninfected food types, suggesting that cleaning is linked directly to the time spent feeding regardless of the food type.

Nonstarved ladybirds tended to spend more time walking than either searching or standing still. Control ladybirds (no aphids), regardless of starvation level, spent more time walking or standing still and very little time searching. In general, ladybirds in the presence of uninfected aphids spent more time searching than those in the presence of infected aphids.

*Response time after encounter (Table 2).* Significant differences in after encounter times were found among aphid food types ( $F_{4,90} = 4.15, P = <0.001$ ) and between starvation regimes ( $F_{2,90} = 38.12, P < 0.001$ ), and an interaction between the two existed ( $F_{8,90} = 4.05, P < 0.001$ ).

When presented with dead, uninfected aphids, all ladybirds, regardless of starvation level, took the same time to respond after an encounter. All 48-hr-starved ladybirds took longer to respond to an encounter than either nonstarved or 24-hr-starved ladybirds. In general, 24-hr-starved ladybirds made faster responses than either nonstarved or 48-hr-starved ladybirds except when presented with dying infected aphids when response times lengthened with increasing starvation.

**TABLE 1**  
Mean Percentage of Time (*p*) Spent Feeding

Aphid food type	Ladybird starvation level		
	Nonstarved (SEM = 0.342; <i>n</i> = 6)	24-hr-starved (SEM = 0.342; <i>n</i> = 6)	48-hr-starved
Dead uninfected	65 ( <i>m</i> = 0.26)	71 ( <i>m</i> = 0.39)	88 ( <i>m</i> = 0.88; SEM = 0.265; <i>n</i> = 10)
Live uninfected	10 ( <i>m</i> = -0.93)	78 ( <i>m</i> = 0.56)	77 ( <i>m</i> = 0.52; SEM = 0.279; <i>n</i> = 9)
Dying infected	4 ( <i>m</i> = -1.41)	87 ( <i>m</i> = 0.83)	67 ( <i>m</i> = 0.30; SEM = 0.296; <i>n</i> = 8)
Dead infected	25 ( <i>m</i> = -0.49)	7 ( <i>m</i> = -1.11)	77 ( <i>m</i> = 0.53; SEM = 0.296; <i>n</i> = 8)
Sporulating infected	1 ( <i>m</i> = -2.35)	1 ( <i>m</i> = -2.32)	70 ( <i>m</i> = 0.38; SEM = 0.265; <i>n</i> = 10)

*Note.* Mean (*m*) and standard error (SEM) are on log-ratio scale, and sample size (*n*) is given in parentheses. Percentages are obtained from *m* using the back transformation.  $p = 10^m / (1 + 10^m)$ .

TABLE 2

Mean Times (Sec, Logged Base 10 after Adding Offset of 1) Spent Between Encounter and Response

Aphid food type	Ladybird starvation level		
	Nonstarved ( <i>n</i> = 6; SEM = 0.135)	24-h-starved ( <i>n</i> = 6; SEM = 0.135)	48-hr-starved ( <i>n</i> = 10; SEM = 0.104)
Dead uninfected	0.630	0.439	0.676 ( <i>n</i> = 10; SEM = 0.104)
Live uninfected	0.501	0.381	0.970 ( <i>n</i> = 9; SEM = 0.110)
Dying infected	0.130	0.710	1.355 ( <i>n</i> = 8; SEM = 0.117)
Dead infected	0.397	0.000	0.779 ( <i>n</i> = 8; SEM = 0.117)
Sporulating infected	0.310	0.000	0.839 ( <i>n</i> = 10; SEM = 0.104)

*Different types of mouthpart encounter responses.* When the log ratios for each treatment were compared by ANOVA, food types and starvation regimes were both highly significant overall ( $F_{4,86} = 17.31$ ,  $P < 0.001$  and  $F_{2,86} = 13.55$ ,  $P < 0.001$ , respectively), and there was also a significant interaction ( $F_{8,86} = 3.25$ ,  $P = 0.003$ ). Backtransforming the mean log ratios gives the approximate percentages of encounters that resulted in feeding (Table 3).

The interaction is strongly influenced by the food type differences for 24-hr-starved ladybirds and the high value for dead infected aphids for nonstarved ladybirds. Ladybirds showed proportionally more "ignore" encounters than "feed" encounters for dying infected and sporulating infected aphids at all times. Apart from live uninfected aphids (nonstarved ladybird) this was reversed for other treatments where they had proportionally more "feeding" encounters.

### Vectoring Study

Aphids became infected with *E. neoaphidis* following exposure to ladybirds inoculated with conidia of this

TABLE 3

Mean Log Ratio of Feed/Ignore Encounters

Aphid food types	Ladybird starvation level		
	Nonstarved ( <i>n</i> = 6)	24-hr-starved ( <i>n</i> = 6)	48-hr-starved
Dead uninfected	0.028 (51.6)	0.953 (90.0)	0.594 (79.7, <i>n</i> = 10)
Live uninfected	-0.719 (16.0)	0.464 (74.4)	0.398 (71.4, <i>n</i> = 9)
Dying infected	-0.954 (10.0)	-1.091 (7.5)	-0.217 (37.8, <i>n</i> = 8)
Dead infected	-0.001 (49.9)	-0.191 (39.2)	0.376 (70.4, <i>n</i> = 8)
Sporulating infected	-1.029 (8.6)	-1.546 (2.8)	-0.125 (42.8, <i>n</i> = 10)

*Note.* Percentage of encounters that resulted in feeding out of the total encounters (feeding plus ignored) are shown in parentheses; SEMs obtained as for Table 1 are 0.3610 (*n* = 6), 0.2796 (*n* = 10), and 0.3229 (*n* = 10 vs *n* = 6).

fungus (original aphids on new plants = 10.3% infected) or plants which had been foraged upon by these inoculated ladybirds (new aphids on original plants = 10.8% infected). No aphids died from *E. neoaphidis* infection when only uninoculated ladybirds were present, or when no ladybird at all was present. A very small proportion of aphids in both these last two treatments became infected with a *Conidiobolus* sp.

The locations (median) of the time courses of infection of the two aphid populations (original aphids on new plants and new aphids on original plants) were significantly different. The Mann-Whitney statistic was  $U = 120$  ( $p < 0.01$ ). Estimates of the medians were 4.25 days for original aphids on new plants and 4.74 days for new aphids on original plants, suggesting that aphids received the inoculum at different times relative to their introduction to the plants.

In no experiments did ladybirds themselves succumb to *E. neoaphidis* infection.

### DISCUSSION

Under laboratory conditions, *C. septempunctata* fed on *A. pisum* infected with *E. neoaphidis*. Infected aphids were perceived, particularly by 48-hr-starved individuals, as acceptable food sources. This is a potentially antagonistic interaction, inoculum of *E. neoaphidis* being removed from the environment of new hosts, thus limiting the development of epizootics. This antagonistic interaction is, however, likely to be limited because only 48-hr-starved individuals spent a substantial proportion of their time feeding on infected prey. In addition, they took longer to consume sporulating aphids compared to uninfected aphids, only occasionally consuming a cadaver entirely (on these occasions they were also seen to graze the surrounding halo of conidia). This slowness of feeding, compared to uninfected dead and living aphids which were usually entirely and rapidly consumed (R. Pluke unpublished data), was also seen in other infected treatments, but to a lesser extent. The ladybirds, in addition to finding infected aphids less palatable, may experience physical difficulty in consuming infected aphids. The feeding methods of some carnivorous coccinellids have been described (Hodek, 1973; Richards and Goletsos, 1991) and many, including *C. septempunctata*, show two methods of feeding. The first is by extraintestinal digestion, whereby enzymes are injected into the prey and the resultant digestive material is sucked out and consumed. The second method is by general mastication and consumption of the prey. Both methods are often used together by the same ladybird. The extensive internal colonization of the aphid by fungal hyphae, even in the living but infected treatments, may make the food item more difficult to masticate in the normal way, and may be resilient to the digestive enzymes produced. This would result in slow consump-

tion and more frequent abandonment of partially consumed prey. These fractions of sporulating cadavers do, however, continue sporulating (H. E. Roy, unpublished data) and thus continue to produce inoculum for further transmission.

The potentially most antagonistic feeding interaction between *C. septempunctata* and *E. neoaphidis* occurs because the ladybirds fed for similar proportions of their time on dying infected and living uninfected aphids. This was particularly clear in ladybirds starved for 24 and 48 hr. Nonstarved ladybirds fed little except on uninfected dead aphids, presumably because these were an irresistible food source, equivalent in palatability to living uninfected aphids, but requiring no effort to capture. The 24-hr-starved ladybirds actually spent a slightly, though not significantly, greater proportion of their time feeding on the living infected aphids compared with living uninfected aphids. Dying infected aphids, being moribund, might be considered easier to catch and thus might be fed on preferentially; however, the percentage of encounters resulting in feeding, at any starvation level, were always higher for living uninfected aphids compared to dying infected aphids (Table 3). In addition, within any starvation level, ladybirds always had longer after-encounter times in response to dying infected aphids (Table 2). These observations suggest that some decision making occurred and that for all ladybirds, the decision on whether to feed or not on dying infected aphids took longest to make and usually resulted in a response other than to feed.

Direct feeding of ladybirds on infected aphids is not, therefore, likely to represent a significant limitation to the development of epizootics of *E. neoaphidis*, particularly because in the field ladybirds are most likely to have choices of food items within any aphid population, and unless very hungry they are likely to feed preferentially on the more suitable uninfected aphids.

During the feeding experiments described here the more common results of continual feeding on unsuitable prey, such as reduced longevity and fecundity (Hodek, 1993), were not studied. These sublethal effects of *C. septempunctata* feeding on *E. neoaphidis*-infected aphids require further study. However, direct susceptibility of *C. septempunctata* to *E. neoaphidis* infection was never observed, confirming the limited host range of the fungus (Glare and Milner, 1989).

Ladybirds feeding on sporulating cadavers (or foraging/feeding in close proximity to sporulating cadavers) became contaminated with the actively discharged conidia of *E. neoaphidis*. These conidia possess preformed mucus making them adhesive. By adhering to the ladybird cuticle, particularly on the numerous hairs around the mandibles, they have the potential to be passively carried or vectored to other aphid populations. From the analysis done on activities other than

feeding, 24- and 48-hr-starved ladybirds (except in the control) spent more time cleaning compared to those that were not starved. These are also the treatments for which more feeding occurred, so it seems likely that cleaning is linked to feeding. In the literature, feeding on unpalatable food has been linked to persistent mouthpart grooming (Nishida and Fukami, 1989) but this was not observed in the present study. There was no greater proportion of time spent cleaning after feeding on the infected food types (even the sporulating infected treatment) than in the uninfected treatments. So, *E. neoaphidis* conidia attaching to the ladybird cuticle are unlikely to receive excessive grooming, which may improve the chances of these conidia remaining attached and being vectored to other aphid populations. Some predators which have fed on unsuitable prey, e.g., the coccinellid *Adalia decempunctata* L. feeding on the aphid *Megoura viciae* Buckton, have been reported to vomit after ingestion (Dixon, 1958); this response was not observed in the work reported here, suggesting limited detrimental effects on the beetle.

Vectoring of *E. neoaphidis* by contaminated ladybirds to susceptible aphids and the initiation of infection in those aphids was demonstrated on whole plants in laboratory experiments. Transmission occurred both to aphids present on the plant at the same time as the ladybird, and to aphids placed onto plants that had been foraged upon by contaminated ladybirds. Further work is required to determine whether the primary conidia on the ladybird cuticle were dislodged, directly infecting aphids, or whether they remained attached, discharging secondary conidia infective to the aphids. Previously, vectoring of plant pathogenic fungi (Nemeye *et al.*, 1990; Gillespie and Menzies, 1993) and entomopathogenic fungi (Schabel, 1982; Poprawski *et al.*, 1992) by other insects has been described, though this is the first record of a predator passively carrying entomopathogenic fungi to hosts. However, there are also examples in which vectoring of entomopathogenic fungi does not occur (Akalach *et al.*, 1992; Furlong and Pell, 1996); indeed, in the work described here only 10–11% of target insects became infected. The level of contamination with conidia in this work may also be higher than ladybirds may encounter in nature, though this has not been quantified. Vectoring may play only a small role in the epizootiology of *E. neoaphidis*, although an initial 10% infection level could still easily initiate a larger epizootic under suitable conditions. The low levels of infection with *Conidiobolus* sp. were probably caused by conidia of this species found naturally occurring on the soil and leaves. The high humidities during the experiment would have encouraged infection.

Behavior other than feeding and cleaning by ladybirds in the presence of infected or uninfected aphid

food items also has relevance to pathogen vectoring potential. Hungry ladybirds (48-hr-starved) spent most of this remaining time searching, in contrast to unstarved ladybirds which spent more time walking. Searching ladybirds make numerous turns, which keeps them within a limited foraging area, whereas walking ladybirds make fewer turns, taking them further from their original foraging sites to new environments and/or aphid colonies. Intensive searching within an infected aphid population would thus encourage pickup of *E. neoaphidis* by ladybirds and encourage transmission within the aphid population. The very presence of the predator may enhance infection levels within the aphid population. Greater aphid escape movements in response to the predators (and the associated release of alarm pheromone) would improve the chances of uninfected aphids to contact inoculum and thus cause an increase in infection levels. This has been observed in similar systems (Hockland *et al.*, 1986; Furlong and Pell, 1996). Walking (and/or migration) by ladybirds, which occurs when they are not hungry or when there are insufficient numbers of aphids to retain hungry individuals in a population, has the potential to transfer or vector inoculum to other aphid colonies. Natural movement of insects has previously been exploited to aid pathogen dispersal. For instance Peng *et al.* (1992) demonstrated that honeybees inoculated with the fungal biocontrol agent *Gliocladium roseum* Link:Bainier while exiting from the hive carried the fungus to strawberry flowers, where it was able to suppress levels of the plant pathogen *Botrytis cinerea* Pers.:Fr. Vectoring of *E. neoaphidis* by *C. septempunctata* could also be manipulated in a similar way using semiochemicals (behavior-modifying compounds). These chemicals have the potential to influence the movement of insects, including natural enemies within farmland ecosystems (Nordlund *et al.*, 1981). Work is currently underway at IACR-Rothamsted to develop management strategies that not only exploit this ability to move insects within and between crops (stimulo-diversionary tactics; Powell *et al.*, 1990, 1993) but also use them to vector pathogens to pest populations and initiate infection (Pell *et al.*, 1993; Furlong *et al.*, 1995).

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