

Adaptations of *Coccinella magnifica*, a myrmecophilous coccinellid to aggression by wood ants (*Formica rufa* group).

II. Larval behaviour, and ladybird oviposition location¹

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Abstract. Like adults, larvae of the myrmecophilous ladybird *Coccinella magnifica* are rarely attacked by *Formica rufa* ants on foraging trails, but more often attacked on ant-tended aphid colonies. Larvae of the non-myrmecophile *Coccinella septempunctata*, to which *C. magnifica* was compared, were more readily attacked by ants in both situations. To avoid or counter ant aggression when foraging for aphids, *C. magnifica* larvae exhibit three main tactics: they may feed on un- or non-tended aphids; when feeding on tended colonies they often carry prey away, minimising time spent there; and if attacked, they use limited chemical defence. No novel behaviours were found in *C. magnifica* larvae, not occurring to some extent in *C. septempunctata*. *Coccinella magnifica* eggs were laid distant to ant-tended aphids, although closer to un- or non-tended aphids.

INTRODUCTION

Because they are soft-skinned, and thus easily damaged or killed, many ladybird larvae are poorly adapted to coexistence with ants (e.g. Banks, 1962; Bach, 1991). Homoptera form the diet of many ladybird taxa (Hodek, 1996) and a large prey resource is potentially available to such ladybirds in the form of Homoptera tended by ants. However, the aggressive defence of tended Homoptera by attendant ants exerts a strong deterrent effect on predatory ladybirds (e.g. Jiggins et al., 1993). Ovipositing ladybird females are likely to avoid colonies of ant-tended Homoptera, because of the risk of attack (Banks & Macaulay, 1967; J.J. Sloggett, unpub. data), and larvae encountering ant-tended colonies can be attacked and driven from the colony, suffering possible injury or death in the process (e.g. Banks, 1962; Bradley, 1973; Vinson & Scarborough, 1989). Additionally, some predatory ant species also take ladybirds and their larvae as prey (e.g. Mariau & Julia, 1977; Sloggett et al., 1999).

However, the larvae of some ladybird taxa have circumvented the problems posed by ants. Some taxa, which encounter ants at times, possess defensive spines or waxy coverings (Richards, 1980; Völkl & Vohland, 1996). Furthermore, a few specialist myrmecophiles have evolved within the Coccinellidae (Sloggett, 1998). Mostly these appear to be larval myrmecophiles, since either the adult has not been recorded with ants, or is known to be attacked by them (e.g. Silvestri, 1903; Wheeler, 1911; Harris, 1921; Pope & Lawrence, 1990; Völkl, 1995). In these cases slow, cautious movement (Völkl, 1995), a waxy covering (Silvestri, 1903; Wheeler, 1911; Harris, 1921), flattened shape (Pope & Lawrence, 1990; Völkl,

1995) or chemical mimicry of Homoptera (Ozenascheck, 1997) are known or likely important adaptations of the larvae to circumvent or counter ant aggression. The effectiveness of such adaptations is testified to by the existence of two species believed to predate ants, rather than Homoptera (Harris, 1921; Pope & Lawrence, 1990).

Coccinella magnifica Redtenbacher (= *C. distincta* Faldermann) is an aphidophagous myrmecophile associated with members of the *Formica rufa* group and closely allied species, at least in northwestern Europe (see Sloggett et al., 2002). Adults and larvae of this species are recorded feeding on ant-tended aphids, although also sometimes on untended or non-tended aphids (Donisthorpe, 1919–1920; Pontin, 1959; Godeau, 1997; Sloggett et al., 2002). *Coccinella magnifica* is unusual amongst myrmecophilous coccinellids in a number of respects. First, all life history stages of this aphidophagous species co-occur with ants (Donisthorpe, 1919–1920; J.J. Sloggett, pers. obs.). Second, *C. magnifica* is the only known myrmecophilous species in the subfamily Coccinellinae. Its larvae are typical of this group, possessing no waxy covering or other obvious morphological adaptation to ants. In fact they are similar to the larvae of other non-myrmecophilous *Coccinella* species, its close relatives.

Formica rufa group ants apparently behave aggressively to ladybirds and their larvae both on and off their colonies of tended aphids (review in Sloggett & Majerus, 2000). Observations in both the laboratory and field have indicated that, although the larvae of *C. magnifica* are sometimes antennated or palpated by *F. rufa* group ants, they are rarely attacked by them, unlike the larvae of other ladybird species (Donisthorpe, 1919–1920; Majerus, 1989; Manica, 1996; Godeau, 1997). Arnold et

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al. (unpublished, see Majerus, 1994, p. 151) have observed that *C. magnifica* larvae placed on colonies of *Aphis fabae* Scopoli tended by the ant *Lasius niger* (L.) are attacked by the ants, but the larvae ignore these attacks, continuing to feed and behave normally. *Coccinella magnifica* is not associated with *L. niger* naturally (Sloggett et al., 2002).

Previous work has shown that the likelihood of *Formica rufa* L. ant attack on adult *C. magnifica* is context-dependent: whilst *C. magnifica* adults are infrequently attacked on ant foraging trails, where they frequently occur, they are often attacked when on colonies of ant-tended aphids. As a consequence adult *C. magnifica* often resort to a combination of physical and behavioural defence when predated ant-tended aphids (Sloggett et al., 1998). In this paper it is demonstrated that the probability of *F. rufa* attack on larvae is also context-dependent. We consider how *C. magnifica* larvae successfully forage and circumvent the problem of ant aggression on colonies of tended aphids. This presents a much greater risk to the soft-bodied larva than to physically well-defended adult. We also address how the aggression of aphid-tending *F. rufa* could influence spatial aspects of adult *C. magnifica* oviposition.

In much of this work, *C. magnifica* larvae are compared to those of its congener *Coccinella septempunctata* L. The two species are known to be closely related on the basis of both morphology and DNA sequence data (Iablokoff-Khnzorian, 1982; G.D.D. Hurst, J.H. Gvd. Schulenburg & J.J. Sloggett, unpub. data) and are ecologically similar in a number of respects (Sloggett et al., 1998, 2002). However *C. septempunctata* is not a myrmecophile, although the adults of this species will sometimes visit colonies of ant-tended aphids when prey is scarce in late summer (Sloggett & Majerus, 2000). *Coccinella septempunctata*, therefore, is a good phylogenetically controlled model for the ancestor of *C. magnifica* before myrmecophily evolved, and has been used as such in a number of earlier studies (see Sloggett, 1998; Sloggett et al., 1998; Sloggett & Majerus, 2000).

MATERIALS AND METHODS

General considerations

The experiments and observations described here were carried out at Oxshott Heath, Surrey England. Additional observations on the location of eggs and larvae were carried out at Esher Common, some one mile distant from Oxshott Heath. General descriptions of the field sites are given by Sloggett et al. (1998). The ant *Formica rufa* is common at both sites.

The majority of work was carried out between April and July of 1998, with a few replicates of experiments on larval behaviour on ant-tended aphid colonies being carried out in June 1999 (see below for details). During spring and early summer, *C. magnifica* eggs and larvae are commonly found at the study sites associated with *F. rufa*. *Coccinella septempunctata* larvae are not found in the vicinity of *F. rufa* nests, although they are to be found on herbaceous plants nearby (see Sloggett & Majerus, 2000).

Experiments using ant-tended aphid colonies were conducted on Scots pine, *Pinus sylvestris* L., using ant-tended *Cinara* aphid colonies. Two *Cinara* species occur at Oxshott Heath on

P. sylvestris: *Cinara pilosa* (Zetterstedt) (often described as *C. pinea* (Mordwilko) by many British authors: C. I. Carter, personal communication), from April to July, and *Cinara pini* (L.), from April to October. Of the two species, *C. pini* is the more intensively tended by ants, whereas *C. pilosa* tends to form looser aggregations, which are sporadically attended (see Völkl & Kroupa, 1997, on *C. pini* and *C. pinea*). The *Cinara* species used in individual trials were not recorded, but the majority of colonies used, if not all, were likely to be *C. pini*, since this forms better, more easily defined colonies which are easier to work with.

Coccinella magnifica larvae used in experiments were laboratory reared from ovipositing *C. magnifica* adults, or from egg clutches, collected at Oxshott Heath. They were fed on a diet of pea aphids, *Acyrtosiphon pisum* (Harris), initially in 90 mm Petri dishes for each clutch of larvae, later in 140 mm Petri dishes for up to eight larvae. There is no difference in the behaviour of *F. rufa* towards naturally occurring and laboratory bred *C. magnifica*, on ant trails at least (Manica, 1996). *Coccinella septempunctata* larvae were a mixture of laboratory bred individuals, reared in the same way as *C. magnifica*, and individuals collected near the field sites and at Mickleham, near Dorking, in Surrey. All experiments used final (fourth) instar larvae.

Experiments comparing *C. magnifica* larvae with *C. septempunctata* larvae, and the effect of context (ant-tended aphid colonies vs. ant trails) were conducted using matched replicates, testing both a *C. magnifica* and a *C. septempunctata* larva on the same trail or ant colony consecutively. Additionally aphid colonies were tended by ants from the same nest as the ants on the foraging trails for each matched replicate in the experiment on context dependence. The order of trials within a replicate was randomised. Within matched replicates on the same *F. rufa*-tended aphid colony, a gap of at least 15 minutes was left between trials. Each larva used in experimental replicates originated from a different female or egg clutch, although larvae in different treatments within a replicate were usually related. Thus pseudoreplication through the use of related individual larvae was avoided.

Formica rufa aggression toward *C. magnifica* and *C. septempunctata* larvae in the presence and absence of ant-tended aphids

To compare the likelihood of attack by *F. rufa* on *C. magnifica* and *C. septempunctata* larvae on foraging trails and colonies of ant-tended aphids, the same method was used as that previously used for adults of the two species (Sloggett et al., 1998). Larvae were released individually, either onto an *F. rufa* foraging trail, on the ground, or near a colony of tended aphids. The number of *F. rufa* workers encountering each larva until it was attacked was recorded and a score allotted on that basis. Thus a score of five would indicate that the first four ants that encountered the larva did not attack it and the fifth did. If a larva was not attacked after 10 encounters it was allotted a score of > 10 or unattacked. A larva was considered attacked if an ant attempted to bite it or spray it with formic acid. Larvae that left the trail or aphid colony before they had been attacked or 10 encounters were recorded were returned to the trail or aphids. A total of 15 replicates were carried out between April and July 1998.

Some larval behaviour on ant trails was also recorded, as was the behaviour of some wild *C. magnifica* larvae observed.

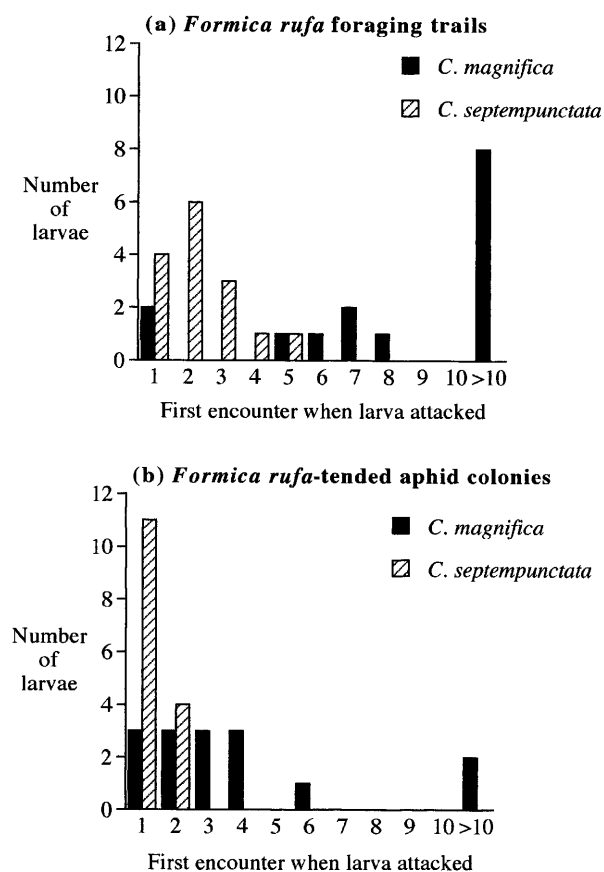


Fig. 1. Frequency distribution of *F. rufa* attacks on the larvae of *C. magnifica* and *C. septempunctata* on foraging trails and tended aphid colonies.

Behaviour of ants and larvae of *C. magnifica* and *C. septempunctata* on ant-tended aphid colonies

Larvae that had been starved from the early morning (> 3 hours) were placed near individual colonies of *F. rufa*-tended aphids and allowed to walk onto the colony. Each individual was recorded as attacked or unattacked by the ants whilst on the colony. A larva was only recorded as unattacked if it was not attacked by any of the ants it encountered whilst on the colony, or if it failed to encounter any ants. Larvae were not returned to the aphid colony if they failed to encounter ants, unlike the adults of Sloggett et al. (1998).

The number of aphids eaten by each larva was recorded, as was other larval behaviour including chemical defence. The time larvae spent on the colony, up to a maximum of 30 minutes was also recorded. Nineteen replicates were carried out between April and July 1998; a further three were carried out in June 1999.

Field observations on the occurrence of *C. magnifica* eggs and larvae

During the experiments, records were kept of the occurrence of naturally occurring *C. magnifica* egg clutches and larvae at Oxshott Heath and Esher Common. The distance of each *C. magnifica* egg clutch from the nearest colony of *F. rufa*-tended aphids and from the nearest untended/non-tended aphids was measured. Measurements were to the nearest centimetre. The distribution of larvae in relation to ant trails and colonies of ant-tended aphids was also recorded.

Analysis and reporting of results

Ordinal data was analysed using non-parametric tests (Wilcoxon Signed Ranks Test for Paired Samples and the Mann-Whitney Test: Zar, 1999), due to its non-normal distribution. Tests were all two-tailed. Frequency data (as 2×2 tables) was analysed using chi-square tests, using the Yates' correction for continuity: data was considered suitable for chi-square analysis since the mean expected value of all tables was greater than 6 (Zar, 1999).

Some observations for larvae similar to those previously analysed fully for adults (Sloggett et al. 1998) are reported here anecdotally, specifically the behaviour of larvae on ant trails and some aspects of larval and ant behaviour on colonies of aphids. In the case of larvae on ant trails it was difficult to make a large number of semi-independent observations after larvae had been attacked by ants and reflex bled, making an analysis similar to that of Sloggett et al. (1998) impossible. On colonies of tended aphids, ant densities were much lower than when adult ladybirds were studied and attacks on larvae were often of a sporadic nature, unlike those on the adults (J.J. Sloggett, personal observation). This makes statistical analysis of some aspects of behaviour highly problematic (for example, larval escape behaviour in the face of ant attack).

RESULTS

Formica rufa aggression toward *C. magnifica* and *C. septempunctata* larvae in the presence and absence of ant-tended aphids

Formica rufa workers attacked *C. magnifica* larvae significantly less than they attacked *C. septempunctata* larvae both on ant trails and on colonies of ant-tended aphids (Fig. 1. Two-tailed Wilcoxon Signed Ranks Test, counting a score of > 10 as 11: trails - $n = 15$ non-ties, $T^+ = 117$, $T^- = 3$, $P < 0.001$; tended aphids - $n = 11$ non-ties + 4 ties, $T^+ = 66$, $T^- = 0$, $P < 0.001$).

However, *F. rufa* aggression towards *C. magnifica* larvae was much greater on colonies of ant-tended aphids than on ant trails (Fig. 1. Two-tailed Wilcoxon test: *C. magnifica*: $n = 12$ non-ties + 3 ties, $T^+ = 74$, $T^- = 4$, $P < 0.005$). The same was also true for *C. septempunctata* larvae ($n = 9$ non-ties + 6 ties, $T^+ = 45$, $T^- = 0$, $P = 0.005$).

Observations of the behaviour of ladybird larvae on ant foraging trails

On trails, *F. rufa* workers often displayed no visible reaction to a *C. magnifica* larva or sometimes tapped it with their antennae. Many *C. magnifica* larvae remained motionless for long periods on ant foraging trails. When moving, *C. magnifica* larvae were, at times, observed to stop on encountering ants, as do *C. magnifica* adults (Sloggett et al., 1998). *Coccinella septempunctata* larvae were rapidly attacked. In response, they frequently produced copious quantities of reflex blood, the characteristic chemical defence of ladybird adults and larvae (Daloze et al., 1995). To some extent reflex bleeding deterred ants from further attacks, although frequently *C. septempunctata* larvae were surrounded and ultimately carried off by the ants, presumably serving as prey (see Sloggett et al. 1999). At times moving *C. septempunctata* larvae stopped in response to an encounter with an ant, like *C. magnifica* larvae.

Table 1. Comparison of *C. magnifica* and *C. septempunctata* interactions with *F. rufa* on colonies of ant-tended aphids.

	<i>Coccinella magnifica</i>	<i>Coccinella septempunctata</i>	Statistical test
N = 22 paired replicates			
Probability of encounter and attack by ants			
Larva did not encounter ants	6	2	$\chi^2 = 1.37$, 1df, NS ^a
Larva did encounter ants, but was not attacked	8	2	$\chi^2 = 5.24$, 1df, $P < 0.025^{\text{a,b}}$
Total larvae unattacked	14	4	$\chi^2 = 7.62$, 1df, $P < 0.01^{\text{a}}$
Larvae attacked	8	18	
Probability of injury or death to larva			
Larva seized	1	3	$\chi^2 = 0.27$, 1df, NS ^c
Larva dragged from colony	0	1	$\chi^2 = 0$, 1df, NS ^c
Larval feeding behaviour on colony			
Feeding on tended aphids	15	14	$\chi^2 = 0$, 1df, NS
Number of aphids eaten (mean \pm SE)	1.64 \pm 0.35	1.00 \pm 0.23	Wilcoxon Signed Ranks Test: n = 15 nonties + 7 ties, $T^+ = 90$, $T^- = 30$, $0.10 > P > 0.05$
Carrying an aphid	11	1	$\chi^2 = 10.49$, 1df, $P < 0.002^{\text{d}}$
Larval chemical defence			
Visible reflex bleeding	2	15	$\chi^2 = 5.95$, 1df, $P < 0.02^{\text{e}}$
No visible reflex bleeding, but ant wiping mouthparts	3	0	
Total chemical defence	5	15	$\chi^2 = 0.43$, 1df, NS ^e
Length of time on colony (medians) ^f	218 sec	242 sec	Wilcoxon Signed Ranks Test: n = 21 non-ties, $T^+ = 132$, $T^- = 99$, NS

^aThree independent chi-square tests have been used to analyse these data, since ant behaviour and larval behaviour effects may be independent.

^bData in which larvae did not encounter ants have been excluded from the chi-square test, thus sample sizes are 16 for *C. magnifica* and 20 for *C. septempunctata*.

^cChi-square tests are based on the total sample size (i.e. $n = 22$ for both species), rather than on the number of individuals attacked.

^dChi-square tests are based on the number of individuals which fed, thus the sample sizes are 15 individuals for *C. magnifica* and 14 individuals for *C. septempunctata*.

^eChi-square tests are based on the number of larvae attacked, thus the sample sizes are 8 for *C. magnifica* and 18 for *C. septempunctata*.

^f $n = 21$ for both species, as one paired replicate where a *C. septempunctata* was captured by the ants has been excluded. Time does not include aphid consumption if the larva carried the aphid from the colony and ate it elsewhere.

Behaviour of ants and larvae of *C. magnifica* and *C. septempunctata* on ant-tended aphid colonies

Ant and larval behaviour on tended aphid colonies is summarised and analysed in Table 1. *Coccinella magnifica* larvae were significantly less likely to be attacked by *F. rufa* than the larvae of *C. septempunctata* during a visit to a colony of tended aphids. This effect arose due to two factors. First, fewer *C. magnifica* larvae were directly encountered by ants during visits to aphid colonies, although the effect is not significant. Second, when *C. magnifica* were encountered by ants during a visit to an aphid colony, fewer were attacked.

In general *C. magnifica* larvae appeared to move more slowly than those of *C. septempunctata*. Both *C. magnifica* and *C. septempunctata* larvae readily fed on tended aphids, although their mode of feeding was somewhat different. *Coccinella septempunctata* larvae began feeding rapidly on encountering aphids and would often continue to feed at the site until attacked. On the other hand, *C. magnifica* larvae frequently picked up aphids and carried

them in their jaws. Typically, a larva would seize an aphid at the edge of a colony and slowly back away from the colony along the branch or a pine needle to a distance of several centimetres. In two cases of 11 larvae moved forward with an aphid, and in one case a larva that had been backing away with an aphid, dropped from the branch with it on being attacked by an ant. This behaviour was observed when ants were both present and absent from the immediate vicinity of larvae. Aphid-carrying behaviour similar to that of *C. magnifica* was only observed for *C. septempunctata* once, and in this case the larva may have been endeavouring to maintain a firm grip on the substrate rather than trying to leave the aphid colony. The difference in the occurrence of this behaviour in the two species was highly significant.

No significant differences were found in the length of time larvae of the two species spent on the aphid colonies. This probably arises for several reasons. *Coccinella septempunctata* larvae were rapidly expelled from the colony when encountered by *F. rufa* and *C. magnifica* larvae

minimise the time spent on the colony by carrying aphids away from the colony to eat. Furthermore both species often fed for some while before encountering ants, and this additional time period, present for both species, acts to further reduce any differences between them in the data.

There was a non-significant trend for *C. magnifica* larvae to consume more aphids than *C. septempunctata*. It is worth noting that the data probably underestimate the difference, since an aphid was counted as eaten when a larva began eating it, rather than when it was entirely consumed. *Coccinella septempunctata* larvae were frequently interrupted, and deterred from further feeding by ant attack, whereas this was rarely the case for *C. magnifica*.

Under ant attack, *C. septempunctata* larvae usually produced copious quantities of reflex blood (83% of attacked larvae). In contrast, attacked *C. magnifica* larvae reflex bled to deter ants visibly on two occasions (25%) and the quantity produced was visibly far less than that of *C. septempunctata* larvae. However, in three further cases ants were observed wiping their mouthparts, for up to five minutes after attacking *C. magnifica* larvae, without any visible reflex bleeding. It is unclear whether they were responding to minute quantities of reflex blood not visible to the observer, or whether the larva is generally distasteful to the ant. The number of cases of reflex bleeding visible to the observer is significantly higher in *C. septempunctata*: If the extra *C. magnifica* cases, where reflex bleeding may have occurred but was not visible, are included the difference between the two species is not significant.

In general, for both *C. magnifica* and *C. septempunctata*, chemical defence appeared to be a very effective deterrent. Only three *C. septempunctata* larvae were seized by the ants and of these only one failed to escape and was carried from the colony by the ants. Ants were clearly repelled by *C. magnifica* reflex bleeding: Only single *C. magnifica* larva was seized by an *F. rufa* worker. This individual did not reflex bleed and no mouthpart wiping was observed on the part of the ant afterwards, although the larva escaped.

After reflex bleeding, *C. septempunctata* larvae typically ran from the aphid colony, to evade the ants. *Coccinella magnifica* larvae also sometimes ran from the colony, but in several cases ant attacks were abandoned and the larvae remained where they were. On occasions larvae of both species dropped from the branch after an attack. There did not appear to be any differences in the tendencies of the two species to run or drop, unlike those reported for the adults under ant attack (Sloggett et al., 1998). Running was most common.

Field observations on the occurrence of eggs and larvae

Seven clutches of *C. magnifica* eggs were discovered in the field in 1998. Three were found at Oxshott Heath on Scots pine. Ant tended aphid colonies here consisted of the two *Cinara* species, *C. pilosa* and *C. pini*, and untended aphids were *C. pilosa* without ants and the non-tended *Schizolachmus pineti* (F.). Four clutches were

found at Esher Common on spruce (*Picea* sp.) with the tended aphid *Cinara piceicola* Cholodkovsky and non-tended aphid *Elatobium abietum* (Walker). At both sites all egg clutches were found to be closer to un- or non-tended aphids (mean \pm SE, 4.4 ± 1.8 cm) than to ant-tended aphid colonies (43.3 ± 8.4 cm) (Two-tailed Wilcoxon Signed Ranks Test of egg clutch distances from tended vs. un-/non-tended aphids: $n = 7$ non-ties, $T^+ = 28$, $T^- = 0$, $P < 0.02$).

In 1998, a total of 76 larvae of all instars were observed at Oxshott Heath and Esher Common. Of 49 larvae observed at Oxshott Heath, 31 (63%: second to fourth instar) were observed apparently foraging on *F. rufa* trails or nearby low growing vegetation in the absence of ant-tended aphids. At Oxshott Heath, *C. pilosa* aphids are often also observed on ant trails, presumably having fallen from overhanging branches of Scots pine (see Kloft, 1953) and a *C. magnifica* larva was observed feeding on such prey on an ant trail. Other non-tended aphids occur on surrounding herbaceous plants here (J.J. Sloggett, personal observation). Eighteen larvae (37%: first to fourth instar) were observed either on, or in the near vicinity of colonies of ant-tended aphids: 13 were observed on Scots pine; some of these may have been foraging on untended *C. pilosa* or the non-tended *S. pineti*. The other five were observed on gorse, *Ulex europaeus* L., associated with *Aphis ulicis* Walker, tended by *F. rufa*, and *Myrmica ruginodis* Nylander. *Coccinella magnifica* larvae, as well as adults, were observed associated with colonies tended by both ants, although *C. magnifica* is not a regular associate of *Myrmica* species.

At Esher Common, spruce was examined again, later in the season, after the discovery of egg clutches there. In excess of 27 larvae were present on a single spruce tree: The non-tended *E. abietum* was not recorded at this time; therefore it appears these larvae were feeding on the *F. rufa*-tended *C. piceicola*, which was present. No *C. magnifica* larvae were found on ant trails. The aphid *C. pilosa* was not present on ant trails at this site.

DISCUSSION

The problems of ant aggression faced by *C. magnifica* larvae appear to be very similar to those faced by adult *C. magnifica* (Sloggett et al., 1998). That is, although ants rarely attack *C. magnifica* larvae in the absence of tended aphids, they are much more likely to attack the larvae on colonies of tended aphids. This is probably because the ants tending colonies of aphids display ownership behaviour (Way, 1963), perhaps responding to a change in aphid behaviour in the presence of an intruder (Nault et al., 1976), in addition to or in place of their predatory instincts exhibited away from aphid colonies (Way, 1963; Sloggett et al., 1999).

Coccinella magnifica larvae, like the adults, probably possess some form of chemical inhibition of *F. rufa* aggression, based on extreme repellence (Sloggett, 1998; J.J. Sloggett, unpublished data; see also Dixon, 2000, p. 30, and below). This provides an extremely effective counter to predatory ant aggression when tended aphids

are not present, for example on ant foraging trails. However, as for the adult, it is less effective on colonies of ant-tended aphids. It is clearly not the case that *C. magnifica* larvae are not attacked by ants whereas the adults are, as has been previously suggested (Godeau & Hemptinne, cited by Dixon, 2000, pp. 105–106). Larvae, like adults, are infrequently attacked by *F. rufa* on trails, and more often attacked on colonies of *F. rufa*-tended aphids.

Faced with potential attack on colonies of *F. rufa*-tended aphids, how do the soft-bodied larvae of *C. magnifica* forage successfully then? The solution appears to be threefold: *C. magnifica* larvae may simply not forage on ant-tended aphids; if they do they minimise the time spent on the aphid colony; and if they are attacked they use limited chemical defence to deter the attack.

Often *C. magnifica* larvae do not forage on ant-tended aphids, but on untended or non-tended aphids, thus avoiding the heightened aggression of aphid-tending *F. rufa*. This is indicated by the occurrence at Oxshott Heath of numbers of larvae on or near ant-foraging trails, probably feeding on stray *C. pilosa* and non-tended aphids on herbaceous plants. At Esher Common, where unlike Oxshott Heath, there were no stray untended *C. pilosa*, larvae were not found on ant trails. This suggests that the occurrence of larvae on trails is not accidental, for example as a consequence of falling from vegetation. Furthermore, the boundary between aphids that are ant-tended and those that are not is not invariable, and for much of spring and early summer there is an excess of available facultatively tended aphids, such as *C. pilosa*, from which *F. rufa* group ants obtain honeydew (Wellenstein, 1952; J.J. Sloggett, personal observation). As a consequence many colonies of aphids are only sporadically attended by ants. *Coccinella magnifica* larvae undoubtedly exploit such colonies.

The larvae also clearly sometimes feed on well-tended aphid colonies, such as those of *C. pini* and *C. piceicola*. When feeding on ant-tended aphids two strategies of *C. magnifica* larvae are apparent. First, *C. magnifica* larvae exhibit a characteristic aphid carrying behaviour, typically picking up an aphid from the edge of colony of tended aphids and backing away from the colony with it. This behaviour serves to minimise the time in which the larva is exposed to aggressive aphid-tending ants. If all else fails, and a larva is attacked, it can use chemical defence. It appears that *C. magnifica* larvae have, however, minimised the cost of chemical defence. Unlike *C. septempunctata* larvae, those of *C. magnifica* do not copiously reflex bleed, losing large quantities of liquid and defensive alkaloid. Very little reflex blood appears to be produced by *C. magnifica* larvae in response to ant attack: In some cases none was visible to the observer. Perhaps *C. magnifica* larvae often rely on their characteristic repellent counter to ant aggression as a protection and do not reflex bleed at all. Certainly some role is played by this in minimising aphid-tending *F. rufa* attacks on *C. magnifica* larvae, since although the larvae are attacked, it is at a lower frequency than *C. septempunctata* larvae.

It is also perhaps noteworthy that, rather than coccinelline and precoccinelline, alkaloids characteristic of the chemical defence of other *Coccinella* species (Daloze et al., 1995; King & Meinwald, 1996), the major alkaloid of *C. magnifica* is hippodamine (Lognay, cited by Dixon, 2000, p. 30), which is present in only minor quantities in *C. septempunctata* (Daloze et al., 1995). The full significance of this finding in relation to *C. magnifica*'s association with ants remains to be assessed, although preliminary experiments indicate that *C. magnifica* larval chemical defences are more repellent to ants than those of *C. septempunctata* (J.J. Sloggett unpublished data).

Relatively slow movement also undoubtedly plays a part in avoidance of ant attack by *C. magnifica* larvae. It has been suggested that *C. magnifica* larvae have slightly shorter legs than *C. septempunctata* (Dixon, 2000, p. 69). This seems a likely adaptation against capture by ants on colonies of ant-tended aphids. Unlike some other myrmecophilous coccinellid larvae, such as *Platynaspis luteorubra* Goeze, which remain in very close association with ant-tended aphid colonies (Völkl, 1995), the highly mobile larvae of *C. magnifica* move readily between aphid colonies: thus their legs are not shorter due to limited use.

As with the adult ladybirds, there appear to be no behaviours unique to *C. magnifica* larvae. Most *C. magnifica* behaviour was observed at least once in *C. septempunctata* larvae. In its evolving myrmecophily, the expression of certain behaviours of *C. magnifica*, such as aphid carrying, have been enhanced and whereas the expression of others, such as reflex bleeding, have been reduced. Such modifications of behavioural expression are undoubtedly far more common than the evolution of entirely novel behaviours. The case of *C. magnifica* and *C. septempunctata* bears noteworthy similarities to the case of the myrmecophilous chrysopid *Chrysopa slossonae* Banks and its non-myrmecophilous sibling species *Chrysopa quadripunctata* Burmeister, in this respect (see Milbrath et al., 1993).

Feeding on sporadically-tended, untended or non-tended aphids is also a tactic followed by adult *C. magnifica* in the spring and early summer. Adult *C. magnifica* have also often been recorded on ant trails (Morris, 1888; Champion, 1903; Donisthorpe, 1919–1920; J.J. Sloggett, personal observation). However ant-tended aphids are of greater importance to adult *C. magnifica*, since these predominate in late summer, after the larvae have metamorphosed. Adult *C. magnifica* are better protected by their tougher cuticle, and therefore are in less danger from ant attack than the soft-bodied larvae. Furthermore, adults typically use purely physical and behavioural defence under ant attack and do not have to resort to chemical defences, which need replacing after use (Sloggett et al., 1998). Thus feeding on ant-tended aphids is undoubtedly less costly for the adults than for the larvae of *C. magnifica*.

From the data presented here, it appears that female *C. magnifica* oviposit close to untended or non-tended aphids, but distant from ant-tended aphids. It had been

previously been suggested that *C. magnifica* oviposits at a distance from aphid colonies, although it remained unclear whether un- or non-tended aphids were considered (Godeau & Hemptinne, cited by Dixon, 2000, p. 106). Although the relatively clumped distribution of ant-tended aphids relative to non-tended species makes the finding that *C. magnifica* egg clutches are laid closer to un- or non-tended aphids intrinsically likely, even were clutch distributions random, it does seem likely that female *C. magnifica* do oviposit away from colonies of ant-tended aphids to avoid ant aggression towards themselves or their eggs. In the absence of nearby tended aphids *C. magnifica* eggs are rarely attacked by *F. rufa* group ants, like *C. magnifica* adults and larvae (Godeau, 1997; J.J. Sloggett, unpublished data). However, it is not known whether *F. rufa* would attack *C. magnifica* eggs occurring in the immediate vicinity of tended aphids, although this seems probable. The finding that eggs are frequently laid near to un- or non-tended aphids is consistent with the view that *C. magnifica* larvae often avoid ant aggression by not feeding on ant-tended aphids. This seems particularly likely for neonate larvae, which will initially encounter un- or non-tended aphids in their search for food. The first instar larvae of *C. magnifica* are arguably at greatest risk from aggression by aphid tending ants, due to their very small size.

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