Leucopis annulipes larvae (Diptera: Chamaemyiidae) use a furtive predation strategy within aphid colonies

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Key words. Predation strategy, active-searching predator, furtive predator, Chamaemyiidae, Leucopis annulipes, Cecidomyiidae, Aphidoletes aphidimyza, Coccinellidae, Harmonia axyridis, Acrystosiphon pisum

Abstract. A laboratory experiment was conducted to determine whether the predation strategy of larvae of the aphidophagous silver fly Leucopis annulipes Zetterstedt (Diptera: Chamaemyiidae) is furtive, as are larvae of the aphidophagous midge Aphidoletes aphidimyza Rondani (Diptera: Cecidomyiidae), or active like aphidophagous ladybird beetle larvae (Coleoptera: Coccinellidae). The impact of L. annulipes larvae on pea aphid, Acrystosiphon pisum (Harris) (Hemiptera: Aphididae), colony disturbance was evaluated and compared with that of the furtive predator A. aphidimyza and the active-searching ladybird beetle Harmonia axyridis Pallas. Key results indicate that aphids showed significantly fewer defensive acts (dropping, walking away, wriggling) in the presence of L. annulipes larvae than of ladybird beetle larvae. Furthermore, the impact of L. annulipes larvae on aphid colony disturbance was similar to that of A. aphidimyza and to a control treatment without predators. These results clearly indicate that L. annulipes larvae use a furtive predation strategy.

INTRODUCTION

Most aphidophagous predators, such as ladybirds and lacewing larvae, use an active-searching strategy that elicits aphid defensive behaviour (Dixon, 1958; Losey & Denno, 1998b; Lucas & Brodeur, 2001). One known exception is the furtive predation strategy used by larvae of Aphidoletes aphidimyza Rondani (Diptera: Cecidomyiidae), or active like aphidophagous ladybird beetle larvae (Coleoptera: Coccinellidae). The impact of L. annulipes larvae on pea aphid, Acrystosiphon pisum (Harris) (Hemiptera: Aphididae), colony disturbance was evaluated and compared with that of the furtive predator A. aphidimyza and the active-searching ladybird beetle Harmonia axyridis Pallas. Results indicate that aphids showed significantly fewer defensive acts (dropping, walking away, wriggling) in the presence of L. annulipes larvae than of ladybird beetle larvae. Furthermore, the impact of L. annulipes larvae on aphid colony disturbance was similar to that of A. aphidimyza and to a control treatment without predators. These results clearly indicate that L. annulipes larvae use a furtive predation strategy.

Most aphidophagous predators, such as ladybirds and lacewing larvae, use an active-searching strategy that elicits aphid defensive behaviour (Dixon, 1958; Losey & Denno, 1998b; Lucas & Brodeur, 2001). One known exception is the furtive predation strategy used by larvae of Aphidoletes aphidimyza Rondani (Diptera: Cecidomyiidae) (Lucas et al., 1998; Hindayana et al., 2001), living within apple aphid colonies (Bouchard et al., 1982; Turner, 1997) observed that 1st and 2nd instar larvae of Leucopis ninae Tanasijtshuk & L. gaimarii Tanasijtshuk tend to stay within the aphid colony where their eggs were laid, while the 3rd instar larvae (the last before pupation) are more mobile and even move between aphid colonies and plants. Such mobility has never been reported for A. aphidimyza.

In Quebec, Canada, silver fly Leucopis spp. (Chamaemyiidae: Diptera) larvae are commonly encountered within apple aphid colonies (Bouchard et al., 1982; Tournear et al., 1992). However, very little is known about the ecology of most members of this group. Since Leucopis spp. larvae are usually found within undisrupted aphid colonies, it is possible that their hunting strategy is similar to that of A. aphidimyza. Tracewski (1983) observed that Leucopis sp. nr. albipuncta eggs are usually laid in group of 2–3 under apple leaves in New Hampshire. As for A. aphidimyza (Kozár et al., 1994; Stewart & Walde, 1997; Brown, 2004; Fréchette et al., 2008), their abundance is highly correlated with aphid density (Tracewski, 1983). In many systems Leucopis spp. are observed only when aphid densities reach moderate to high densities (Tracewski, 1983; Carroll & Hoyt, 1984; Fox et al., 2004). However, significant behavioural differences between Leucopis spp. and A. aphidimyza larvae may also exist. For instance, Leucopis spp. larvae are probably more active than A. aphidimyza larvae. In barley, Gaimari & Turner (1997) observed that 1st and 2nd instar larvae of Leucopis ninae Tanasijtshuk and L. gaimarii Tanasijtshuk tend to stay within the aphid colony where their eggs were laid, while the 3rd instar larvae (the last before pupation) are more mobile and even move between aphid colonies and plants. Such mobility has never been reported for A. aphidimyza.

The aim of this experiment was thus to determine whether the predation strategy of Leucopis annulipes Zetterstedt matched more closely the definition of furtive or active-searching predation strategy. The impact of L. annulipes larvae on pea aphid, Acrystosiphon pisum (Harris) (Hemiptera: Aphididae), colony disturbance was thus compared to that of A. aphidimyza larvae, which are known as furtive predators, and Harmonia axyridis Pallas (Coleoptera: Coccinellidae) larvae, which are active-searching predators.

MATERIAL AND METHODS

Biological material

Leucopis annulipes larvae were collected in an apple orchard located in Saint-Bruno-de-Montarville, near Montreal, QC, Can-
ada, in September 2006. This species was identified by Stephen D. Gaimari (California Department of Food and Agriculture) and voucher specimens are deposited in the National Museum of Natural History, Washington, DC (USNM). Leucopis annulipes larvae were found on apple trees near colonies of *Aphis pomi* De Geer and *or Aphis spireicola* Pagenstecher (Hemiptera: Aphididae). Captured individuals were then reared in the laboratory mainly following Gaimari & Turner (1996b) methodology. Both adults and larvae were kept in the same plastic framed cages (35 × 35 × 35 cm) covered with muslin. Larvae were reared on broad bean plants, *Vicia faba* L., infested with either *A. pismum* or *Aphis fabae* Scopoli (Hemiptera: Aphididae). New plants were added about once a week. Discarded plants were cut and kept in the cages for about 2 weeks in order to allow adult emergence from pupae. Adults were fed a mixture of honey and yeast spread on the outer surface of the cage in order to avoid adults becoming stuck in this diet. Cages were held at 23–25°C, RH 24–30%, 16L : 8D.

The *A. aphidimyza* larvae used in this experiment came from the commercial supplier Pant-Prod Québec (Laval, Qc, Canada). As for *L. annulipes*, adults and larvae of *A. aphidimyza* were reared in the same cages (35 × 35 × 70 cm). Larvae were reared on broad bean plants infested with both *A. pismum* and *A. fabae*. New plants were added about once a week. As *A. aphidimyza* pupae develop in the soil, pots in which discarded plants grew were kept in the cages for 3 weeks. Adults were fed a mixture of water and commercial sugar (1/10) provided on a cotton wool. Conditions for rearing were the same as for *L. annulipes*.

The Asian ladybird beetle *H. axyridis* used in the experiment came from a stock culture maintained in the laboratory, which is refreshed yearly with field-captured individuals. Adults were kept in cages (35 × 35 × 35 cm) along with broad bean plants infested with *A. pismum* and *A. fabae*. Moreover, adults were also fed with sugar water, crushed pollen, and *Sitotroga cerealella* Olivier eggs (Lepidoptera: Gelechiidae). Ladybird beetle egg masses were isolated in Petri dishes and larvae reared therein fed on an artificial diet of crushed pollen and *S. cerealella* eggs.

### Impact of three predators on aphid colony disturbance

Experiments were performed on broad bean plants at 23–25°C, RH 24–30%, 16L : 8D. One aphid colony was introduced into a clip-cage on the abaxial surface of a leaf on each experimental plant at least 24 h before the beginning of the experiment. The clip cage was then removed, and aphid colonies were standardized to about 12–14 *A. pismum* individuals aged 2–4 days. After colony standardisation, a delay of 10 min was allowed before the beginning of the experiment. During this 10-min period, the colony was schematized, i.e. each individual aphid was given a number and its position determined. *A. pismum* was chosen for this experiment since this aphid species shows a high level of defense which is readily observed (Nelson & Rosenheim, 2006).

One predator per experimental plant was then introduced near the petiole of the aphid infested leaf, marking the beginning of the observation period. Three experimental treatments using a different predator were compared, that is (1) *A. aphidimyza*, (2) *L. annulipes*, and (3) *H. axyridis*. Moreover, a control treatment without a predator was also recorded. Each of the 4 treatments were replicated 25 times. In order to eliminate the potential effect of predator size on aphid colony disturbance, development stages were chosen so that the predators were of similar size, i.e. 1st and early 2nd instar *H. axyridis* larvae, and 3–4 mm long *A. aphidimyza* and *L. annulipes* larvae, which corresponds to a 3rd instar *L. annulipes* larvae (Sluss & Foute, 1971, 1973; Tracewski, 1983; Gaimari & Turner, 1996a). A controversy still exists about the number of *A. aphidimyza* larval stages (see Lucas et al., 1998). Observations were made over a 45-min period. Predators that did not interact with the aphid colony were discarded and replaced.

Aphid defensive behaviour was observed following the introduction of predators, and classified in one of the three categories: (1) walking away from the feeding site, (2) dropping off the plant and (3) wriggling and kicking. For each aphid defensive behaviour observed, it was noted whether it was a consequence of a direct interaction with the predator (following physical contact) or an indirect interaction (without physical contact between aphids and the predator). Indirect interaction could result from aphid reaction to alarm pheromone, physical contact with conspecifics, and / or plant-borne vibrations caused by the predator. Also, for each predator, the attack success was also noted in terms of the number of prey consumed / the number of contacts with prey.

### Statistical analysis

As aphid colony size varied from 12–14, the number of defensive acts observed was divided by the initial number of individuals in the colony. The mean number of defensive acts / individual for each colony were then compared using Kruskal-Wallis tests since the data were not normally distributed (tested with Shapiro-Wilk). For the comparison of total defensive acts, the number of reactions in the control treatment that would have been attributed to defensive behaviour (walking away, dropping, wriggling and kicking) in any other treatment were recorded. For the comparison of both direct and indirect defensive behaviour, the control treatment was excluded from the analysis since aphid reactions could not be segregated into direct and indirect interactions in the absence of predators. The proportions of each defensive act were compared between predators using a χ² analysis computed by hand (Zar, 1999). The proportions of successful attacks were arcsin square root transformed and compared using a Kruskal-Wallis test. Calculations were made using the statistical software JMP (SAS Institute, 2001).

### RESULTS

#### Defensive behaviour

Every *H. axyridis* and *L. annulipes* individual observed ate only one aphid per aphid colony. *A. aphidimyza* larvae were sometimes observed surrounded by 2 dead aphids.

When all defensive behaviour resulting from both direct and indirect interactions with predators are pooled,
a significant difference is observed between treatments (Kruskall-Wallis; \( \chi^2 = 57.46, \text{d.f.} = 3, P < 0.001 \)) (Fig. 1). The total number of defensive acts / aphid was significantly higher in the presence of \( H. \) axyridis larvae than in all the other treatments (\( P < 0.05 \)), which were not different from one another (\( P > 0.05 \)).

The number of defensive acts resulting from direct interactions was significantly different between treatments (Kruskall-Wallis; \( \chi^2 = 20.48, \text{d.f.} = 2, P < 0.001 \)) (Fig. 2). Direct interaction with \( H. \) axyridis elicited more defensive behaviour than with the 2 other predators (\( P < 0.05 \)). The number of defensive acts resulting from direct interaction was not significantly different between \( L. \) annulipes and \( A. \) aphidimyza (\( P < 0.05 \)).

The number of defensive acts elicited by indirect interactions was also significantly different between treatments (Kruskall-Wallis; \( \chi^2 = 47.88, \text{d.f.} = 2, P < 0.001 \)) (Fig. 3). Their number was greater in the presence of \( H. \) axyridis larvae than in the 2 other treatments (\( P < 0.05 \)). However, there were no differences between the \( L. \) annulipes and \( A. \) aphidimyza treatments (\( P > 0.05 \)).

**Types of defensive behaviour**

In the control treatment, no defensive behaviour was observed with the exception of 4 aphids, which were observed walking, a behaviour that would have been classified as a ‘Walking away’ and a defensive act in any of the 3 other treatments. Among the 3 predator treatments, no significant difference was observed in the proportion of the different defensive behaviour types (\( \chi^2 = 6.062, P > 0.05 \)) (Table 1). This indicates that the different types of aphid defensive behaviour were not associated with predator identity.

**DISCUSSION**

The results of this experiment clearly indicate that the predation strategy of \( L. \) annulipes larvae is similar to that of \( A. \) aphidimyza larvae. Unlike active-searching predators, \( L. \) annulipes larvae are furtive predators and do not disrupt aphid colonies.

Among the active-searching predators, different predator species induce different levels of aphid defensive behaviour (Losey & Denno, 1998b; Nelson & Rosenheim, 2006). For example, Nelson & Rosenheim (2006) recorded that the ratio of disturbed : consumed \( A. \) pisum on alfalfa \( M. \) sativa \( L. \) was 19 : 2 when the predator was \( N. \) sp. (Heteroptera: Naiidae) and 71 : 1 when the predator was \( H. \) convergens Guerin de Méneville (Coleoptera: Coccinellidae). Losey & Denno (1998b) also record that adult \( C. \) septempunctata \( L. \) (Coleoptera: Coccinellidae) disturb \( A. \) pisum...

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colonies more than heteropteran predators. The last authors propose that predator body size, searching behaviour, and consumption rate differences may explain the different impacts on the aphid colonies. Losey & Denno (1998b) also propose that the differences in the aphid response to predator type may depend on the volume of alarm pheromone released, the level of substrate vibration produced by the predators or a combination of these two factors.

In our study, since predator body size was standardised, this factor should not have affected the results. However, the searching behaviour of *H. axyridis* larvae differs from that of the two dipters: *H. axyridis* larvae are campodeiform and probably create more leaf vibration than the veriform *A. aphidimyza* and *L. annulipes* larvae, which move by crawling. Also, *H. axyridis* larvae move faster within the colony and have a lower attack success, generating more disturbance than the slow moving and more efficient furtive predators. Attack success should, however, not be considered as a key factor since Lucas & Brodeur (2001) found that aphid colonies are significantly more disrupted by *Chrysoperla rufilabris* Burmeister (Neuroptera: Chrysopidae) 1st instar larvae than by *A. aphidimyza* larvae, even though both have similar attack successes.

Within a single species, Dixon (1958) observed that the ratio of dropping : walking shown by *Microlophium evansi* (Theobald) (Hemiptera: Aphididae) aphids when approached by an adult *Adalia decempunctata* (L.) (Coleoptera: Coccinellidae) was greater than when approached by a 2nd instar larva. He proposed that this might be due to a decrease in the ratio of the speed of the aphid : speed of the predator when *A. decempunctata* is older, making the Walking away response of aphids less effective. This is supported by the fact that early and late 1st instar larvae, which are of similar size but differ in speed of movement, elicited different defensive behaviour patterns: a greater proportion of aphids escaped predation by *Dropping* when approached by the faster late instar larvae than the early instar larvae. However, the author also stressed that different coccinellid instars with a similar speed of movement also elicited different defensive behaviour patterns, suggesting that size may also play a role.

In our experiment, no significant differences were observed in the proportions of aphid defensive behaviour types elicited by the three predators, which suggests that aphids show similar behaviour when reacting to active-searching and furtive predators. This contrasts with other studies that have found different types of defensive behaviour in response to different predator species. For example, Brodsky & Barlow (1986) have demonstrated that *A. pismum* had a greater tendency to drop than back away from an approaching adult of the ladybird beetle *Adalia bipunctata* (L.) (Coleoptera: Coccinellidae), but showed the opposite tendency when approached by a larva of the syrphid *Megasyrphus corollae* (Fab.) (Diptera: Syrphidae). They suggest that backing away is effective in the presence of a slow moving predator such as syrphid larvae. However, it may not be effective when approached by a fast moving predator such as ladybird beetles, in which situation dropping could be more effective. The difference between our results and those presented by Brodsky & Barlow (1986) could be attributed to the fact that *A. aphidimyza* and *L. annulipes* triggered very few defensive acts, lowering the probability of finding a statistical difference between treatments. A higher number of replicates may have resulted in results similar to those of Brodsky & Barlow (1986).

The furtive predation strategy of *A. aphidimyza* is thought to have a double advantage. First, *A. aphidimyza* larvae have a low dispersal capacity (see Wilbert, 1973) and survival would be greatly compromised if they would have to search for a new aphid colony in order to complete the development. Second, *A. aphidimyza* larvae suffer from intraguild predation by other aphidophagous predators (Lucas et al., 1998; Hindayana et al., 2001). However, Lucas & Brodeur (2001) demonstrated that intraguild predation on *A. aphidimyza* larvae by the larvae of the lacewing *C. rufilabris* is reduced in denser aphid colonies. These authors demonstrated that *A. aphidimyza* larvae benefit from a dilution effect in large aphid colonies.

*Leucopis annulipes* larvae could benefit from the same double advantage of furtive predation. As for *A. aphidimyza* larvae, *Leucopis* spp. 1st and 2nd instar larvae also have a low dispersal capacity. Although 3rd instar may have a higher mobility (Gaimari & Turner, 1997), the probability of finding a new aphid colony in an architecturally complex environment such as an apple tree is probably low. Although movement between aphid colonies by 3rd instar larvae is recorded in grassland by Gaimari & Turner (1997), the possibility of completing development within a single aphid colony would nevertheless increase *L. annulipes* larvae probability of survival. This should be tested experimentally. Also, *Leucopis* spp. larvae are occasionally victims of intraguild predation by ladybird beetle and lacewing larvae (Sluss & Foote, 1973), and some species are known to exhibit cannibalism (Gaimari & Turner, 1997). *Leucopis annulipes* larvae may thus lower their predation risk by benefiting from a dilution effect as is the case for *A. aphidimyza* (Lucas & Brodeur, 2001). However, the susceptibility of *L. annulipes* larvae to intraguild predation in aphid colonies of different sizes remains to be tested.

From a biological control point of view, the aphid colony disturbance caused by active-searching predators may be an advantage, particularly when aphid displacement enhances their mortality risk, for instance through synergistic predation (Losey & Denno, 1998a). As a result, the real mortality rate inflicted by colony-disruptive predators could be higher than their simple consumption rate. As such, active-searching predators could be more effective for the biological control of aphid species with a high tendency to drop or walk away when attacked. However, even though the strategy adopted by the furtive predators seems a priori less appropriate for a knock-down effect on aphid colonies, the role they play...
in the long term regulation of aphid populations can be important (Meadow et al., 1985).

ACKNOWLEDGEMENT. We would like to thank S.D. Gaimari, California Department of Food & Agriculture, for Leucopis annulipes identification and rearing advice, L. Voynaud for technical advice and A. Sentis for technical assistance. We are also grateful to two anonymous reviewers for helpful comments on the manuscript.

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Received September 18, 2007; revised and accepted November 5, 2007.