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Dropping behaviour of larvae of aphidophagous ladybirds and its effects on incidence of intraguild predation: interactions between the intraguild prey, *Adalia bipunctata* (L.) and *Coccinella septempunctata* (L.), and the intraguild predator, *Harmonia axyridis* Pallas

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Abstract. 1. Two experiments were performed in the laboratory to assess the behaviour of dropping from a host plant as a defence against intraguild predation in aphidophagous ladybird larvae.

2. In the first experiment, encounters were observed on bean plants between fourth instars of the intraguild predator species, *Harmonia axyridis*, and first instars of two other ladybird species, *Adalia bipunctata* (L.) and *Coccinella septempunctata* (L.). The percentages of first instars of the latter two species that dropped from the plant in response to attack differed dramatically, with 47.5% of *C. septempunctata* first instars dropping vs. 0% of *A. bipunctata*.

3. In the second experiment, first instars of *A. bipunctata* or *C. septempunctata* and a fourth instar of *H. axyridis* were allowed to forage together on bean plants for 3 h. During this time, 44.3% of *C. septempunctata* larvae dropped from the plant, but less than 2% of *A. bipunctata* larvae did so. In contrast, 95.0% of *A. bipunctata* larvae fell victim to intraguild predation by *H. axyridis* vs. only 54.5% of *C. septempunctata* larvae.

4. The significance of dropping behaviour of ladybird larvae as a defence against intraguild predation, and the relationship of dropping behaviour to species-specific habitat affinity of ladybirds, is discussed.

Key words. *Adalia bipunctata*, *Coccinella septempunctata*, *Harmonia axyridis*, ladybird beetle.

Introduction

Aphidophagous ladybird species often co-occur at aphid colonies, thereby forming a guild of predators (e.g. Maredia *et al.*, 1992; Lamana *et al.*, 1996; Brown & Miller, 1998; Sakuratani *et al.*, 2000; Wright & DeVries, 2000; Burkness *et al.*, 2001). While sharing aphids as prey, guild members may at times engage in intraguild predation and consume one another (Cottrell & Yeargan, 1998). For example, larvae of *Propylea japonica* (L.) tend to be the victims of intraguild predation by larvae of *Harmonia axyridis* Pallas. Consequently, larvae of *P. japonica* are less likely to complete their development when larvae of *H. axyridis* are present (Sato *et al.*, 2003).

Larval ladybirds are also likely to have some protection from intraguild predation by larvae of other, co-occurring species in the same ladybird guild. Often this protection seems to be chemically based (e.g. Agarwala & Dixon, 1992; Agarwala *et al.*, 1998). For instance, habitat preferences of

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Adalia bipunctata (L.) and *Coccinella septempunctata* (L.), both common species in Europe, overlap to some extent (Hemptinne *et al.*, 2000). Larvae of *A. bipunctata* are chemically well protected from intraguild predation by *C. septempunctata* (Agarwala & Dixon, 1992; Agarwala *et al.*, 1998 Hemptinne *et al.*, 2000; Sato & Dixon, 2004). Body size also affects the incidence of intraguild predation, as large species are less likely to be intraguild prey of small species than vice versa (Lucas *et al.*, 1998). The relatively large larvae of *C. septempunctata* are therefore likely to be protected physically from intraguild predation by the smaller larvae of *A. bipunctata*. Hence, it is suggested that intraguild predation is unlikely between these two species when they co-occur as members of the same ladybird guild.

Coccinella septempunctata also co-occurs at aphid colonies with *H. axyridis* at a certain time of the year. In general, their habitat preferences seem to vary (Osawa, 1991); *C. septempunctata* is more likely to occur on grasses, while *H. axyridis* seems to be abundant on shrubs. However, from mid-spring to early summer, these two species regularly co-dominate the ladybird guild in shrub habitats, such as *Hibiscus* trees (Yasuda & Shinya, 1997; Sato, 2001). Larvae of *H. axyridis* are very aggressive larvae that attack a variety of types of prey in addition to aphids (Sato, 2001). They are also well protected chemically from intraguild predation by *C. septempunctata* (Sato & Dixon, 2004). If intraguild predation is generally unlikely between co-occurring, co-dominant ladybird species, then larvae of *C. septempunctata* are also likely to be protected in some way from intraguild predation by larvae of *H. axyridis*. However, larvae of the two species are similar in body size (Sato, 2001). In addition, larvae of *C. septempunctata* are less protected chemically from intraguild predation by larvae of *H. axyridis* than vice versa (Sato & Dixon, 2004). That is, neither chemical protection nor body size differences seem to be important in this regard. Thus, the mechanism by which larvae of *C. septempunctata* may avoid excessive intraguild predation by larvae of *H. axyridis* is unknown.

In the present study, dropping behaviour was examined as a possible mechanism by which larvae of *C. septempunctata* may avoid becoming intraguild prey of *H. axyridis*. Thus, the tendency of larvae of *C. septempunctata* to drop from a plant when attacked by larvae of *H. axyridis* was assessed and compared with that of the chemically more protected species, *A. bipunctata*.

Materials and methods

Ladybird culture

Adults of the three species, *A. bipunctata*, *C. septempunctata*, and *H. axyridis*, were collected near Logan, Utah, U.S.A. These adults were maintained in groups of several pairs in Petri dishes (15 cm in diameter) with an excess of pea aphids, *Acyrthosiphon pisum* (Harris). Eggs were removed and kept in a Petri dish until they hatched. Larvae of *A. bipunctata* and *C. septempunctata* were used in experiments within 24 h of the egg hatch. In these experiments, larvae of *H. axyridis* were tested as intraguild predators of the hatchling larvae. The *H. axyridis* larvae were fed an excess of the pea aphids daily until they moulted to fourth-instar larvae, and were then starved for 24 h before being used in the experiments. All animals were reared in an incubator kept at 20 °C with photoperiod of LD 16:8 h.

Experimental arena

As an experimental arena for both of the following experiments, a 20-cm tall bean plant, *Vicia faba* (L.), was fixed to the centre of a Petri dish (15 cm in diameter). The Petri dish was fully filled with water to prevent ladybird larvae that were placed on the plant from emigrating by crawling off the plant, and to catch and trap larvae that dropped from the plant.

Experiment 1; Behaviour of first instars when attacked by an intraguild predator

A single first instar of either *A. bipunctata* or *C. septempunctata* was placed on a pea plant and allowed to settle for 20 min. Subsequently, a single fourth instar of *H. axyridis* was introduced onto the same pea plant, and the behaviour of the two larvae was monitored until the fourth instar of *H. axyridis* attacked the first instar. The behaviour of the first instar (i.e. whether or not it dropped from the plant) was recorded when it was attacked. In total, 40 first instars were tested for each of *A. bipunctata* and *C. septempunctata*. In this experiment, no first instars of either of these species dropped from the pea plants before they were attacked by fourth instars of *H. axyridis*. In addition, no ladybird larvae emigrated from the plants.

Experiment 2; Effects of dropping behaviour of larvae on incidence of intraguild predation

In this experiment, the effects of dropping behaviour of first instars on the incidence of intraguild predation were examined. Ten first instars of either *A. bipunctata* or *C. septempunctata* were placed with a fourth instar of *H. axyridis* on the same pea plant. The larvae were observed every 10 min for the first hour after being placed on the plant, and every 30 min for 2 h thereafter (i.e. 10 observations of larvae were made over 3 h). The number of first instars still present on the pea plant was noted at every observation. In addition, the number of first instars that had dropped from the plant and were trapped in the dish of water beneath the plant was recorded at the conclusion of the experiment (no water-trapped larvae succeeded in leaving the experimental arena). The number of first instars that were lost to intraguild predation was then calculated by subtracting the total of the number of larvae trapped in the water dish and the number still on the plants at the conclusion of the experiment from the number present initially (i.e. 10). This experiment was replicated seven times for each species.

Statistical analysis

In the first experiment, the frequency with which first instars of *A. bipunctata* vs. *C. septempunctata* dropped from the pea plants was compared using a χ^2 test. In the second experiment, the average percentages of first instars of the two species that were '*present on the plant*', '*water-trapped*', and '*lost to intraguild predation*' were compared using a Mann–Whitney *U*-test.

Results

Experiment 1; Behaviour of first instars when attacked by an intraguild predator

When attacked by a fourth instar of *H. axyridis*, 47.5% of first instars of *C. septempunctata* dropped from the bean plant while 52.5% remained on the plant despite the attack. In contrast, no individuals of *A. bipunctata* dropped from the plant ($\chi^2_1 = 25.4$, $P < 0.0001$).

Experiment 2; Effects of dropping behaviour of larvae on incidence of intraguild predation

The average percentages of first instars of *A. bipunctata* and *C. septempunctata* that remained on a bean plant decreased in similar and rapid fashion after the larvae were placed on the plant along with a fourth instar of *H. axyridis* (Fig. 1). For example, more than 80% of first instars of both species had disappeared from the plants an hour after being placed on the plant (Mann–Whitney $U = 16$, $P > 0.05$ for the comparison between the two species). At the conclusion of the experiment, less than 5% of first instars of both species remained on the plant (Mann–Whitney $U = 17$, $P > 0.05$ for the comparison between the two species).

Although the rate of disappearance from the plant was very similar for first instars of *A. bipunctata* and *C. septempunctata*, the fate of larvae that disappeared differed between the two species (Fig. 2). Thus, less than 2% of first instars of *A. bipunctata* dropped from the plant whereas more than 40% of first instars of *C. septempunctata* did so (Mann–Whitney $U = 0$, $P < 0.01$). In contrast, almost all first instars of *A. bipunctata* were preyed upon by the fourth instar of *H. axyridis* during the 3 h over which the experiment was conducted, whereas only approximately half of the first instars of *C. septempunctata* fell victim to intraguild predation (Mann–Whitney $U = 0$, $P < 0.01$).

Discussion

The experiments presented here demonstrate that first instars of *C. septempunctata* were more likely to drop from a plant when attacked by a fourth instar of *H. axyridis* than were first instars of *A. bipunctata*, and consequently the former larvae were less likely to fall prey to intraguild predation by the larvae of *H. axyridis*. In some species of ladybirds, morphological features such as spines (Yasuda *et al.*, 2001) and waxy covering (Agarwala & Yasuda, 2001) serve to protect larvae from intraguild predation by larvae of other species. Larvae of *C. septempunctata* are not very spiny, however, and they lack a waxy covering such as that of *Scymnus* species (Agarwala & Yasuda, 2001). Thus, the readiness with which first instars of *C. septempunctata* drop from a plant when attacked is likely to be a major way of avoiding intraguild predation by fourth-instar larvae of *H. axyridis*.

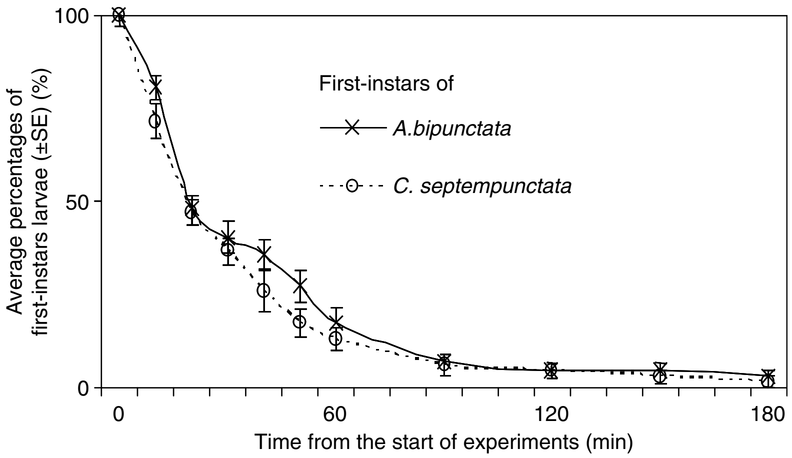

Fig. 1. Temporal changes in the average percentages of first instars of *Adalia bipunctata* and *Coccinella septempunctata* that remained on a bean plant after being placed on the plant with a fourth instar of *Harmonia axyridis*. $n = 7$ trials, each with ten first instars placed simultaneously on the plant, for each species.

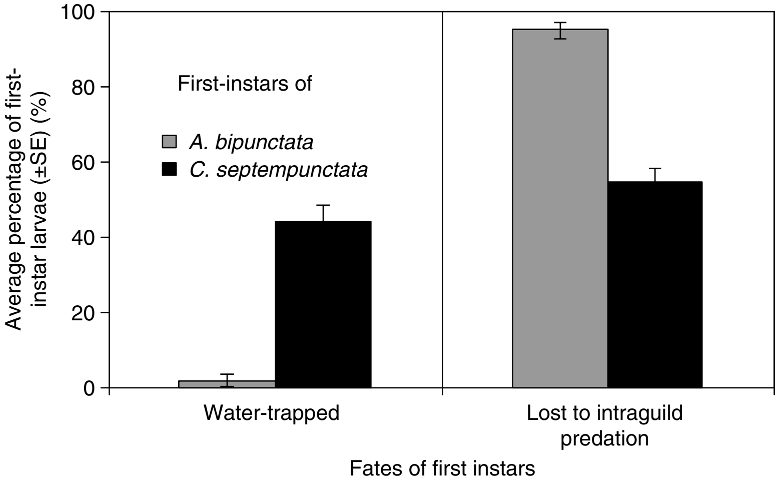

Fig. 2. Average percentages of first instars of *Adalia bipunctata* and *Coccinella septempunctata* that were water-trapped and lost to intraguild predation during a 3-h period after being placed together with a fourth instar of *Harmonia axyridis* on a bean plant. $n = 7$ trials, each with ten first instars placed simultaneously on the plant, for each species.

In contrast to larvae of *C. septempunctata*, larvae of *A. bipunctata* do not appear to drop readily from plants as means of avoiding intraguild predation. Rather, chemical protection is known to be a defence against intraguild predation in this species (Agarwala & Dixon, 1992; Agarwala *et al.*, 1998 Hemptinne *et al.*, 2000; Sato & Dixon, 2004). In general, ladybird species have species-specific alkaloids (Pasteels *et al.*, 1973) that can deter intraguild predation by other ladybird species. However, this chemical protection is effective only in those cases where the larvae are toxic to their intraguild predator. *Adalia bipunctata* is no more toxic to *H. axyridis* than vice versa (Sato & Dixon, 2004). Thus, the high incidence of intraguild predation of first instars of *A. bipunctata* observed in this study suggests that the chemical protection of this species is ineffective against *H. axyridis.*

The difference in dropping behaviour between larvae of *A. bipunctata* and *C. septempunctata* seems likely to reflect the difference in their habitat preferences. Although these two species overlap to some extent in their habitat preferences (Hemptinne *et al.*, 2000), they also show differences. *Adalia bipunctata* occurs most abundantly on trees (Mills, 1979; Sakuratani *et al.*, 2000), whereas *C. septempunctata* is more likely to be found on grasses (Osawa, 1991). In aphidophagous ladybirds, periods of starvation directly affect survival and development of larvae (Kawauchi, 1979). Larvae that drop from a plant must find another plant with aphids, and in general, doing so will be much more difficult and dangerous for a tree-dwelling larva than for a larva that lives on herbaceous plants. For example, among other considerations, a larva that has dropped to the ground and must locate a new tree or shrub is likely to be exposed to predators such as ground beetles (Winder, 1990; Schellhorn & Andow, 1999) for longer periods of time than a larva associated with denser herbaceous vegetation. Thus, the cost of dropping behaviour is probably much greater for an arboreal species such as *A. bipunctata* than for a herbaceous species such as *C. septempunctata.* Larvae of *A. bipunctata* are better able to prevent untimely dropping than are larvae of *C. septempunctata* because the former larvae have an enlarged anal gland that enables them to stick to plants (S. Sato, pers. obs.).

Chemical protection also probably costs larvae energetically. In general, ladybird larvae often exude a yellowish or reddish fluid when disturbed; this 'reflex bleeding' has long been regarded as a defence against their predators (Dixon, 2000). Gill and Moore (1998) showed that larvae that reflex bleed at regular intervals during their development tend to take longer to develop and grow to smaller adult size compared with conspecifics that do not reflex bleed. Hence, if chemical protection of larvae from intraguild predation depends mainly on their reflex bleeding, then there is probably a high energetic cost associated with such chemical protection. For primarily herbaceous species such as *C. septempunctata*, dropping behaviour therefore may be a more advantageous form of defence than chemical protection. Thus, proximate differences in morphology, and ultimate differences in 'cost', probably account for the difference in dropping behaviour between the two species.

Interestingly, the chemical protection that *A. bipunctata* may use (instead of defending itself behaviourally by dropping from the plant) appears to be ineffective when it shares a plant with the intraguild predator *H. axyridis.* In general, both *A. bipunctata* (Mills, 1979) and *H. axyridis* (Osawa, 1991) are more likely to occur on shrubs than in herbaceous habitats. Larvae of these two species occasionally interact with one another in shared habitat in Japan (Sakuratani *et al.*, 2000). Therefore larvae of *A. bipunctata* might be expected to be well defended against intraguild predation by those of *H. axyridis.* However, historically these ladybirds did not overlap in their geographic distributions; only recently they have come into contact with each other and begun interacting (e.g. Kajita *et al.*, 2000). Thus, the vulnerability of *A. bipunctata* larvae to intraguild predation by *H. axyridis* likely reflects an absence of coevolution that is generally important in the acquisition of defensive adaptations enabling ladybird larvae to avoid intraguild predation by other species.

In conclusion, ladybird larvae may frequently become victims of intraguild predation by the aggressive larvae of *H. axyridis* if they do not drop from a plant when attacked by this predator. However, the survival of species that drop from their host plants when attacked by intraguild predators is likely to differ between arboreal- and herbaceous-dwelling ladybirds. Especially among herbaceous-dwelling ladybirds, some dropping larvae are likely to migrate to other host plants and survive. For example, larvae of *C. septempunctata* are often observed on the ground in the field (Yasuda & Shinya, 1997), suggesting that movement between host plants occurs frequently. Thus, it is important to take into account dropping behaviour of ladybird larvae when considering the potential interactions among species in aphidophagous guilds. The fact that the dropping behaviour of larvae is a marked feature of some ladybirds suggests that it is adaptive for these species. However, the fate of larvae after dropping is still unknown, and needs to be determined by additional experiments.

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