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ORIGINAL ARTICLE

Vulnerability of larvae of two species of aphidophagous ladybirds, *Adalia bipunctata* Linnaeus and *Harmonia axyridis* Pallas, to cannibalism and intraguild predation

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Abstract

Vulnerability of larvae of two species of aphidophagous ladybirds, *Adalia bipunctata* Linnaeus and *Harmonia axyridis* Pallas, to cannibalism and intraguild predation was assessed in the laboratory. In the first experiment, a first instar of one of the two above species was kept with a fourth instar of the other species in a Petri dish. The number of times each first instar larva was encountered by the fourth instar larva and the fate of the first instar was determined over a period of 10 min. The fourth instar larvae captured and killed all the first instar larvae of their own species at the first encounter. However, when presented with fourth instar larvae of the other species the first instar larvae of *A. bipunctata* and *H. axyridis* were encountered 6.4 ± 1.3 ($n = 10$) and 19.4 ± 2.1 ($n = 10$), respectively. In this experiment no first instar larvae of *H. axyridis*, whereas all those of *A. bipunctata*, were killed.

Key words: cannibalism, chemical protection, Coccinellidae, intraguild predation, predatory ladybirds.

INTRODUCTION

The larvae and adults of the ladybird species exploiting the same aphid colonies form an aphidophagous ladybird guild (Yasuda & Shinya 1997; Dixon 2000; Sato 2001). Intraguild predation can be a common cause of death in such assemblages, but the members are not all equally vulnerable to intraguild predation. For example, larvae of *Propylea japonica* Thunberg and *Harmonia axyridis* Pallas vary in their vulnerability to intraguild predation; larvae of the former species are much more likely to be attacked and eaten than *vice versa* (Sato *et al.* 2003). Consequently, larvae of *P. japonica* often fail to complete their development when larvae of *H. axyridis* are present. If this is generally true, then protection from intraguild predation would increase ladybird larval survival and facilitate coexistence within a guild. However, what determines the vulnerability of larvae to intraguild predation is poorly understood.

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Given the potential for frequent intraguild predation, it is likely that ladybird larvae will have evolved some means of protection from intraguild predation, which enables co-occurring species to complete their development. In fact, several ways of avoiding intraguild predation have been reported for aphidophagous ladybird guilds (Volkl & Vohland 1996; Agarwala & Yasuda 2001; Sato *et al.* 2003). One important way is chemical protection (Agarwala & Dixon 1992; Agarwala *et al.* 1998; Cottrell & Yeargan 1998; Hemptinne *et al.* 2000; Sato & Dixon 2004).

For example, ladybirds produce species-specific alkaloids (Pasteels *et al.* 1973; Dixon 2000). In some species, these alkaloids are toxic to other species and their consumption can adversely affect larval survival and development (e.g. Agarwala & Dixon 1992). Therefore, larvae should avoid consuming other ladybird species when their preferred prey, aphids, is available. In fact, larvae are reluctant to consume eggs of other species and this reluctance reflects their chemical protection (Agarwala & Dixon 1992). In the field, aphids often become scarce before the larvae complete their development (Yasuda & Shinya 1997) and at such times the incidence of intraguild predation is likely to increase (Dixon

2000). That is, chemical protection can be an important means of avoiding intraguild predation. However, the effectiveness of chemical protection against intraguild predation is poorly understood.

As a result of a recent invasions two species of aphidophagous ladybirds, *Adalia bipunctata* (L.) and *H. axyridis*, co-occur in similar habitats in both North America and Japan (e.g. Sakuratani *et al.* 2000; Brown 2003). In a previous study no first instar larvae of *A. bipunctata* that ate eggs of *H. axyridis* survived, whereas larvae of the latter species can eat and develop on a diet of eggs of the former species. This suggests that *H. axyridis* is chemically more protected from intraguild predation by *A. bipunctata* than *vice versa* (Sato & Dixon 2004). In the present study, the effectiveness of the chemical protection of the first instar larvae of these two species against attack by fourth instar larvae of their own and the other species was determined in the laboratory.

MATERIALS AND METHODS

Adults of *A. bipunctata* and *H. axyridis* were collected within a few kilometers of Utah State University (Logan, Utah, USA). Pairs were kept in Petri dishes (9.0 cm in diameter), and provided daily with an excess of pea aphid *Acyrthosiphon pisum* Harris. Egg clusters were removed and kept in Petri dishes until the eggs hatched. All first instars used as prey in the following two experiments were used within 12–24 h of hatching. Some first instars were fed an excess of pea aphids until they molted to the fourth instar. They were then starved for 24 h before being used as predators in the experiments. In some species, larvae are reluctant to consume their kin (Agarwala & Dixon 1993; Pervez *et al.* 2005). Therefore, in the experiments prey and predator did not have the same parents. These animals were reared in an insectary kept at 20°C with a photoperiod 16 h light : 8 h dark, and the experiments were conducted at room temperature. The results were compared using a Mann–Whitney *U*-test.

In the experiment, the effectiveness of the chemical protection of the larvae of *A. bipunctata* and *H. axyridis* against cannibalism and intraguild predation was assessed. A first instar larva of *A. bipunctata* or *H. axyridis* was placed in a Petri dish (3 cm in diameter) along with a fourth instar larva of either their own or the other species. Each first instar larva was observed until it was either captured and killed, or for 10 min if not captured and killed, and the number of encounters with the fourth instar larva was recorded. The number of replicates for each species was ten.

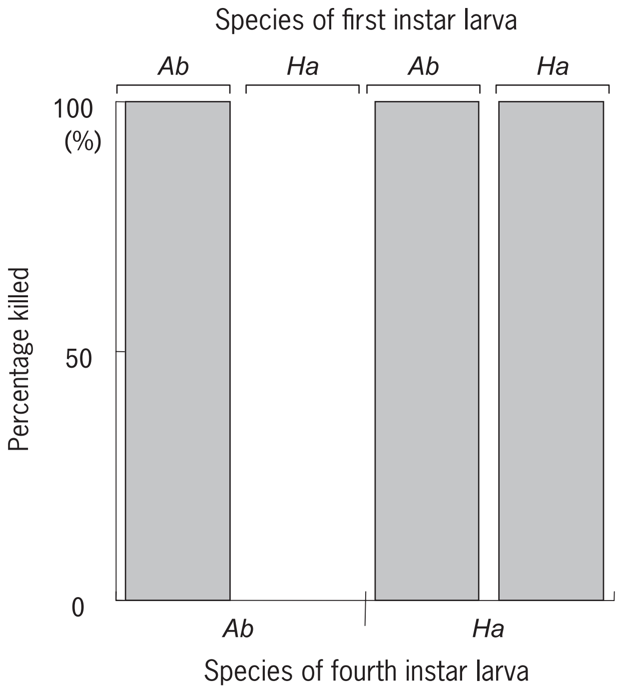

Figure 1 Percentages of first instar larvae of *Adalia bipunctata* (Ab) and *Harmonia axyridis* (Ha) that were killed when each species was kept with a fourth instar larva of either its own or the other species.

RESULTS

In the case of cannibalism all the first instar larvae of both species were captured and killed (Fig. 1) at the first encounter (Fig. 2). That is, in the present experiment, the first instar larvae of both species are vulnerable to cannibalism by fourth instar larvae. In the case of intraguild predation all the first instar larvae of *A. bipunctata* were killed (Fig. 1) and the average number of encounters increased to 6.4 ± 1.3 ($n = 10$) (Mann–Whitney $U = 0.0$, $P < 0.0001$, Fig. 2), whereas none of the larvae of *H. axyridis* was killed (Fig. 1) and the number of encounters increased to 19.4 ± 1.3 ($n = 10$) (Mann–Whitney $U = 6.0$, $P < 0.001$, Fig. 2). This indicates that both species are better protected against predation by each other's species than by cannibalism, more so in *H. axyridis* than *A. bipunctata*.

DISCUSSION

First instar larvae are much smaller and less mobile than fourth instar larvae; for example, in *A. bipunctata* and *H. axyridis*, fourth instar larvae weigh more than 50 times the first instar larvae (S. Sato, unpubl. data, 2004). As the vulnerability of a ladybird depends on

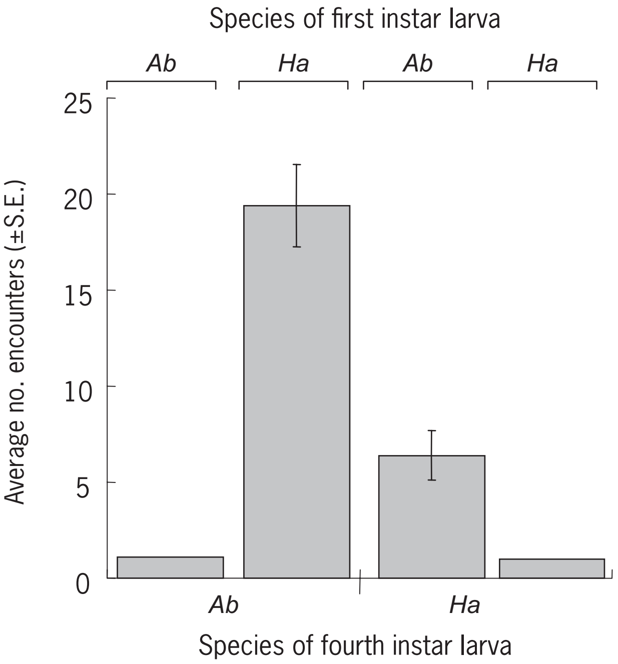

Figure 2 Average number of encounters with a first instar larva of *Adalia bipunctata* (Ab) and *Harmonia axyridis* (Ha) when each species was kept with a fourth instar larva of either its own or the other species. The number of encounters is the number of times a first instar larva of *A. bipunctata* or *H. axyridis* was encountered by a fourth instar larva of either the same or the other species.

its size and mobility relative to the cannibal (Dixon 2000), it is not surprising that first instar larvae are highly vulnerable to cannibalism by fourth instar larvae. In fact, in the present study the first instar larvae of both species were readily killed by fourth instar larvae of their own species, which suggests that first instar larvae of these species were vulnerable to cannibalism by fourth instar larvae. That is, their vulnerability in terms of size and mobility appears to determine their vulnerability to cannibalism. Thus, it would be expected that the first instar larvae of both *A. bipunctata* and *H. axyridis* are highly vulnerable to intraguild predation by fourth instar larvae of the other species, as the first instar larvae of both species are much smaller than fourth instar larvae of the other species (e.g. a fourth instar larva of *A. bipunctata* weighs about 50 times more a first instar larva of *H. axyridis*) (S. Sato, unpubl. data, 2004).

However, ladybirds contain species-specific alkaloids, which tend to be toxic to other species and provide some protection from intraguild predation (Agarwala & Dixon 1992; Agarwala *et al.* 1998; Cottrell & Yeargan 1998; Hemptinne *et al.* 2000; Sato & Dixon 2004). That is, any decrease in vulnerability to predation relative to cannibalism is likely to depend on their chemical protection. In a previous study all the first instar larvae of *A. bipunctata* died before molting after consuming eggs of *H. axyridis*, whereas half of the first instar larvae of *H. axyridis* molted after consuming eggs of *A. bipunctata* (Sato & Dixon 2004). This result suggests that *H. axyridis* is more chemically protected from intraguild predation by *A. bipunctata* than *vice versa*. In the present study, first instar larvae of *A. bipunctata* were also more vulnerable to intraguild predation than those of *H. axyridis*. That is, the vulnerability of first instar larvae of *A. bipunctata* and *H. axyridis* to intraguild predation by fourth instar larvae of the other species reflects their toxicity for the other species.

How larvae use their chemical defense to avoid intraguild predation is poorly understood. It may be via the yellowish fluid that they exude when attacked, which is referred to as reflex bleeding (Majerus & Kearns 1989). In some species the species-specific alkaloid makes up about 5% of the fresh weight of this fluid (de Jong *et al.* 1991). In addition, larvae of *A. bipunctata* and *H. axyridis* reflex bleed when attacked by a predator (S. Sato, unpubl. data, 2004). Thus, reflex bleeding may be the mechanism by which the first instar larvae in this study avoided predation by fourth instar larvae. The amount of fluid that a ladybird produces in a single reflex bleeding event can be up to 20% of their total body weight (de Jong *et al.* 1991), which suggests that the availability of this defense is probably limited. However, the effect of reflex bleeding on the incidence of predation was not recorded in the present study.

In conclusion, results of the present study reveal that the larvae of *A. bipunctata* and *H. axyridis* are protected to different degrees from predation. As the larvae of both species appear to be equally vulnerable to cannibalism the additional protection from predation may be due to differences in the effectiveness of their chemical protection. The fact that first instar larvae of *H. axyridis* were rarely killed and eaten by fourth instar larvae of *A. bipunctata* suggests that chemical protection may be a very efficient way of avoiding intraguild predation. In contrast, chemically vulnerable first instar larvae of *A. bipunctata* were all killed by *H. axyridis*, suggesting that poor chemical protection form intraguild predation determines their poor survival. In Japan, the first *A. bipunctata* were reported in 1993 (Sakuratani *et al.* 2000), but they have not become widely distributed (Toda & Sakuratani 2006). Our results may account for the current distribution of *A. bipunctata* in Japan. In addition, the fact that first instars of both species are vulnerable to cannibalism suggests that ladybirds are well adapted to cannibalism, as suggested by previous studies (Osawa 1992; Joseph *et al.* 1999; Yasuda & Ohnuma 1999; Snyder *et al.* 2000; Michaud & Grant 2004; Perry & Roitberg 2005; Omkar *et al.* 2007). Since avoidance of intraguild predation is important for larval survival it is important to consider the ways in which ladybirds are protected against intraguild predation, including chemical protection, when seeking to understand the dynamics of ladybird guilds and their interactions with aphids.

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