Effects of native ladybirds on oviposition of the exotic species, *Adalia bipunctata* (Coleoptera: Coccinellidae), in Japan

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Abstract
Effects of native ladybird females of *Harmonia axyridis* Pallas and *Coccinella septempunctata* L. on oviposition behavior of the exotic species, *Adalia bipunctata* (L.), were assessed by pairing females of the exotic species with conspecific or heterospecific females. Pairs of females were provided with aphids in excess for 30 days, during which time there were no significant differences in weight, rates of mortality, or selections of sites for oviposition among the treatments. However, females of *A. bipunctata* laid significantly fewer eggs over the 30-day experimental period when paired with females of *H. axyridis* or *C. septempunctata* than when paired with conspecifics, even though aphids were abundantly available in all three cases. The number of eggs laid by a female of *A. bipunctata* paired with conspecifics gradually declined over the 30-day experimental period, while egg production declined especially rapidly during the second ten-day period when females were paired with females of *H. axyridis* and *C. septempunctata*. Thus, egg production by females of the exotic species was reduced in the presence of the native ladybirds.

Key words: Coccinellidae; oviposition; reproductive strategy; native species; introduced species

INTRODUCTION

How and where females choose to lay their eggs plays an important role in the fitness of their offspring, as the suitability of the site of oviposition directly affects the survival of eggs and hatching larvae. Therefore, selection will favor females that lay eggs in the most profitable sites for their offspring (Hocking, 1967; Resetarits and Wilbur, 1989). Assessment of potential oviposition sites may be especially critical for newly introduced species because gravid females and their offspring must compete successfully in new environments against well-adapted individuals of native species.

Female aphidophagous ladybirds appear to evaluate aphid colonies carefully in selecting oviposition sites. For example, it is well known that they lay eggs near the aphid colony (Evans and Dixon, 1986), and that they avoid laying eggs when conspecific larvae are already present (Hemptinne et al., 1992). Recently, the exotic two-spotted ladybird, *Adalia bipunctata* (L.), has become established at the port town of Osaka (Sakuratani, 1994), but it does not appear to have succeeded in expanding its distribution (Sakuratani et al., 2000). Studies of mechanisms responsible for failure of this introduced ladybird in Japan have focused on intraguild predation between larvae of this species and native ladybirds (Kajita et al., 2000). Adult ladybirds do not prey on each other, but may interact in negative fashion in a variety of other ways. Reproduction by females of the exotic ladybird may be inhibited through interaction with native Japanese ladybirds. Such inhibition might arise simply from strong competition for food, but it may also arise because females of *A. bipunctata* must compete for oviposition sites and/or because they avoid laying eggs when females of native species are present.

Such interactions between native and exotic ladybirds involving reproductive inhibition have not previously been studied. Here, we carried out laboratory experiments to understand the effects of the native Japanese ladybirds, *Coccinella septempunctata* L. and *Harmonia axyridis* Pallas, on the oviposition behavior of the introduced species, *A.*

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bipunctata. Our objective was to assess the reproductive interaction between native and introduced ladybirds, which might be another potential mechanism responsible for the limited establishment and distribution of *A. bipunctata* in Japan.

MATERIALS AND METHODS

The experiment was carried out at constant temperature ($22 \pm 1°C$) and photoperiod (L16 : D8) from March to December in 2000. In preparation, groups of four second instars of *A. bipunctata*, *H. axyridis*, or *C. septempunctata* were reared to adulthood under these conditions in plastic containers (12 cm in diameter and 4.5 cm in height, or 9 cm diameter and 1 cm in height). Fresh pieces of bean plants bearing an excess supply of pea aphids, *Acyrthosiphum pisum* (Harris), were provided each day. Newly moulted adults of *A. bipunctata* were sexed and paired, and allowed to mate for 24 hours. Females were then paired with already reproductively active females of *H. axyridis* and *C. septempunctata* that had molted 20 to 25 days previously and were laying eggs in large numbers each day. Two females were placed together in a plastic container (11 cm in diameter and 8 cm in height), according to the following three treatments: (1) two females of *A. bipunctata* (19 replicates), (2) one female of *A. bipunctata* and one female of *H. axyridis* (17 replicates), (3) one female of *A. bipunctata* and one female of *C. septempunctata* (16 replicates). Two stems of bean plant (7 cm in height and bearing three leaves) with excess numbers of aphids were provided daily, thereby preventing egg predation from occurring. Had such egg predation occurred, it would have been evident from the yellow residue left on the substrate at the point of egg attachment; such yellow residue, however, was never observed, during the experiment.

The numbers of eggs laid by females of *A. bipunctata* were counted daily for 30 days (i.e., up through the period in a female's life of maximum egg production, H. Yasuda, unpublished data). Because the eggs of *A. bipunctata* are smaller than those of *H. axyridis* or *C. septempuctata*, we could easily distinguish them. From these data, the total numbers of eggs laid during the first, second, and third ten-day period of the experiment (i.e., first ten-day experimental period: from 1st to 10th day; second ten-day period: 11th to 20th day; and third

ten-day period: 21st to 30th day) were determined. The oviposition sites for these eggs were also recorded daily by examining leaves (both the dorsal and ventral sides of leaves) and other substrates (the underside of the container lid, and the inner walls of the container). Females of *A. bipunctata* were weighed on the 1st, 10th, 20th and 30th day of the experiment.

The cumulative numbers of eggs laid by *A. bipunctata* females over 30 days, and during each ten-day experimental period, were analyzed by ANOVA (for the treatment of two females of *A. bipunctata*, the total number of eggs laid by both females was divided by two). The Fisher's PLSD multiple range test was used to compare among the treatments. Change in weight of *A. bipunctata* females over time was analyzed by repeated-measures ANOVA. The percentage of eggs laid by *A. bipunctata* at each oviposition site and the survival rate of the females were analyzed by χ^2 test.

RESULTS

The number of eggs laid by *A. bipunctata* females over a 30-day period differed significantly among treatments (ANOVA; $F_{2,36} = 5.28$, $p < 0.001$, Fig. 1). Females of *A. bipunctata* laid fewer eggs when paired with females of *H. axyridis* or *C. septempunctata* than when paired with conspecifics (Fisher's PLSD, Ab+Ab vs Ab+Ha: $p < 0.05$; Ab+Ab vs Ab+Cs: $p < 0.01$; Ab+Ha vs Ab+Cs:
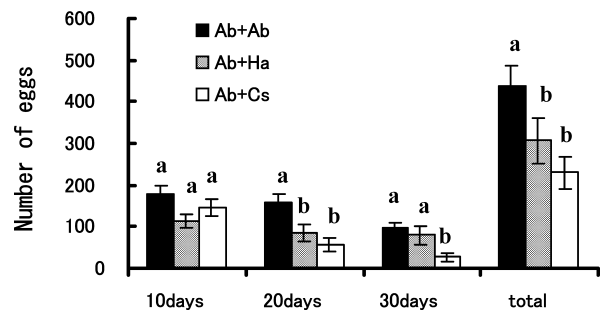

Fig. 1. The mean numbers of eggs ($\pm$one standard error) that a female of *A. bipunctata* (Ab) laid when kept with another conspecific female or with a female of *H. axyridis* (Ha) or *C. septempunctata* (Cs). Mean cumulative numbers of eggs laid per treatment are given for the first ten days of the experiment ("10 days"), the second and third ten days ("20 days" and "30 days"), and the full 30-day period ("total"). Differences in means among treatments, as determined by the Fisher's PLSD, are indicated by different letters.

Table 1.   The mean wet weight ($\pm$SE mg) of females of *A. bipunctata* (Ab) with a conspecific or heterospecific female on days 1, 10, 20 and 30 during the experiment

Treatment	Day			
	1	10	20	30
Ab+Ab	14.58$\pm$0.41	14.49$\pm$0.5	15.07$\pm$0.38	13.83$\pm$0.31
Ab+*H. axridis*	13.72$\pm$0.65	14.29$\pm$0.74	13.83$\pm$0.68	13.6$\pm$0.67
Ab+*C. septempunctata*	14.0$\pm$0.6	14.37$\pm$0.72	14.37$\pm$0.76	13.64$\pm$0.68

$p>0.05$). Females of *A. bipunctata* paired with *H. axyridis* tended to lay fewer eggs than females paired with conspecifics in first ten days of the experiment, although the difference in the number of eggs between treatments was only marginally significant (ANOVA; $F_{2,36}=2.83$, $p=0.07$, Fig. 1). The numbers of eggs laid by *A. bipunctata* paired with *H. axyridis* or *C. septempunctata* were significantly reduced in the second ten-day period compared to the numbers of eggs laid by females paired with conspecifics (ANOVA, $F_{2,36}=7.12$, $p<0.01$; Fisher's PLSD, Ab+Ab vs Ab+Ha: $p<0.01$, Ab+Ab vs Ab+Cs: $p<0.001$, Ab+Ha vs Ab+Cs: $p>0.05$, Fig. 1). In the third ten-day period, females of *A. bipunctata* paired with females of *C. septempunctata* laid especially few eggs (ANOVA, $F_{2,36}=4.43$, $p<0.05$; Fisher's PLSD, Ab+Ab vs Ab+Ha: $p>0.05$; Ab+Ab vs Ab+Cs: $p<0.01$; Ab+Ha vs Ab+Cs: $p<0.05$, Fig. 1).

There was no significant difference in choice of oviposition sites by females of *A. bipunctata* among the treatments (Ab+Ab: on leaf 54%, at other sites 46%; Ab+Ha: on leaf 51%, at other sites 49%; Ab+Cs: on leaf 51%, at other sites 49%; $\chi^2=0.24$, df=2, $p=0.89$). Most *A. bipunctata* eggs (for all treatments combined) were laid on the underside of leaves (44.1%) or of the container lid (36.5%), with fewer laid eggs on the dorsal surfaces of leaves (6.9%) or on the inner walls of the container (12.5%). The wet weights of females of *A. bipunctata* did not differ significantly among treatments or over time (repeated-measures ANOVA: effect of treatment, $F_{2,29}=0.45$, $p>0.05$; effect of time, $F_{3,87}=2.14$, $p>0.05$; interaction of treatment$\times$time, $F_{6,87}=0.78$, $p>0.05$, Table 1). There was also no significant difference in the mortality rates of *A. bipunctata* females over the 30-day period among the treatments (13.2% with conspecifics, 16.7% with Ha, and 25.0% with Cs;

$\chi^2=4.99$, df=2, $p=0.08$).

DISCUSSION

The number of eggs laid by *A. bipunctata* females decreased significantly when these females occurred with heterospecific females of *H. axyridis* and *C. septempunctata* versus with conspecific females. Aphids were provided in excess throughout the experiment, and there were no significant differences in weight or mortality rate of *A. bipunctata* females among the three treatments. It therefore appears that females of *A. bipunctata* were not limited in their egg production by aphid availability in the presence of heterospecific females. The number of eggs produced by all *A. bipunctata* females tended to decline gradually over the 30-day period; however, the decline in egg production was apparent even early in the experiment when females were paired with heterospecifics (Fig. 1). This suggests that females of *A. bipunctata* were inhibited from laying eggs by the presence of other females that could act as intraguild predators of their offspring (Kajita et al., 2000).

Similar responses have been recorded in other organisms. Females of the tree frog *Hyla chrysoscelis*, for example, were inhibited from laying eggs in the presence of predators and competitors, and they discriminated among potential egg-laying sites based on the presence of these other species (Resetarits and Wilbur, 1989). In addition, female parasitoids spent less time in aphid patches that included predatory ladybirds (Taylor et al., 1998), and they responded to the presence of a ladybird larva by reducing the number of eggs laid in the patch (Takizawa et al., 2000).

Selection of suitable sites for oviposition by gravid ladybirds is critical for the survivorship of their offspring (e.g., Evans, 2003 and references

therein). Schellhorn and Andow (1999), for example, studied differences in the oviposition behavior of *A. bipunctata* and *Coleomegilla maculata lengi* Timberlake. Each species had its own specific oviposition behavior, with important implications for strength of cannibalism and interspecific predation. It is also well known that adult ladybirds tend to avoid laying eggs upon contact with oviposition-deterring semiochemicals in larval tracks of conspecifics and heterospecifics (e.g., Hemptinne and Dixon, 2000; Yasuda et al., 2000; Hemptinne et al., 2001; Ruzicka, 2001) or in ladybird feces (Agarwala et al., 2003). In our experiment, we placed adults in new containers each day. Nonetheless, females of *A. bipunctata* may have encountered such semiochemicals associated with newly produced feces and/or the presence of *H. axyridis* or *C. septempunctata*, and they may consequently have been inhibited from laying eggs.

In this study, the ovipostion sites selected by females did not differ among the treatments. In the field, a small sized ladybird, *Propylea japonica* Mulsant, which is often the victim of intraguild predation by *H. axyridis*, tended to avoid staying on plants with the intraguild predator (Agarwala et al., 2003). The lack of difference in oviposition site among treatments in this study might be the result of the experimental setting, an enclosed and small container in which females had difficulty finding suitable oviposition sites not exposed to intraguild predators such as *C. septempunctata* and *H. axyridis*.

Although *A. bipunctata* has become established in Japan (Sakuratani, 1994), its distribution remains very limited (Sakuratani et al., 2000). In contrast, *C. septempunctata* and *H. axyridis* have become very abundant and broadly distributed throughout the continent following their introductions to North America, and they appear to be negatively interacting with native ladybird species (LaMana and Miller, 1996; Brown and Miller, 1998; Colunga-Garcia and Gage, 1998; Lucas et al., 2002; Michaud, 2002; Brown, 2003; Nault and Kennedy, 2003; Evans, 2004; Snyder et al., 2004; Yasuda et al., 2004). Elliott et al. (1996) reported population declines of *A. bipunctata* and *C. transversoguttata richardsoni* Brown in midwestern field crops following the establishment of *C. septempunctata*. Similarly, it is becoming increasingly difficult to find *A. bipunctata* in Minnesota

following the establishments of both *C. septempunctata* and *H. axyridis* (D. A. Andow, personal communication). Intraguild predation between larvae is thought to be one of the potential mechanisms of adverse effects of *C. septempunctata* and *H. axyridis* on *A. bipunctata* in Japan (Kajita et al., 2000) and on native ladybird species in North America (Cottrell and Yeargan, 1998; Michaud, 2002; Snyder et al., 2004; Yasuda et al., 2004). There are no studies yet, however, to understand the reproductive interaction involving inhibition of egg-laying such as studied here. Given that *A. bipunctata* and *H. axyridis* overlap almost completely in their use of habitats (i.e., arboreal habitats), not only larval interactions but also reproductively-based adult interactions between these species would be more intense than between *A. bipunctata* and other species in Japan and North America.

This is the first report to show the potential for negative reproductive interactions between native and introduced ladybirds. It is uncertain at present whether such negative reproductive interaction functions to limit the distribution and to contribute to the decline in abundance of *A. bipunctata* in Japan and in North America, respectively. Therefore, further studies are needed to understand more fully the reproductive interactions between ladybird species in large-scale settings in which females may choose oviposition sites among as well as within habitat patches occupied to varying extents by other ladybird species.

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