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Control of *Myzus persicae* and *Lipaphis erysimi* (Hemiptera: Aphididae) by adults and larvae of a flightless strain of *Harmonia axyridis* (Coleoptera: Coccinellidae) on non-heading *Brassica* cultivars in the greenhouse

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Abstract The green peach aphid [*Myzus persicae* (Sulzer)] and turnip aphid [*Lipaphis erysimi* (Kaltenbach)] are economically important pests with a worldwide distribution. We have evaluated the efficacy of releasing adults and larvae of a flightless strain of the multicolored Asian lady beetle (*Harmonia axyridis* Pallas) as a control measure against these aphids on plants of non-heading *Brassica* cultivars. Both adults and larvae of *H. axyridis* were observed to be effective biocontrol agents, markedly decreasing the numbers of aphids. The residence duration of adults was longer than that of larvae. The proportion of non-marketable plants damaged by aphids was lower in plots into which either adults or adults and larvae of *H. axyridis* had been released. These results suggest that both adults and larvae of this flightless strain of *H. axyridis* are effective in controlling aphids on plants of non-heading *Brassica* cultivars.

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Introduction

The green peach aphid, *Myzus persicae* (Sulzer), is a polyphagous and economically important pest with a worldwide distribution (Vorburger et al. 2008). The turnip aphid, *Lipaphis erysimi* (Kaltenbach), is one of the most important pests of *Brassica* leafy vegetables worldwide (Blande et al. 2008), especially in the USA (Liu and Chen 2001), Japan (Nagasaka et al. 2003), and India (Dutta et al. 2005). High densities of these aphids cause actively growing leaves to curl, thereby forming pockets and folds that provide shelter to the aphids and, consequently, protection against insecticide treatments (Liu and Chen 2001). In addition, aphid control has become less effective due to the evolution of insecticide resistance in natural populations. Hence, alternative control methods, such as biological control, are desirable.

Komatsuna, *Brassica rapa* var. *perviridis* L. H. Bailey, and Mizuna, *Brassica rapa* var. *nipposinica* (L. H. Bailey) Kitam, are non-heading *Brassica* cultivars. In Japan, plants of these cultivars can harvested about one month after sowing between spring and fall. Because most of the non-heading *Brassica* cultivars are considered to be 'minor crops' (production <30,000 t year^{-1}) in Japan, few pesticides have been registered for the control of aphids on them. Thus, additional pesticides need to be registered or other methods of control need to be developed and applied.

The release of augmented numbers of the multi-colored Asian lady beetle *Harmonia axyridis* Pallas (ladybird beetle), either by translocation or by mass rearing and release, has been a widely used approach to control aphids (Dixon 2000). However, even in greenhouses, adult beetles tend to fly away from the crop soon after release and, consequently, they are viewed as unreliable biological control agents (Hagen 1962; Hämäläinen 1977; Marples et al. 1993; Dreistadt and Flint 1996; Dixon 2000; Minoretti and Weisser 2000). To improve the efficacy of this augmentative biological control approach, a flightless strains of the ladybird beetle was developed by artificial selection (Tourniaire et al. 2000; Seko et al. 2008). When released as an augmentative biological control measure, adults of this flightless strain were shown to be effective against *Phorodon humuli* (Schrank) on hops (Weissenberger et al. 1999) and *Aphis gossypii* Glover on eggplants (Seko et al. 2008). To date, the efficacy of adults of the flightless strain against aphids attacking plants of non-heading *Brassica* cultivars has never been investigated. The efficacy of larvae of the flightless strain in controlling aphids has also not been studied. The release of larvae of the flightless strain, rather than adults, may be advantageous in that the costs of rearing ladybird beetles could be avoided (Seko 2009). In addition, it may be possible to extend the duration of effective control due to the presence over a longer time of both larval and adult stages. However, despite its acknowledged benefits as a biological control agent, *H. axyridis* is becoming known as a case of biological control gone awry. Both in North America and Europe, the ladybird beetle is considered an invasive species that impacts non-target arthropods and fruit production and is a nuisance when overwintering in houses (Brown et al. 2008; Koch and Galvan 2008). These problems are unlikely to arise in Japan, where *H. axyridis* is a native species.

The aim of the study reported here was to establish a program for the biological control of aphids on plants of non-heading *Brassica* cultivars grown in the greenhouse using a flightless strain of *H. axyridis*. To achieve this goal, we first investigated the effectiveness of adults and second instar larvae of flightless *H. axyridis* against aphids on plants of two non-heading *Brassica* cultivars. We then compared the residence duration of the adults and the second instar larvae. Finally, we investigated whether the release of flightless *H. axyridis* reduced aphid damage on plants of a non-heading *Brassica* cultivar.

Materials and methods

Aphids and ladybird beetles

Two aphid species, *Myzus persicae* and *Lipaphis erysimi,* were used for the experiments. *M. persicae* was collected from Qing-geng-cai (*Brassica rapa* var. *chinensis*) plants grown at the Research Institute of Environment, Agriculture and Fisheries, Osaka Prefectural Government (O-REAF; Habikino, Osaka, Japan, 34.5°N,135.6°E) in April 2008. *M. persicae* cultures were then initiated and maintained at 20°C under a 16/8-h (light/dark) photoperiod on fava beans, *Vicia faba*. *L. erysimi* was collected from *Brassica campestris* var. *amplexicaulis* at O-REAF in November 2009. *L. erysimi* was used directly for experiments without maintenance in the laboratory.

Adults (<seven days old) and second instars of the flightless strain of *Harmonia axyridis* were provided from the stock culture of Agrisect (Tokyo, Japan) that was originally established by artificial selection for reduced flight ability at the National Agricultural Research Center for the Western Region of Japan (Seko and Miura 2009).

Biocontrol experiments

Release experiments in greenhouses containing *B. rapa* var. *perviridis* and *B. rapa* var. *nipposinica* were conducted at O-REAF from 2008 to 2009. The lateral opening sections in all greenhouses were covered by 1.0-mm mesh net to keep out natural enemies of aphids. The experimental details are summarized in Table 1.

Trial 1

On average, 8.3 individuals of *M. persicae* were transferred to each seedling of *B. rapa* var. *perviridis* and 2.7 individuals of *M. persicae* were transferred to each seedling of *B. rapa* var. *nipposinica* using

Table 1 Experimental design of the *Harmonia axyridis* release experiments

Trial	Period	Plant	Infestation date	Transplant date	Release date	Release rate	Plot size		Temperature (°C)		
							Area (m^2)	n[a]	Mean	Maximum	Minimum
1	7–28 November (2008)	*Brassica rapa* var. *perviridis*	3 November	4 November	7 November	40 (2 adults m^{-2})	2.0 × 10.0	220	13.7	35.0	2.2
		B. rapa var. *nipposinica*				40 (2 adults m^{-2})	2.0 × 10.0	220	13.7	35.0	2.2
2	20 March –10 April (2009)	*B. rapa* var. *perviridis*	16 March	17 March	20 March	40 (2 adults m^{-2})	2.0 × 10.0	220	15.7	39.3	1.4
3	13 November–4 December (2009)	*B. rapa* var. *perviridis*	11 November	12 November	13 November	40 (2 adults m^{-2})	2.0 × 10.0	244	13.6	37.4	1.3
					13 and 20 November	200 (10 larvae m^{-2})	2.0 × 10.0	244	13.3	36.7	0.8

[a] Number of seedlings

brushes. *B. rapa* var. *perviridis* seedlings with two leaves were transplanted into two plots, and the *B. rapa* var. *nipposinica* seedlings with two leaves were transplanted into a separate two plots. All plots were laid out in different greenhouses. Each plot consisted of two ridges 100 cm apart. Two rows of seedlings were planted on each ridge at 15-cm spacing. Forty adults of *H. axyridis* were released onto one of the plots of *B. rapa* var. *perviridis* and another 40 were released onto one of the *B. rapa* var. *nipposinica* plots. The release density of adults was determined based on the recommendations for the commercially-sold *H. axyridis* strain "Nami-top" in Japan. *H. axyridis* adults were not released onto the remaining plots, which served as experimental controls. The numbers of *M. persicae* and *H. axyridis* on 80 randomly selected plants in each plot were counted at intervals of three or four days. The temperature in each greenhouse was measured hourly using an Ondotori thermo-recorder, type TR-72U (T&D Corp, Tokyo, Japan).

Trial 2

This experiment was similar to trial 1 except that only *B. rapa* var. *perviridis* was used as the host plant species. On average, 2.7 individuals of *M. persicae* were transferred to each plant.

Trial 3

This experiment was similar to trial 2 except that *L. erysimi* was used as the target aphid species, both adults and larvae of *H. axyridis* were released, and the seedlings were planted at 12.5-cm spacing. On average, 1.6 individuals of *L. erysimi* were transferred to each plant. In the larval release plot, 200 larvae of *H. axyridis* were released into the plot on 13 November ("Larva I") and again on 20 November 2009 ("Larva II"). The release density of larvae was determined based on the production cost of the larvae. The production cost of one adult was estimated to be equal to that of ten second instars (T. Seko et al., unpublished). Thus, a total of 20 individuals were released per m^2. As data from a preliminary experiment had revealed that larvae tended to disperse faster than adults, they were released twice with a one-week interval between releases.

Two-way analysis of variance (ANOVA) was performed to compare the incidence of aphids among greenhouses, with dates as replicates, treatment as a fixed effect, and stock nested in treatment as a random effect. The statistical analyses were performed using JMP ver. 6.0.3 software (SAS Institute 2005).

Residence duration of *H. axyridis* adults and larvae

The residence duration of adults and larvae of the flightless strain of *H. axyridis* were compared by counting the numbers of ladybird beetles on all *B. rapa* var. *perviridis* plants on four consecutive days following each release day (Adults, Larva I, and Larva II) in November 2009 in trial 3. The proportion of resident ladybird beetles among "Adults", "Larva I", and "Larva II" four days after each release was compared using the χ^2 test. If the differences were significant, multiple comparisons were performed using Tukey's test. The statistical software package R ver. 2.10.1 (R Development Core Team 2009) was used for the statistical analyses.

Damage assessment

To compare the damage to *B. rapa* var. *perviridis* plants caused by *L. erysimi* among the adult plot, the larva plot and the control plot, we recorded the numbers of non-marketable plants with curled leaves in all plots in trial 3 on 8 December 2009. The proportion of non-marketable plants among the plots was compared using the χ^2 test. If the differences were significant, multiple comparisons were performed using Tukey's test (R Development Core Team 2009).

Results

Biocontrol experiments

Figure 1 shows the numbers of aphids and ladybird beetles in greenhouse-grown *B. rapa* var. *perviridis* (Fig. 1a) and *B. rapa* var. *nipposinica* in trial 1 (Fig. 1b), *B. rapa* var. *perviridis* in trial 2 (Fig. 1c), and *B. rapa* var. *perviridis* in trial 3 (Fig. 1d). In all trials, the density of aphids in the release plots was similar to that in control plots immediately prior to the beetle releases (7 November in Fig. 1a, b, 20 March in Fig. 1c, 13 November in Fig. 1d). In the first trial, 21 days after the release of adult *H. axyridis*, the number of *M. persicae* in the release plot of *B. rapa* var. *perviridis* was only about 2% of that in the control plot (Fig. 1a), with the number of *H. axyridis* adults observed per 80 plants varying between 3 and 12 during this same period. Also in the first trial, 21 days after the release of adult *H. axyridis*, the number of *M. persicae* in the release plot of *B. rapa* var. *nipposinica* was about 7% of that in the control plot (Fig. 1b), with the number of *H. axyridis* adults observed per 80 plants varying between 1 and 13 during this same period.

In the second trial, the number of *M. persicae* in the release plot of *B. rapa* var. *perviridis* 17 days after the release of adult *H. axyridis* was about 11% of that in the control plot (Fig. 1c). However, by 21 days after release, the number had recovered to about 28% of the control value. The number of *H. axyridis* adults observed per 80 plants varied between 11 and 19 during this same period.

In the third trial, the number of *L. erysimi* in the release plot of *B. rapa* var. *perviridis* 14 days after the release of adult *H. axyridis* was about 11% of that in the control plot (Fig. 1d). However, aphid numbers recovered to about 19% of the control value by 21 days after release. The number of *H. axyridis* adults observed per 80 plants varied between 11 and 19 during this same period. Also in the third trial, 21 days after the first release (14 days after the second release), the number of *L. erysimi* in the larval release plot of *B. rapa* var. *perviridis* was about 2% of that in the control plot, with the number of *H. axyridis* larvae observed per 80 plants varying between one and eleven during this period.

Two-way ANOVA revealed significant effects on aphid numbers in all trials as illustrated in Fig. 1a (treatment $F_{1,158} = 167.2$, $P < 0.0001$; stock: $F_{158,948} = 2.0$, $P < 0.0001$; dates: $F_{6,948} = 61.5$, $P < 0.0001$; dates × treatment: $F_{6,948} = 64.7$, $P < 0.0001$) and Fig. 1b (treatment: $F_{1,158} = 278.2$, $P < 0.0001$; stock: $F_{158,948} = 1.7$, $P < 0.0001$; dates: $F_{6,948} = 71.6$, $P < 0.0001$; dates × treatment: $F_{6,948} = 64.9$, $P < 0.0001$) for trial 1, in Fig. 1c (treatment: $F_{1,158} = 229.4$, $P < 0.0001$; stock: $F_{158,948} = 1.5$, $P < 0.001$; dates: $F_{6,948} = 61.6$, $P < 0.0001$; dates × treatment: $F_{6,948} = 52.4$, $P < 0.0001$) for trial 2, and in Fig. 1d (treatment:

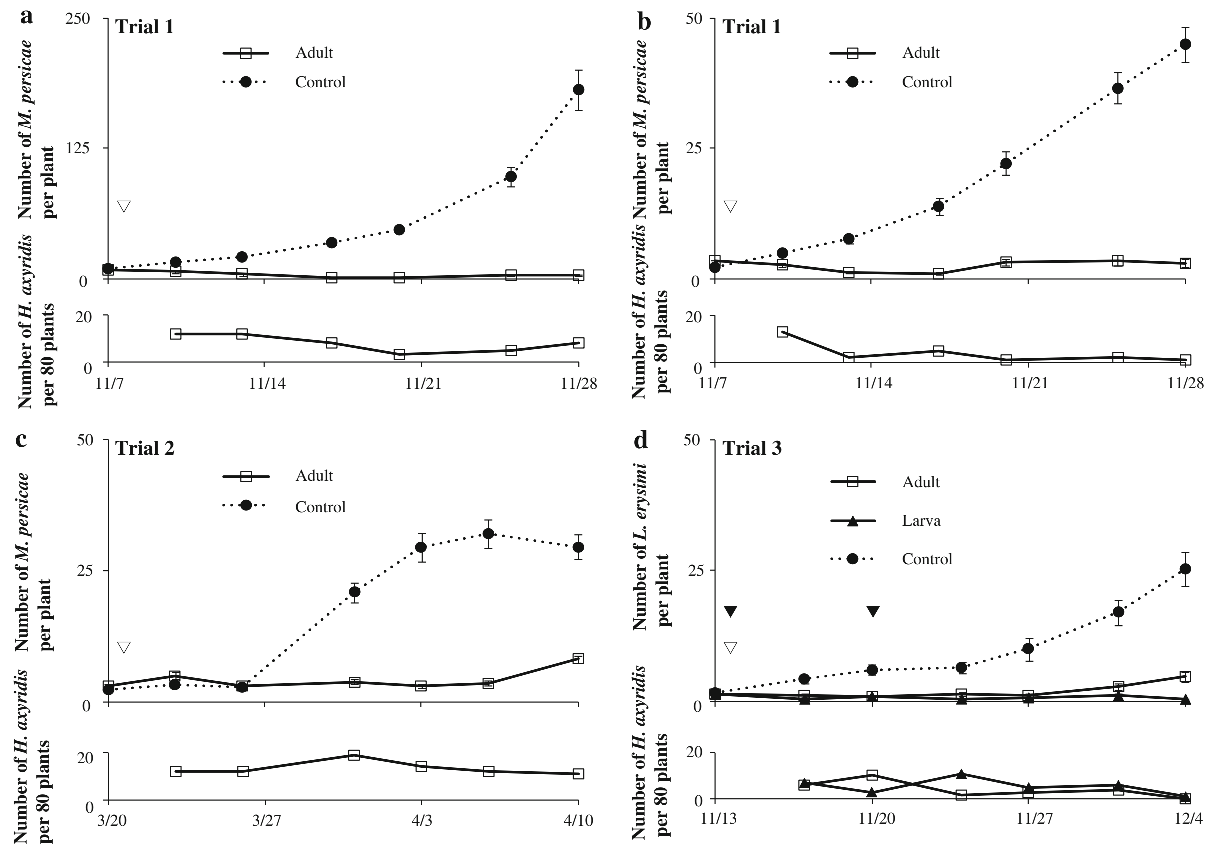

Fig. 1 Changes in the number of aphids per plant and the number of ladybird beetles per 80 plants in greenhouse-grown *Brassica rapa* var. *perviridis* (**a**) and *B. rapa* var. *nipposinica* in trial 1 (**b**), *B. rapa* var. *perviridis* in trial 2 (**c**), and *B. rapa* var. *perviridis* in trial 3 (**d**). All values are expressed as the means ± SE. Open inverted triangles Day on which 40 *Harmonia axyridis* adults were released, filled inverted triangles the 2 days on which 200 *H. axyridis* larvae were released. Ladybird beetles were not released into the control plots

$F_{2,237} = 96.4$, $P < 0.0001$; stock: $F_{237,1422} = 1.7$, $P < 0.0001$; dates: $F_{6,1422} = 26.8$, $P < 0.0001$; dates × treatment: $F_{12,1422} = 18.3$, $P < 0.0001$) for trial 3.

In the adult plots, the maximum temperature ranged from 39.3°C to 35.0°C, the mean temperature ranged from 15.7°C to 13.6°C, and the minimum temperature ranged from 2.2°C to 1.3°C during trials 1, 2, and 3. In the larval release plot, the maximum temperature was 36.7°C, the average temperature was 13.3°C, and the minimum temperature was 0.8°C during trial 3.

Residence duration of *H. axyridis* adults and larvae

The percentage of *H. axyridis* remaining in release plots decreased over time in trial 3, and the residence duration of adults was longer than that of larvae at 4 days after release (Adult = 32.5%, Larva (I) = 7.5%, Larva (II) = 12.5%; Fig. 2).

Damage assessments

The proportion of non-marketable plants damaged by aphids in trial 3 was highest in the control plot and lowest in the adult and larva plots (Table 2).

Discussion

In comparison with the control plots lacking ladybird beetles, the number of aphids was reduced in the plots where *H. axyridis* flightless adults were released (Fig. 1) and the proportion of plants damaged by

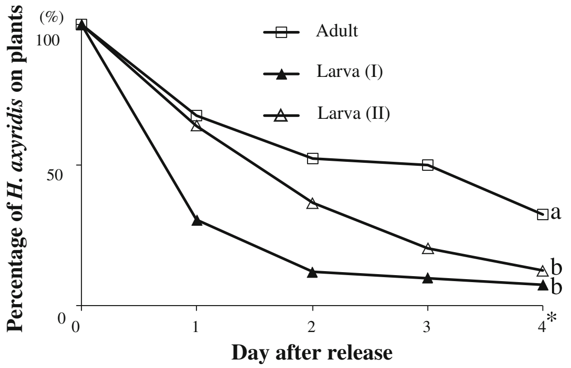

Fig. 2 Residence duration of adults and larvae of flightless *H. axyridis* that were released onto *B. rapa* var. *perviridis* grown in the greenhouse. Forty adults were released into the Adult plot. Larva (I) and Larva (II) refer to the release of 200 larvae into the same plot on two separate occasions. Asterisk A statistical analysis was conducted on residence data on day 4 after release. Different letters indicate significant differences according to Tukey's test ($P < 0.001$)

Table 2 Proportion of non-marketable plants damaged by *Lipaphis erysimi* in trial 3 following releases of either adults or larvae of *Harmonia axyridis*

Plot	Number of plants	Percentage of non-marketable plants (± SE)[a]
Adult	224	2.7 ± 1.1 a
Larva	230	2.1 ± 1.0 a
Control	223	23.3 ± 2.8 b

[a] Percentages followed by different lowercase letters are significantly different by Tukey's test ($P < 0.001$)

L. erysimi was lower (Table 2). These results can easily be interpreted as indicating predation of aphids by flightless adults that remained on the plants for a long period (Fig. 2). Seko et al. (2008) reported that adults of the flightless ladybird beetle strain remained longer on eggplants than adults of the control (non-selected) strain. However, although these adults would lay several hundreds of eggs, the eggs would unlikely contribute to the suppression of aphid densities on plants of non-heading *Brassica* cultivars as these are harvested about one month after sowing. Overall, our results suggest that the release of two flightless adults per m^2 is effective in controlling aphids at an initial aphid density of 1.4–7.8 individuals per plant of a non-heading *Brassica* cultivar. Such aphid densities are not unusual on commercially grown non-heading *Brassica* cultivars. In addition, the plant spacing in our trials was similar to those found in commercial settings.

Aphid numbers were also suppressed in the larval release plot relative to the control plot (Fig. 1d). However, a smaller proportion of released larvae was present in plots four days after release in comparison to the proportion of released adults four days after release (Fig. 2). A similar tendency was reported for normal *H. axyridis* larvae released as a control measure for *Aphis gossypii* Glover on strawberry, *Fragaria* × *ananassa* Duch. cv. Toyonoka (Kitagami and Ohkubo 1998), with about 22% of *H. axyridis* larvae remaining seven days after release. These decreased numbers could be a result of natural mortality, escape from the greenhouse, cannibalism (e.g., Dixon 2000), or predation by other predators, such as spiders (Kuroda and Miura 2003). Overall, these results suggest that the release of ten larvae of the flightless strain per m^2 on two occasions is an effective control measure against aphids present an initial aphid density of 1.4 individuals per plant of non-heading *Brassica* cultivars. The production cost of one adult has been estimated to be equal to that of ten second instar larvae (T. Seko et al., unpublished). From this point of view, the release of second instar larvae would be more cost effective that the release of adults.

In Japan, *Aphidius colemani* Viereck (Hymenoptera: Aphidiidae) is the most intensively used biological control agent against aphids. For leaf vegetables, however, the release of ladybird beetles, rather than parasitoids, may be advantageous because parasitoids would produce mummies which may reduce the commercial value of leaf vegetables. Further experiments are needed to compare the effectiveness of parasitoids with that of ladybird beetles in various vegetable types.

Our results confirm the effectiveness of adults and larvae of a flightless strain of *H. axyridis* as a biological control measure against two aphid species. However, many other pest species of non-heading *Brassica* cultivars occur in the field. If these are to be controlled by pesticides, the effects of the pesticides on the mortality of *H. axyridis* should be investigated. In addition, the effectiveness of adults and larvae of the flightless strain for the control of aphids on non-heading *Brassica* cultivars in open fields remains to be investigated.

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