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SHORT COMMUNICATION

Control of *Aphis gossypii* and *Myzus persicae* (Hemiptera: Aphididae) by a flightless strain of *Harmonia axyridis* (Coleoptera: Coccinellidae) on green pepper plants in open fields

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Abstract

The cotton aphid *Aphis gossypii* Glover and the green peach aphid *Myzus persicae* (Sulzer) are economically important pests with a worldwide distribution. We evaluated the efficacy of releasing a flightless adult strain of the multicolored Asian lady beetle *Harmonia axyridis* (Pallas) as a control measure against these aphids on green pepper plants in open fields. Flightless *H. axyridis* adults were observed on the green pepper plants in the releasing plots throughout the experimental period and were found to be effective biocontrol agents, markedly decreasing the numbers of aphids. These results suggest that adults of this flightless strain of *H. axyridis* are effective in controlling aphids on green pepper plants in open fields.

Key words: aphids, biological control, flightless natural enemy, lady beetle.

The cotton aphid *Aphis gossypii* Glover and the green peach aphid *Myzus persicae* (Sulzer) are polyphagous and economically important pests with a worldwide distribution (Vorburger *et al*. 2008). 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 & Chen 2001). In addition, chemical aphid control has become less effective due to the evolution of insecticide resistance in natural populations. Hence, alternative control methods, including biological control, are desirable.

In Japan, biological control, particularly augmentative release, has been carried out in greenhouses (Taguchi 2006), but not in open fields (Ohno 2003) because released natural enemies could escape from the targeted crops in an open field. To decrease the likelihood of escape of a natural enemy of aphids, a flightless strain of the multicolored Asian ladybird beetle *Harmonia axyridis* (Pallas) was developed by artificial selection (Seko *et al*. 2008).

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The green pepper *Capsicum annuum* var. *angulosum* is generally cultivated in open fields in Japan. Its most important pests are two aphids, *A. gossypii* and *M. persicae*. However, because the green pepper is considered to be a "minor crop" (production < 30 000 t per year) in Japan, few pesticides have been registered for the control of these aphids. Thus, additional pesticides need to be registered or other methods of control need to be developed and applied. When released as an augmentative biological control measure, adults of the flightless strain of *H. axyridis* have been shown to be effective against *A. gossypii* on eggplant plants in open fields and on non-heading *Brassica* in greenhouses (Seko *et al*. 2008; Adachi-Hagimori *et al*. 2011). However, to the best of our knowledge, the efficacy of flightless *H. axyridis* adults against aphids attacking green pepper plants has not yet been investigated in open fields.

The aim of the present study was to establish a program for the biological control of aphids on green pepper plants grown in open fields using a flightless strain of *H. axyridis*. To achieve this goal, we investigated the effectiveness of *H. axyridis* adults against aphids on plants of several green pepper cultivars in open fields.

Release experiments in open fields were conducted at the Wakayama Research Center of Agriculture, Forestry

and Fisheries, Agricultural Experimental Station (WAS) (Wakayama, Japan, 34.2°N, 135.3°E) in 2009 and 2010.

The experimental details are summarized in Table 1. We conducted two release experiments, trials 1 and 2. Each experiment involved two plots, one in which adults of the flightless strain of *H. axyridis* were released and a control plot that received no treatment. Several density levels of aphids and several density levels and times of release of *H. axyridis* were used to evaluate whether *H. axyridis* is effective in controlling the aphids *A. gossypii* and *M. persicae* in open fields of cultivated green peppers. Green pepper seedlings (cv. "Kishu-shishito" in 2009 and "Aoi-shishito" in 2010) were planted in plots on 20–23 April each year at WAS. Each plot (38.4 m^2; 4.8 m × 8.0 m) consisted of three rows of plants with 1.6 m spacing between the rows and 60 cm between plants so that each plot contained 39 plants. They were trained following the Japanese traditional system.

In trial 1, approximately 200 and 300 apterous adult aphid *A. gossypii* were attached to leaves near the ground in each plot on 30 Apr and 2 May 2009, respectively. The *A. gossypii* had been collected from green pepper plants grown at WAS just before attaching. The other aphid, *M. persicae*, occurred naturally. The density of aphids per plant was adjusted by hand to about 30 individuals immediately prior to the first release of *H. axyridis* on 7 May 2009. Two, three and three flightless adults per plant were released in the experimental plot on 7, 14 and 21 May 2009, respectively.

In trial 2, both aphids occurred naturally. The density of aphids per plant was adjusted by hand to about 60 individuals immediately prior to the release of three flightless *H. axyridis* adults per plant in the experimental plot on 27 May 2010.

Adults of the flightless strain of *H. axyridis* (<7 days old) were provided from stock culture (Agriset Inc., 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 & Miura 2009).

The numbers of aphids, their mummies, *H. axyridis* and other predators of aphids on all leaves of 14 to 39 plants were counted at intervals of three or four days. The temperature and relative humidity in each field were recorded by self-registering thermohygrograph. During the experiments, the mean temperatures in trials 1 and 2 were 20.5°C (min. 5.4°C and max. 39.9°C) and 20.9°C (min. 8.9°C and max. 34.0°C), respectively.

The Mann–Whitney *U*-test was used to determine the significance of the differences in the density of aphids between release and control plots using SPSS software 13.0J for Windows (SPSS Japan, Inc. 2004).

Changes in the numbers of aphids and *H. axyridis* after the release of flightless adults are shown in Figures 1 and 2 for trials 1 and 2, respectively. In trial

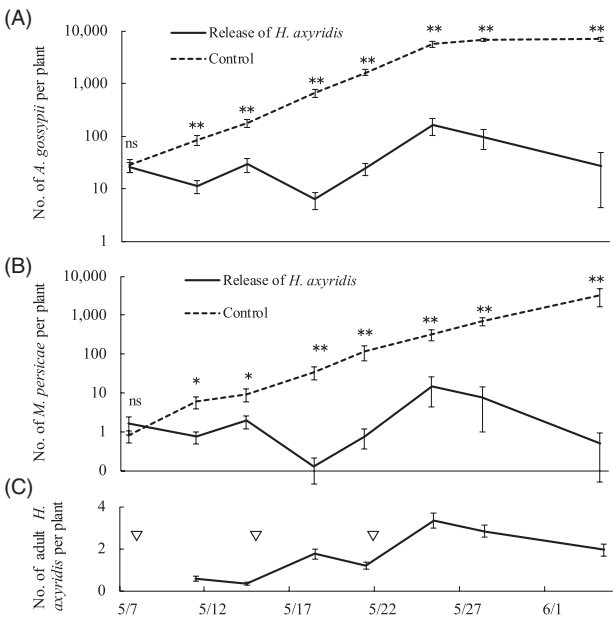

Figure 1 Changes in the number of (A,B) aphids and (C) *Harmonia axyridis* per plant in trial 1, 2009. Horizontal axis shows date (month/day). All values are expressed as means ± SE. Introduction of *H. axyridis* adults is shown (▽). *Harmonia axyridis* were not released into the control plot. *, significant difference between the test and control plots ($P < 0.05$); **, significant difference between the test and control plots ($P < 0.01$); ns, no significant difference between test and control plots (Mann–Whitney *U*-test).

Table 1 Profiles of *Harmonia axyridis* release experiments in a *Capsicum annuum* var. *angulosum* field

Trial	Period	Cultivar	Transplant date	*H. axyridis*		Temperature (°C)		
				Release date	Release rate per plant	Mean	Max.	Min.
1	7 May–4 June 2009	Kishu-shishito	20 April	7 May 14 May	2 adults 3 adults	20.5	39.9	5.4
2	27 May–21 June 2010	Aoi-shishito	23 April	21 May 27 May	3 adults 3 adults	20.9	34.0	8.9

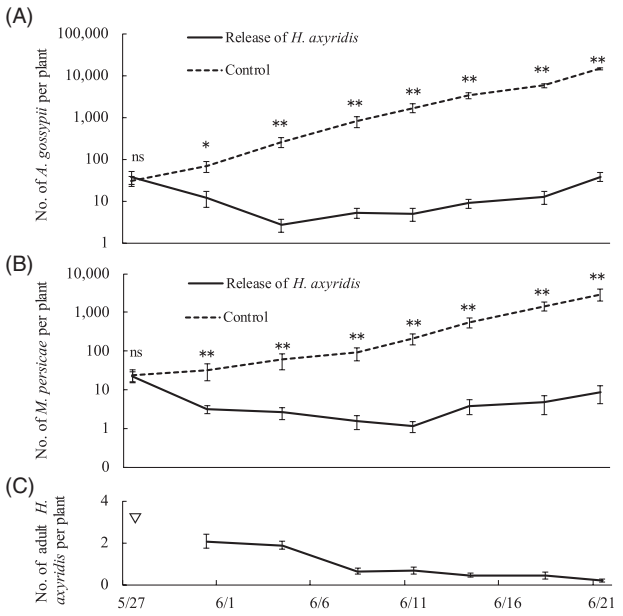

Figure 2 Changes in the number of (A,B) aphids and (C) *Harmonia axyridis* per plant in trial 2, 2010. Horizontal axis shows date (month/day). All values are expressed as the means ± SE. Introduction of *H. axyridis* adults is shown (▽). *Harmonia axyridis* were not released into the control plot. *, significant difference between the test and control plots ($P < 0.05$); **, significant difference between the test and control plots ($P < 0.01$); ns, no significant difference between test and control plots (Mann–Whitney *U*-test).

1, the density of *A. gossypii* in the test plot was almost as high as that in the control plot just before release (7 May). Soon after release (11 May), fluctuations in aphid density showed different trends in the two plots, with the density decreasing in the test plot. Surprisingly, at 11 days after release, the test plot showed a reduction of aphid density to less than 1% of that in the control plot. Over the duration of the whole experiment, the reduction trends of the density of *M. persicae* were the same as those for *A. gossypii* in the test plot. At 18 days after release (25 May), the density of aphids recovered in the test plot then decreased.

In trial 2, the density of *A. gossypii* in the test plot fluctuated to a lower level than that in the control plot, as in trial 1. Also, at 12 days after release, the test plot showed a reduction of aphid density to less than 1% of that in the control plot, and over the duration of trial 2, the reduction trends of the density of *M. persicae* were the same as those of *A. gossypii* in the test plot.

There were significant differences in the number of aphids between the test and control plots at each examined date in both trials ($P < 0.05$, Mann–Whitney *U*-test; Figs 1,2). The tolerable density of aphids on green pepper plants is not clearly understood. However, the present results indicate that a low density of aphids was maintained. Seko *et al.* (2008) also report that in open fields of cultivated eggplants, the number of *A. gossypii* was suppressed in plots that contained the same flightless strain of *H. axyridis* compared to control plots without *H. axyridis*.

Figures 1C and 2C show the residence period of the flightless adults of *H. axyridis* that were released in each plot. In both trials 1 and 2, flightless adults of *H. axyridis* were observed in the test plots throughout the entire duration of the experiment but were not observed in the control plots. The number of resident flightless adults and the resident periods of *H. axyridis* in the present study were almost the same as those observed in an eggplant experiment in an open field (Seko *et al.* 2008) and in non-heading *Brassica* cultivars in a greenhouse (Adachi-Hagimori *et al.* 2011).

In open fields, indigenous predators play an important role in suppressing aphids (Van Den Berg *et al.* 1997; Wells *et al.* 2001; Ito *et al.* 2005). In the present study, only a small number of mummies and predators of aphids were observed throughout the entire duration of the experiment (Tables 2 and 3). Therefore, natural enemies other than flightless *H. axyridis* are considered not to have affected the density of aphids during the present experiments.

The present results strongly suggest that flightless *H. axyridis* adults are effective against *M. persicae* and *A. gossypii* as a biological control agent in open fields planted with green peppers. It remains to be determined, however, how frequently and at what densities flightless *H. axyridis* should be released to achieve the optimum control.

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Table 2 Mean number of natural enemies of aphids per plant in trial 1, 2009

Species	Stage	Plots	Mean number of natural enemies per plant (mean ± SE); date							
			7 May	11 May	14 May	18 May	21 May	25 May	28 May	4 June
Orius spp.	Adults	Release of	0	0	0	0	0	0	0	0.05 ± 0.05
	Larvae	*H. axyridis*	0	0	0	0	0	0	0	0
	Adults	Control	0	0	0	0	0	0	0	0
	Larvae		0	0	0	0	0	0	0	0
Syrphidae spp.	Larvae	Release of *H. axyridis*	0	0	0	0	0	0.05 ± 0.05	0	0
	Larvae	Control	0	0	0	0	0	0.10 ± 0.10	0.05 ± 0.05	0.30 ± 0.15
Mummy by parasitoid		Release of *H. axyridis*	0.03 ± 0.03	0	0	0	0.03 ± 0.03	0.20 ± 0.09	0.30 ± 0.16	0
		Control	0	0.08 ± 0.04	0.10 ± 0.06	0.87 ± 0.23	0.87 ± 0.27	4.30 ± 1.60	10.70 ± 7.36	12.70 ± 3.28
Aphidoletes aphidimyza	Larvae	Release of *H. axyridis*	0	0	0	0	0	0	0	0
	Larvae	Control	0	0	0	0	0	0	0.40 ± 0.28	0
Coccinella septempunctata	Adults	Release of	0	0	0	0	0	0	0	0
	Larvae	*H. axyridis*	0	0	0	0	0	0	0.05 ± 0.05	0.30 ± 0.21
	Adults	Control	0	0	0	0	0	0.20 ± 0.09	0.05 ± 0.05	0
	Larvae		0	0	0	0	0	0.10 ± 0.07	0.85 ± 0.29	0.70 ± 0.30
Scymnus hoffmanni	Adults	Release of	0	0	0	0	0	0	0	0
	Larvae	*H. axyridis*	0	0	0	0	0	0	0	0
	Adults	Control	0	0	0	0	0	0	0	0
	Larvae		0	0	0	0	0	0	0	0
Propylaea japonica	Adults	Release of	0	0	0	0	0	0	0	0
	Larvae	*H. axyridis*	0	0	0	0	0	0	0	0
	Adults	Control	0	0	0	0	0	0	0	0
	Larvae		0	0	0	0	0	0	0	0

Table 3 Mean number of natural enemies of aphids per plant in trial 2, 2010

Species	Stage	Plots	Mean number of natural enemies per plant (mean ± SE); date							
			27 May	31 May	4 June	8 June	11 June	14 June	18 June	21 June
Orius spp.	Adults	Release of	0	0	0.03 ± 0.03	0.03 ± 0.03	0.07 ± 0.05	0.10 ± 0.06	0.37 ± 0.10	0.87 ± 0.27
	Larvae	*H. axyridis*	0	0	0	0	0	0	0.07 ± 0.05	1.47 ± 0.45
	Adults	Control	0	0.03 ± 0.03	0	0	0.07 ± 0.05	0.14 ± 0.07	0.13 ± 0.09	0.43 ± 0.23
	Larvae		0	0	0	0	0	0	0.07 ± 0.07	0
Syrphidae spp.	Larvae	Release of *H. axyridis*	0	0	0	0	0	0	0	0.07 ± 0.07
	Larvae	Control	0.03 ± 0.03	0	0	0.07 ± 0.05	0.07 ± 0.05	0.03 ± 0.03	0.47 ± 0.19	1.86 ± 0.61
Mummy by parasitoid		Release of *H. axyridis*	0.53 ± 0.17	0.50 ± 0.16	0.77 ± 0.18	0.37 ± 0.11	0.23 ± 0.10	0.10 ± 0.07	0.17 ± 0.08	0.07 ± 0.07
		Control	0.37 ± 0.17	1.37 ± 0.47	3.23 ± 0.93	3.40 ± 0.81	4.03 ± 1.05	6.48 ± 2.71	15.87 ± 3.54	12.43 ± 2.74
Aphidoletes aphidimyza	Larvae	Release of *H. axyridis*	0	0.03 ± 0.03	0	0	0	0	0	0
	Larvae	Control	0.07 ± 0.05	0	0	0.03 ± 0.03	0.27 ± 0.27	2.83 ± 1.10	8.00 ± 2.43	15.57 ± 4.61
Coccinella septempunctata	Adults	Release of	0	0	0	0	0	0	0	0
	Larvae	*H. axyridis*	0	0	0	0	0	0	0	0
	Adults	Control	0	0	0.03 ± 0.03	0.07 ± 0.05	0.03 ± 0.03	0	0	0
	Larvae		0	0	0	0	0	0	0	0
Scymnus hoffmanni	Adults	Release of	0	0	0	0	0	0	0	0
	Larvae	*H. axyridis*	0	0	0	0	0.03 ± 0.03	0	0	0
	Adults	Control	0	0	0	0	0	0	0	0
	Larvae		0	0	0.10 ± 0.06	0	0	0	0	0
Propylaea japonica	Adults	Release of	0	0.03 ± 0.03	0.03 ± 0.03	0.03 ± 0.03	0	0	0.03 ± 0.03	0.13 ± 0.09
	Larvae	*H. axyridis*	0	0	0	0	0	0	0.07 ± 0.05	0
	Adults	Control	0	0	0	0	0	0	0.07 ± 0.07	0
	Larvae		0	0	0	0	0	0	0	0

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