Appl. Entomol. Zool. 38 (2): 271–274 (2003)

Comparison of the effectiveness of two methods for releasing *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae) against *Aphis gossypii* Glover (Homoptera: Aphididae) on cucumbers in a greenhouse

Tsuyoshi KURODA[1] and Kazuki MIURA[2,*]

[1] Ehime Agricultural Experiment Station; Hohjo 799–2405, Japan
[2] National Agricultural Research Center for Western Region; Fukuyama 721–8514, Japan

(Received 12 June 2002; Accepted 28 February 2003)

Abstract
The control of *Aphis gossypii* on cucumbers was evaluated by releasing egg mass sheets or flightless adults of *Harmonia axyridis*. In experiment one, 1, 5 and 10 egg mass sheets per plant in each greenhouse were released only one time. The density of aphids in the greenhouses receiving egg mass sheets of *H. axyridis* was lower than that without the sheets. Within 5 days after release, the greenhouses receiving 5 egg mass sheets and 10 egg mass sheets per plant showed reduction of aphid densities to less than 1% of that without the sheets. In experiment 2, five egg mass sheets and 5 flightless adults per plant were released in each greenhouse three times at about one week intervals. The density of aphids in greenhouses receiving flightless adults showed fluctuations to a lower level than in the greenhouses receiving egg mass sheets.

Key words: *Harmonia axyridis*; *Aphis gossypii*; cucumber; biological control; releasing methods

INTRODUCTION

The cotton aphid, *Aphis gossypii* Glover, is a serious pest of the cucumber, *Cucumis sativus* L., in greenhouses in Japan (Matsuzaki and Kiritani, 1972; Matsuzaki, 1974; Nozato, 1993). Development of alternative control methods including biological control is needed to cope with the development of insecticide resistance in *A. gossypii*. *Harmonia axyridis* (Pallas) is a common predator of aphids, so the possibility of using this predator in a biological control program has been investigated (Dixon, 2000). In Japan, *H. axyridis* has been marked as a candidate for an indigenous biological control agent against aphids.

There are three types of release methods for *H. axyridis*; egg masses, larvae and adults (Tedders and Schaefer, 1994; Ferran et al., 1998; Trouve et al., 1997; Kitagami and Ohkubo, 1998; Tourniaire et al., 1999). Egg mass sheets and flightless adults will soon be sold as biological control agents in Japan. The flightless adults are not a mutagen and selection of their progeny for flightlessness (Tourniaire et al., 1999) but created by an artificial breeding method developed by the Japanese company Cats Agrisystems. The mechanism of flightless is not a hereditary factor. However, how to utilize *H. axyridis* against the cotton aphid on cucumbers in greenhouses has never been determined.

Here, we attempted to compare the suppressive effects of *H. axyridis* against the cotton aphid on cucumbers in greenhouses by two release methods, egg mass sheets and flightless adults.

MATERIALS AND METHODS

Biological materials. Egg mass sheets and flightless adults of *H. axyridis* were from the stock culture at Sankei Chemical Co. Ltd. and Cats Agrisystems, respectively. One egg mass on a paper sheet (5 cm×5 cm) included 20.4±7.2 (average±SD) eggs of *H. axyridis*.

Experiment 1 (Exp. 1): Fluctuation of aphid population with and without egg mass sheets of *H. axyridis*. Six to 10 cucumber plants (cv. 'Frontier') with fifteen leaves were planted in each of four greenhouses (5.4 m×10.0 m) covered with 0.6 mm nets on 21 Apr. 1999 at the Ehime Agricultural Experiment Station, Hohjo, Ehime Prefecture. Cucumbers were planted in two rows of three to five plants each and were trained by the Japanese traditional system.

* To whom correspondence should be addressed at: E-mail: miurak@affrc.go.jp

Two apterous aphids were attached on leaves near the ground by a brush on 19 May 1999. The density of aphids per plant was adjusted to about 50 individuals just before the release of *H. axyridis*. The number of egg mass sheets released per plant in each greenhouse was 1, 5 and 10. No *H. axyridis* were released in the control greenhouse. Egg mass sheets were set on the leaves of cucumber plants using staples on 28 May 1999.

The number of aphids and *H. axyridis* on all leaves of all plants were counted at intervals of two or three days. On 31 May, the number of *H. axyridis* was not counted because most hatching. Temperature and relative humidity in each greenhouse were recorded by self-registering thermohygrographs. During the experiment, the mean temperature and relative humidity were 24.3°C (Min. 20.9°C and Max. 28.0°C in mean temperature) and 74.3% R.H. (Min. 56.7% R.H. and Max. 90.4% R.H. in mean relative humidity).

Experiment 2 (Exp. 2): Fluctuation of aphid population after release of egg mass cards and flightless adult *H. axyridis*. Twenty cucumber plants (cv. 'Frontier') with fifteen leaves were planted in each of two greenhouses (4.5 m×13.0 m) covered with 0.4 mm nets on 12 Sept. 2000 at the National Agricultural Research Center for Western Region, Fukuyama, Hiroshima Pref. Cucumbers were planted in two rows of 10 plants each and were trained by the traditional Japanese system.

Five apterous aphids were released on leaves near the ground with a brush on 27 Sept. 2000. The density of aphids per plant was adjusted to about 80 individuals just before the release of *H. axyridis*.

Five egg mass sheets and 5 flightless adults per plant were released in each greenhouse. Egg mass sheets were set on leaves of cucumber plants and flightless adults were set between each hill on the 4, 12 and 19 Oct. 2000.

The number of aphids and *H. axyridis* on all leaves of all the plants were counted at about one week intervals. Temperature and relative humidity in the greenhouses were recorded by self-registering thermohygrographs. During the experiment, the mean temperature and relative humidity were 17.6°C (Min. 11.3°C and Max. 23.9°C in mean temperautre) and 77.1% R.H. (Min. 27.0% R.H. and Max. 99.0% R.H. in mean relative humidity).

RESULTS

Exp. 1: Fluctuation of aphid population with and without egg mass sheets of *H. axyridis*

Changes in the number of aphids and *H. axyridis* after releasing egg mass sheets of *H. axyridis* are shown in Table 1. Just before release (28 May), the density of aphids with egg mass sheets was almost as high as without egg mass sheets. Until 3 days after the release (31 May), fluctuations in aphid density showed the same trend in every greenhouse. Then the density of aphids decreased in all greenhouses except the greenhouse without egg mass sheets. In particular, the density of aphids in greenhouses receiving five or ten egg mass sheets per plant decreased remarkably as compared to those without egg mass sheets. Five days after release, the greenhouses receiving 5 egg mass sheets and 10 egg mass sheets per plant showed a reduc-

Table 1. Changes in the number of aphids and ladybirds per plant in Exp. 1. Egg mass sheets were set on 28 May.

Plots	Species	May		June		
		28 Mean±SD	31 Mean±SD	2 Mean±SD	4 Mean±SD	8 Mean±SD
One egg mass sheet	Aphids	41.7±6.7	64.7±25.9	35.6±31.1	54.6±62.3	239.0±212.8
	Ladybirds	—	—[a]	10.8±5.7	1.5±1.2	0.5±0.8
Five egg mass sheets	Aphids	47.5±2.3	70.0±21.8	1.7±3.6	0.2±0.4	29.5±48.1
	Ladybirds	—	—[a]	17.0±4.1	2.3±1.2	1.7±0.5
Ten egg mass sheets	Aphids	48.2±2.9	37.2±32.1	0.5±0.5	0.8±1.6	37.0±32.4
	Ladybirds	—	—[a]	28.2±2.10	3.7±3.8	1.8±1.2
Control	Aphids	35.8±16.8	50.2±38.3	101.6±79.8	384.0±341.7	1,437.6±1,139.6

[a] Not counted because near hatching.

Table 2. Changes in the number of aphids and ladybirds per plant in Exp. 2. Egg mass sheets and flightless adults were set on 4, 12 and 19 Oct.

Plots	Species	Oct.						
		4 Mean±SD	6 Mean±SD	11 Mean±SD	16 Mean±SD	18 Mean±SD	23 Mean±SD	26 Mean±SD
Flightless adult	Aphids	85.1±17.6	60.4±68.5	4.6±5.2	6.0±9.4	13.4±23.5	14.1±19.8	5.3±8.2
	Ladybirds	—	3.0±1.8	0.9±1.3	1.7±1.7	1.1±1.2	2.8±1.4	2.8±2.1
Egg mass sheet	Aphids	81.5±24.0	174.3±78.0	20.9±13.8	33.6±23.7	51.5±30.2	91.0±60.3	90.2±96.3
	Ladybirds	—	25.3±11.3	8.4±5.6	4.6±3.7	1.7±1.6	30.8±17.8	8.4±3.6

Plots	Species	Nov.			
		2 Mean±SD	9 Mean±SD	16 Mean±SD	28 Mean±SD
Flightless adult	Aphids	3.8±6.3	1.4±3.3	2.6±7.5	3.3±7.8
	Ladybirds	2.2±1.6	1.5±1.2	1.3±1.7	1.0±0.9
Egg mass sheet	Aphids	54.1±70.1	28.4±31.5	30.7±22.1	14.1±9.5
	Ladybirds	2.8±1.9	1.6±1.4	1.2±1.2	0.6±1.0

tion of aphid densities to less than 1% of that without egg mass sheet. However, 11 days after release (8 June), the density of aphids recovered.

In every greenhouse, the number of larvae of *H. axyridis* decreased as the days passed. Eleven days after release (8 June), there were more than 1.0 larvae per plant in the greenhouses receiving 5 and 10 egg mass sheets per plant, but there were less than 1.0 larvae per plant in the greenhouse receiving one egg mass sheet per plant. Pupae of *H. axyridis* were found 11 days after release (8 June).

Exp. 2: Fluctuation of aphid population after release of egg mass cards and flightless adult *H. axyridis*

Changes in the number of aphids and *H. axyridis* depending on the release methods are shown in Table 2. The density of aphids in the greenhouses receiving flightless adults fluctuated to a lower level than that in greenhouses receiving egg mass sheets, although the density of aphids in each greenhouse was almost the same at the start of the experiment. During the experiment, more than 1.0 adult of *H. axyridis* per plant were recognized in greenhouses receiving five flightless adults per plant. However, in greenhouses receiving five egg mass sheets per plant, there was less than 1.0 larvae of *H. axyridis* per plant 36 days after release (9 Nov.). In greenhouses receiving egg mass sheets, *H. axyridis* pupae were observed 19 days after release (23 Oct.) and the adults were recognized 29 days after release (2 Nov.).

DISCUSSION

The results in Exp. 1 indicated that the larvae of *H. axyridis* decreased the density of aphids. In particular, the suppression of aphid density in greenhouses receiving 5 or 10 egg mass sheets per plant was more effective than in greenhouses receiving 1 egg mass sheet per plant. Mogi (1969) reported that the maximum number of prey, *Aphis craccivora* Koch, eaten by 1st , 2nd, 3rd and 4th instars of *H. axyridis* larvae per day were about 3, 12, 50 and 120, respectively. The decrease in aphid density in the treated greenhouses compared with that in the untreated greenhouse can easily be explained as predation by *H. axyridis*. However, a comparison between the present experiment and Mogi's experiments was difficult because of differences in the prey species and conditions of the experiments.

Five days after release of egg mass sheets, the number of the larvae of *H. axyridis* decreased rapidly. Escape from the greenhouse, natural mortality of larvae, cannibalism (e.g., Dixon, 2000) and other the factors may be the reasons for this

decrease. Moreover, most the larvae pupated by 11 days after release. The recovery of the aphid population 11 days after release is likely due to the above-mentioned reasons. On the other hand, the recovery of the aphid population in Exp. 2 was not observed when egg mass sheets of *H. axyridis* were released several times. Thus, we recommend the release of egg mass sheets of *H. axyridis* at several times. As a result, there is no period in which only the pupal stage of *H. axyridis* and lower densities of the larvae occur in the greenhouse.

From the results of Exp. 1, the release of egg mass sheets of *H. axyridis* effectively suppressed the density of aphids. Consideration must be paid to the density of *H. axyridis* when utilizing this species because the extent of suppressive effectiveness is affected by the release density and also initial density of aphids.

In Exp. 2, the density of aphids in the greenhouse receiving flightless adults fluctuated to a lower level than in the greenhouses receiving egg mass sheets. The reason is likely that predation capacity in the adult stage is higher than in the larval stages (Miura and Nishimura, 1980) and flightless adults remained on the crops longer (Ferran et al., 1998). On the other hand, the suppressive effect of egg mass sheet release in Exp. 2 was higher than in Exp. 1, because egg mass sheets were released several time in Exp. 2. Also the short daylength during Exp. 2 may induce diapause of flightless adults, because we could not observe any eggs or larvae in this greenhouse. However, release of flightless adults of *H. axyridis* has a greater suppressive effect on the density of aphids than that of egg mass sheet release.

Under the conditions of the present experiments, release of flightless adults was more effective to suppress the aphid population, although release of egg mass sheet was also effective.

ACKNOWLEDGEMENTS

We wish to thank Drs. M. Morishita, Wakayama Research Center of Agriculture, Forestry and Fisheries and M. Ohtaishi, National Agricultural Research Center for Western Region, for their critical reading of the original manuscript and Sankei Chemical Co. Ltd. and Cats Agrisystems for the supply of *H. axyridis.* We are grateful to two anonymous reviewers for their helpful comments which improved this manuscript.

REFERENCES

Dixon, A. F. G. (2000) *Insect Predator-Prey Dynamics. Ladybird Beetles & Biological Control.* Cambridge University Press, Cambridge, 257 pp.

Ferran, A., L. Giuge, R. Tourniaire, J. Gambier and D. Fournier (1998) An artificial non-flying mutation to improve the efficiency of the ladybird *Harmonia axyridis* in biological control of aphids. *BioControl* 43: 53–64.

Kitagami, T. and N. Ohkubo (1998) Release of larvae of *Coccinella septempunctata* and *Harmonia axyridis* in greenhouse for controlling *Aphis gossypii* on strawberry. *Proc. Kanasai Pl. Proc.* No. 40: 151–152 (in Japanese).

Matsuzaki, M. (1974) Occurrence of aphids in greenhouse and its problems. *Plant Protect.* 28: 241–246 (in Japanese).

Matsuzaki, M. and K. Kiritani (1972) Topics of greenhouse pests. *Agric. Hort.* 47: 794–800 (in Japanese).

Miura, T. and S. Nishimura (1980) The larval period and predacious activity of an aphidophagous coccinellid, *Harmonia axyridis* Pallas. *Bull. Fac. Agr. Shimane Univ.* 14: 144–148 (in Japanese with English summary).

Mogi, M. (1969) Predation response of the larvae of *Harmonia axyridis* Pallas (Coccinellidae) to the different prey density. *Jpn. J. Appl. Entomol.* Zool. 13: 9–16 (in Japanese with English summary).

Nozato, K. (1993) Behavioral traits of *Aphis gossypii* Glover alatae (Homoptera: Aphididae), in relation to its flight and reproduction, and effects of natural enemies on the survival of the aphid. *Sci. Bul. Fac. Agric., Kochi Univ.* 37: 121–129 (in Japanese with English summary).

Tedders, W. L. and P. W. Schaefer (1994) Release and establishment of *Harmonia axyridis* (Coleoptera: Coccinellidae) in the Southeastern United States. *Entomol. News* 105: 228–243.

Tourniaire, R., A. Ferran, J. Gambier, L. Giuge and F. Bouffault (1999) Locomotor behavior of flightless *Harmonia axyridis* Pallas (Col., Coccinellidae). *J. Insect Behav.* 12: 545–558.

Trouve, C., S. Ledee, A. Ferran and J. Brun (1997) Biological control of the damson-hop aphid, *Phorodon humuli* (Hom.: aphididae), using the ladybeetle *Harmonia axyridis* (Col.: Coccinellidae). *Entomophaga* 42: 57–62.