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Residence period of a flightless strain of the ladybird beetle *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae) in open fields

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ABSTRACT

Mark-release experiments were conducted to compare the residence period of *Harmonia axyridis* adults between flightless (selected) strain and control (nonselected) one in small-scale open fields with cultivated eggplants. A flightless strain of *H. axyridis* was established by artificial selection for reduced flight ability of adults in a laboratory population. Two flightless or control adults per eggplant were released at the center of each experimental plot. Few adults of the control strain were recorded in plots after the release day. In contrast, adults of the flightless strain remained longer on eggplants compared with the control strain in the open fields, even under conditions of low aphid density and a small-scale area. The number of *Aphis gossypii* was suppressed in plots that contained the flightless strain compared with plots that contained the control strain. These results suggest that the flightless strain of *H. axyridis* is effective against *A. gossypii* as a biological control agent in open fields with cultivated eggplants.

1. Introduction

Augmentative biological control has been performed in open fields with cultivated crops, vegetables and fruits around the world (e.g., Hoy et al., 1990; Smith, 1996; Heinz et al., 1999). Dispersal of predators or parasitoids from selected release points should be great enough to allow discovery of potential hosts, on the other hand, a propensity of parasitoids or predators to disperse can result in reduced effectiveness in controlling pests (Jung et al., 2004; Bellamy et al., 2004; Corbett and Rosenheim, 1996; Keller et al., 1985; Hougardy and Mills, 2006; Andow and Prokrym, 1991).

Ladybirds have been widely used to control aphids through augmentation by translocation or mass rearing and release (Dixon, 2000). The use of ladybirds in augmentative biological control has been hampered by the tendency of adults to disperse (Marples et al., 1993; Hagen, 1962; Dreistadt and Flint, 1996; Dixon, 2000). The coccinellid *Harmonia axyridis* Pallas has been utilized in augmentative biological control in Asia (e.g., Seo and Youn, 2000), Europe (Trouve et al., 1997; Ferran et al., 1996), and North America (e.g., LaRock and Ellington, 1996). However, *H. axyridis*, in common with some other aphidophagous ladybirds, is a good flyer (Hodek et al., 1993; Obata, 1986; Tourniaire et al., 2000a). Marples et al. (1993) suggested that if ladybirds were poor flyers they would remain in a crop for longer and provide the potential for more long-term control. Tourniaire et al. (2000b) produced a homozygous flightless strain by artificial selection from a laboratory population of *H. axyridis*. Adults of this strain have normal elytra and wings but drop almost vertically when they attempt to fly (Tourniaire et al., 2000b). It was effective for augmentative biological control of the damson hop aphid *Phorodon humuli* Schrank in open fields with cultivated hops (Weissenberger et al., 1999). The flightless strain can contribute to aphid control in biological control program (Tourniaire et al., 2000a,b), but the effectiveness has not been reported in open fields with cultivated crops, vegetables and fruits except for Weissenberger et al. (1999).

In Japan, augmentative release has been performed in greenhouse (Taguchi, 2006) but not in open fields (Ohno, 2003). Cultivated areas of open fields containing eggplant crops cover less than 1 ha in 95% of farm households in Japan (MAFF, 2005). The use of flightless ladybirds can be effective to control aphids in open fields with such small-scale areas, but not been examined yet. This study investigated the potential of a flightless strain of *H. axyridis* as biological control agents in open fields. Firstly, we produced a flightless strain of *H. axyridis* by the artificial selection of a population collected from the field. Secondly, in open fields with cultivated eggplants, we researched the residence period of *H. axyridis* and evaluated the effectiveness of this strain for control of the cotton aphid, *Aphis gossypii* Glover.

2. Materials and methods

2.1. Selection of flightless adults

Artificial selection for reduced flight ability of *H. axyridis* was conducted on a laboratory population to produce a flightless strain.

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Eighty adults of *H. axyridis* were collected in Fukuyama (western Japan, Lat. 34°28′N, Long. 133°23′E) during late April 2003 and were allowed to mate and lay eggs on tissue paper at a density of 40 individuals per plastic square container (16 × 22 × 8 cm). Thirty egg clusters of *H. axyridis* were collected, and 130 hatchlings were randomly selected from these egg clusters (the first generation). Larvae were reared at a density of 20 individuals per circular plastic container (diameter 10 cm, height 5.5 cm).

After emergence, male and female adults of *H. axyridis* were reared separately to avoid mating. Seven-day-old adults were measured for flight distance using the flight mill system, which can accommodate 12 individuals at a time. Flight distance, which is an indicator of flight ability, was automatically logged in the computer unit (KV-700, Keyence, Ltd., Osaka, Japan) of the flight mill system. A small amount of bond (Zero time wide, Loctite, Ltd., Tokyo, Japan) was applied to the left elytra of 48 adults of each sex. Each adult was attached to the tip of the flight mill rotor (10 cm in length, 0.23 g in weight). They were allowed to fly for 1 h under a light regime. Adults that recorded shorter flight distances (bottom 30%) were selected and mated in plastic square containers. Egg clusters were collected from selected adults. The same procedure as described above was repeated in the next generation.

An isogenic line was established from selected adults from the seventh generation to promote homozygosis. Four adults that recorded shorter flight distances (bottom 25% in selected adults) from each sex were selected, and four mating pairs were established. Three pairs of them could not lay fertilized eggs, on the other hand, only one pair succeeded to produce offspring. During the eighth and ninth generations, flight distance was not measured because the population size was less than 10 individuals. The selection on flight ability of *H. axyridis* was resumed from the 10th generation onward. The procedure and population size per generation were same as those of before seventh generation. This line is referred to as ‘flightless’ strain. Because of a lack of diet in the laboratory, in the fourth and 13th generations, the strain was maintained with a population size of 24 males and 39 females in the fourth generation, and 25 males and 20 females in the 13th generation with no artificial selection.

Another line from same adults that were used to establish the flightless strain was maintained with randomly selected offspring, thereby imposing no artificial selection on flight ability. Twenty egg clusters of *H. axyridis* were collected, and 80 hatchlings were randomly selected from these egg clusters. Larvae were reared at a density of 20 individuals per circular plastic container. After emergence, 40 Adults (20 males and 20 females) were selected randomly and mated in plastic square containers. The same procedure as described above was repeated in the next generation. This line is referred to as the ‘control’ strain. The flight distance of the control strain was measured in the twentieth and 30th generations. Because the flight distance of both flightless and control strains was not normally distributed in all generations (Shapiro-Wilk test, $P < 0.001$), Mann–Whitney U test was conducted to compare the flight ability between the flightless and control strains.

This experiment was conducted under LD 16:8 at 25 °C and 40–60% RH. In the laboratory, larvae and adults of *H. axyridis* were reared on pea aphids *Acyrthosiphon pisum* Harris, which were raised on broad bean seedlings in plastic cages (26 × 18 × 13 cm).

Take-off behavior of the flightless strain in the 20th and 30th generations was measured in the greenhouse (3 × 3 × 2.5 m) on warm sunny days. Temperature was recorded in the greenhouse by a thermo recorder (TR-72S, T and D, Ltd., Nagano, Japan). Mean temperatures were 31.6 °C (Min. 27.8 °C and Max. 37.0 °C) in the 20th generation, and 29.5 °C (Min. 25.1 °C and Max. 32.0 °C) in the 30th generation. A wooden stick (diameter 0.5 cm, height 60 cm) was placed vertically in the center of the area. An adult was released at the bottom of the stick at once. Most of the adults released at the bottom of the stick climbed to the top and then jumped off. The points at which adults of each strain either landed on the ground immediately (i.e. they could not fly) or on the wall and the ceiling (i.e. they could fly) were recorded. Fisher’s exact test was used to compare the proportion of adults that could not fly between the flightless and control strains.

2.2. The residence period of flightless adults

Mark-release experiments were conducted to compare the residence period of adults of *H. axyridis* between the flightless and control strains in small-scale open fields with cultivated eggplants. Seven-day-old adults of each strain were marked with a felt pen on their right elytra to identify individuals that were released at the age of 8–13 days. The release of *H. axyridis* were carried out at the National Agricultural Research Center for Western Region, Fukuyama, Hiroshima Prefecture (Lat. 34°50′N, Long. 133°38′E) in May and October 2007, and Hyogo Prefectural Technology Center for Agriculture, Forestry and Fisheries–Agricultural Technology Institute, Kasai, Hyogo Prefecture (Lat. 34°92′N, Long. 134°89′E) in September 2007. Table 1 shows conditions during the mark-release experiment in these open fields with cultivated eggplants.

Eggplant seedlings (cv. ‘Chikuyô’) with five leaves were transplanted in two plots on 9 May 2007 in Fukuyama. Each plot consisted of two rows of plants with 80 cm spacing between rows and 50 cm between plants so that each plot contained 30 plants. The incidence of *Aphis gossypii* was observed within one week after the transplantation. Sixty flightless and control adults in the 31st generation (30 males and 30 females, respectively) were released on the ground at the center of the experimental plot on 15 May 2007. The number of *H. axyridis* on all plants and the ground of the entire plot was counted every day after the release. The number of *A. gossypii* on plants, which indicate the suppressive effect of *H. axyridis*, was counted every three days. Six leaves were randomly selected per eggplant to measure the number of *A. gossypii*.

The same experiment was conducted from mid-October to mid-November in Fukuyama. Transplanting of eggplant seedlings occurred on 10 October 2007, and *A. gossypii* was observed the next day of the transplantation. Adults of *H. axyridis* in the 35th generation (15 males and 15 females) were released on 12 October 2007. Six leaves were randomly selected per eggplant to measure the number of *A. gossypii*.

Fifteen eggplant seedlings (cv. ‘Senryô’) with 10 leaves were planted in each of the two plots on 14 September 2007 in Kasai. Eggplants were planted in one row of 15 plants. The incidence of *A. gossypii* was also observed within one week after the transplantation. Thirty adults in the 35th generation were released in the center of the plot on 25 September 2007. Six leaves were randomly selected per eggplant to measure the number of *A. gossypii*.

3. Results

3.1. Selection of the flightless strain

Fig. 1 shows the response to the artificial selection of the flight distance in adults of *H. axyridis*. Flight ability of *H. axyridis* responded to the selection, and the response was similar in both sexes. From the 23rd generation, the flight distance of most adults in the flightless strain recorded nearly 0 m (Fig. 1). These adults have normal elytra and wings, but the rotor of the flight mill system did not spin because they could not flap their wings.

The flight distance of the control strain on both male and female was significantly higher than that of the flightless strain, respectively, in the 20th and 30th generations (Table 2, $P < 0.001$). All adults dropped on the ground in the 30th generation of the flightless strain (Table 2).

Table 1
Conditions during the mark-release experiment in the three open fields with cultivated eggplants

Place	Experimental period	Day of the transplantation of eggplant seedlings	Release day of *H. axyridis*	Plot size		Temperature (°C)		
				Area	n[a]	Mean	Max.	Min.
Fukuyama	14 May–10 June	9 May	15 May	4.5 × 13.0 m	30	19.7	29.7	11.8
Fukuyama	12 October–15 November	10 October	12 October	4.5 × 13.0 m	30	15.8	26.5	5.1
Kasai	20 September–10 October	14 September	25 September	2.0 × 13.0 m	15	23.0	31.7	14.2

[a] Number of eggplant seedlings.

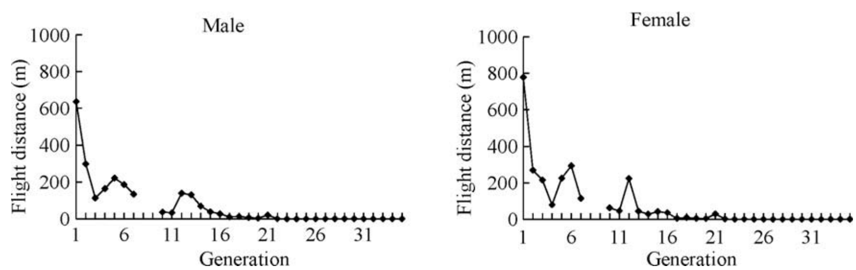

Fig. 1. Response to artificial selection for reduced flight distance (median) for *H. axyridis* male (left) and female (right) adults over 35 generations of selection. An isogenic line was established from selected adults in the seventh generation. During the eighth and ninth generations, flight distance was not measured because the population size in the line was less than 10 individuals. In the fourth and 13th generations, the line was maintained as a small population size with no selection, because of a lack of *A. pisum* in the laboratory.

3.2. The residence period of the flightless adults

Fig. 2 shows the residence period of *H. axyridis* adults that were released in each plot. Few adults of the control strain were recorded in the plots after the release day. Adults of the flightless strain remained longer in the plots compared with those of the control strain. All adults of *H. axyridis* observed in all plots had marked elytra. The density of *A. gossypii* tended to be lower in plots that contained the flightless strain compared with plots that contained the control strain in Fukuyama (Fig. 3). Few *A. gossypii* was observed in both plots after September 27 in Kasai (Fig. 3).

4. Discussion

In this study, some adults of the control strain of *H. axyridis* tended to fly immediately after their release. There were few individuals in the plots after 1 day. This suggests that adults of the control strain rapidly disperse from the release areas. On the other hand, adults of the flightless strain remained longer on eggplants than those of the control strain in open fields, even under conditions of low aphid density and a small-scale area. The number of *A. gossypii* was suppressed in plots that contained the flightless strain compared with plots that contained the control strain. In Kasai, although the residence period of the flightless strain was

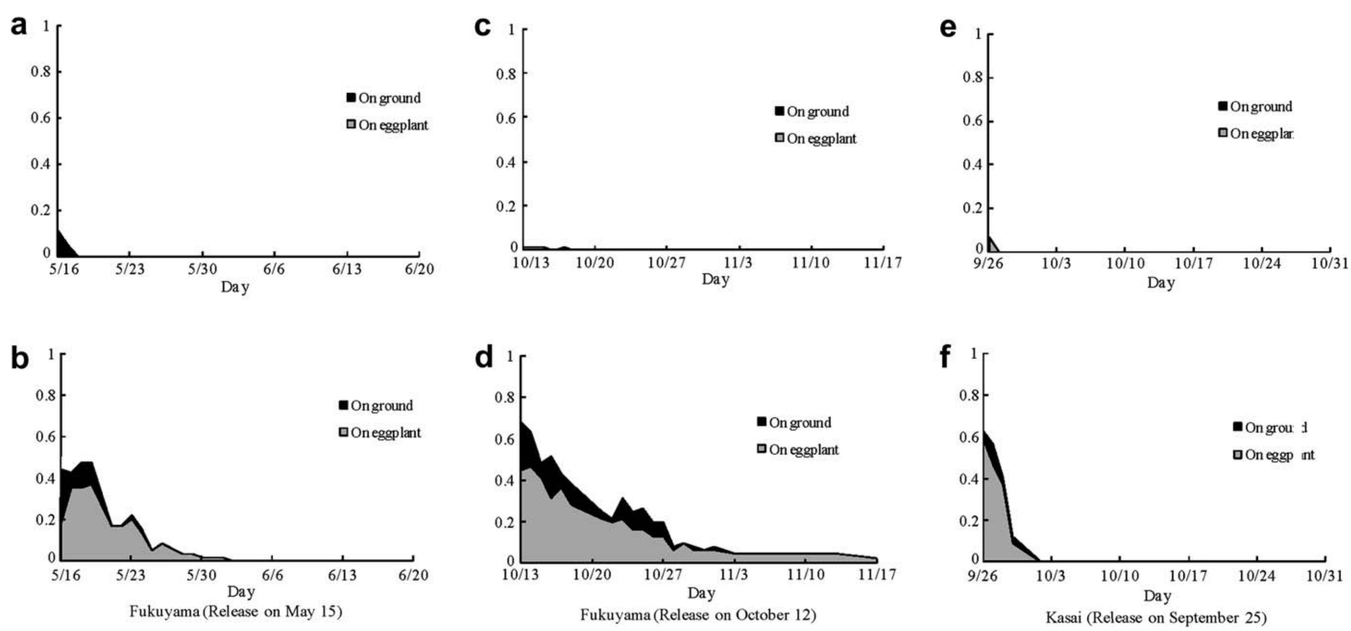

Fig. 2. The residence period of *H. axyridis* adults that were released in open fields with cultivated eggplants for (a) the control strain and (b) the flightless strain on 14 May–10 June in Fukuyama, for (c) the control strain and (d) the flightless strain on 12 October–15 November in Fukuyama, and for (e) the control strain and (f) the flightless strain on 20 September–10 October in Kasai. The longitudinal axis shows the daily residence rate of *H. axyridis* adults, i.e. the proportion of the number of released adults.

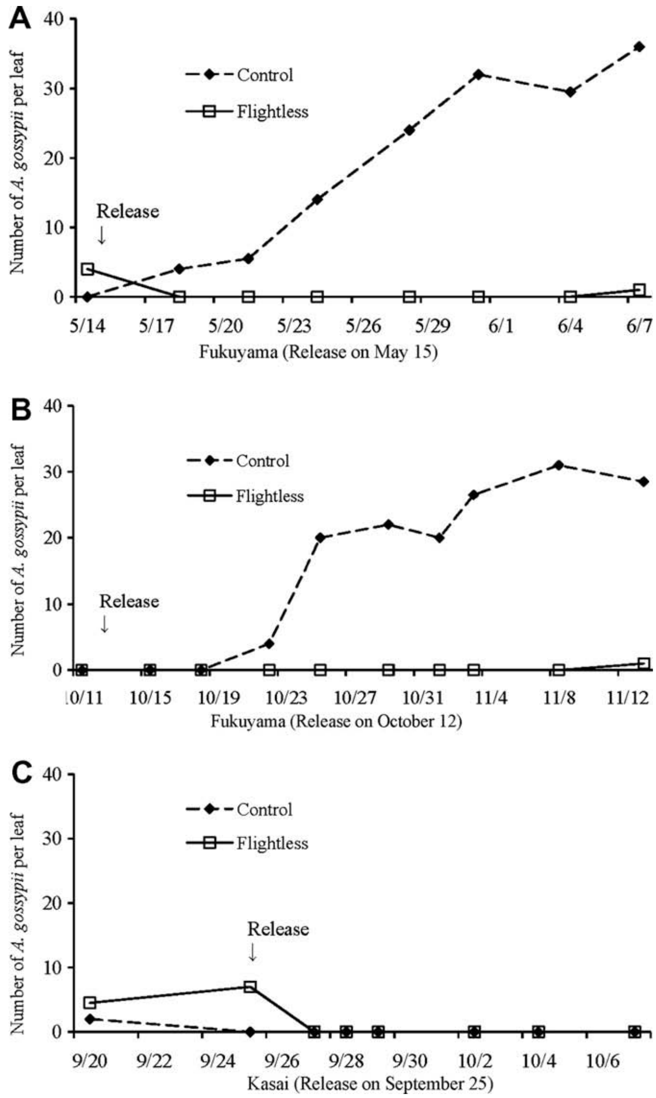

Fig. 3. Comparison of the number of *A. gossypii* (median) per leaf on plants between plots which released the flightless and control strains in open field with grown eggplants for (A) the research on 14 May–10 June in Fukuyama, for (B) the research on 12 October–15 November in Fukuyama, and for (C) the research on 20 September–10 October in Kasai.

shorter, the density of *A. gossypii* approached zero after three days from the release. These results suggest that the flightless strain of *H. axyridis* is effective against *A. gossypii* as a biological control agent in open fields with cultivated eggplants.

Inbreeding (the mating of close relatives) increases the frequency of homozygotes (Hopper et al., 1993; Hoy, 1985; Falconer and Mackay, 1996). Artificial selection for reduced flight ability in a population of *H. axyridis* can lead to the decline of survival and reproductive characteristics by exposing deleterious recessive alleles. Tourniaire et al. (2000b) suggested that characteristics with fitness did not differ between a flightless strain and control one in the 15th generation of *H. axyridis*. On the other hand, in the flightless strain which was used in our study, the survival rate of larvae and fecundity were lower than those of the control strain significantly in 28th generation (Seko and Miura, unpublished). In our mark-release experiment, few eggs and larvae of *H. axyridis* were observed in plots with released adults of the flightless strain. This might reflect the inbreeding effect for reproductive characteristics in the flightless strain. The management of genetics of the flightless strain for avoiding inbreeding depression, e.g., hybridization before release between iso-genic lines (Hopper et al., 1993), should be performed to control aphid populations sufficiently.

Introduced species of ladybirds might adversely affect the abundance of rare species of prey and native species of ladybird (Dixon, 2000; Koch, 2003). *H. axyridis* is recognized as invasive alien species in Europe and America (Brown et al., 2008; Koch and Galvan, 2008). van Lenteren et al. (2008) discussed that *H. axyridis* should not have been used as a biological control agent in Northwest Europe because the risks of its release are manifold. Though *H. axyridis* is a native species in Asian range, the release of large numbers of flightless *H. axyridis* might affect the abundance of wild *H. axyridis* and the frequency of individuals with genes for flightlessness in a population. An environmental risk assessment for the flightless strain of *H. axyridis* should be examined in both laboratory and field.

In open fields, indigenous predators play a role in suppressing aphids (Van Den Berg et al., 1997; Ito et al., 2005; Wells et al., 2001). In our study, potential natural enemies of aphids such as oligota beetles (Scheller, 1984; Sunderland and Vickerman, 1980), spiders (Zhang, 1992) and frogs (Hirai, 2007) were observed in Kasai. The presence of these indigenous predators might explain the suppression of *A. gossypii* in the control treatment before the release of *H. axyridis* in Kasai (Fig. 3). On the other hand, though larvae of the lady beetle *Coccinella septempunctata* were observed in the flightless and control treatments from late October to early November in Fukuyama, it was not enough to suppress the number of *A. gossypii* in the control treatment (Fig. 3). Indigenous predators of *A. gossypii* tend to increase rapidly from late May and disappear in early November in open fields with cultivated Okura in western Japan (Shimoyakawa, 2002). The release of the flightless strain of *H. axyridis* can be effective to control *A. gossypii* in environmental conditions or seasons that do not suit indigenous predators.

Augmentative biological control is constrained by the cost of producing large numbers of ladybirds (Dixon, 2000) and by the lower profit per site with vegetables grown in open fields compared with those grown in greenhouses (Ohno, 2003). It is possible to reduce the cost of biological treatments with *H. axyridis* by releasing young larvae of the flightless strain because mass rearing to the more voracious fourth instar larvae and adults would be unnecessary. However, its effectiveness might not be maintained over longer periods of time because larvae of *H. axyridis* tend to disperse quickly when an aphid density is low (Ferran et al., 1996).

Table 2
Comparison of flight distance and proportion of flightless adults between the flightless and control strains

Flight character	Strain	20th Generation				30th Generation			
		n	Male	*n*	Female	*n*	Male	*n*	Female
Flight distance (m)	Control	30	431.1 (2.7–2059.8)	30	395.6 (1.3–2691.0)	30	54.1 (0–2137.9)	30	173.5 (3.6–1868.5)
	Flightless	48	6.5 (0–958.9)***	48	5.65 (0–998.8)***	48	0 (0–0.9)***	48	0 (0–0.9)***
Proportion of flightless adults	Control	31	0.16	33	0.09	30	0.27	30	0.27
	Flightless	45	0.69***	46	0.74***	30	1.00***	30	1.00***

Flight distance (median(range)): ***$P < 0.001$, Mann–Whitney U test; proportion of flightless adults: ***$P < 0.001$, Fisher's exact test.

Further experiments considering the release development stage and the release density of the flightless strain are needed to establish a cost-effective biological control strategy in open fields with cultivated eggplants.

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