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Influence of aphid–host plant pairs on the survivorship and development of the multicolored Asian ladybird beetle: implications for the management of vegetation in rural landscapes

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Abstract Although the value of noncrop vegetation for biological control has been extensively studied in agricultural landscapes, there are few reports on how it functions mechanistically. When focusing on the pest control function provided by noncrop vegetation, tritrophic interactions among a predatory natural enemy, its prey, and the prey's host plant need to be examined. In Japan, the multicolored Asian ladybird beetle, *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae), an aphidophage, serves as a natural pest control agent in agricultural production, although the species' introduction into Europe and North America for pest control has had a negative impact on native ecosystems. In the present study, 33 aphid–plant pairs from an agricultural landscape in the eastern Kanto region of Japan were examined experimentally for initial larval survivorship and development of *H. axyridis*. Significant differences were found among plant–aphid pairs with regard to these parameters. In addition, the larval survivorship of *H. axyridis* was not consistently determined by host plant or aphid species alone but was context-dependently influenced by the aphid–plant combination. Some alien host plants showed positive effects on the ladybird beetle. Others, however, served as hosts for unsuitable prey species, such as the competitive alien plants *Solidago canadensis* L. and *Robinia pseudoacacia* L., which are the host plants of *Uroleucon nigrotuberculatum* (Olive) and *Aphis craccivora* Koch, respectively. These findings suggest that various noncrop plants could be

managed to promote ladybird beetle populations in rural landscapes.

Keywords Natural enemy · Noncrop vegetation · Tritrophic interaction · *Harmonia axyridis* · Aphid

Introduction

Costanza et al. (1997) estimated the ecosystem service of biological control to be worth at least US \$417 billion per year at a worldwide scale. Bianchi et al. (2006) noted that noncrop vegetation in agricultural landscapes serves as refugia for natural enemies, thus preventing pest outbreaks in farmland in large proportions of various ecosystems. Few studies, however, have reported on how this function of noncrop vegetation is maintained. Gaining a better understanding of these mechanisms could lead to ecologically and economically optimized management of agricultural landscapes. When studying the pest control function provided by noncrop vegetation, it is important to examine tritrophic interactions among a predatory natural enemy, its prey, and the prey's host plant (Agrawal 2000).

Many ladybird beetles are polyphagous, and some of them feed on pest aphids, functioning as a natural pest control agent (Hodek and Honěk 1996). The abundance of ladybird beetles is affected by prey aphid quality as well as quantity, because some pairs of aphids and their host plants are toxic for ladybird beetles (e.g., Hokusima and Kamei 1970; Pasteels 2007). With some exceptions, most aphid species are monophagous or oligophagous (Eastop 1986), which means that a prey aphid population depends strongly on its host plant's distribution. In recent years, noncrop vegetation in agricultural landscapes has been reduced or simplified due to land-use intensification or abandonment of land management (Stoate et al. 2001). In addition, the replacement of noncrop vegetation is sometimes triggered by the invasion of competitive alien plants (e.g., Mabey 1998;

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Blaustein 2001). We still understand little about how these influences on noncrop vegetation affect the population of beneficial ladybird beetles through changes in prey aphid quality and quantity.

The multicolored Asian ladybird beetle, *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae), is native to eastern Asia and is a representative species of ladybird beetle in warm temperate regions of Japan (Hukusima and Kamei 1970; Koch 2003). The species was intentionally introduced into Europe and North America for pest control, which has unfortunately resulted in negative impacts on native ecosystems (Koch 2003; Brown et al. 2008). In warm temperate areas of Japan, this native beetle functions as a natural pest control agent in agricultural production without any noticeable negative impact. In this climate, evergreen forests composed mainly of Fagaceae and Lauraceae develop as the climax community (Fukushima 2005). However, a complex mosaic of various vegetation types is arranged in the landscape depending on the pattern of natural resource use during the country's long history of agriculture (Washitani 2003). In such an agricultural landscape, where land-use intensification or land abandonment around farmland has been modest, candidate host plants for aphids are diverse and include various noncrop vegetation patches such as well-developed evergreen forest, early- and mid-successional deciduous forest, ornamental hedges, and herbaceous vegetation. A variety of aphid species is also expected to inhabit such an agricultural landscape, resulting in numerous combinations of prey aphids and host plants in the region. Although some intensive studies have examined the effects of selected aphid–plant pairs on *H. axyridis* (Hukusima and Kamei 1970; Okamoto and Sato 1973; Takizawa et al. 2000; Ueno 2003; Fukunaga and Akimoto 2007), an extensive survey has not been performed. A better understanding of how prey quality is influenced by both the aphid species itself and its host plant throughout the seasons is essential for the effective management of noncrop vegetation for the reproduction of *H. axyridis*.

In the present study, we examined the effects of 33 aphid–plant pairs from an agricultural landscape in the eastern Kanto region of Japan on the initial larval survivorship and development of *H. axyridis*. In addition to survival, the developmental rate of *H. axyridis* larvae is important, because a longer larval stage may result in prey shortages, predation by other animals, and even cannibalism within the species (Osawa 1992; De Clercq et al. 2003). In order to investigate such a wide variety of candidate aphid–plant pairs, we adopted an experimental procedure in which observations were limited to 5 days after hatching. This approach allowed us to screen out less-suitable aphid–plant pairs for younger ladybird beetle larvae. We investigated three questions. First, are there significant differences among aphid–plant pairs with regard to survivorship of *H. axyridis* larvae tested during the same experimental period? Second, if such significant differences in the survivorship exist, are they due to the aphid species, the plant species,

or both? Finally, are there significant differences among aphid–plant pairs with regard to the larval developmental rate of *H. axyridis*? Based on the answers to these questions, we discuss some implications for the management of vegetation in agricultural landscapes from the perspective of using *H. axyridis* as a natural pest control agent.

Materials and methods

Collecting and breeding of *H. axyridis*

Adults of *H. axyridis* were collected from *Vicia angustifolia* L., *Rhaphiolepis indica* (L.) Lindl., *Sorghum* sp., and *Salix integra* Thunb. between June 2008 and May 2009 in the experimental field of the National Institute for Agro-Environmental Sciences, Tsukuba, Japan (36°1'30"N, 140°6'57"E). They were held individually in transparent plastic containers (5-cm diameter × 3-cm height) without sex identification. A piece of folded filter paper (20 cm²; Advantec No. 1, Toyo Roshi Kaisha, Tokyo, Japan) was moistened with a drop of distilled water and placed in each container for oviposition. As a food source lyophilized drone pupa powder (ca. 15 mg; Agrisect, Tokyo, Japan) was provided, modifying an established artificial rearing method (Okada and Matsuka 1973). The containers were kept in a dark chamber (SLI-400, Eyela, Tokyo, Japan) at 24°C and checked daily for oviposition. The procedure for rearing consecutive generations of *H. axyridis* in continuous darkness was described by Nijima (1991). When eggs were laid on the filter paper or the walls of the container, the beetle was transferred to a new container with a piece of folded, moistened filter paper and lyophilized drone pupa powder for the next oviposition. The eggs were kept in the chamber until hatching (ca. 3 days). The first-instar larvae were placed individually on a piece of circular filter paper (5-cm diameter; Advantec No. 1) in a new container as they started dispersing from the egg clusters (ca. 1 day after hatching). Female beetles that had ceased laying eggs were fed three aphid species, *Indomegoura indica* (van der Goot), *Greenidea nigra* (Maki), and *Chaitophorus saliapteris* Shinji, to stimulate oviposition.

Aphids

Aphids to be tested were added to the containers with first-instar larvae of *H. axyridis*. Aphids were not identified as larvae or adults. They were brushed off carefully from plant material with a paintbrush either immediately after collection or after being kept alive with the host plant in the chamber for 1 or 2 days. The containers were kept in the dark chamber at 24°C for 5 days and checked every 24 h to ascertain whether larvae were alive and the larval instar. In this study, instar composition served as an index of development because it reflects the duration of each instar. An excess of aphids

was provided daily, if needed, in order to avoid a shortage of prey.

We tested 26 species of aphids and 29 species of host plants (33 aphid–host plant pairs in total; Table 1). Between 30 and 34 *H. axyridis* larvae were used per treatment. Every aphid–plant pair found in and around the experimental field was evaluated for the following three criteria: (1) the host plant was distributed in typical noncrop vegetation in the region, including secondary forests composed of evergreen or deciduous trees, forest margins, planted zone such as hedge, and forb-, grass-, or reed-dominated herbaceous vegetation along roadsides, riversides and in open sites; (2) the aphid colony was exposed (i.e., not in galls or formed under bark); and (3) the size of the colony appeared to be sufficient to feed about 30 *H. axyridis* larvae for 5 days. All pairs that satisfied these criteria were used for the experiment. Each aphid–plant pair was collected from the same location.

Statistical analyses

To minimize the confounding effect that may have been caused by the adaptation of *H. axyridis* to a seasonally

changing environment, statistical tests among aphid–plant pairs for the survivorship and developmental rate of *H. axyridis* larvae were conducted within the same experimental periods: period 1, June 2008; period 2, July 2008; period 3, September 2008; period 4, October 2008; period 5, early May 2009; period 6, late May 2009. A survival curve was estimated for each of the 33 experiments to evaluate how each aphid–plant pair affected the survivorship of *H. axyridis* larvae using the Kaplan–Meier method (Kaplan and Meier 1958). Significant differences in survivorship were tested among all pairs in the same experimental period using a log-rank test. To evaluate aphid–plant pairs from a larval development perspective, rank sums of larval instar composition (frequency of first, second, third, and fourth instars) at day 5 were compared among pairs in the same experimental period using the Wilcoxon rank-sum test. For three aphid–plant pairs (exp. 1, 16, 21), no *H. axyridis* larvae survived at day 5, and these pairs were eliminated from the comparisons of instar composition. *P* values ($P < 0.05$) were corrected for multiple tests within the same experimental period using false discovery rate control (Verhoeven et al. 2005) for both the log rank tests on survivorship and the Wilcoxon rank-sum tests on instar

Table 1 Pairs of host plant and its parasitizing aphid tested for suitability as prey for the ladybird beetle *Harmonia axyridis*

Exp. no.	Host plant	Family	Habitat ^b	Aphid	Date ^c
1	<i>Aeschynomene indica</i> L.	Fabaceae	A	<i>Aphis craccivora</i> Koch	8 September 2008
2	<i>Bidens pilosa</i> L. ^a	Asteraceae	C	<i>Aphis spiraeicola</i> Patch	7 October 2008
3	<i>Brassica juncea</i> (L.) Czern. ^a	Brassicaceae	E	<i>Brevicoryne brassicae</i> (Linnaeus)	23 May 2009
4	<i>Castanea crenata</i> Sieb. et Zucc.	Fagaceae	D, F	<i>Lachnus tropicalis</i> (van der Goot)	6 October 2008
5	<i>Castanopsis cuspidata</i> Schottky	Fagaceae	D, F	<i>Eutrichosiphum pasaniae</i> (Okajima)	6 July 2008
6	<i>C. cuspidata</i>	Fagaceae	D, F	<i>Eutrichosiphum shiicola</i> Takahashi	6 October 2008
7	<i>C. cuspidata</i>	Fagaceae	D, F	<i>L. tropicalis</i>	6 July 2008
8	<i>Celastrus orbiculatus</i> Thunb.	Celastraceae	D	<i>Toxoptera citricida</i> (Kirkaldy)	5 July 2008
9	<i>Eleusine indica</i> (L.) Gaertn.	Poaceae	C	<i>Hysteroneura setariae</i> (Thomas)	10 September 2008
10	<i>Euscaphis japonica</i> (Thunb.) Kanitz	Staphyleaceae	D	<i>Indomegoura indica</i> (van der Goot)	20 June 2008
11	<i>Hemistepta lyrata</i> Bunge	Asteraceae	C	<i>Aphis solanella</i> Theobald	24 May 2009
12	<i>H. lyrata</i>	Asteraceae	C	<i>Uroleucon cephalonopli</i> (Takahashi)	16 June 2008
13	<i>Humulus japonicus</i> Sieb. et Zucc.	Cannabaceae	B, D, E	<i>Phorodon humuli japonensis</i> Takahashi	7 October 2008
14	<i>Lithocarpus edulis</i> Nakai	Fagaceae	D, F	<i>Greenidea nigra</i> (Maki)	9 September 2008
15	<i>Metaplexis japonica</i> Makino	Asclepiadaceae	D	<i>Aphis nerii</i> Boyer de Fonscolombe	10 September 2008
16	<i>Nerium indicum</i> Mill.	Apocynaceae	F	<i>A. nerii</i>	8 September 2008
17	<i>Oenothera biennis</i> L. ^a	Onagraceae	B, C	<i>Aphis oenotherae</i> Oestlund	24 May 2009
18	<i>Phragmites australis</i> (Cav.) Steud.	Poaceae	A	<i>Hyalopterus pruni</i> (Geoffroy)	3 July 2008
19	<i>Picris hieracioides</i> L.	Asteraceae	C	<i>Uroleucon picridis</i> (Fabricius)	10 May 2009
20	<i>Pyracantha coccinea</i> M. Roem. ^a	Rosaceae	F	<i>T. citricida</i>	9 September 2008
21	<i>Robinia pseudoacacia</i> L. ^a	Fabaceae	D, E, F	<i>A. craccivora</i>	8 June 2008
22	<i>Rosa multiflora</i> Thunb.	Rosaceae	B	<i>Sitobion ibarae</i> (Matsumura)	7 May 2009
23	<i>Rosa wichuraiana</i> Crép.	Rosaceae	E	<i>S. ibarae</i>	4 May 2009
24	<i>Rumex crispus</i> L. ^a	Polygonaceae	B, C	<i>Aphis rumicis</i> Linnaeus	5 May 2009
25	<i>Salix integra</i> Thunb.	Salicaceae	A, E	<i>Chaitophorus saliapteris</i> Shinji	8 September 2008
26	<i>Smilax china</i> L.	Smilacaceae	D	<i>Impatiens impatiens</i> (Shinji)	4 July 2008
27	<i>Solidago canadensis</i> L. ^a	Asteraceae	B, C	<i>Uroleucon nigrotuberculatum</i> (Olive)	16 June 2008
28	<i>Sonchus asper</i> (L.) Hill ^a	Asteraceae	C	<i>Uroleucon sonchi</i> (Linnaeus)	5 May 2009
29	<i>Sonchus oleraceus</i> L.	Asteraceae	C	<i>Uroleucon formosanum</i> (Takahashi)	11 September 2008
30	<i>Spiraea thunbergii</i> Sieb. ex Blume	Rosaceae	F	<i>A. spiraeicola</i>	7 September 2008
31	<i>Typha latifolia</i> L.	Typhaceae	A	<i>Schizaphis scirpi</i> (Passerini)	4 July 2008
32	<i>Vicia angustifolia</i> L.	Fabaceae	C	<i>A. craccivora</i>	4 May 2009
33	<i>V. angustifolia</i>	Fabaceae	C	<i>Megoura crassicauda</i> Mordvilko	4 May 2009

^aPlants that have been intentionally or unintentionally introduced into Japan since 1867

^bA wetland, B abandoned field, C roadside, D forest or forest margin, E riverside, F planted zone

^cDate when feeding experiments were started

composition. All statistical analyses were performed using the R version 2.8.1 (R Development Core Team 2008).

Results

Differences in the survival rates of *H. axyridis* larvae

The survivorship of *H. axyridis* larvae varied among aphid–plant pairs (Fig. 1). Significant differences in

survival rates were observed in all experimental periods except period 4, in which almost all larvae survived for 5 days irrespective of the aphid–plant pairs.

The two experiments using *Aphis craccivora* Koch (exp. 32) and *Megoura crassicauda* Mordvilko (exp. 33) resulted in significantly different survival rates for *H. axyridis* larvae (Fig. 1e), in spite of the fact that these aphid species had been parasitizing a common host plant, *V. angustifolia*. This result demonstrates that the host plant does not solely determine the prey aphid

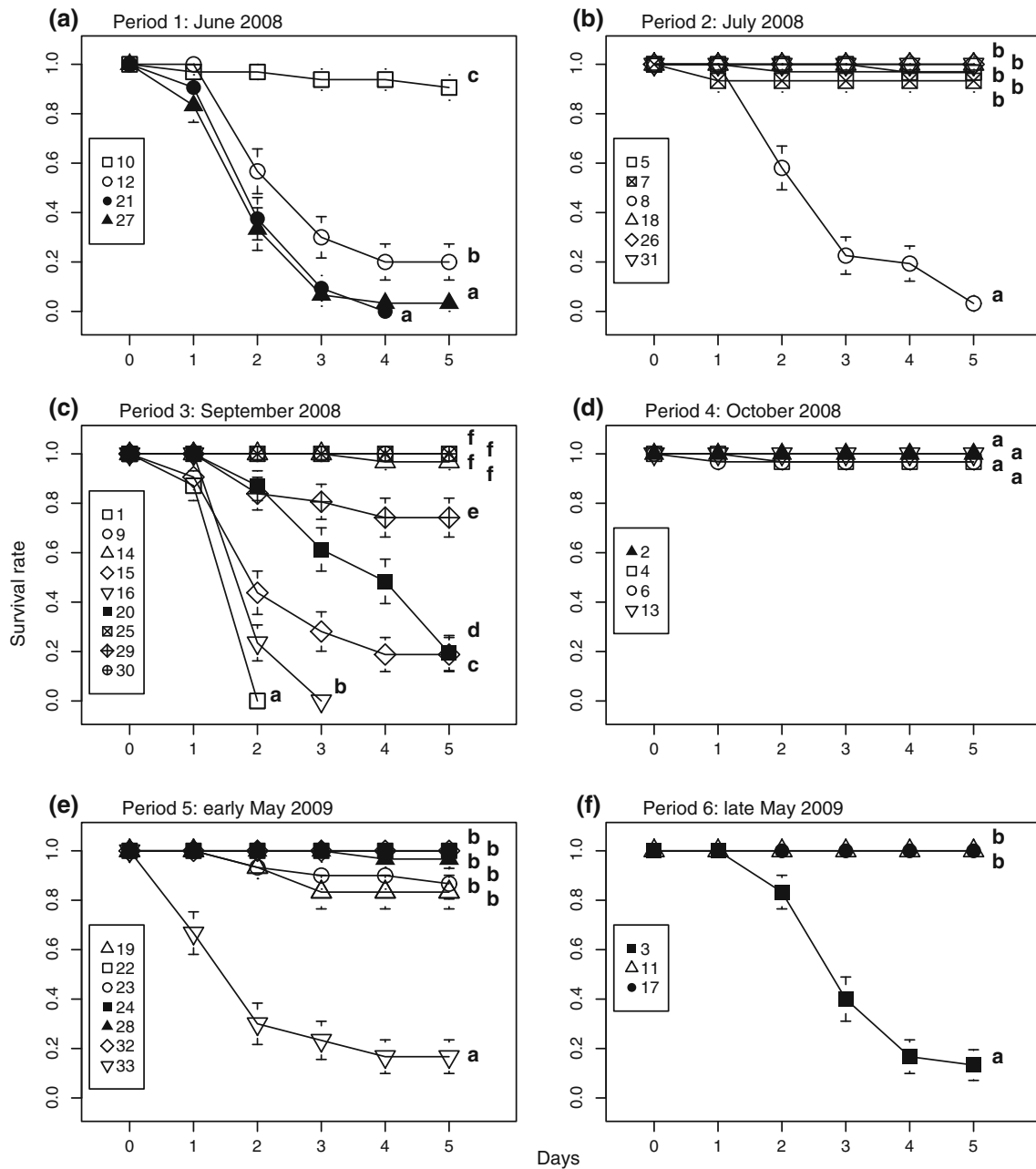


Fig. 1 Estimated 5-day survival curves of *Harmonia axyridis* larvae feeding on 33 aphid–plant pairs in six experimental periods. Numbers next to symbols correspond to the experiment numbers (for details, see Table 1). Survival curves within each experimental

period with the same letters are not significantly different (log-rank test corrected by false discovery rate control, $P > 0.05$). A closed symbol means that the host plant is an alien species

quality for the survival of *H. axyridis* larvae. Although between-period comparisons could not be analyzed in the present study, contrasting results between two aphid species (exp. 12, period 1; exp. 11, period 6) on a common host–plant species seem to support this interpretation. Moreover, two experiments with a common aphid, *Aphis nerii* Boyer de Fonscolombe, parasitizing the host–plant species *Metaplexis japonica* Makino (exp. 15) and *Nerium indicum* Mill. (exp. 16) resulted in significantly different survival rates for *H. axyridis* larvae (Fig. 1c). This observation indicates that the prey aphid species alone does not necessarily determine the larval survivorship of *H. axyridis*. Although in different experimental periods, survival rates in three experiments with a common aphid, *A. craccivora* (exp. 1, 21, 32), seem to support the aphid-independence of larval survivorship. Therefore, the survival of *H. axyridis* larvae is not consistently determined by host–plant or aphid species alone, but is context-dependently influenced by the aphid–plant combination.

Differences in the larval instar compositions of *H. axyridis*

The larval instar compositions at day 5 were significantly different in five of the six experimental periods (Fig. 2). For example, larvae fed on *Uroleucon picridis* (Fabricius) parasitizing *Picris hieracioides* L. (exp. 19) and *Uroleucon sonchi* (L.) parasitizing *Sonchus asper* (L.) Hill (exp. 28) predominated at the second instar at day 5, whereas those fed on *A. craccivora* parasitizing *V. angustifolia* (exp. 32) were mainly fourth-instar larvae (Fig. 2e).

Discussion

Numerous species of aphids that parasitize a variety of seasonally changing host–plant species serve as potential food sources for the young larvae of *H. axyridis* (Fig. 1). Because the majority of aphid species emerge and form colonies during limited periods, to maintain reproductive populations of this beneficial insect it is important to have a variety of noncrop species around farmland. Many studies of the pest control functions of noncrop vegetation have shown that landscape composition does matter (for a review, see Bianchi et al. 2006), but the underlying mechanisms have not been clarified. Furthermore, many landscape ecology studies on biocontrol issues have treated presumably heterogeneous noncrop vegetation as homogeneous areas (e.g., Marino and Landis 1996; Roschewitz et al. 2005). By examining numerous tritrophic interactions among ladybird beetles, aphids, and their host plants, the present study aimed to clarify the mechanisms underlying the relationship between the structure of agricultural landscapes and biocontrol functions.

The results of our investigation of 33 aphid–plant pairs suggest that the survival rates of *H. axyridis* larvae were not related to the origin (native or alien) of the host plants. For example, experiments with the invasive alien tree *Robinia pseudoacacia* L. (exp. 21) and invasive alien perennial *Solidago canadensis* L. (exp. 27) resulted in low survivorship of *H. axyridis* larvae in period 1 (Fig. 1a), but the experiment with the invasive alien biennial *Oenothera biennis* L. (exp. 17) resulted in the highest survivorship and fastest development of the larvae in period 6 (Figs. 1e, 2e). Based on these inconsistent results, it is not possible to generalize that alien plant invasion always leads to a weakened pest control function in agricultural landscapes. At the same time, the invasion of alien plants poses a wide variety of environmental risks, so we cannot simply promote the aphid–plant pairs that enhanced the initial growth of the ladybird beetle larvae, as discussed in more detail below.

The quality of aphids as prey for *H. axyridis* depends on both the aphids and their host plants. Aphid species alone do not determine it, as indicated by the case of *A. nerii* parasitizing different host–plant species, *M. japonica* and *N. indicum* (exp. 15, 16), and that of *A. craccivora* parasitizing *Aeschynomene indica* L., *R. pseudoacacia*, and *V. angustifolia* (exp. 1, 21, 32). Our observations were consistent with those of Hukusima and Kamei (1970), who reported that adults and larvae of *H. axyridis* did not survive feeding on *A. craccivora* on seven host plants including *R. pseudoacacia*, whereas they could feed on *A. craccivora* parasitizing *V. angustifolia*. Likewise, plant species alone does not determine the quality of aphids as prey, as demonstrated by the case of *A. craccivora* and *M. crassicauda* parasitizing *V. angustifolia* (exp. 32, 33) and that of *Aphis solanella* Theobald and *Uroleucon cephalonopli* (Takahashi) parasitizing *Hemistepta lyrata* Bunge (exp. 11, 12). The toxicity of aphids derived from the host plant was reviewed by Pasteels (2007). For example, concentrations of toxins in *Brevicoryne brassicae* (Linnaeus) proved to be much higher than those in the host plants *Brassica napus* L. and *Sinapis alba* L., probably because the aphids store toxins in their bodies for chemical defense (Francis et al. 2001). In contrast, concentrations of cardenolides in *A. nerii* were less than those in the host plant *Nerium oleander* L. because portions of the highly toxic compounds are excreted with honeydew (Rothschild et al. 1973; Malcolm 1990). These mechanisms for adjusting the concentration of toxic compounds in their bodies might play a role in the varying toxicity levels among aphid species that parasitize identical host–plant species.

The present study aimed to provide an overview of the effect of aphid–plant pairs on *H. axyridis* survivorship and developmental rate and was designed to examine as many pairs as possible. The beetle larvae were observed only for 5 days (see Appendix), because feeding fourth instars until pupation requires numerous aphids, which would have drastically narrowed the number of pairs available for the experiments. Any

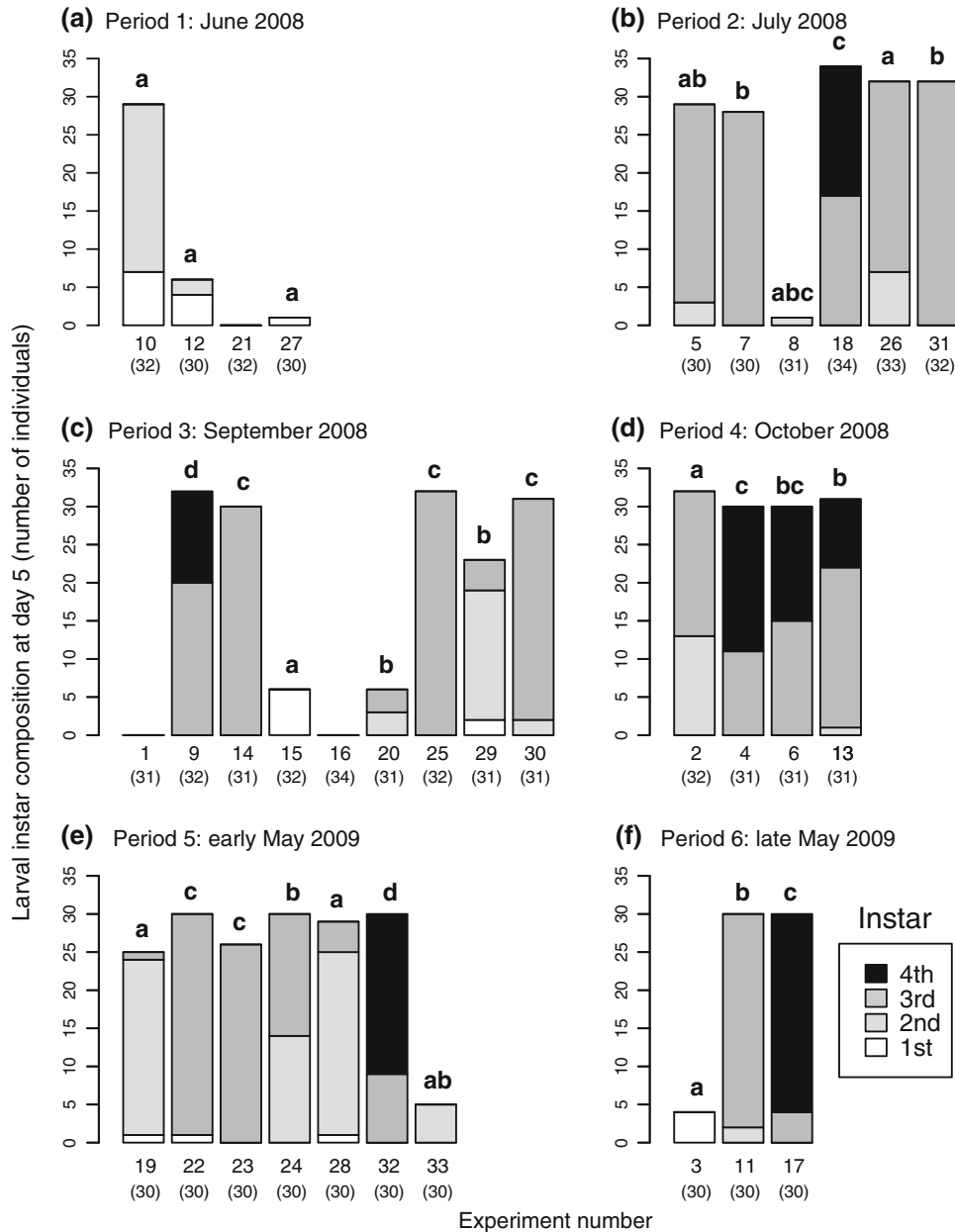


Fig. 2 Day 5 instar composition of surviving *H. axyridis* larvae feeding on 33 aphid–plant pairs in six experimental periods. Bars within each experimental period with the same letters are not significantly different (Wilcoxon rank-sum test corrected by false

discovery rate control, $P > 0.05$). Sample size is given in parentheses below each experiment number (for details, see Table 1)

unfavorable influences of these aphid–plant pairs that are detectable only after emergence, such as sterility or shorter life span, would thus not have been detected in this study. Therefore, it is inappropriate to conclude that aphids from the pairs resulting in high survival rate and rapid development in our experiments are “suitable” prey. To establish whether such aphids are in fact suitable prey, the aphid–plant pairs must be tested on *H. axyridis* throughout the beetle’s entire life cycle.

The highly positive effect of the alien *Aphis oenotherae* Oestlund parasitizing the alien *O. biennis* on

H. axyridis survivorship and development (exp. 17; Figs. 1f, 2f) raises questions regarding the responses of native predators to exotic prey and host plants. It is a controversial issue whether this aphid–plant pair is desirable in vegetation around farmland. The main concern is how *H. axyridis* has adapted to *A. oenotherae* parasitizing *O. biennis*. If the traits of *A. oenotherae* as prey are well within the feeding capacity of *H. axyridis*, then it would serve as an appropriate prey without any serious problems. However, if the prey traits were within the feeding capacity of only some individuals in the

H. axyridis population, then invasion of this pair into the landscape would place a selection pressure on the predator (Carlsson et al. 2009). In that case, the presence of *O. biennis* would not necessarily favor *H. axyridis* in the long term due to its potential effect on the genetic diversity of *H. axyridis*. At the same time, however, this example demonstrates excellent adaptation by *H. axyridis* to an alien aphid species. Such adaptability could be a reason why this ladybird beetle became firmly established in Europe and the United States after its introduction.

It would be reasonable to regard the aphid–plant pairs resulting in low survival rates as “unsuitable” prey for *H. axyridis*, because any lethal effect on the early larval stages will decrease the number of emerging individuals. With regard to the management of vegetation in rural landscapes, we discuss here some host plants that resulted in low survival rates of *H. axyridis*. The importance of each species in a management plan should be determined by its potential to dominate a vast area of noncrop vegetation. *Solidago canadensis* and *R. pseudoacacia* are more likely to dominate the landscape than other host plants from the pairs resulting in low survival rate for *H. axyridis*: *Celastrus orbiculatus* Thunb., *Pyracantha coccinea* M. Roem., *M. japonica*, *N. indicum*, *A. indica* L., and *Brassica juncea* (L.) Czern. Although *H. lyrata* and *V. angustifolia* resulted in low survival rates with some aphids, when paired with *A. solanella* and *A. craccivora*, respectively, the survival rates of *H. axyridis* were high. *Solidago canadensis* and *R. pseudoacacia* were introduced intentionally into Japan from North America in the late nineteenth century and have proven to be highly invasive. The former spread vigorously after World War II and has become one of the most common alien invasive plants in Japan

(Hattori 2002). The latter was planted in Japan as an early colonizer of disturbed lands, a roadside tree, and a nectar source for honeybees, and the species frequently spreads from planted areas (Maekawa 2002). In our experiments with *S. canadensis* and *R. pseudoacacia*, the survival rate was almost nil by day 5. Therefore, the dominance of these two plants, which is occasionally observed in rural landscapes in Japan, could decrease the value of noncrop vegetation as a prey source for *H. axyridis*.

To more fully evaluate the quality of vegetation around farmland as reproduction sites for predators and their prey, other principal species of the guild also should be investigated. For example, in the Kanto region of Japan, other ladybird beetle species, such as *Coccinella septempunctata* Linnaeus and *Propylaea japonica* (Thunberg), serve as natural pest control agents and could be examined using the methods of the present study. Investigating the relationships among numerous ladybird beetles, aphids, and host plants would help to elucidate the mechanisms underlying the abundance of ladybird species in different types of vegetation. By devising appropriate plans that account for aphid species and their host plants, landscapes can be managed to produce greater numbers of ladybird beetles, which would decrease crop damage caused by aphids.

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Appendix

See Table 2

Table 2 The numbers of 1st, 2nd, 3rd, and 4th instars, and dead larvae of *Harmonia axyridis* fed on aphids on a variety of plant species for 5 consecutive days

Exp. no.	N	(Numbers of 1st, 2nd, 3rd, and 4th instars, and dead larvae)				
		Day 1	Day 2	Day 3	Day 4	Day 5
1	31	(27, 0, 0, 0, 4)	(0, 0, 0, 0, 31)	(0, 0, 0, 0, 31)	(0, 0, 0, 0, 31)	(0, 0, 0, 0, 31)
2	32	(32, 0, 0, 0, 0)	(29, 3, 0, 0, 0)	(4, 28, 0, 0, 0)	(0, 31, 1, 0, 0)	(0, 13, 19, 0, 0)
3	30	(30, 0, 0, 0, 0)	(25, 0, 0, 0, 5)	(12, 0, 0, 0, 18)	(5, 0, 0, 0, 25)	(4, 0, 0, 0, 26)
4	31	(30, 1, 0, 0, 0)	(1, 29, 0, 0, 1)	(0, 14, 16, 0, 1)	(0, 2, 28, 0, 1)	(0, 0, 11, 19, 1)
5	30	(30, 0, 0, 0, 0)	(2, 28, 0, 0, 0)	(1, 29, 0, 0, 0)	(0, 23, 6, 0, 1)	(0, 3, 26, 0, 1)
6	31	(30, 0, 0, 0, 1)	(5, 25, 0, 0, 1)	(0, 16, 14, 0, 1)	(0, 2, 28, 0, 1)	(0, 0, 15, 15, 1)
7	30	(28, 0, 0, 0, 2)	(12, 16, 0, 0, 2)	(0, 28, 0, 0, 2)	(0, 20, 8, 0, 2)	(0, 0, 28, 0, 2)
8	31	(30, 1, 0, 0, 0)	(5, 13, 0, 0, 13)	(0, 7, 0, 0, 24)	(0, 5, 1, 0, 25)	(0, 1, 0, 0, 30)
9	32	(32, 0, 0, 0, 0)	(5, 27, 0, 0, 0)	(0, 29, 3, 0, 0)	(0, 0, 32, 0, 0)	(0, 0, 20, 12, 0)
10	32	(31, 0, 0, 0, 1)	(30, 1, 0, 0, 1)	(23, 7, 0, 0, 2)	(11, 19, 0, 0, 2)	(7, 22, 0, 0, 3)
11	30	(30, 0, 0, 0, 0)	(21, 9, 0, 0, 0)	(2, 28, 0, 0, 0)	(0, 22, 8, 0, 0)	(0, 2, 28, 0, 0)
12	30	(30, 0, 0, 0, 0)	(17, 0, 0, 0, 13)	(9, 0, 0, 0, 21)	(6, 0, 0, 0, 24)	(4, 2, 0, 0, 24)
13	31	(31, 0, 0, 0, 0)	(2, 29, 0, 0, 0)	(0, 22, 9, 0, 0)	(0, 2, 29, 0, 0)	(0, 1, 21, 9, 0)
14	31	(31, 0, 0, 0, 0)	(7, 24, 0, 0, 0)	(0, 31, 0, 0, 0)	(0, 2, 28, 0, 1)	(0, 0, 30, 0, 1)
15	32	(29, 0, 0, 0, 3)	(14, 0, 0, 0, 18)	(9, 0, 0, 0, 23)	(6, 0, 0, 0, 26)	(6, 0, 0, 0, 26)
16	34	(34, 0, 0, 0, 0)	(8, 0, 0, 0, 26)	(0, 0, 0, 0, 34)	(0, 0, 0, 0, 34)	(0, 0, 0, 0, 34)
17	30	(30, 0, 0, 0, 0)	(0, 30, 0, 0, 0)	(0, 9, 21, 0, 0)	(0, 0, 30, 0, 0)	(0, 0, 4, 26, 0)
18	34	(34, 0, 0, 0, 0)	(2, 32, 0, 0, 0)	(0, 20, 14, 0, 0)	(0, 0, 34, 0, 0)	(0, 0, 17, 17, 0)
19	30	(30, 0, 0, 0, 0)	(28, 0, 0, 0, 2)	(16, 9, 0, 0, 5)	(4, 21, 0, 0, 5)	(1, 23, 1, 0, 5)

Table 2 continued

Exp. no.	N	(Numbers of 1st, 2nd, 3rd, and 4th instars, and dead larvae)				
		Day 1	Day 2	Day 3	Day 4	Day 5
20	31	(31, 0, 0, 0, 0)	(15, 12, 0, 0, 4)	(2, 17, 0, 0, 12)	(0, 13, 2, 0, 16)	(0, 3, 3, 0, 25)
21	32	(29, 0, 0, 0, 3)	(12, 0, 0, 0, 20)	(3, 0, 0, 0, 29)	(0, 0, 0, 0, 32)	(0, 0, 0, 0, 32)
22	30	(30, 0, 0, 0, 0)	(5, 25, 0, 0, 0)	(1, 28, 1, 0, 0)	(1, 6, 23, 0, 0)	(1, 0, 29, 0, 0)
23	30	(30, 0, 0, 0, 0)	(4, 24, 0, 0, 2)	(1, 19, 7, 0, 3)	(1, 2, 24, 0, 3)	(0, 0, 26, 0, 4)
24	30	(30, 0, 0, 0, 0)	(23, 7, 0, 0, 0)	(10, 20, 0, 0, 0)	(0, 24, 6, 0, 0)	(0, 14, 16, 0, 0)
25	32	(32, 0, 0, 0, 0)	(2, 30, 0, 0, 0)	(0, 32, 0, 0, 0)	(0, 1, 31, 0, 0)	(0, 0, 32, 0, 0)
26	33	(32, 1, 0, 0, 0)	(5, 27, 0, 0, 1)	(3, 26, 3, 0, 1)	(0, 15, 17, 0, 1)	(0, 7, 25, 0, 1)
27	30	(25, 0, 0, 0, 5)	(10, 0, 0, 0, 20)	(2, 0, 0, 0, 28)	(1, 0, 0, 0, 29)	(1, 0, 0, 0, 29)
28	30	(30, 0, 0, 0, 0)	(30, 0, 0, 0, 0)	(19, 11, 0, 0, 0)	(6, 23, 0, 0, 1)	(1, 24, 4, 0, 1)
29	31	(31, 0, 0, 0, 0)	(26, 0, 0, 0, 5)	(15, 10, 0, 0, 6)	(4, 19, 0, 0, 8)	(2, 17, 4, 0, 8)
30	31	(31, 0, 0, 0, 0)	(22, 9, 0, 0, 0)	(0, 31, 0, 0, 0)	(0, 24, 7, 0, 0)	(0, 2, 29, 0, 0)
31	32	(31, 1, 0, 0, 0)	(1, 31, 0, 0, 0)	(0, 10, 22, 0, 0)	(0, 1, 31, 0, 0)	(0, 0, 32, 0, 0)
32	30	(30, 0, 0, 0, 0)	(3, 27, 0, 0, 0)	(0, 14, 16, 0, 0)	(0, 0, 30, 0, 0)	(0, 0, 9, 21, 0)
33	30	(20, 0, 0, 0, 10)	(9, 0, 0, 0, 21)	(6, 1, 0, 0, 23)	(1, 4, 0, 0, 25)	(0, 5, 0, 0, 25)

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