Effects of the goldenrod aphid, *Uroleucon nigrotuberculatum* on the reproductive performance of the predatory lady beetles, *Coccinella septempunctata bruckii* and *Propylea japonica* (Coleoptera: Coccinellidae)

(セイタカアワダチソウヒゲナガアブラムシがナナホシテントウおよびヒメカメノコテントウの繁殖特性に及ぼす影響)

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By

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APPROVAL

This dissertation entitled **"Effects of the goldenrod aphid, *Uroleucon nigrotuberculatum* on the reproductive performance of the predatory lady beetles, *Coccinella septempunctata bruckii* and *Propylea japonica* (Coleoptera: Coccinellidae)",** submitted by Adema Abdellahi Barry in partial fulfillment of the requirements for degree of Doctor philosophy in Agriculture (Entomology) has been approved on the recommendation of

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DEDICATIONS

I dedicate this work to my wife MARIATA SOW and my two beloved daughters FATIMATA BARRY and NAFISSATA BARRY.

To my parents for their prayers, eternal love and support.

To my brothers and sisters.

TABLE OF CONTENTES

LIST OF TABLES

LIST OF FIGURES

Abstract

The goldenrod aphid, *Uroleucon nigrotuberculatum* (Olive) is a specialist aphid of Solidago, *Solidago altissima* L., which was introduced into Japan in early 1990s from North America. Solidago also is an exotic invasive plant from North America, which has become widely spread around the arable land in abandoned fields and riparian areas in Japanese agroecosystem. Solidago and the goldenrod aphid have set a new habitat for the native aphidophagous lady beetles. Since the Solidago patches harbor heavy infestation of the goldenrod aphid in late spring and early summer, they attract the aphidophagous lady beetles emigrant from vetch, *Vicia angustifolia* (Koch) patches depleted of vetch aphid, *Megoura crassicauda* (Mordvilko. Therefore, any effects of the goldenrod aphid on the fitness of these lady beetles may alter the biological control service they are offering to the agroecosystem. Despite extensive literatures on the negative effects of introduced predators on their native guilds, there are still scanty of studies on the potential negative effects of novel exotic prey on the native predator performance. Thus, it is pertinent to study the impacts of this new prey aphid on the reproductive performance of two main aphidophagous lady beetles, *Coccinella septempunctata bruckii* Mulsant and *Propylea japonica* (Thunberg) (Coleoptera: Coccinellidae).

Studies were conducted to evaluate the effects of the goldenrod aphid on these lady beetles reproductive performance by addressing: 1) The effects of the goldenrod aphid on the development and survival of larvae of *C. septempunctata bruckii* and *P. japonica* in comparison with *M. crassicauda* as well as the fecundity of the adults on both prey aphids; 2) The defensive function of red droplets secreted from the cornicles of the goldenrod aphid and their deadly effects on the predator larvae; 3) The foraging behavior of these lady beetles on native prey aphid *M. crassicauda*

and the novel prey, the goldenrod aphid as well as their prey preference; and 4) Seasonal occurrence and population dynamics of these lady beetles in both Solidago patches and vetch patches during 2 years, and the ovarian dynamics of *C. septempunctata bruckii* in response to the prey aphids in different habitats.

I found that the goldenrod aphid delays the development of both lady beetles larvae, causes high larval mortality, and inhibits the reproduction of *C. septempunctata bruckii* adults but maintain them and supports reproduction of *P. japonica* at very low rate. The novel prey affects the larvae of the lady beetles physically by smearing them with sticky secretions which gum up the mouthparts, or prevent a successful molting. Larvae of *C. septempunctata bruckii* can learn to avoid preying on the goldenrod aphid, while larvae of *P. japonica* are not selective for prey aphids and thus prey randomly on the different aphids. From early spring, the lady beetles community on vetch patches consists mainly of reproductively active females of *C. septempunctata bruckii* with mature ovarioles. *Propylea japonica* occurs at late April but remains lower in number than *C. septempunctata bruckii*. In Solidago patches from early May, *C. septempunctata bruckii* adults and larvae are dominant, while *P. japonica* becomes more prevalent from mid-May. However, the *C. septempunctata bruckii* adults in Solidago patches were not reproductively active as their ovarioles were undeveloped.

These results revealed that the goldenrod aphid is not an essential prey aphid for *C. septempunctata bruckii* or it does not support the reproduction of *C. septempunctata bruckii* but its occurrence during prey aphid depletion in vetch patches serves to maintain the newly emerged adults even though, it hinders the adults to realize second generation in early summer. However, *P. japonica* can realize a generation with low reproductive success on Solidago patches. In conclusion, the

presence of Solidago and its specialist aphid, the goldenrod aphid has positive and negative impacts on the metapopulation of both *C. septempunctata bruckii* and *P. japonica* in the agroecosystem. Although the goldenrod aphid in Solidago patches maintains the lady beetles adults, it may act as an ecological trap for predatory lady beetles.

Chapter 1 General introduction

Anthropogenic activities mediated by the advanced technology have conveyed remarkable changes on the agroecosystems worldwide. The sophisticated means of transport have facilitated commercial exchanges of goods and animals, alien organism propagules as well as disease vectors between far apart geographical areas (Tatem et al. 2006; Hulme 2009; Banks et al. 2015). Many of these transported organisms have established, spread and invaded the new geographical areas. The invasive species are those species that were introduced intentionally or unintentionally into a new range and have become abundant and expanded their geographical range where they exert negative impacts on the new communities of the local ecosystem (Richardson et al. 2000; Colautti and MacIsaac 2004; Yamanaka et al. 2015), they jeopardize the biodiversity of ecosystem (Mcgeoch et al. 2010) and may hinder biocontrol of pest outbreak by decreasing the richness and disturbing the natural habitats of the native natural enemies fauna (Clavero and Garcia-Berthou 2005; Smith and Gardiner 2013).

Propagules of plants and herbivore insects have been introduced to several new geographical areas unintentionally by ships with soil or water ballasts, hitchhiking in different commodities and by accidental anthropogenic transport (Lockwood et al. 2007; Pratt and Center 2012). The introduced exotic plants can form new habitats (Harvey and Fortuna 2012) and set a suitable condition for its specialist herbivores from the place of origin to invade the new range once they get their way through (Memmott et al. 2000) since this new range is often free of specialist antagonists of the exotic herbivores (i.e., natural enemies hypothesis) (Maron and Vila 2001; Vilà et al. 2005). However, these herbivores may become a valuable source of alternative prey for local predators in an altered habitat by anthropogenic activities (Snyder and Evans 2006; Twardochleb

et al. 2012). As an alternative prey, the exotic species can also bear threat to the native predator species. They can be an evolutionary trap or ecological trap when they are toxic prey or unsuitable hosts with same cues of the suitable native prey or hosts (e.g., Kokko and Sutherland 2001; Schlaepfer et al. 2005; Robertson et al. 2013). An evolutionary trap is a disassociation between the cues that an organism uses to make behavioral or reproductive decision and the fitness outcomes due to a rapid alteration in the local environment by human activity (Schlaepfer et al. 2002), while ecological trap is a special case of an evolutionary trap which occurs when an organism choose a poor quality habitat because it delivers similar cues associated with a high quality habitat (Kokko and Sutherland 2001). Thus, conservation and enhancement of native natural enemies should take in consideration trap effects of exotic organisms in the new range.

Biological control and invasion of exotic organisms

Biological control is the use of living organisms - the populations of natural enemies - to suppress pest populations to lower densities permanently or temporarily (Van Driesche et al. 2008). Introduction of beneficial organisms for biological control of invading pests is an important route for the intentionally introduced invasive species. Organisms of different taxa have been used worldwide in biological control of weed, herbivore pests, etc. For instance, about 2000 biological control agent species have been introduced worldwide to control exotic adventive weeds and invader pests (van Lenteren et al. 2006). Since 1888 after the spectacular success of vedalia lady beetle, *Rodolia cardinalis* (Mulsant) (Coleoptera: Coccinellidae) introduced from Australia into California for the control of cottony cushion, *Icerya purchase* Maskell, many lady beetles have been introduced into different parts of the globe for controlling permanently new exotic pests of

the same place of origin, what is termed "Classical biological control" (Dixon 2000). The introduction of exotic natural enemies to control new invaded pests was considered as inevitable alternative risk of using synthetic pesticides (Thomas and Willis 1998). However, many negative effects of introduced natural enemies on the native ones and on the non-target arthropods have been proclaimed (e.g. Boettner et al. 2000; Kajita et al. 2006; Brown et al. 2011). It follows that biological control practitioners and researchers have encouraged using and enhancing native natural enemies through habitat manipulation, a practice known as "conservation biological control." Yet, less interest has been given to the impact of an exotic alternative prey or host on native biological control agents in their persistence and pest control outcomes which is a field of interest for conservation biological control practitioners and researchers.

Conservation biological control

Conservation biological control is the protection and enhancement of native biological control agents by modification of the environment or by cultural practices (Eilenberg et al. 2001). Actually, conservation biological control might be the oldest form of biological control practiced by Chinese 900 AD by placing nest of predaceous ant, *Oecophylla smaragdina* F. on citrus trees to control defoliator insects (Ehler 1999). It may be the most practical control tactic for farmers because it relies on practices which are familiar to them (e.g., Planting flowering plants, farmscaping, etc.) (Ohno, personal communication). Conservation biological control depends on providing food subsidy resources, alternative habitats and prey to the native natural enemies, particularly, the generalist which uses more than one food source to complete their life cycles (Tscharntke et al. 2007). Thus, the diversity within a landscape can greatly affect communities of herbivores and

their natural enemies within an agricultural crop (Grez et al. 2014). Because conservation biological control works at community level and includes assemblage of natural enemies, it is crucial to understand how both local and landscape processes affect long term stability and resilience of biological control services provided by native predators (Bianchi et al. 2006; Tscharntke et al. 2007). So that any attempt for sustainable and effective biological control services from multitude of native natural enemies, their conservation and enhancement should be considered at landscape level. The tenet that introduced natural enemies have more ability to use the available resources (i.e., prey or hosts) than the native natural enemies (their guilds) (Morrison and Hay 2011; Chapple et al. 2012; Roy et al. 2012), and thus have potential to become invaders, has fostered intensive researches on the negative effects of the exotics natural enemies on non-target species and on native guilds at the expense of the role of a novel invasive prey or a food source on the populations of native enemies and their conservation. In this dissertation, I discussed the potential negative impacts of a novel prey aphid, the goldenrod aphid, *Uroleucon nigrotuberculatum* (Olive) (Homoptera: Aphididae) on the life history parameters and the reproductive performance of *Coccinella septempunctata bruckii* Mulsant and *Propylea japonica* (Thunberg) (Coleoptera: Coccinellidae).

Lady beetles as biocontrol agents

Lady beetles (Coleoptera: Coccinellidae) are small, 1-10mm long, usually round or oval shaped insects, often having bright red, orange, or yellow color and black spots. Approximately more than 4500 species of lady beetles have been described worldwide (Roy et al. 2013). Lady beetles are holometabolous (i.e., complete metamorphosis), passing through: eggs, larva, pupa and adult

stages during their life cycle. Predatory lady beetles can be divided into two main groups according to their prey: (1) aphidophagous lady beetles which prey mainly on aphid and (2) coccidophagous which prey mainly on coccids. The aphidophagous lady beetles are key predators and play an important role in biological control of aphids. For example, *C. septempunctata bruckii* and *P. japonica* are among the main aphidophagous lady beetles in Japan (Taki et al. 2013), and they exert a considerable role in regulating aphid populations. *Coccinella septempunctata bruckii* has two generations intermitted by an estivation period in summer and hibernation in winter, while *P. japonica* did not estivate and can realize 2-3 generations per year (Kawauchi 1997). The overwintered adults immigrate into patches of vetch, *Vicia inguistifolia* (Koch) infested with vetch aphid, *Megoura crassicauda* Mordvilko (Homoptera: Aphididae) in early spring (from March) where they breed their first generation. Duration of larval development of lady beetles depends on the ambient temperature, prey abundance and prey quality (Nedvĕd and Honĕk 2012). Generally, upon the lady beetles are reaching adult stage in a natal habitat, the ephemeral colonies of prey aphids become depleted, consequently, the newly emerged adults have to find new suitable habitats harboring abundant prey aphids in the adjacent crop fields or the non-crop vegetation. Therefore, complex and diverse landscape must support more stable and permanent population of lady beetles since such habitat can harbor varied aphid populations both in time and space, and the generalist lady beetles found in a complex and diverse agroecosystem provide more durable biological control service (Tscharntke et al. 2007). Since lady beetles prey on various aphid species with different nutritional qualities and defensive response (Dixon 2000; Hodek and Evans 2012), the quality of prey aphid in the non-crop vegetation may define their biological control service outcomes in the adjacent crop fields. Moreover, the non-crop vegetation can be a sink or a source of the lady beetles for therein intermingled arable land. For instance, when the non-crop vegetation

5

is a suitable habitat for reproduction of lady beetles, it splits over the newly emerged lady beetles into the adjacent crop fields. On the other hand, the lady beetles attracted into an unsuitable non-crop vegetation habitat around crop field may sink in, particularly when this habitat is a specific host for a toxic prey aphid which impair the reproductive success of the lady beetles.

The goldenrod aphid, *Uroleucon nigrotuberculatum* and Solidago patches a new habitat for aphidophagous lady beetles in Japan

The goldenrod aphid, *U. nigrotuberculatum* is a large red aphid, monoecious and holocyclic in Japan, but in some areas it is also anholocyclic, which was introduced from North America four decades ago (Sugimoto and Matsumoto 2000). It is a very common aphid in Solidago patches throughout Japan from later spring to early summer. Goldenrod, *Solidago altissima* (Asteraceae) hereafter referred to as Solidago, is an invasive plant introduced from North America since later 1890s (Osada 1976). It was introduced as an ornamental plant or flower for bees, and has naturalized and widely spread in Japanese agroecosystem around roadsides and in abandoned fields (Takafuji 1980; Etterson et al. 2008). It constitutes the most widely spread invasive alien plant in riparian area of Japan (Miyawaki and Washitani 2004). The goldenrod aphid affects the community of arthropod which co-occurs with it at the same season as well as that temporally separately occurring arthropod community (i.e., it is a keystone species on Solidago). Solidago and the goldenrod aphid system forms a new habitat for aphidophagous lady beetles in Japanese agroecosystems where they have been well established (Mito and Uesugi 2004). In southern part of Japan, the goldenrod aphid infests heavily Solidago patches from late April to early June. Despite, the goldenrod aphid is a noxious prey for the Asian multicolored lady beetle, *Harmonia*

axyridis (Pallas) (Kamo and Tokuoka 2011), it is common to observe this lady beetle, as well as other Japanese lady beetles such as *C. septempunctata bruckii* and *P. japonica* preying on this aphid in early summer. To my knowledge, no ecological study on the influence of this prey aphid on both *C. septempunctata bruckii* and *P. japonica* in the Japanese agroecosystem has been reported. Therefore, in this dissertation I investigated the ecological function and effects of Solidago and its specialist aphid, the goldenrod aphid on *C. septempunctata bruckii* and *P. japonica*.

Interaction between predator-prey in non-crop vegetation drives the biological control services of native predators

Non crop vegetation is the space where the main interactions between herbivores and their natural enemies that lead to a biological control service in the surrounding crop fields interplay (Gardiner et al. 2009). Generalist predators like lady beetles depend on the structure and composition of the non-crop vegetation for their reproduction, shelter and overwintering or estivation (Caballero-lópez et al. 2012; Amaral et al. 2013). This vegetation harbors different prey aphids, and provides non prey food to the lady beetles during the period of scarce prey in crop vegetation (Taki et al. 2013). Moreover, it affects the interaction between the lady beetles (i. e., the intraguild predation) (Amaral et al. 2015). Therefore, alteration of the non-crop vegetation composition by anthropogenic activity or by replacement of native plants by invasive exotic plants disturbs the natural habitat and the trophic link in the arthropod communities, which may affect the life history of lady beetles in those communities.

An agroecosystem dominated by a monoculture crop receives low biological control service and is vulnerable to insect pest outbreak because such environment cannot support and sustain the natural enemies during the off farming period (Nicholls and Altieri 2007). Similarly, replacement of natural vegetation by invasive plants simplifies plant community and removes the trophic link associated with the replaced plants; their herbivores and the generalist predators of that herbivores (Hartley et al. 2010; Berthon 2015). Consequently, the predator populations will diminish because they must emigrate in seeking for habitats that provides their need of food. However, the new invasive plants may add new herbivores to the arthropod communities which can be an alternative prey for the native predators (Yoshioka et al. 2014). In such case, there are two possible outcomes of the new interaction: (1) the new herbivores are suitable prey for the native predators and can support their reproduction, the predators regulate the population of the new herbivores and prevent them being invasive; (2) the new herbivores are toxic prey for the native predators both adults and larvae, or toxic for the larvae but can maintain the adults even though it did not support their reproduction. In the latter case, the new herbivores proliferate and become invasive in the new range. For instance, the goldenrod aphid has become a widely spread and established herbivore in Solidago patches in Japan. In other words, the native natural enemies including aphidophagous lady beetles could not halt the way of the goldenrod aphid invasion. Now, the question is to what extent this aphid affects the life history and conservation of the native lady beetles in Japan?

The main questions of this dissertation

Biological control practitioners and researchers have been focusing on the negative effects of competition between natives and exotic lady beetles introduced as biological control agents in

classical biological control programs. For instance, introduction of *C. septempunctata* and *H. axyridis* into North America for aphid control has affected the native populations of lady beetles and thus has fostered studies on their replacement of the native lady beetles in the past two decades (Kenis and Auger-Rozenberg 2009; Roy et al. 2012; Smith and Gardiner 2013; Turnipseed et al. 2014). Similarly in Japan, the introduction of the alien lady beetles, *Adalia bipunctata* has started attracting the interest of some scientists (Toda and Sakuratani 2006). However, there is no such interest on the negative impacts of a new prey associated with an established exotic plant in the new region. In Japan, Solidago patches form a main part of the non-crop habitat in the agroecosystem. The presence of its specialist aphid, the goldenrod aphid attracts the native lady beetle into Solidago patches. It is important to study the impacts of this new habitat on the native lady beetles, particularly, *C. septempunctata bruckii* and *P. japonica*. This aphid has become a main prey of the aphidophagous lady beetles in early summer in the agroecosystem. Although it has been reported to be noxious to *H. axyridis* larvae and adults, no report on its effects on the other two main aphidophagous lady beetles native to Japan such as *C. septempunctata bruckii* and *P. japonica*. In this dissertation, I studied the effects of the goldenrod aphid on the reproductive performance of these two lady beetles as well as the implication of the invasion of an exotic plant and its herbivores insects on the conservation of native predators. I tackled this subjects through seeking answers to these four questions, which are constituting four chapters of this dissertation.

1- The effects of the goldenrod aphid on the life parameters and the reproductive performance of *C. septempunctata bruckii* and *P. japonica*: in this chapter, I studied the effects of this prey aphid on the lady beetles larval development, survival, adult weight, reproduction and survival in the laboratory. I also studied the fecundity of the adults on Solidago patches in the field.

2- The goldenrod aphid, *Uroleucon nigrotuberculatum* defensive response to the attack of these lady beetles and its impact on their fitness by investigating the effects of the goldenrod aphid defense by secretion of red gluing liquid from its cornicles on the survival of lady beetle larvae.

3- The implication of defensive response and unsuitability of *U. nigrotuberculatum* on alteration of prey preference and foraging behavior of *C. septempunctata bruckii* and *P. japonica*.

4- The seasonal occurrence of the goldenrod aphid on Solidago patches and the lady beetle population dynamics as well as ovarian dynamics of *C. septempunctata bruckii*.

I discussed the implication of the system Solidago-goldenrod aphid on the conservation of *C. septempunctata bruckii* and *P. japonica*, hence proving biological control service for regulating aphid in crop fields.

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Chapter 2 Prey aphid inhibits development and reproduction of *Coccinella septempunctata bruckii* and *Propylea japonica* (Coleoptera: Coccinellidae) but maintains adults[1]

Abstract

I compared the development and survival of *Coccinella septempunctata bruckii* Mulsant and *Propylea japonica* (Thunberg) on *Uroleucon nigrotuberculatum* (Olive) with those of the same species raised on *Megoura crassicauda* Mordvilko. *Uroleucon nigrotuberculatum* significantly prolonged the development time and drastically reduced the survival of the larval stages of both predators. Adult body weight of *C. septempunctata bruckii* and females of *P. japonica* raised on *M. crassicauda* was substantially heavier. No females of *C. septempunctata bruckii* raised on *U. nigrotuberculatum* laid eggs. Only 15% of *C. septempunctata bruckii* females collected from *Solidago altissima* L. patch laid eggs for 48h when fed on *U. nigrotuberculatum*. In contrast, females fed on *M. crassicauda* were highly fecund. Some *P. japonica* females raised on *U. nigrotuberculatum* laid a few eggs discontinuously for a short period. Approximately 90% of *P. japonica* adult females collected from the Solidago patch and fed on *U. nigrotuberculatum* laid a few eggs, but females fed on *M. crassicauda* laid substantially more eggs. These results suggest that patches of the introduced plant Solidago, a natural host of the introduced aphid, *U.*

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nigrotuberculatum, are not adequate habitats for reproduction but may provide lady beetle adults with alternative prey on which to survive when suitable prey are scarce.

Introduction

Non-crop vegetation is a site of interaction between herbivores and their natural enemies; it provides food, shelter, and refuge for natural enemies, and prevents outbreaks of the pest in farmland located in a variety landscapes (Bianchi et al. 2006). Aphidophagous lady beetles (Coleoptera: Coccinellidae) depend on prey aphids in non-crop vegetation during early spring to early summer when prey aphids in crop vegetation are scarce (Bianchi and van der Werf 2004; Taki et al. 2013).

Landscape vegetation can be displaced by competitive exotic plants, which can interact in new ways with native herbivores but are often followed by exotic herbivores that specialize on these plants in their regions of origin (Hierro et al. 2005; Memmott et al. 2000; Mitchell et al. 2006). Invasive plants can also fragment the habitat of native arthropods and thus form structural barriers that restrict the ability of predators to find prey (Harvey and Fortuna 2012); they can also add novel prey for native predators (Snyder and Evans 2006). For instance, goldenrod, *Solidago altissima* L., was introduced into Japan in the late 1890s from North America (Osada 1976), has since displaced herbaceous vegetation in Japan. Goldenrod infested with aphid, *Uroleucon nigrotuberculatum* (Olive) in Japan was first found in the early 1990s (Sugimoto and Matsumoto 2000). This exotic aphid is one of the most important herbivores in Solidago patches, where heavy infestation by this aphid in late spring to early summer is shaping the communities of predators such as lady beetles (Ando et al. 2011).

Several studies have focused on how exotic lady beetles affect native lady beetles and on the role of exotic lady beetles in suppressing pest aphids (Hoki et al. 2014; Kajita et al. 2006; Losey et al. 2012). However, there is insufficient research on the effect of alien invasive plants with their specialist herbivores on generalist predator communities in the new ranges. Kamo and Tokuoka (2011) reported the noxious effect of *U. nigrotuberculatum* on *Harmonia axyridis* (Pallas), although its effect on other important Japanese aphidophagous lady beetles such as *Coccinella septempunctata bruckii* Mulsant and *Propylea japonica* (Thunberg) has not been examined. Here, I examined the effect of *U. nigrotuberculatum* on *C. septempunctata bruckii* and *P. japonica* by comparing their development time, survival, and reproduction when fed on this exotic prey aphid with those when they were fed on a major native prey aphid, *Megoura crassicauda* (Mordvilko).

Materials and methods

Plant and insect cultures

Seeds of broad bean, *Vicia faba* L. were germinated in vermiculite. One-day-old seedlings were uprooted and suspended through wire mesh on the top of a polypropylene pot containing tap water so the roots hung in the water. The pots were placed in acrylic cages (30 cm × 30 cm × 30 cm) in the laboratory at 25 °C with a 16L: 8D h photoperiod. The seedlings were replaced every 8 to 10 days to maintain quality. The seedlings were used to maintain the culture of *M. crassicauda* in laboratory. Colonies of *M. crassicauda* were initiated in the laboratory from a single apterous adult collected from vetch *Vicia angustifolia* (Koch). *Uroleucon nigrotuberculatum* aphids were collected every day from Solidago patches because of the difficulty of rearing them under laboratory conditions.

Adults of *C. septempunctata bruckii* and *P. japonica* were collected from vetch patches infested with *M. crassicauda* on the Kibana campus (31°49.604′N; 131°24.736′E) of the University of Miyazaki, Miyazaki Prefecture, Kyushu, Japan. The coccinellids were paired, and each pair was kept in a polystyrene breeding dish (100 mm diameter, 40 mm high) with a meshed hole on the cap. They were fed ad libitum *M. crassicauda* and held in an incubator at 23 °C with a relative humidity of >70% and a 14L: 10D h photoperiod.

Effects of prey aphids on larval development, survival and adult weight of *C. septempunctata bruckii* and *P. japonica*

Eggs laid by the lady beetles were transferred on a fine camelhair brush into Petri dishes (ø 50 mm) containing wet filter paper, and incubated at 23 °C until hatching. Thirty newly hatched (24-h-old) larvae of *C. septempunctata bruckii* and *P. japonica* were reared individually in breeding dishes. The larvae were provided ad libitum with *M. crassicauda* or *U. nigrotuberculatum* (at all development stages) on pieces of their host plant to maintain aphid survival and quality. The prey and their plants were changed every 24 h, when I recorded the stage of development and survival of the coccinellid larvae until adult eclosion. An electronic balance (ViBRA HT) with a precision of 0.1 mg was used to weigh newly emerged adults before feeding them.

Effects of prey aphids on fecundity of *C. septempunctata bruckii* and *P. japonica*

Newly emerged (24-h-old) lady beetles were sexed and paired, and each pair was placed in a breeding dish and fed ad libitum with the same prey aphids they were raised on. I raised 14 pairs

of *C. septempunctata bruckii* on *M. crassicauda* and 6 on *U. nigrotuberculatum*; and 20 pairs of *P. japonica* on *M. crassicauda* and 11 on *U. nigrotuberculatum*. Because of the ephemerality of aphid colonies in nature and the importance of early reproduction on the fecundity of lady beetles (Dixon and Agarwala 2002), I recorded the number of eggs laid every 12 h during the first 30 days of reproduction. Numbers of occasionally cannibalized eggs were estimated from the yellow marks left where the egg patch was laid.

Effects of *U. nigrotuberculatum* on oviposition by *C. septempunctata bruckii* and *P. japonica*

I collected 20 mature adults of *C. septempunctata bruckii* and *P. japonica* from a Solidago patch on the Kibana campus. I selected the lady beetles with dark elytron color which seemed to be mature. The *C. septempunctata bruckii* adults were fed on 20 aphids and the *P. japonica* adults were fed on 10, collected daily from Solidago patches. I recorded the numbers of eggs laid every day for 10 days.

Statistical analysis

The development and pre-oviposition time of lady beetles on the prey aphids of each species were compared by use of Student's *t*-test. Kaplan–Meier estimated survival curves for the larvae of each species on each prey were compared by use of log-rank test. All statistical analyses were performed in JMP v. 10 software (SAS Institute 2012).

Results

Effects of prey aphids on the development, survival and adult weight of *C. septempunctata bruckii* and *P. japonica*

Mean duration of the 1st to 4th instar of *C. septempunctata bruckii* raised on *U. nigrotuberculatum* were significantly longer than those of *C. septempunctata bruckii* raised on *M. crassicauda* (1st instar, $t = 4.41$, $p < 0.001$; 2nd, $t = 5.28$, $p < 0.001$; 3rd, $t = 5.08$, $p < 0.001$; 4th, $t = 5.46$, $p < 0.001$; Table 2-1). There were no significant differences in duration of the pre-pupal or pupal stage, but the total duration of the pre-imaginal stages was longer on *U. nigrotuberculatum* ($t = 14.37$, $p < 0.001$). Adults raised on *U. nigrotuberculatum* were significantly lighter than those raised on *M. crassicauda* (female, $t = -5.20$, $p < 0.001$; male, $t = -4.27$, $p < 0.01$). Mortality of the larvae was significantly higher on *U. nigrotuberculatum* than on *M. crassicauda* ($\chi^2 = 23.45$, $p < 0.001$); >50% of larvae on *U. nigrotuberculatum* died within the first 2 weeks (Fig. 2-1).

Mean durations of the 1st to 4th instar of *P. japonica* raised on *U. nigrotuberculatum* were significantly longer than those of *P. japonica* raised on *M. crassicauda* (1st instar, $t = 5.23$, $p < 0.001$; 2nd, $t = 3.26$, $p < 0.01$; 3rd, $t = 3.41$, $p < 0.01$; 4th, $t = 2.97$, $p < 0.01$; Table 2-2). There were no significant differences in durations of the pre-pupal or pupal stage, but the total duration of the pre-imaginal stages was significantly longer when raised on *U. nigrotuberculatum* ($t = 4.28$, $p < 0.01$). Females raised on *U. nigrotuberculatum* were significantly lighter than those raised on *M. crassicauda* ($t = -4.72$, $p < 0.001$), but the weights of males did not differ ($t = -1.98$, $p > 0.05$). Survival was significantly greater for larvae of *P. japonica* raised on *M. crassicauda* than those raised on *U. nigrotuberculatum* ($\chi^2 = 14.96$, $p < 0.001$): for those raised on *U. nigrotuberculatum* mortality was highest during the first week (Fig. 2-2).

Effects of prey aphids on fecundity of *C. septempunctata bruckii* and *P. japonica*

For 30 days after eclosion, none of the 6 females of *C. septempunctata bruckii* raised on *U. nigrotuberculatum* laid eggs, although they mated and 67% of adults survived (Table 2-3). However, the 14 females raised on *M. crassicauda* had a pre-oviposition period of 16.1 ± 1.44 days and laid more than 1000 eggs during the first 30 days, or 35.2 ± 1.3 eggs per day (Fig. 2-3).

Only 3 of the 11 females of *P. japonica* raised on *U. nigrotuberculatum* laid eggs, with a pre-oviposition period of 15.7 ± 1.20 days and 64% of adults survived for 30 days after emergence (Table 2-3). However, all 20 females of *P. japonica* raised on *M. crassicauda* laid eggs, with a pre-oviposition period of 6.2 ± 0.84 days. The mean pre-oviposition period of *P. japonica* differed significantly between the two prey ($t = 6.85$, $p < 0.01$). During the first 30 days, those raised on *M. crassicauda* laid a total of 233 eggs (8 eggs/day; Fig. 2-4a), but those raised on *U. nigrotuberculatum* laid only 40 eggs (1 egg/day; Fig. 2-4b).

Effects of *U. nigrotuberculatum* on oviposition by *C. septempunctata bruckii* and *P. japonica*

Among 20 females of *C. septempunctata bruckii* collected in Solidago patches, only 3 females laid few eggs when fed on *U. nigrotuberculatum* for 48 h (Fig. 2-5). They preyed on an average of 10.2 ± 0.8 aphids/day. In contrast, about 90% of *P. japonica* females laid eggs, intermittently during 10 days of observation. They preyed on an average of 3.9 ± 0.2 aphids/day.

Discussion

The development times of *C. septempunctata bruckii* and *P. japonica* were affected by the prey aphid provided. Both species grew more slowly on *U. nigrotuberculatum* than on *M. crassicauda*. Adults of *C. septempunctata bruckii* and adult females of *P. japonica* raised on *M. crassicauda* were heavier than those raised on *U. nigrotuberculatum*. Larval mortality was significantly higher on *U. nigrotuberculatum*. Different effects of aphid prey species on the development time, adult fresh weight, and survival of lady beetles have been reported in other studies (Kalushkov and Hodek 2004; Omkar and Srivastava 2003; Tsaganou et al. 2004; Zhang et al. 2012) and are attributed to differences in aphid nutritional quality acquired from the host plant (Giles et al. 2002; Rattanapun 2012) or to the effects of plant secondary metabolites used by aphids to defend against predators (Francis et al. 2001).

Interestingly, in the present study some of *C. septempunctata bruckii* larvae reached adult eclosion. The *C. septempunctata bruckii* females raised on *U. nigrotuberculatum* did not lay eggs, and although the larvae suffered severe mortality, the emerged adults could be maintained on this prey more than 30 days. In contrast, Kamo and Tokuoka (2011) reported that all larvae of *H. axyridis* fed on *U. nigrotuberculatum* died within a few days and the adults died within 10 days. Thus, this aphid could be considered as an alternative prey for *C. septempunctata bruckii* according to the classification of prey aphids by Hodek (1996). The effects of unsuitable prey on adults may be different from that on larvae. Michaud (2000) found that green citrus aphid, *Toxoptera citricidus* (Kirkaldy) hinders the larval development of *C. septempunctata bruckii* while supporting the reproduction of the adults. Alternatively, unsuitable prey might supplement an insufficiency of suitable prey; for instance, alfalfa weevil *Hypera postica,* (Gyllenhal) can

maintain *C. septempunctata* and improves egg production by females given insufficient suitable prey (Evans et al. 2004). Furthermore, because of the seasonal changes in aphid species composition and abundance in agroecosystems, lady beetles have to prey on different aphid species including unsuitable or toxic aphids (Evans and Toler 2007; Gardiner et al. 2009) such as *U. nigrotuberculatum,* which affected larval development and survival as well as adult reproduction, but may support the survival of *C. septempunctata bruckii* adults till more suitable prey is found.

Although *U. nigrotuberculatum* reduced the survival of *P. japonica* larvae, it supported a low reproductive rate. Some *P. japonica* females raised on *U. nigrotuberculatum* laid a few eggs, indicating that this prey was nutritionally poor. This result agrees with the result that *P. japonica* can lay eggs at varying rates when preying on various aphids (Tang et al. 2013). In addition, numerous studies have reported that *P. japonica* can successfully develop and reproduce on different food types (e.g., Hamasaki and Matsui 2006). Zhang et al. (2007) have reported that *P. japonica* is euryphagous and can lay eggs even on a diet of whitefly, *Bemisia tabaci (Gennadius).*

Only a few mature females of *C. septempunctata bruckii* collected from Solidago patches laid eggs for 48h when fed on *U. nigrotuberculatum.* Because the adults raised on this aphid in the laboratory did not lay eggs at all, I suggest that these mature females' eggs could be matured while they preyed on other suitable aphids before immigration into Solidago patches. The presence of hatchling larvae of *C. septempunctata bruckii* in Solidago patches infested with *U. nigrotuberculatum* from late April to mid-May might be due to the migration of gravid females from depleted vetch patches. Unlike *C. septempunctata bruckii* females, almost all *P. japonica* females collected from Solidago patches laid eggs in the laboratory, albeit few. This suggests that the euryphagous *P. japonica* can exploit *U. nigrotuberculatum* better, or because it can use lower-quality food to produce eggs (Hamasaki and Matsui 2006).

Coccinella septempunctata bruckii seems to be more sensitive than *P. japonica* to prey quality for egg production. Because both *C. septempunctata bruckii* and *P. japonica* in Japan have a short evolutionary history with *U. nigrotuberculatum*, which was unintentionally introduced into Japan about 30 years ago (Sugimoto and Matsumoto 2000), the inhibition of egg production in *C. septempunctata bruckii* raised on *U. nigrotuberculatum* cannot be explained as an adaptive or evolutionary strategy, because the females must interact with novel prey for a long time to evolve refraining oviposition on this deadly prey for their offspring. Further studies of the relationship between the suitability of prey aphids for lady beetle larval development and survival and their suitability for predator egg production are needed. Nevertheless, my results suggest that *U. nigrotuberculatum* is an important prey for maintaining newly emerged *C. septempunctata bruckii* and *P. japonica* migrating from depleted vetch patches during early summer, when suitable prey aphids are scarce. Solidago patches may have positive implications for conservation of *C. septempunctata bruckii* and *P. japonica* in agroecosystems, even though Solidago patches infested with *U. nigrotuberculatum* are not suitable for adults of *H. axyridis* survival (Kamo and Tokuoka 2011). In this context, more studies on the ecological impact of Solidago patches on lady beetle populations are needed.

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Table 2-1 Development time and adult fresh weight (mean ± SE) for *C. septempunctata bruckii* raised on *U. nigrotuberculatum* and *M. crassicauda*

Prey aphid	Duration of each larval stage (days)				Pre-pupa (days)	Pupa (days)	Total pre-imaginal period (days)	Adult weight (mg)	
	1st instar	2nd instar	3rd instar	4th instar				Female	Male
U. nigrotuberculatum	3.7 ± 0.43a	5.9 ± 0.75a	5.7 ± 0.70a	7.4 ± 0.45a	1.0 ± 0.00a	5.0 ± 0.00a	26.9 ± 0.75a	22.73 ± 1.49b	17.42 ± 1.99b
M. crassicauda	1.8 ± 0.11b	1.9 ± 0.06b	2.1 ± 0.07b	4.2 ± 0.38b	1.1 ± 0.06a	5.1 ± 0.07a	15.8 ± 0.16b	31.96 ± 0.96a	28.03 ± 1.47a

Means within a column followed by different letters are significantly different (Student's t test: $p < 0.05$)
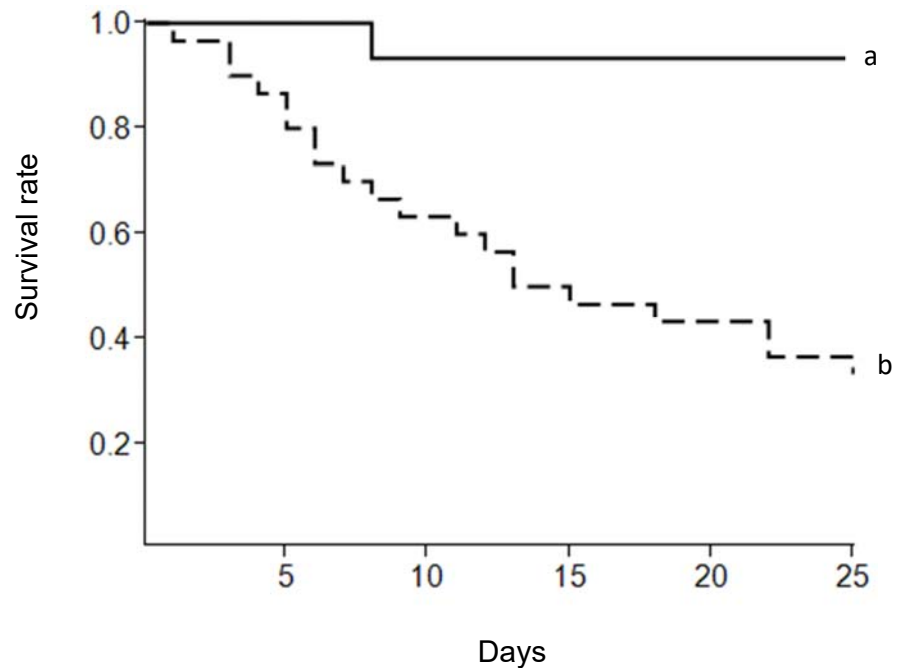
Figure 2-1 Kaplan-Meier survival curves for *C. septempunctata bruckii* larvae raised on *M. crassicauda* (solid line) and on *U. nigrotuberculatum* (dashed line). Survival curves are significantly different (Log-Rank test: $p < 0.05$, n = 30)

Table 2-2 Development time and adult fresh weight (mean ± SE) of *P. japonica* raised on *U. nigrotuberculatum* and *M. crassicauda*

| Prey aphid | Duration of each larval stage (days) | | | | Pre-pupa (days) | Pupa (days) | Total pre-imaginal period (days) | Adult weight (mg) | |
	1st instar	2nd instar	3rd instar	4th instar				Female	Male
U. nigrotuberculatum	2.4 ± 0.23a	3.3 ± 0.47a	3.2 ± 0.37a	4.9 ± 0.61a	1.1 ± 0.07a	3.86 ± 0.10a	17.9 ± 0.77a	5.97 ± 0.18b	4.20 ± 0.67a
M. crassicauda	1.2 ± 0.07b	1.6 ± 0.10b	1.7 ± 0.10b	2.6 ± 0.12b	1.0 ± 0.04a	3.9 ± 0.04a	12.1 ± 0.08b	7.23 ± 0.20a	5.67 ± 0.32a

Means within a column followed by different letters are significantly different (Student's *t*-test: $p < 0.05$)
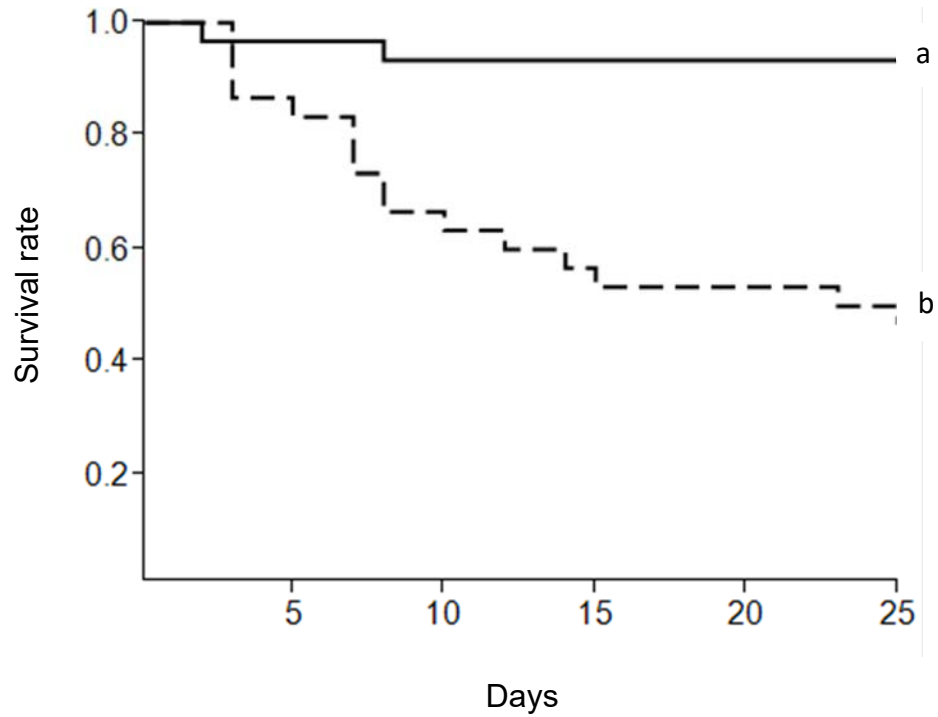
Figure 2-2 Kaplan-Meier survival curves for *P. japonica* larvae raised on *M. crassicauda* (solid line) and on *U. nigrotuberculatum* (dashed line). Survival curves are significantly different (Log-Rank test: $p < 0.05$, n = 30)

Table 2-3 Pre-oviposition period (mean ± SE), percentage of adult surviving and percentage of females laying eggs for *C. septempunctata bruckii* and *P. japonica* raised on *U. nigrotuberculatum* (Un) and *M. crassicauda* (Mc)

Lady beetle	Prey aphid	Pre-oviposition [a] period (day)	% of Survival [b] during 30 days	% of female laying eggs
C. septempunctata	Un	-	67b	0
	Mc	16.1±1.44	100a	100
P. japonica	Un	15.7±1.20b	64b	27
	Mc	6.2 ± 0.84a	100a	100

[a] Means within a column followed by different letters are significantly different (Student's *t*-test, $p < 0.05$)

[b] Percentages within a column followed by different letters are significantly different (Log-Rank test; $p < 0.05$)
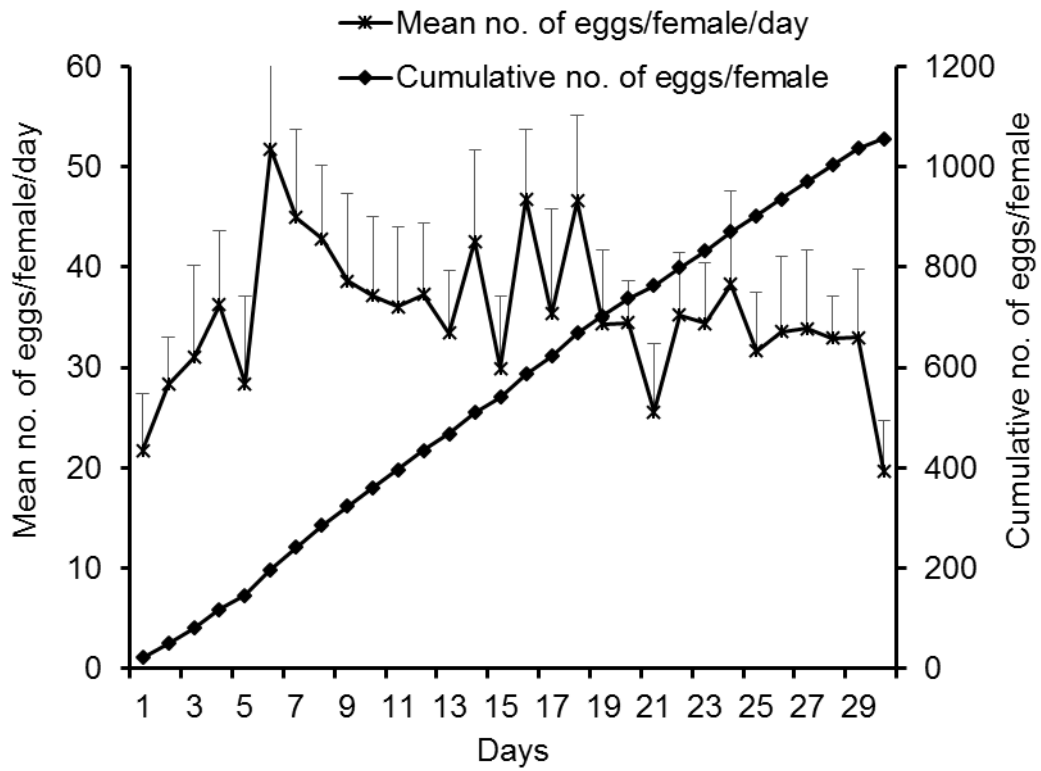
Figure 2-3 Daily egg production (mean + SE) and cumulative number of eggs laid during the 30 days after starting reproduction by *C. septempunctata bruckii* females raised on *M. crassicauda* (n = 14)
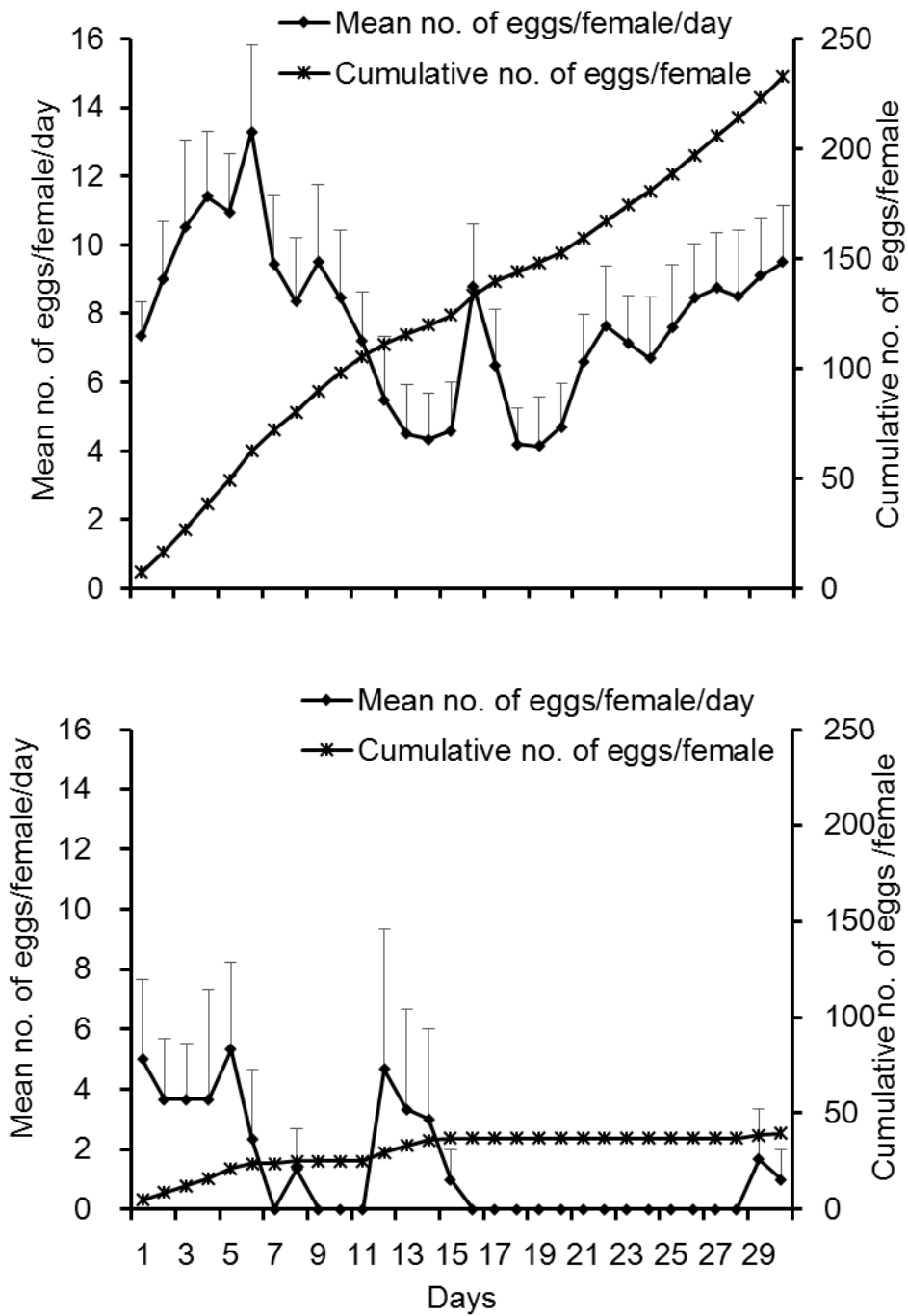
Figure 2-4 Daily egg production (mean + SE) and the cumulative number of eggs laid during the 30 days after starting reproduction by *P. japonica* females raised on (a) *M. crassicauda* (n= 20) and (b) *U. nigrotuberculatum* (n= 3)
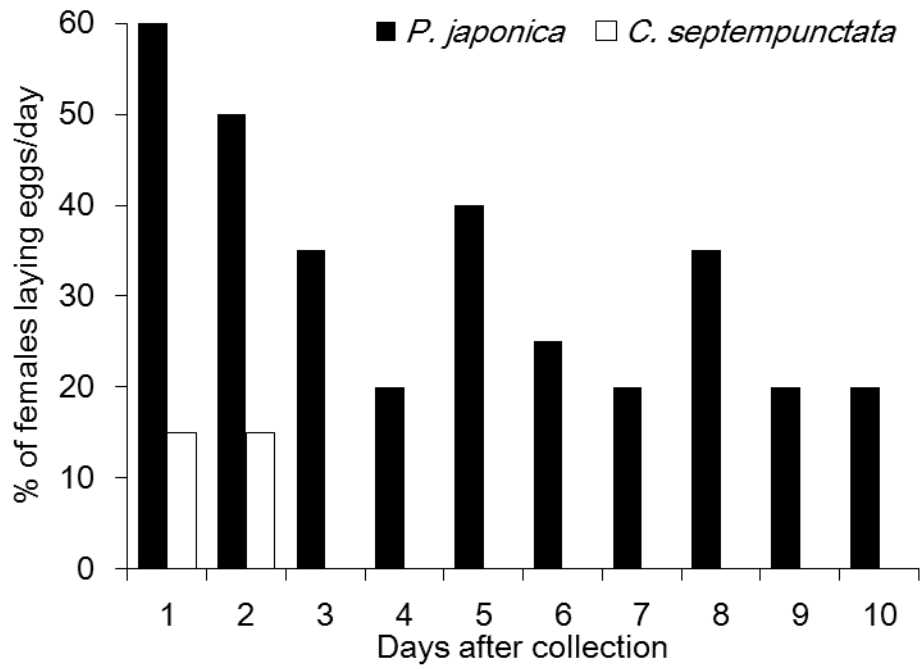
Figure 2-5 Percentage of egg-laying females (n = 20) collected in Solidago patches and fed on *U. nigrotuberculatum* in the laboratory

Chapter 3 Cornicle secretions of *Uroleucon nigrotuberculatum* (Homoptera: Aphididae) as the last bullet against lady beetle larvae[2]

Abstract

Aphids have evolved various defense strategies against natural enemies, including secretions from their cornicles. I assessed the defensive function of cornicle secretions by the goldenrod aphid, *Uroleucon nigrotuberculatum* (Olive), against larvae of the lady beetles *Coccinella septempunctata bruckii* Mulsant and *Propylea japonica* (Thunberg) (Coleoptera: Coccinellidae). The aphid secreted red droplets from its cornicles when attacked by the larvae. Two-thirds of the *C. septempunctata bruckii* larvae and 46.7% of the *P. japonica* larvae that preyed on the aphids died before reaching the pre-pupal stage. The secretions caused molting failure when smeared on the larvae's heads or glued to the larvae's mouthparts, killing 56.7% of *C. septempunctata bruckii* larvae and 36.7% of *P. japonica* larvae. Second instar larvae were affected mostly. About 40% of 3rd and 4th instar larvae of *C. septempunctata bruckii* vomited soon after ingesting the aphids. In the field, up to 40% of 1st and 2nd instar larvae were smeared with red secretions. My results show that these cornicle secretions are an effective and active defense against earlier instars of coccinellid larvae.

Introduction

Gregarious insects face a high risk of predation because of their physical or chemical conspicuousness to their natural enemies, although the group-living habit is likely to increase individual fitness. Among group-living herbivorous insects, aphids have developed various defense strategies against predators and parasitoids, including chemical and induced behavioral defenses, such as alarm pheromones and subsequent dispersal or avoidance of predation (reviewed by Vandermoten et al. 2012), or dropping off the host plant by the pea aphid, *Acyrthosiphon pisum* Harris (e.g. Hoki et al. 2014; Nelson and Rosenheim 2006).

Aphids secrete sticky droplets from their cornicles upon attack by natural enemies (Dixon 1958). The droplets dispense alarm pheromones that alert neighbors of the presence of enemies (Mondor et al. 2000). Most studies of cornicle secretions have focused on the alarm pheromones and their protective effects on aphid colonies (e.g. Mondor and Messing 2007). Consequently, physical defense by cornicle droplets has been overlooked. In fact, secreted droplets contain triglycerides, which coalesce and dry on contact with predators or parasitoids (Greenway and Griffiths 1973). Moreover, the droplets may gum up natural enemies' mouthparts or glue them to their prey (Edwards 1966).

The goldenrod aphid, *Uroleucon nigrotuberculatum* (Olive), was unintentionally introduced into Japan from North America about 40 years ago (Sugimoto and Matsumoto 2000). Recent studies suggest that this aphid is a noxious prey for Japanese native aphidophagous lady beetles: it delays their larval development, causes very high larval mortality, and inhibits adult reproduction of *Harmonia axyridis* (Pallas) (Kamo and Tokuoka 2011), *Coccinella septempunctata bruckii* Mulsant, and *Propylea japonica* (Thunberg) (Barry and Ohno 2015). In the laboratory and in the

field, I observed that many lady beetle larvae and adults preying on the goldenrod aphid were smeared with red secretions, and I assumed that the aphid actively secretes these droplets as a physical defense against the predators. To evaluate the mortality of lady beetles caused by the droplets, I studied the effects of the cornicle secretions on the larvae of two common lady beetles native to Japan, *C. septempunctata bruckii* and *P. japonica*.

Materials and methods

Insects and plants

Adults of *C. septempunctata bruckii* and *P. japonica* were collected from patches of vetch, *Vicia angustifolia* (Koch) infested with the aphid *Megoura crassicauda* (Mordvilko) on the Kibana campus of the University of Miyazaki, Miyazaki Prefecture, Kyushu, Japan. Pairs of lady beetles were kept in polystyrene breeding dishes (100 mm diameter, 40 mm high) with a meshed hole in the cap. They were held in an incubator at 23°C and >70% RH under a 14:10 h photoperiod and fed *ad libitum* on vetch aphids cultured on bean, *Vicia faba* L. seedlings in the laboratory. Because I could not maintain goldenrod aphid colonies in the laboratory, I collected the aphids from the field prior to each experiment.

Aphid responses to the attacks of lady beetle larvae

To examine the defensive behavior of the goldenrod aphid toward predators, I transferred 20 nymphs (2nd to 4th instar) of the aphid on a twig of *Solidago* into a breeding dish in 30 replicates of each instar of each predator. I introduced a 1-day-starved larva of *C. septempunctata bruckii* or

P. japonica into each dish and observed the aphids' reactions until they were released or consumed. I considered every physical contact between a predator larva and an aphid as an encounter, and an oriented movement of a larva's front legs and head to grasp and subdue an aphid as an attack. I recorded the aphids' responses to the predator and the predator's direction of approach to the prey (front: approaching from aphid's head or rear: approaching from aphid's back).

Effects of secreted red droplets on predator larvae

Thirty newly hatched larvae of *C. septempunctata bruckii* or *P. japonica* were individually transferred into breeding dishes and fed on 20 nymphs of the goldenrod aphid (mixed larval stages) every day until they died or reached the pre-pupal stage. Every 24 h I recorded the numbers of predator larvae with their heads smeared with red droplets, with their mouthparts stuck with prey aphids, or that had died. The dead larvae were examined under a digital microscope for the presence of dried secretions on the head or other sensitive body parts.

Assessment of cornicle secretions on larvae of *C. septempunctata bruckii* in the field

Beginning on 21 March 2015 in a patch of *Solidago* infested with goldenrod aphid near the University of Miyazaki at (31°49′19″N; 131°24′51″E), each week for 5 weeks I counted *C. septempunctata bruckii* larvae on randomly selected *Solidago* plants (n = 75, 144, 126, 104, 76) and assessed aphid secretions on each larval stage. I classified the secretions on a 5-point scale: level 0, no secretions; level 1, few secretions on the predator's head; level 2, thin layer of secretions on head or mouthparts; level 3, thick layer of secretions on head and mouthparts; and level 4, prey

44

is stuck to the predator. Because few larvae were found during the 5th survey, these data were excluded.

Statistical analysis

Difference in the aphid defensive response to the predator species at each developmental stage was analyzed by using generalized linear model (GLM) with binomial error distribution. The effects of attack direction on the defensive response and mortality caused by cornicle secretions were analyzed with Fisher's exact test. The mortality rate of the two lady beetles species' larvae were compared by using survival analysis with Log-Rank test. These tests were performed with JMP (SAS Institute Inc 2012). To examine the relationship between larval stage and smearing levels in the field, a generalized linear model with multinomial error distribution was used. This analysis was performed with R software package (R Core Team 2015).

Results

Responses to the goldenrod aphid to attack by lady beetle larvae

The aphids secreted red droplets from their cornicles when they were attacked by either species of lady beetle (Fig. 3-1), at similar rates toward both predator species at all four predator instar stages as shown by the GLM with binomial error distribution (L1, $\chi^2 = 0.20$, $p = 0.66$; L2, $\chi^2 = 2.14$, $p = 0.14$; L3, $\chi^2 = 0.07$, $p = 0.80$; and L4, $\chi^2 = 0.74$, $p = 0.39$; Fig. 3-2). Secretion of the droplets did not cause the predators to release the prey aphid immediately, although nearby aphids became agitated. All 30 larvae of each instar of both predator species seized an aphid upon first contact.

The predators caught aphids from the front and the rear similarly ($p > 0.05$, Fisher's exact test). The direction of attack did not affect the frequency of red droplet secretions (*C. septempunctata bruckii*, $p = 0.79$; *P. japonica*, $p = 0.59$, Fisher's exact test). Around 40% of 3rd and 4th instar larvae (43.3% and 36.7%, respectively) of *C. septempunctata bruckii* vomited dark liquid soon after they consumed their first prey aphid (Fig. 3-3), but none of the 1st or 2nd instar larvae did so or released their prey. Only one 2nd instar larva of *P. japonica* released its prey and vomited dark liquid.

Effects of secreted red droplets on predatory larvae

Some predators died with their heads completely covered with red secretions, which prevented molting; some starved to death with prey stuck in their mouthparts; and some died without secretions (Fig. 3-4). Overall, 66.7% (20/30) of *C. septempunctata bruckii* and 46.7% (14/30) of *P. japonica* larvae died before reaching the pre-pupal stage (Fig. 3-5). Of *C. septempunctata bruckii*, 56.7% (17/30) died with a smeared head, stuck prey, or both, but 10% (3/30) (all 1st instar) died of unknown causes, which may be because of the failure of the neonate larvae to catch prey aphid. Of *P. japonica*, 36.7% (11/30) died with a smeared head, stuck prey, or both, but 10% (3/30) (all 1st instar) died of unknown causes. Death associated with the secretions was significantly higher than death by unknown causes (*C. septempunctata bruckii*, $p < 0.001$; *P. japonica*, $p < 0.001$, Fisher's exact test). Second instar larvae were affected the most: 41.2% (7/17) of *C. septempunctata bruckii* and 54.5% (6/11) of *P. japonica* died. There was no significant difference between the larval survival rates of these two lady beetle species ($\chi^2 = 1.71$, $p = 0.19$, Log-Rank test).

Assessment of cornicle secretions on larvae of *C. septempunctata bruckii* in the field

In the field, the 1st and 2nd instar larvae of *C. septempunctata bruckii* suffered most from the secretions, being more frequently head covered completely by secretions (level 3) or stuck to prey (level 4) (Fig. 3-6). Overall, 32.7% (16/49) of 1st instar larvae and 39.1% (27/69) of 2nd instar larvae were thus affected. The GLM analysis of the effects of larval stage on smearing level revealed that the 4th instar larvae were less affected by high smearing level of the goldenrod aphid (Table 3-1). No 4th instar larvae were observed having head completely covered by secretions; 4th instar larvae were most frequently free of any secretions. I observed three dead 1st instar and one dead 2nd instar larvae having head completely covered by secretions in the field.

Discussion

Generally, the defensive function of droplets secreted from cornicles has been considered to be dispensing alarm pheromones, which benefit clone-mates (e.g. Mondor and Roitberg 2004; Nault and Bowers 1974). In many aphid species, the secretions enable the neighbors in a colony to escape an attack by natural enemies. Unlike most aphid defensive behaviors performed collectively, such as twitching and kicking (Hartbauer 2010) and dropping off (Francke et al. 2008), under attack goldenrod aphids only secreted droplets, and neighbors did not show any escape behavior. This result strongly suggests that the cornicle secretions of this species do not contain alarm pheromones. Similarly, Joachim et al. (2013) reported that not all the cornicle secretions of pea aphid attacked by lady beetle or lacewing contain alarm pheromones. Instead, the goldenrod aphid appears to have evolved different defense tactics. Our laboratory and field observations revealed that lady beetle larvae daubed with the droplets suffered high mortality. In some cases, the

secretions disturbed the larvae's molting and thus led to their death. Therefore, droplet secretion by the goldenrod aphid is an effective physical defense.

In contrast to the indirect effect of alarm pheromones on natural enemies (Wu et al. 2010), the sticky droplets physically discourage further predation. Dried secretions glued the ecdysial cleavage line (epicranial suture), from which the cuticle splits when an insect molts (Simpson 2013), causing molting failure and death; or gummed up the larvae's mouthparts; or glued them to their prey, causing death by starvation. In contrast to alarm pheromones, which are very volatile and may disperse quickly, sticky droplets have long-term mechanical effects on the predator if daubed on sensitive body parts such as head, mouthparts, and antennae. In the present study, such daubing mostly caused the death of 1st and 2nd instar larvae of both predator lady beetles. Similarly, cornicle secretions of the soybean aphid, *Aphis glycines* Matsumura are deadly to *Orius insidiosus* Say (Hemiptera: Anthocoridae) (Butler and O'Neil 2006), and smearing of cornicle secretions by a cereal aphid, *Sitobion avenae* (Fabricius) killed *Aphidius rhopalosiphi* de Stefani-Perez (Hymenoptera: Braconidae) (Wu et al. 2010).

The cornicle secretions were less effective against 3rd and 4th instar larvae. One reason may be that as predator size increase, more secretions are needed. Another may be that the predators learn to cope with the defense strategy. For example, the hypothesis of alternation between acceptance and aversion proposed by Toft (1997) may explain how later-stage larvae avoid the aphid defense by feeding intermittently on few aphids.

Although the lady beetle larvae seized the prey aphids upon encounter, some 3rd and 4th instar larvae of *C. septempunctata bruckii* released their prey and vomited a few minutes later. This vomiting behavior indicates that the goldenrod aphid is an emetic prey (Dixon 1958), which poses a high cost on predators (Higginson et al. 2011). Such toxicity is generally due to either

sequestration of toxins from host plants or *de novo* synthesis of toxins as a second-line defense against predators (Fukunaga and Akimoto 2007) and as a first-line defense, toxic prey display warning coloration (aposematism). However, only one 2nd instar larva of *P. japonica* vomited after preying on this aphid; this result suggests that larvae of *P. japonica* are more able to prey on the goldenrod aphid.

The experimental arena can enforce certain behaviors that are difficult to confirm under natural conditions. However, my field survey results agreed with the laboratory results. In particular, high proportions of 1st and 2nd instar larvae were covered with secretions or were glued to prey. Thus, the cornicle secretions observed on the predators in the laboratory were not an artifact caused by the arena size. The laboratory results therefore suggest that substantial part of 1st and 2nd instar larvae will die when preying on the goldenrod aphid in the field too.

In conclusion, the results reveal that smearing by cornicle secretions plays an important role in protection of aphid colonies against early-stage larvae of lady beetles, and that this aphid is emetic to 3rd and 4th instar larvae of *C. septempunctata bruckii*. In addition, the deadly effects of the secretions may support the inclusive fitness of clonal colonies of the goldenrod aphid through increasing the reliability of the conspicuous warning coloration.

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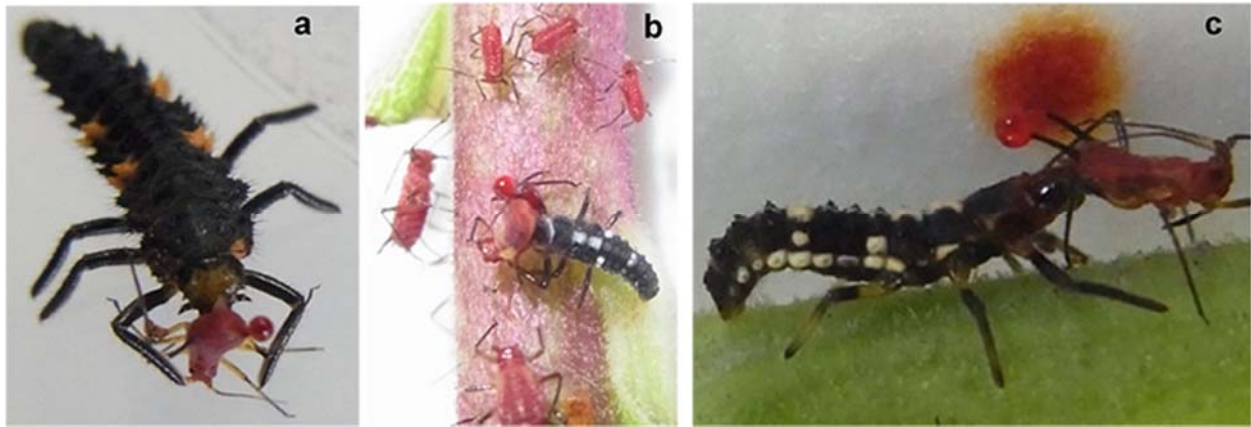
Figure 3-1 Secretion of red droplets from cornicles of the goldenrod aphid attacked by (a) *C. septempunctata bruckii* and (b and c) *P. japonica*
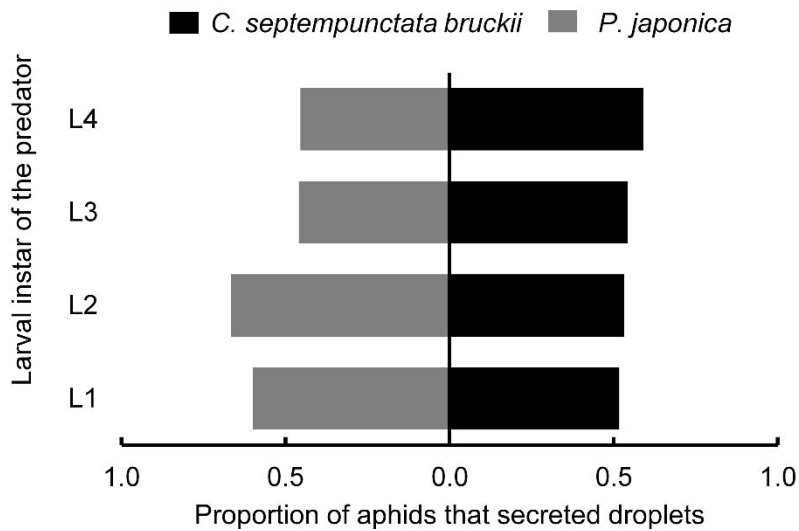
Figure 3-2 Comparison between the responses by the goldenrod aphid to attacks by predators, *C. septempunctata bruckii* and *P. japonica* at each larval instar. The defensive responses at each larval instar were not significantly different (GLM with binomial error distribution, $p > 0.05$, n = 30 for each larval instar)
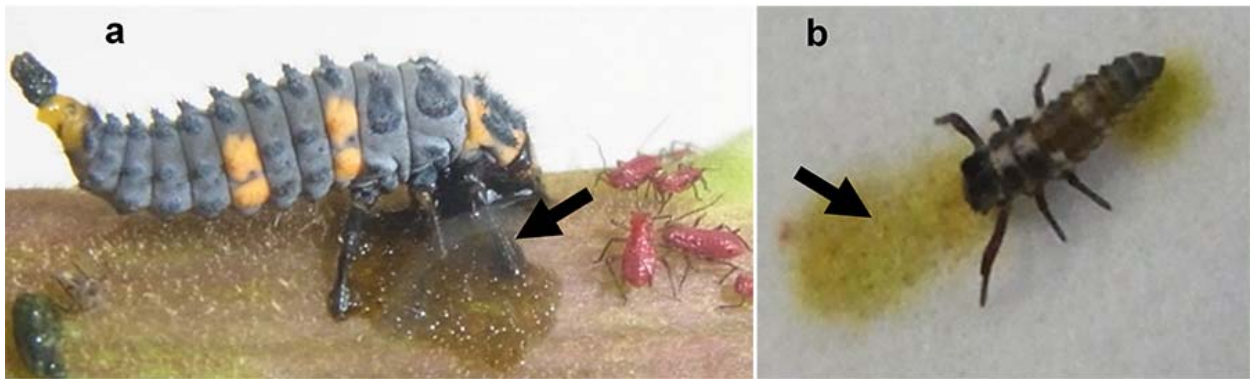
Figure 3-3 Lady beetle larvae vomiting after consuming 1st prey of the goldenrod aphid: (a) 4th instar larva of *C. septempunctata bruckii*; (b) 2nd instar larva of *P. japonica.* Arrows show vomit.
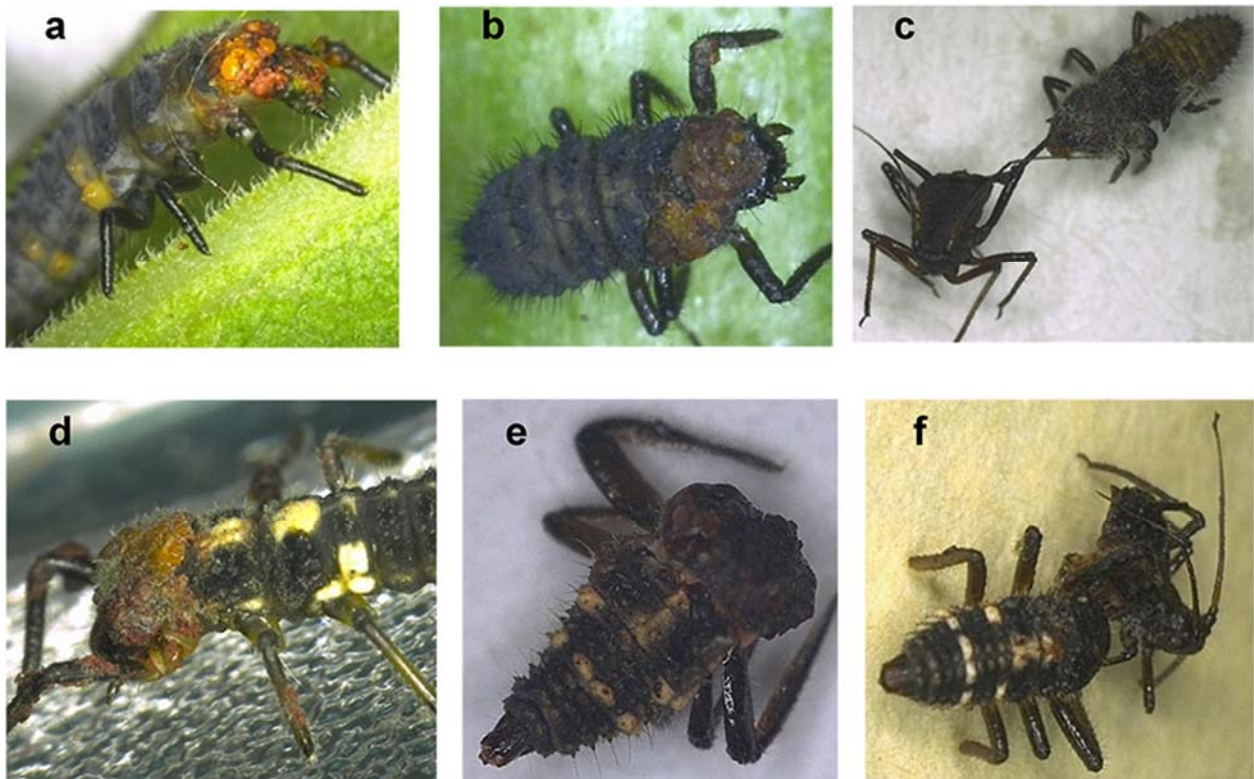
Figure 3-4 Effects of the goldenrod aphid secretion on larvae of (a-c) *C. septempunctata bruckii* and (d-f) *P. japonica:* (a, d) head smeared with defensive secretion; (b, e) dead larvae with dried smearing on their heads; (c, f) aphid stuck on predator's mouthparts
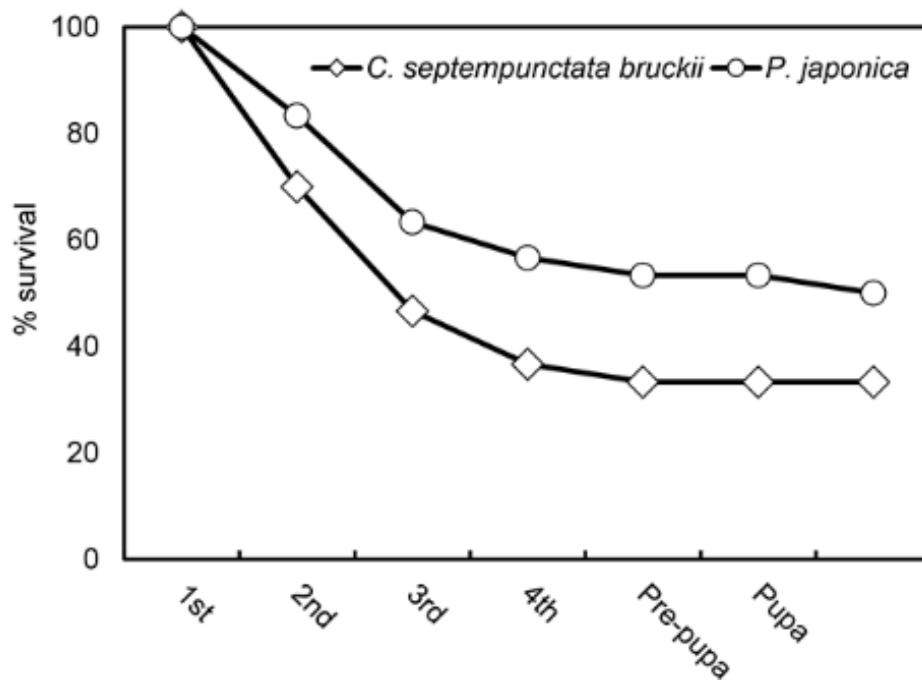
Figure 3-5 Percentage survival of *C. septempunctata bruckii* and *P. japonica* larvae that preyed on the goldenrod aphid. There was no significant difference on the survival of these lady beetles species (Long-Rank test, $p > 0.05$, n = 30 for each species)
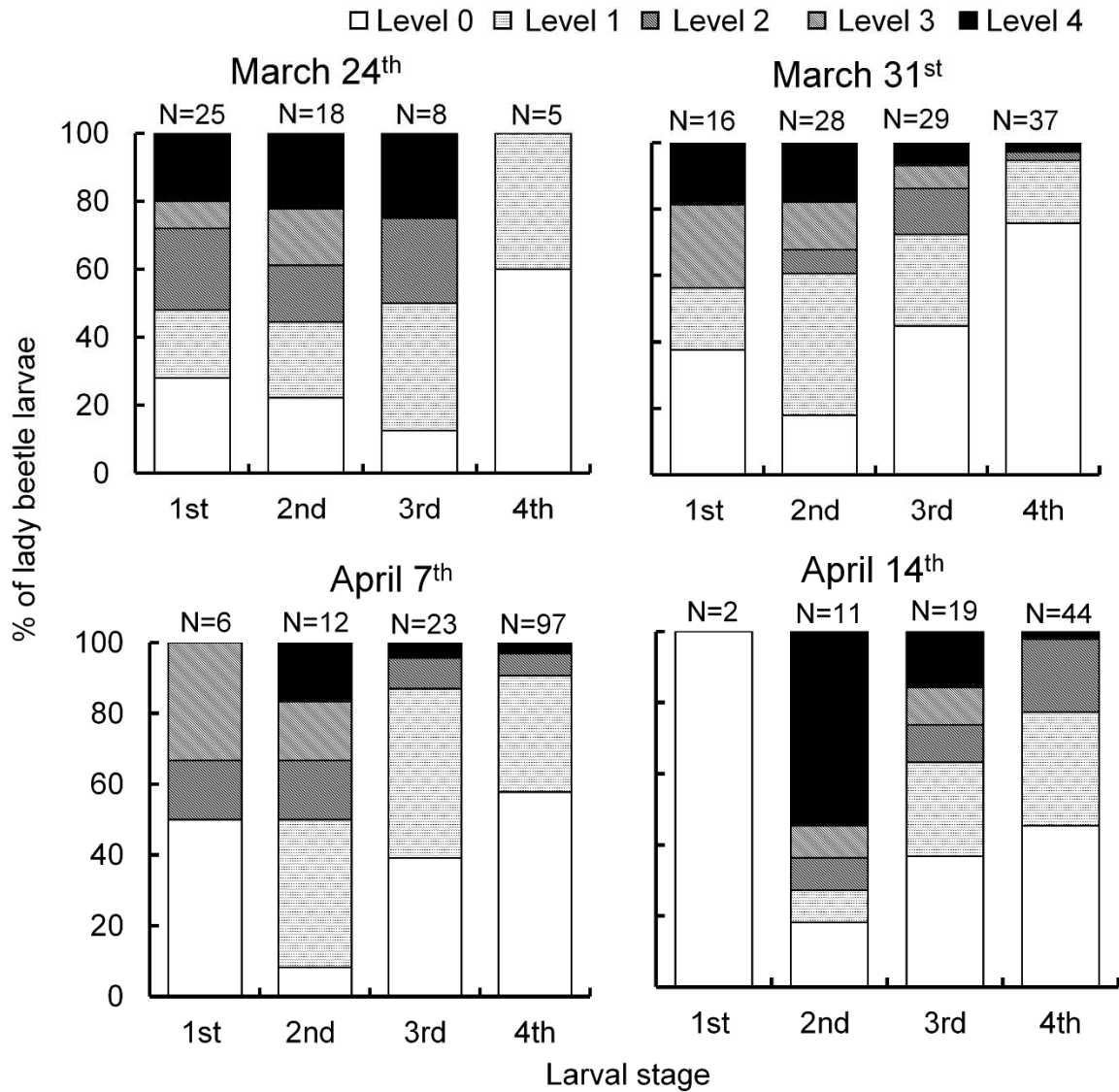
Figure 3-6 Percentage of *C. septempunctata bruckii* larvae with different levels of smearing by the goldenrod aphid secretions in a Solidago patch in 2015: Level 0 no secretions; level 1 few secretions on the head; level 2 thin smearing on the head, level 3 thick smearing on the head; level 4 prey was stuck to the predator

Table 3-1 Summary of generalized linear model with multinomial error distribution of the effects of larval stage on the frequency of each smearing level by the goldenrod aphid

Factor	Estimate	SE	z value	p
Intercept 1	-1.02	0.27	-3.81	<0.0001
Intercept 2	0.41	0.26	1.56	0.1189
Intercept 3	1.17	0.27	4.23	<0.0001
Intercept 4	1.77	0.30	6.07	<0.0001
Second instar	-0.49	0.33	-1.46	0.1447
Third instar	0.53	0.33	1.62	0.1061
Fourth instar	1.42	0.30	4.75	<0.0001

Chapter 4 Alterations in foraging behavior of *Coccinella septempunctata* and *Propylea japonica* mediated by a novel defended prey affect their predatory potential [3]

Abstract

Generalist aphidophagous coccinellids have to cope with novel prey aphids in environments that are changing rapidly because of anthropogenic activity. The goldenrod aphid, *Uroleucon nigrotuberculatum* is a noxious prey, which was recently introduced into Japan. It is a major prey for the native Japanese predators *Coccinella septempunctata bruckii* and *Propylea japonica* in early summer on patches of Solidago. Prey preference of these coccinellids between this aphid and a native suitable prey aphid, *Megoura crassicauda*, as well as their foraging behavior were studied for a better understanding of the negative impacts of the noxious novel aphid. *Coccinella septempunctata bruckii* initially preferred the novel prey, but later on switched to the native prey, *M. crassicauda*; *P. japonica* had no preference for either prey. Feeding time of the first instars of *C. septempunctata bruckii* on *U. nigrotuberculatum* was significantly longer than that of *P. japonica*. The foraging bouts of fourth instars of *C. septempunctata bruckii* were significantly longer on *U. nigrotuberculatum* than on *M. crassicauda*, whereas only the feeding time of fourth instars of *P. japonica* on *U. nigrotuberculatum* was significantly longer than on *M. crassicauda*. Both coccinellids consumed less of *U. nigrotuberculatum* than of *M. crassicauda*. Thus, Solidago patches infested by *U. nigrotuberculatum* may present an ecological trap, especially for *C. septempunctata bruckii*.

Introduction

Generalist aphidophagous coccinellids prey on aphids, which vary widely in suitability (Hodek 1993), nutritional value (Blackman 1967), and passiveness or vulnerability (Dixon 2000). They also have to cope with the introduction of novel exotic prey aphids in their native habitats, particularly in environments undergoing rapid change mediated by anthropogenic activities. Several aphid species have been introduced unintentionally into various regions, where some of them have become invasive. For instance, the goldenrod aphid, *Uroleucon nigrotuberculatum* (Oliver) (Homoptera: Aphididae), has been introduced into Japan from North America in the early 1990s (Sugimoto and Matsumoto 2000) and soybean aphid, *Aphis glycines* Matsumura (Homoptera: Aphididae), into North America probably from Japan in 2000 (Venette and Ragsdale 2004). These introduced aphid species are abundant prey resources and can alter and establish predator–prey interactions with the native aphidophagous coccinellids.

Newly introduced exotic prey species often lacks any evolutionary history with native predators. Therefore, for native predators to include the new prey in their diet and exert biotic resistance against its invasion, the prey must be (1) suitable and similar to native prey; and (2) close to the feeding capability of the native predators, though it may impose some behavioral adaptations on the predators (Carlsson et al. 2009). In addition, under certain circumstances predators switch to the novel prey when it is very abundant or when other prey items are scarce (Jaworski et al. 2013). However, when the new exotic prey is toxic and delivers cues similar to those of a suitable and familiar prey (i.e., deceiving cues), it may become an ecological trap for native predators that fail to undergo the necessary behavioral adaptations (Robertson et al. 2013). Ecological traps are

defined as a low-quality habitat for reproduction and survival, which are wrongly preferred over other available habitats of higher quality (Donovan and Thompson 2001). It has adverse effects on native predators because they fail to soundly select for resources by relying on the cues used to make optimal decisions.

In Japan, goldenrod plant, *Solidago altissima* L. (Asteraceae) (hereafter Solidago) was introduced from North America a century ago, since when it has become the most noticeable exotic plant in the country and forms pure stands in various ecosystems (Takafuji 1980). Since its arrival, *U. nigrotuberculatum* has heavily infested Solidago patches and it has become the main herbivore on Solidago. It plays an important role in shaping the arthropod communities on Solidago patches (Ando et al. 2011). The goldenrod aphid is a large red aphid that emerges from overwintered egges in early March and disappears by early August. It is a noxious prey, as it causes high larval mortality and delayed development of *Coccinella septempunctata bruckii* Mulsant and *Propylea japonica* (Thunberg) (Coleoptera: Coccinellidae) (Barry and Ohno 2015), and *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae) (Kamo and Tokuoka 2011). During early summer (May -July), however, aphidophagous coccinellids are found preying on *U. nigrotuberculatum* in the Miyazaki area of southern Japan, when other aphid species are scarce (Barry, pers. obs.). Although *P. japonica* has a smaller body than *C. septempunctata bruckii*, which limits its prey range (Sloggett 2008), more of its advanced larval stages and pupae were observed in Solidago patches than *C. septempunctata bruckii*, and the larvae were seen to prey on *U. nigrotuberculatum*.

According to the foraging theory of Stephens and Krebs (1986) a generalist predator preferentially utilizes the most profitable prey to maximize its fitness gain. Consequently, if a less profitable prey aphid species or one that is able to defend itself, becomes abundant in a habitat, the

generalist aphidophagous lady beetles must alter their foraging behavior to achieve maximum fitness. Thus, when a predator encounters a novel prey, its fitness gain will depend on its behavioral plasticity for efficient utilization of the new resource or on its innate pre-adaptation attributes (Colares et al. 2015; Tapajós et al. 2016). Although *C. septempunctata* and *P. japonica* are both generalists, *C. septempunctata* thrives mainly on a varied range of aphid species (Hodek and Evans 2012) whereas *P. japonica* can successfully develop and reproduce not only on varied aphid species, but also on other soft bodied insect taxa like whiteflies (Zhang et al. 2007).

Reproductive success of aphidophagous lady beetles depend on the reproduction site choice made by gravid females (Kindlmann and Dixon 1993; Seagraves 2009). Although *U. nigrotuberculatum* inhibits reproduction of *C. septempunctata bruckii* and drastically reduces fecundity of *P. japonica* (Barry and Ohno 2015), gravid beetle females may lay eggs in Solidago patches heavily infested by *U. nigrotuberculatum*. Because the foraging range of the larvae is very limited, they are likely to be more vulnerable to the adverse effects of unsuitable prey. Thus, the interaction between lady beetle larvae and *U. nigrotuberculatum* will depend not only on prey density but also on prey trait (anti-predator traits) (Abrams 2008).

Most studies of new predator–prey trophic interactions have focused on the prey's antipredator, behavioral, and morphological adaptations (e.g., Blake and Gabor 2014; Berthon 2015) and have overlooked or ignored prey-mediated alterations in predator behavior. The reason for this negligence is centered on the tenet of the "life-dinner" principle (Dawkins and Krebs 1979), which purports that the selection pressure on prey is stronger than that on predators. However, if a predator prefers a novel and toxic prey, or is forced under certain environmental conditions to prey on a deadly prey, it is confronted with an ecological trap and it might face the same selection

pressure as a prey. This could be the case for the interaction between aphidophagous coccinellids and the goldenrod aphid in Japan. To test the trap effect of Solidago patches on the native Japanese lady beetles, we studied the prey preferences of the fourth instars of both coccinellids on the novel and noxious prey, *U. nigrotuberculatum* and the suitable native vetch aphid, *Megoura crassicauda* Mordvilko (Homoptera: Aphididae). Barry and Ohno (2015) have reported high performance of *C. septempunctata bruckii* and *P. japonica* on *M. crassicauda*, which is a main prey in spring, when, these lady beetles produce their first generation brood after overwintering. I assumed that the foraging behavior of aphidophagous coccinellids, which has been meticulously investigated (Nakamuta 1983; Murakami and Tsubaki 1984; Ide et al. 2007; Finlayson et al. 2009) may be altered to feed efficiently or avoid predation on a novel and unsuitable prey. Therefore, I also investigated the foraging behavior of *C. septempunctata bruckii* and *P. japon*ica larvae.

Materials and methods

Insects and plants

Adults of *C. septempunctata bruckii* and *P. japonica* were collected from vetch, *Vicia angustifolia* L. at the Kibana campus of the University of Miyazaki to establish a laboratory culture. They were reared on vetch aphids, *M. crassicauda* in the laboratory at 23 °C and L14: D10 h photoperiod. Vetch aphid colonies were cultured on seedlings of broad bean, *Vicia faba*. The aphid culture conditions were 25 ± 1 °C and L16: D8 h photoperiod. Goldenrod aphids, *U. nigrotuberculatum* were collected from Solidago patches near Kaeda vallety 31º 49' 19'' N: 131º 24' 51'' E, Miyazaki, Japan for use in the experiments. Larvae obtained from the laboratory population of lady beetles were used in the following experiments.

Prey preferences

One fourth instar of either *C. septempunctata bruckii* or *P. japonica* that had been starved for 24 h was introduced into an insect breeding dish (100 mm diameter, 40 mm high) containing 60 aphid nymphs (first to third instars) of *M. crassicauda* and *U. nigrotuberculatum* (30 of each). The dish contained no host plant material, establish a random distribution of prey aphids in the arena. I chose the fourth instar because it is the most voracious lady beetle stage. For each coccinellid species, 72 replications were observed. The number of aphids consumed of each prey species was recorded throughout the first 15 min after introduction (early feeding trial), after which the insects were kept in an incubator (23 °C) for 4 h, and then the number of each prey aphids consumed by each larvae was recorded (late feeding trial). Consumed prey aphids were not replaced in the course of the experiment. Preferences between the two prey aphids in the early and late feedings, as well as any switches in preference, were tested by using Manly's preference index (Manly et al. 1972), which is widely used to assess the prey preference by a predator (e.g. Lucas et al. 1997; Soares et al. 2004; Zuharah and Lester 2011) and takes into consideration prey depletion in the course of the experiment. it is given by:

$$\beta_i = \frac{\log p_i}{\sum_{j=1}^{m} \log p_j}$$

where β_i is Manly's beta for prey type i; p_i is the proportion of prey i remaining at each recording time relative to the original input (i = 1, 2, 3, 4, … , m); p_j is the proportion of all prey types together remaining at the recording time relative to the original input (j = 1, 2, 3, 4, …. , m); and m is the number of prey types.

β_i takes values from 0 to 1. In our case, because there is a combination of only two types of prey, $\beta = 0.5$ represents no preference or random sampling of the two prey types. Predators that did not

feed at all during any period of the experiment were excluded from the analysis, because β indices cannot be calculated with zero values.

Foraging behavior on *Uroleucon nigrotuberculatum*

Larvae of *C. septempunctata bruckii* or *P. japonica* at the first, second, third, or fourth instar that had been starved for 24 h were introduced individually into an insect breeding dish (100 mm diameter, 40 mm high) containing 20 *U. nigrotuberculatum* (first to third instar) on a twig of Solidago. For each instar of both species, we observed the foraging behavior of 30 larvae and recorded the time spent in every foraging activity "bout" over 1 h. Observations were conducted at 25 °C and ambient light. The main foraging activities were searching (time spent walking in the breeding dish), feeding (time spent consuming a captured aphid), resting (time spent motionless), and grooming (time spent rubbing the mouthparts or using the front legs to clean up the mouthparts). The number of aphids consumed by every larva of each larval instar was also recorded.

Foraging behavior on *Megoura crassicauda*

Single fourth instar of *C. septempunctata bruckii* or *P. japonica* that has been starved for 24 h was released into an insect-breeding dish containing 20 *M. crassicauda* (first to third instar) on a twig of broad bean. For each lady beetle species, 30 replications were observed for 1 h following the same procedure as in the previous experiment. Foraging bouts (searching time, feeding time, resting time, and grooming time) were recorded as above. These foraging bouts and the number of *M. crassicauda* consumed by each lady beetle species were compared with those of *U. nigrotuberculatum*.

Statistical analysis

The difference in Manly's preference index of the two prey species and the switching preference for each predator species were tested with the Wilcoxon signed rank test because the normality assumption of Student's t-test was not met. Foraging activities of the two ladybird beetle species on *U. nigrotuberculatum* were compared using the Wilcoxon rank test for two samples. The times spent on each foraging bout by fourth-instars of both beetle species on the two prey were compared using Student's t-test, and the number of prey eaten by each coccinellid were compared by using the Wilcoxon rank test for two samples. All analyses were conducted with SPSS ver. 20.0 statistical software (SPSS, Chicago, IL, USA).

Results

Prey preferences

Larvae of *C. septempunctata* preferred *U. nigrotuberculatum* in the early feeding trial, but in the late feeding trial they switched to *M. crassicauda*. Manly's preference index differed significantly between the two prey aphids in both feeding periods (early feeding, $Z = -2.48$, $p = 0.013$; late feeding, $Z = -2.09$, $P = 0.037$; Fig. 4-1a), and the larvae switched their preference between the two feeding periods ($Z = -2.77$, $p = 0.006$; Fig. 4-1a). In contrast, the *P. japonica* larvae preyed randomly on the two prey aphids and showed no preference for either of the prey aphids (early feeding, $Z = -0.263$, $p = 0.793$; late feeding, $Z = -1.057$, $P = 0.291$; Fig. 4-1b), and they did not switch their selection for any aphid between two feeding trials ($Z = -0.284$, $p = 0.776$; Fig. 4-1b).

Foraging behavior on *Uroleucon nigrotuberculatum*

The searching times of first and third instars of *C. septempunctata bruckii* and *P. japonica* were not significantly different (Wilcoxon test, first instars: $Z = -1.310$, $p = 0.190$; third instars: $Z = -0.686$, $p = 0.493$; Fig. 4-2a). However, there were significant differences in searching times between *C. septempunctata bruckii* and *P. japonica*'s second and fourth instars (second instars: $Z = -2.442$, $p = 0.015$; fourth instars: $Z = -2.446$, $p = 0.014$; Fig. 4-2a). The feeding time of first instars of *C. septempunctata bruckii* was significantly longer than that of first instar of *P. japonica* ($Z = -2.04$, $p = 0.044$; Fig. 4-2b). Second instars of *C. septempunctata bruckii* rested significantly longer than those of *P. japonica* ($Z = 2.093$, $p = 0.036$; Fig. 4-2c). We did not observe grooming and resting activities in first instars of *C. septempunctata bruckii*. Fourth instars of *P. japonica* groomed significantly shorter than those of *C. septempunctata bruckii* ($Z = -2.184$, $p = 0.029$; Fig. 4-2d).

Foraging behavior on *Megoura crassicauda*

The searching, feeding, resting, and grooming times of fourth instars of *C. septempunctata bruckii* on *M. crassicauda* were significantly different from those on *U. nigrotuberculatum* (searching: $t = -5.62$, df $= 58$, $p < 0.001$; feeding, $t = 7.67$, df $= 58$, $p < 0.001$; resting, t $= 2.81$, df $= 58$, $p = 0.0078$; grooming, $t = 3.33$, df $= 58$, $p = 0.003$; Fig. 4-3A). For fourth instars of *P. japonica* feeding on *M. crassicauda* and *U. nigrotuberculatum*, the only significant difference was between feeding times ($t = 3.32$, df $= 58$, $p = 0.0016$; Fig. 4-3B). Fourth instars of both *C. septempunctata bruckii* and *P. japonica* consumed significantly more *M. crassicauda* than *U. nigrotuberculatum* (Wilcoxon signed rank test, *C. septempunctata bruckii*: $Z = -4.80$, $p < 0.001$; *P. japonica*: $Z = -2.43$, $p =$

0.015; Fig. 4-4).

Discussion

The coccinellid larvae accepted and consumed *U. nigrotuberculatum* upon encountering it, and *C. septempunctata bruckii* larvae preferred *U. nigrotuberculatum* over *M. crassicauda* during the early feeding trial (in the first 15 min) when they were hungry, just after their release into the breeding dishes. However, after 4 h they had switched their preference to the more suitable prey aphid, *M. crassicauda.* This prey switching behavior by *C. septempunctata bruckii* could be a result of feeding experience on the noxious prey, *U. nigrotuberculatum* and is consistent with the behavior of *Coleomegilla maculata* ssp. *lengi* Timberlake (Coleoptera: Coccinellidae) on a mixed diet of high and low quality food (Boivin et al. 2010). In contrast, the euryphagous *P. japonica* larvae preyed randomly on both prey aphids regardless of their hunger level, because *P. japonica*, as a typical generalist, should be less selective for prey and should have more opportunistic feeding habits (Symondson et al. 2002). Nevertheless, Solidago patches may attract *P. japonica* adults in early summer because of their preference to lay eggs in patches with high density of young prey aphids (Ouyang et al. 2012).

Several factors affect prey preference of natural enemies. They include prey relative abundance (Desneux and O'Neil 2008), olfactory cues (Wang et al. 2015), color (Harmon et al. 1998), nutritional value (Ferrer et al. 2008), and escape or defensive responses (Provost et al. 2006). The aulica phenotype of *H. axyridis* preferred *Myzus persicae* Sulzer over *Aphis fabae* Scopoli at different relative abundance (Soares et al. 2004). Both *C. septempunctata bruckii* and *P. japonica* use visual cues scantily and perceive prey aphids only at a distance of up to 7 mm (Nakamuta

1984; Wang et al. 2015). In addition, *C. septempunctata* did not choose between green and red color morphs of the pea aphid, *Acyrthosiphon pisum* Harris, on a white background (Harmon et al. 1998). Therefore, the switching preference of *C. septempunctata bruckii* larvae for the green aphid, *M. crassicauda*, over the red aphid, *U. nigrotuberculatum*, may be based on a difference in nutritional quality and defensive response rather than only on relative prey abundance or color.

The decision of a predator to reject a toxic prey depends on the level of toxin in its body (i.e., a predator should be more reluctant to attack a noxious prey after having fed on it (Sherratt 2003)). This corroborates my results in so far as *C. septempunctata bruckii* larvae used in the prey preference experiment had been starved for 24 h, and thus their high levels of hunger might have predisposed them to choose the most conspicuous prey, even if it was toxic. However, there is a discrepancy in the literature regarding prey choice by coccinellids. In some reports coccinellids have chosen the less profitable or toxic prey when provided with a mixed diet of suitable and unsuitable or toxic prey (Blackman 1967; Nedvêd and Salvucci 2008), whereas in other reports they have chosen the more suitable prey or did not prefer either (Blackman 1967; Lucas et al. 1997; Dutra et al. 2012). In the present study, experience and prey relative abundance may have played an important role in switching preference of *C. septempunctata bruckii* larvae.

Foraging bouts of a predator depend on prey features, species, mobility, size and defensiveness (Provost et al. 2006). The foraging bouts of *C. septempunctata bruckii* larvae on *U. nigrotuberculatum* were considerably prolonged, especially in the case of first instars, which spent almost 1 h for feeding on a single prey of this aphid and thus did not display resting and grooming activities. Except for the first instar, the various larval stages of *P. japonica* spent similar amounts of time compared with those spent by *C. septempunctata bruckii* feeding on *U. nigrotuberculatum.*

Thus, in consideration of their relative body weights, the larger *C. septempunctata bruckii* must have gained less net energy than the smaller *P. japonica* when both were preying exclusively on this aphid because, as suggested by Sloggett (2008), larger predators consume more prey of the same size than do smaller predators and implicitly have shorter feeding bouts.

It will take predators more time to forage on a toxic or defended prey than on a suitable one due to difficulties subduing the prey and the effects of anti-feeding toxin. Consistently, fourth instar larvae of *C. septempunctata bruckii* consumed four times as many of the native and suitable prey aphid, *M. crassicauda,* as of the novel and noxious *U. nigrotuberculatum.* However, *C. septempunctata bruckii* larvae spent shorter searching time on *U. nigrotuberculatum*, which could be attributable to the fact that *U. nigrotuberculatum* is (1) a conspicuous aphid that is easy to detect (Mappes et al. 2005) and (2) less prone to escape from predator presence (Uematsu et al. 2013) than is a suitable prey such as *M. crassicauda*. The short searching time did not affect the lengthy total time spent foraging on the unsuitable prey; therefore, as expected, *C. septempunctata bruckii* consumed fewer of the noxious aphids. In contrast, the foraging bouts of the fourth instars of *P. japonica* were similar on both *M. crassicauda* and *U. nigrotuberculatum*, with the exception of a significantly longer feeding time on *U. nigrotuberculatum*. Because feeding time is usually the longest foraging bout and its duration markedly affects the number of prey aphids consumed by coccinellids, ultimately, *P. japonica* consumed significantly fewer aphids of *U. nigrotuberculatum* than of *M. crassicauda*. The capability of *P. japonica* to feed successfully on various prey taxa (Hamasaki and Matsui 2006; Zhang et al. 2007) might explain the similarity of its foraging bouts on the various prey aphids with different nutritional values and defensiveness.

In conclusion, larvae of *C. septempunctata bruckii* preferred *U. nigrotuberculatum* initially but

switched to *M. crassicauda*, whereas *P. japonica* larvae did not show any prey preference. Because Solidago patches infested with *U. nigrotuberculatum* occur naturally in early summer, when other suitable habitats such as vetch patches infested with aphids such as *M. crassicauda*, *Aphis craccivora* Koch, and *A. pisum* are depleted, adult coccinellids may immigrate to Solidago patches. As *U. nigrotuberculatum* is unsuitable for their offsprings (Barry and Ohno 2015), they thus fall into an ecological trap. The prolonged feeding bouts of both *C. septempunctata bruckii* and *P. japonica* larvae on *U. nigrotuberculatum* significantly reduced their prey consumption compared with that *on M. crassicauda*. My results suggest that *C. septempunctata bruckii* may be affected more by the ecological trap effects of Solidago patches infested with *U. nigrotuberculatum* in Japanese agro-ecosystem than *P. japonica*. Field studies on habitat preference to obtain more information on the trap effects of Solidago patches infested with *U. nigrotuberculatum* are needed to consider appropriate conservation measures for these coccinellids.

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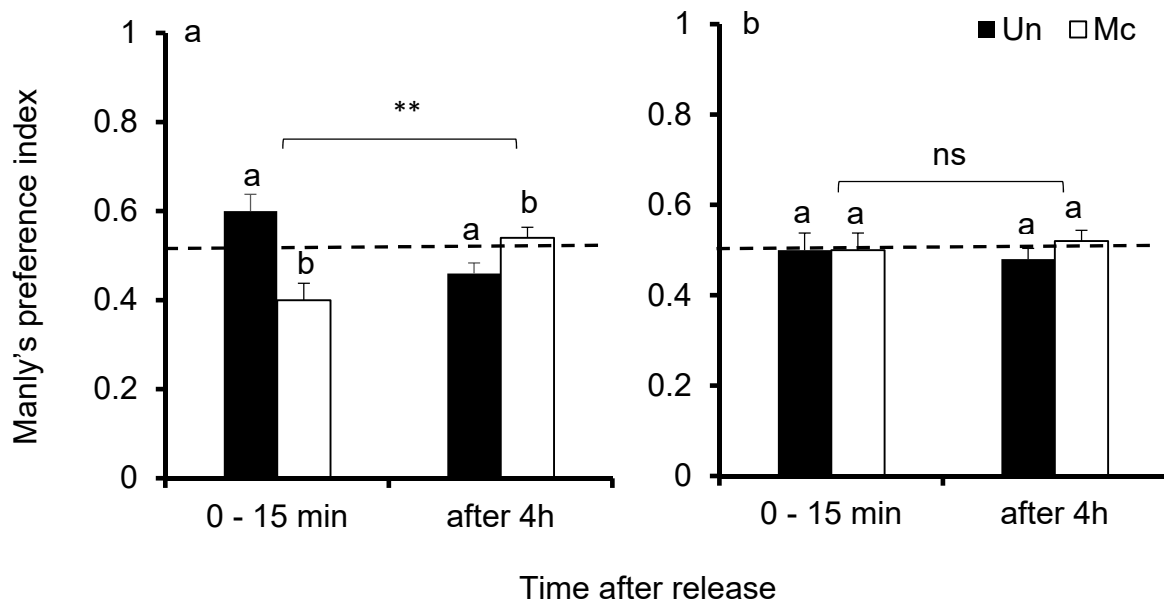
Figure 4-1 Manly's preference index (mean + SE) of fourth instars of (a) *Coccinella septempunctata bruckii* and (b) *Propylea japonica* preying on mixed prey aphids [30 *Uroleucon nigrotuberculatum* (Un); and 30 *Megoura crassicauda* (Mc)]. Means within a period capped with different letters indicate significant differences between prey aphids, and asterisks indicate a switching of preference between the two periods (Wilcoxon signed rank test: **$p < 0.01$; ns: no significant difference)
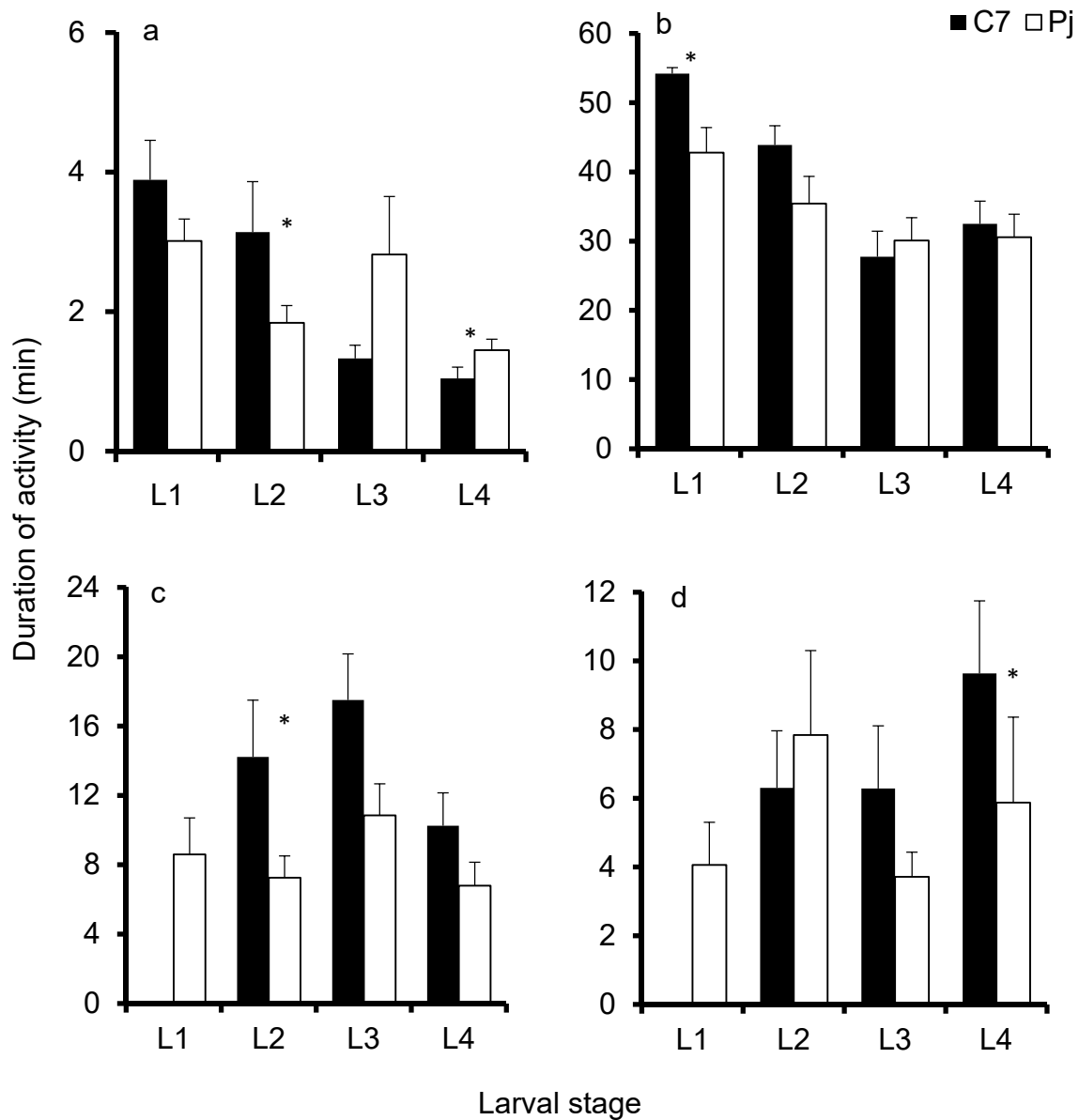
Figure 4-2 Mean (+ SE; n = 30) durations (min) of the main foraging activities of various larval stages of *Coccinella septempunctata bruckii* (C7) and *Propylea japonica* (Pj) on *Uroleucon nigrotuberculatum.* (A) searching, (B) feeding, (C) resting, and (D) grooming. Asterisks indicate significant difference between predator species (Wilcoxon rank test; *p* < 0.05)
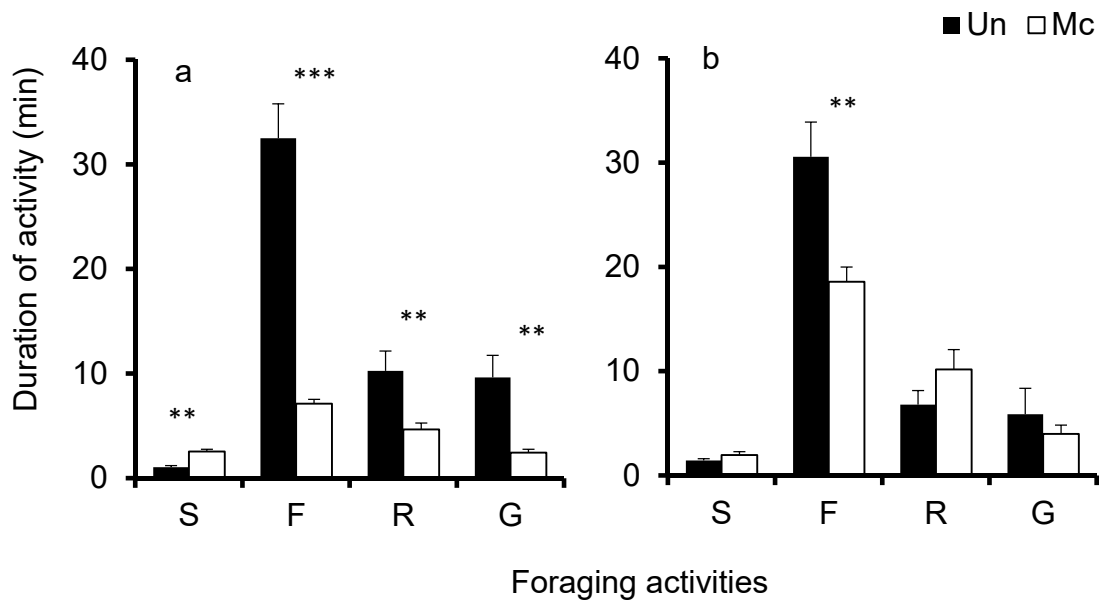
Figure 4-3 Mean (+ SE; n = 30) durations (min) of the main foraging activities (S, searching; F, feeding; R, resting; and G, grooming) of (a) *Coccinella septempunctata bruckii* and (b) *Propylea japonica* fourth instar larvae preying on *Uroleucon nigrotuberculatum* (Un) and *Megoura crassicauda* (Mc). Asterisks above bars denote significant differences between prey aphids (Student's *t*-test; ***$p < 0.001$; **$p < 0.01$)
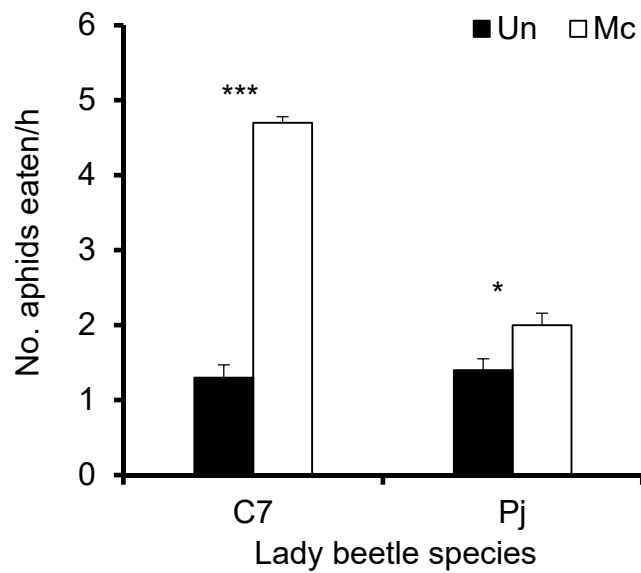
Figure 4-4 Mean (+ SE; n = 30) numbers of each prey eaten in 1 h (Un, *U. nigrotuberculatum* or Mc, *M. crassicauda*) by fourth instars of *C. septempunctata bruckii* (C7) or *P. japonica* (Pj). Asterisks indicate a significant difference between the two prey aphids (Wilcoxon rank test; ***$p < 0.001$ and *$p < 0.05$).

Chapter 5 Seasonal occurrence, population dynamics of *Coccinella septempunctata bruckii, Propylea japonica* and the ovarian dynamics of *C. septempunctata bruckii* in vetch and Solidago patches

Abstract

Lady beetle population dynamics depend on the prey aphid availability and quality in the natural habitat. Complex and various landscape supports abundant and diverse lady beetle communities. I surveyed the population of both *Coccinella septempunctata bruckii* Mulsant, *Propylea japonica* (Thunberg) (Coleoptera: Coccinellidae) and the prey aphids in vetch patches as well as in Solidago patches, I also measured the females' weight and size, and observed ovarian dynamics of *C. septempunctata* in vetch and Solidago patches. Vetch aphid, *Megoura crassicauda* Mordvilko occurred in vetch patches from mid-March until mid-April, while the goldenrod aphid, *Uroleucon nigrotuberculatum* infested Solidago patches from early-May till the early-June. The aphid density in the same patch changed from year to another. The population of lady beetles in vetch patches was almost composed of adults and larvae of *C. septempunctata bruckii*. Both adults and larvae of *C. septempunctata bruckii* were dominant in Solidago patches in early May but *P. japonica* became dominant from mid-May. The size and weight of *C. septempunctata bruckii* females collected from vetch patches were significantly greater than those from Solidago patches. The ovarioles of *C. septempunctata bruckii* collected from vetch patches in March were developed and matured while those in April were undeveloped. The ovarioles of *C. septempunctata bruckii* from Solidago patches in late May were undeveloped, indicating that Solidago patches are not be suitable for the reproduction of *C. septempunctata bruckii*.

Introduction

Diverse and complex natural vegetation around arable lands is a source of natural enemies in the crop fields. For instance, the population dynamics of aphidophagous lady beetles depend mainly on presence and abundance of prey aphids in the natural vegetation. The lady beetle adults move continuously between patches searching for prey aphids, which act as attractant and arrestant for the immigrant lady beetle adults (e.g., Purandare and Tenhumberg 2012; Vandermoten et al. 2012). The residence period of lady beetle adults in a patch depends on prey aphid abundance (e.g., Costamagna and Landis 2007) that increasing the natural habitat around the arable land augments the abundance of lady beetles (Woltz et al. 2012), which can be attained through habitat manipulation and ecological engineering. However, anthropogenic activities and invasion of exotic plants may simplify the natural vegetation in the boundaries of crop fields and decreases the biological control service provided by the native natural enemies.

Introduction of an invasive plant into an agroecosystem alters the trophic link of the local arthropod communities through various ways. It may provide an alternative host for native herbivores or set favorable condition for the invasion of its specialist herbivore from their geographical area of origin (Harvey and Fortuna 2012). For example, introduced Solidago into Japan from North America has been established and forms an important part of the non-crop vegetation in the agroecosystem (Mito and Uesugi 2004). This plant was followed by its specialist aphid, the goldenrod aphid *Uroleucon nigrotuberculatum* in early 1990s (Sugimoto and Matsumoto 2000), consequently Solidago patches form a prey rich habitat for the aphidophagous lady beetles such as *Coccinella septempunctata bruckii* and *Propylea japonica*.

Overwintered adults of *C. septempunctata bruckii* breed their first generation in March, which estivates from June to August, and may reproduce a second generation from September, while *P. japonica* occurs late April and does not estivate, thus it can breed more than 2 generations in a year in Japan (Kawauchi 1997). Vetch patches are the main breeding habitat for the overwintered adults of *C. septempunctata bruckii*, but the newly emerged adults of the first generation and overwintered adults of *P. japonica* are forced by the shortage of prey in vetch patches late April to immigrate into new and prey rich patches. Solidago patches infested with *U. nigrotuberculatum* attract the immigrant lady beetles. However, Kamo and Tokuoka (2011) reported that *U. nigrotuberculatum* has negative effects on *Harmonia axyridis* (Pallas). To evaluate the negative impact of Solidago patches infested with *U. nigrotuberculatum* on population dynamics of *C. septempunctata bruckii* and *P. japonica* in the field. I surveyed the population of both *C. septempunctata bruckii* and *P. japonica* in vetch patches in 2014 but for two seasons 2013 and 2014 in Solidago patches, and I compared the weight, size and ovarian dynamics of *C. septempunctata bruckii* females from vetch and Solidago patches.

Materials and methods

Aphids and lady beetles in vetch and Solidago patches

Lady beetles, *C. septempunctata bruckii* and *P. japonica* occurrence in vetch patches was surveyed from March to late April around Kibana campus at (31º 49' 60''N: 131º 24' 73''E and 31º 49' 22''N: 131º 24' 43''E) in 2014. A twig of 4 cm length was cut off gently into an insect breeding dish (100 mm diameter and 40 mm high) and the number of aphids and alfalfa weevil larvae, *Hypera postica* were counted. Randomly, 17 to 33 plants of vetch were selected depending on the

size of the patch. The number of each of *C. septempunctata bruckii* and *P. japonica* adults and larvae per plant were counted. The adults were counted while standing nearby the vetch plant at a position from which the present lady beetle adults were not disturbed, but the larvae were counted by checking thoroughly the vetch plants.

In Solidago patches infested with *U. nigrotuberculatum*, the lady beetles occurrence was surveyed weekly from early May to early July in 2013 and 2014. In 2013, a Solidago patch near Kiwara field at (31º 50' 45" N: 131º 24' 6" E) Miyazaki, Japan. In 2014, two Solidago patches were surveyed weekly: one at Kibana campus, University of Miyazaki, Miyazaki, Japan and another near Kiwara field at the same position as 2013. Aphid infestation was assessed on every survey day by selecting randomly a hundred plants of Solidago along a 100 m transect and the percentage of infested plants were calculated. The patch quality was evaluated based on the aphid infestation level. Four predefined levels of infestation were used in this survey: level "0" when a plant is free of aphid; level "1" when only few aphid adults are present and just they are initiating reproduction; level "2" when a plant is infested with many young aphid nymphs (young colonies) and only few apterous adults; and level "3" when a plant is heavily infested with aphid nymphs and apterous adults with few alate aphids. On each survey day, the percentage of each aphid infestation level was calculated.

Visual census of adults and larvae of both *C. septempunctata bruckii* and *P. japonica* in the Solidago patches was conducted by standing near to a randomly selected plant and with a minimum possible disturbance of the present lady beetle adults; I counted their number by species and then we checked meticulously for the presence of larvae and recorded their number by species. This procedure was repeated with 100 plants along transects of 100 meters.

Body weight, size and ovarian dynamics of *C. septempunctata bruckii* adults in vetch and Solidago patches

Adult females of *C. septempunctata bruckii* were collected from vetch patches in March to April 2015 and from Solidago patches in late May 2015. Sex of the collected lady beetles were confirmed in the laboratory by checking the ventral side of the last abdominal segment under a loupe (Lens 18X), then they were separated into small vials (4cc) (One female per vial). The lady beetles' fresh body weight was measured by using an electronic balance (precision 0.1mg). After that they were killed and preserved in 70 % alcohol solution until dissection. Prior to dissection, the body length and body width were measured under a digital microscope. The length was expressed as the distance between the head cap and the distal tip of the elytron, while the width was expressed as the ventral width of the largest segment which is the third thoracic segment and the first abdominal segment. The body size, expressed as the ventral area, was calculated using an equation for an ellipse area [$\pi \times 1/2$ (body length) $\times 1/2$ (body width)] (Phoofolo et al. 2007). The females were dissected under binocular in a glass petri dish of (90 mm diameter) containing 0.9% saline solution. The ovaries were cautiously detached off from the female abdomen and gently placed in the saline solution. The matured ovarioles and developing oocytes were counted and the reproductive status of the different populations was observed through the ovarian dynamics. I followed the classification of Sakurai et al. (1983) - there are 5 oogenic stages for *C. septempunctata bruckii* oogenesis- to compare the reproductive status of the two populations. Stage 1 is undeveloped follicle, stage 2 is growing follicle, stage 3 is the pre-vitellogenic, stage 4 is the mid-vitellogenic and stage 5 is the post-vitellogenic and mature eggs.

Statistical analysis

The number of females of *C. septempunctata bruckii* collected from the same habitat at different dates (March 13th and 20th in vetch; May 20th and 25th in Solidago) was pooled to form the sample collected from that given habitat (vetch patches or Solidago patches) because there was no significant different between them. The difference in the weight, size and the number of ovarioles between *C. septempunctata bruckii* females collected from vetch and those collected from Solidago was analyzed by Student's *t*-test for two independent samples. All tests were performed by using SPSS 20.

Results

Aphids and lady beetles in vetch and Solidago patches

Vetch aphid occurred in vetch patches from late March until late April. In early March, the vetch patches were infested mainly with alfalfa weevil larvae. *Coccinella septempunctata bruckii* was the dominant species throughout the survey period and the number of sampled *C. septempunctata bruckii* adults and larvae are shown in table 5-1. They were observed preying on the alfalfa weevil larvae before the occurrence of vetch aphid in vetch patches. However, only few adults and larvae of *P. japonica* were observed in the vetch patches during the survey period.

On May 8th in 2013, the aphid, *U. nigrotuberculatum* was observed in the Solidago patches (Fig. 5-1A). Most of Solidago plants were infested with level "1" and "2" or free from aphids. From May 13th until May 22nd the aphid infestation reached its peak when most plants having level "2" and "3" infestation, but from May 30th aphid colonies started declining drastically so on June

6th the aphid almost disappeared. In 2014 the infestation of Solidago patch in Kiwara field with *U. nigrotuberculatum* was lower than that in 2013 (Fig. 5-1B). On May 8th, 2014 only few plants were infested. From May 15th infested plants with level "1" became dominant. The aphid colonies appeared to collapse after June 5th. However a different intensity of infestation with *U. nigrotuberculatum* was observed in another Solidago patch during the same period at Kibana campus (Fig. 5-1C). From May 4th, the majority of the plants were infested with level "1" and on May 10th the level "2" infestation became dominant. From May 17th, the patch became heavily infested with large colonies of *U. nigrotuberculatum* (level "3"). The aphid colonies collapsed on June 14th (Fig. 5-1).

On May 8th in 2013, *C. septempunctata bruckii* adults were dominant in the Solidago patch in Kiwara field but from May 13th *P. japonica* adults were dominant throughout the survey period. Similarly, the larvae of *C. septempunctata bruckii* were dominant on May 5th and on May 22nd, while on May 13th and from May 30th larvae of *P. japonica* were dominant (Fig. 5-2). In 2014, the lady beetles adults and larvae in Kiwara field were dominated by *P. japonica* throughout the survey period. However, in the Solidago patch at Kibana campus, in May, adults and larvae of *C. septempunctata bruckii* were dominant, but from early June both adults and larvae of *P. japonica* became dominant (Fig. 5-2).

Body weight, size and ovarian dynamics of *C. septempunctata bruckii* adults in vetch and Solidago patches

The females of *C. septempunctata bruckii* sampled from vetch patches were significantly heavier and larger than those sampled from Solidago patches (for fresh weight, $t = 2.21$, $p = 0.03$; for body

size, $t = 2.4$, $p = 0.019$) (Table 5-2). However, the females sampled from both vetch and Solidago patches had same number of ovarioles ($t = -0.6$, $p = 0.57$) (Table 5-2). As shown in Fig. 5-3; the ovariole dynamics of sampled females consisted of 5 different oogenic stages. About 80% of the sampled females from vetch patches in March had matured ovarioles (e.g., stage 4 and 5) and no female with undeveloped ovarioles (stage 1) was sampled (Fig. 5-4). In contrast, in April all the females had undeveloped ovarioles (stage 1). About 95% of the females sampled from Solidago patches in May had undeveloped ovarioles (stage 1), even no female with ovarioles at pre-vitellogenic stage (stage 3) was sampled from Solidago patches (Fig. 5-4).

Discussion

Vetch patches in Japanese agroecosystem are good habitats for the overwintered *C. septempunctata bruckii* from early March because they provide vetch aphid and alfalfa weevil as prey. Despite the alfalfa weevil is not an essential prey for *C. septempunctata* (Hodek and Evans 2012), it may play an important role for sustaining the *C. septempunctata* adults (Evans and Toler 2007), which is advantageous to *C. septempunctata* for better exploiting the egg window (Dixon 2000) by synchronizing their reproduction with the initial phase of vetch aphid infestation. Consistently, the larvae of *C. septempunctata bruckii* increased when vetch aphid increased in vetch patches. Evans and Gunther (2005) reported that *Harmonia axyridis* includes alfalfa weevil in their diet when aphids are scarce but did not reproduce if preying solely on the weevil larvae. During this period of the year, only very few adults of *P. japonica* were observed in the vetch patches. Despite *P. japonica* generally occurs later than *C. septempunctata bruckii*, it remains

reproductively active throughout the summer unlike *C. septempunctata bruckii* which estivate from June to August (Kawauchi 1997).

Actually, both lady beetle species occur in Solidago patches at the same time but *C. septempunctata bruckii* was predominate in the early aphid infestation. Since *U. nigrotuberculatum* belong to the large sized aphid, the predominance of *C. septempunctata bruckii* in the early aphid colonization could be explained by the postulation of Sloggett (2008) that small lady beetles are unable to feed on large size aphid species at low density. In 2014, the aphid infestation in the Solidago patch was less than in 2013 near Kiwara field but the percentage of young colonies (level 2 infestation) was considerably important. This presence of young aphid colonies might have attracted *P. japonica*. The low number of *C. septempunctata bruckii* adults at the end of aphid infestation suggests that Solidago patches are not a good habitat for reproduction, and that the larvae undergo considerable mortality during larval development.

Prey quality and quantity affects body size and weight of lady beetles adults (Hauge et al. 1998; Nielsen et al. 2002; Ferrer et al. 2008; Simelane et al. 2008). Notwithstanding the *C. septempunctata bruckii* females sampled from vetch patches were significantly heavier than those from Solidago patches, the number of ovarioles of both populations from vetch and Solidago patches were same. These results are consistent with that *H. axyridis* ovarioles number was not altered with food quantity (Osawa 2005) and thus number of ovarioles is species specific (Nedvěd and Honěk 2012) and is independent of habitat quality in contrary with the oogenic stages of the ovarioles which depend solely on the habitat quality. The ovarioles of *C. septempunctata bruckii* females collected from vetch patches on 13 and 20 March were mature, while the ovarioles of all sampled females from vetch patches on April 16th were undeveloped. This suggests that these latter

females were the newly emerged first generation of *C. septempunctata bruckii* as the pre-imaginal development time of *C. septempunctata bruckii* preying on vetch takes about 14 to 16 days (Omkar and Srivastava 2002; Barry and Ohno 2015). Interestingly, the ovarioles of females sampled from Solidago patches on May 20th, and 25th which were most likely had been emerged and emigrated from the depleted vetch patches more than one month ago, were undeveloped. Therefore, the prey aphid, *U. nigrotuberculatum* in Solidago patches was not nutritionally suitable enough for maturation of ovarioles, in other word, this aphid is not an essential prey (Hodek and Evans 2012).

In summary, *C. septempunctata bruckii* is the predominant lady beetle species in vetch patches in spring in Miyazaki area, Southern Kyushu, Japan. The overwintered adults dwell vetch patches from early March even before vetch aphid infestation but they do not start ovipositing until prey aphid becomes available. They may prey in that period on alfalfa weevil larvae and some time on other aphid species that may be already available like *Acyrthosiphon pisum* Harris. The ovarioles of the *C. septempunctata bruckii* females are matured in March while the newly emerged adults of the first generation of *C. septempunctata bruckii* in April have undeveloped ovarioles in vetch patches. In Solidago patches, both lady beetles species were present. *Coccinella septempunctata bruckii* were the dominant species in early May but *P. japonica* becomes dominant from mid-May. The ovarioles of *C. septempunctata bruckii* females in Solidago patches are undeveloped. Despite most of the *C. septempunctata bruckii* adults in Solidago patches are coming from the depleted vetch patches and old enough to have mature ovarioles, their ovarioles were undeveloped. Thus, the prey aphid, *U. nigrotuberculatum* is not essential prey, it can maintain the adults but does not support their reproduction. The Solidago patches in Japanese agroecosystems can serve as a bridge habitat for the newly emerged *C. septempunctata bruckii* adults where they can accumulate the necessary fat bodies for aestivation. Therefore, Solidago patches may have a positive impact on *C.*

septempunctata bruckii population but they have an ecological trap effects on the aphidophagous

lady beetles.

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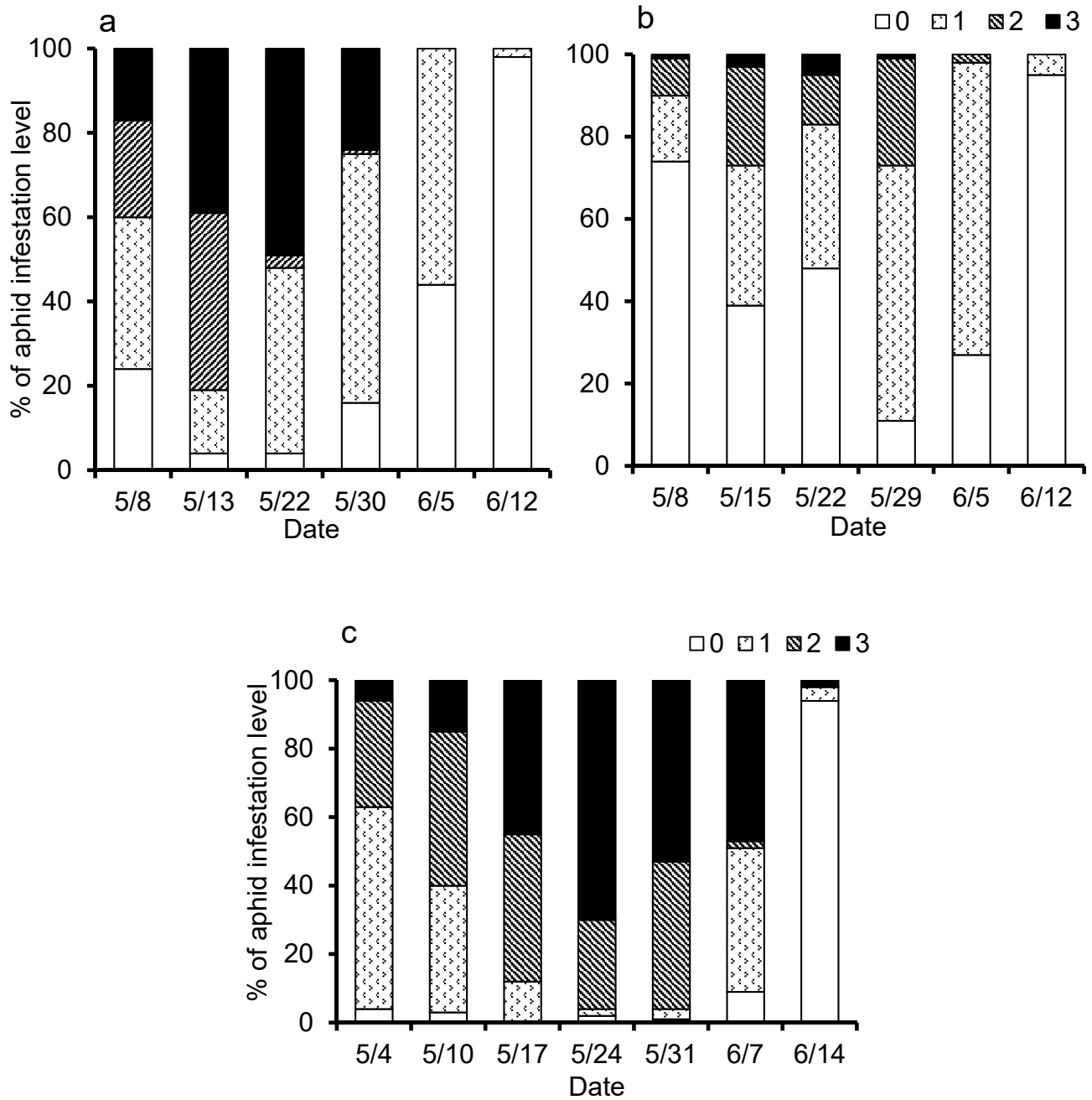
Figure 5-1 Percentage of different levels of *U. nigrotuberculatum* infestation in Solidago patches.

(a) Kiwara in 201, (b) Kiwara in 2014 and (c) Kibana in 2014. Level "0" no aphid, level "1"

few adults, level "2" young colonies and few apterous adults, level "3" heavy infestation with

nymph, apterous adults and alates

Figure 5-2 Percentage of adults and larvae of both of *C. septempunctata bruckii* and *P. japonica* and the percentage infested plants with *U. nigrotuberculatum* in Solidago patches

Table 5-1 Mean number of vetch aphid and alfalfa weevil larvae on a 4 cm of vetch twig in 2014 and the mean (± SD) number of *C. septempunctata bruckii* and *P. japonica* per plant

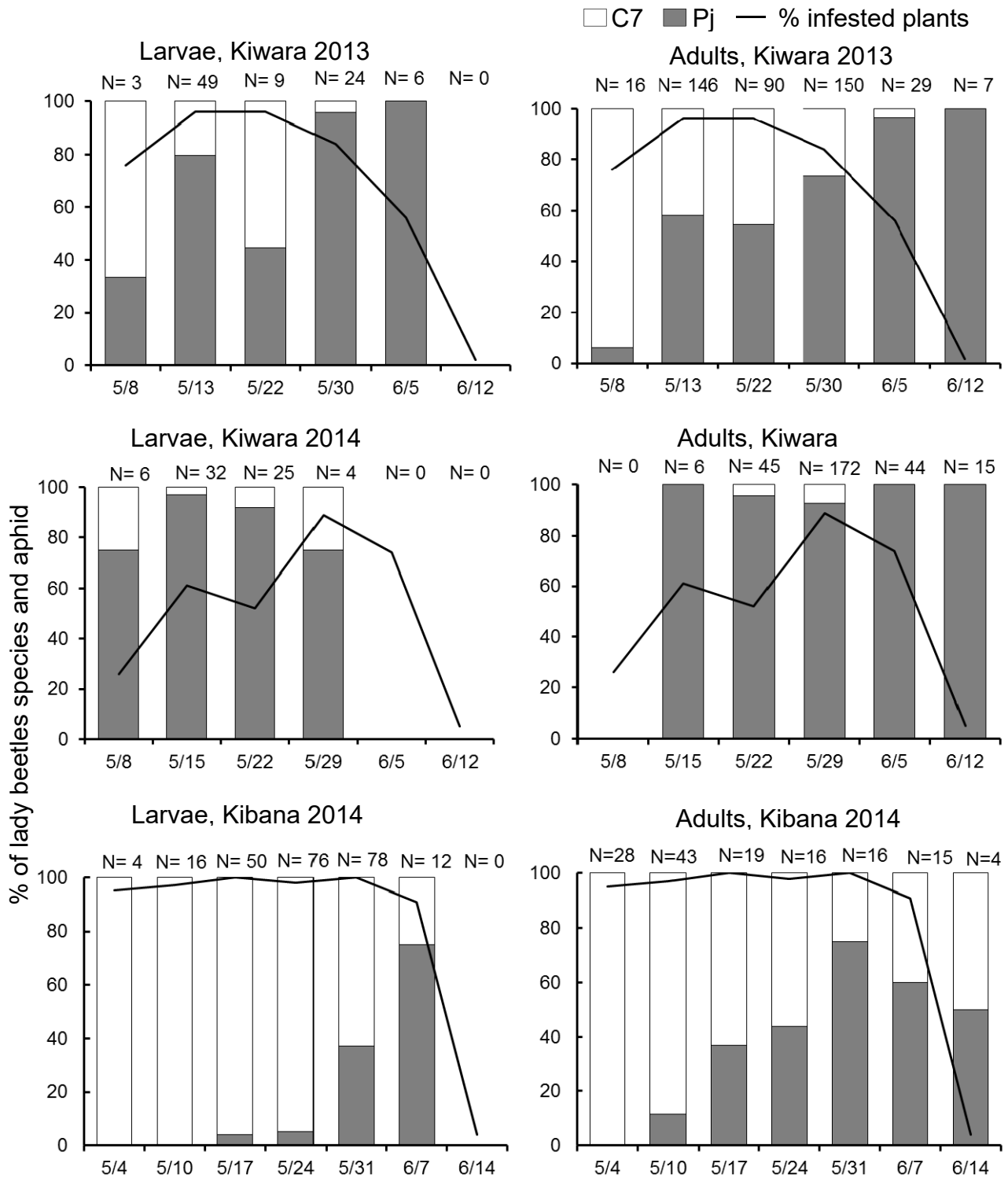| Date	n	Number of aphid/twig	Number of weevil/twig	*C. septempunctata bruckii*		*P. japonica*
				Adults	Larvae	Adults
9-Mar	20	0	2.8	1.3 ± 1.5	0.2 ± 0.4	0
15-Mar	33	0	2.1	2.1 ± 2.3	0.4 ± 0.6	0.1 ± 0.2
22-Mar	33	0.1	1	0.9 ± 1.1	0.5 ± 1.3	0.3 ± 0.6
30-Mar	33	5.4	0.4	2.5 ± 2.0	1.1 ± 1.8	0.1 ± 0.3
5-Apr	26	9.9	0.1	1.3 ± 2.9	2.4 ± 2.3	0
12-Apr	19	20.2	0	1.2 ± 1.7	7.4 ± 10.0	0.3 ± 0.6
19-Apr	17	>50	0	0.8 ± 0.9	4.4 ± 7.8	0.2 ± 0.6
26-Apr	18	>500	0	0.2 ± 0.5	1.8 ± 2.8	0

Table 5-2 Mean (± SE) fresh weight, body area and number of ovarioles of *C. septempunctata bruckii* adults collected from vetch and Solidago patches

Habitat	Vetch	Solidago
Fresh body weight (mg)	46.65±3.77a	36.34±2.78b
Body size (mm^2)	28.11±0.58a	26.10±0.60b
Number of ovarioles	87.2±5.8a	91.1±3.5a

Means in a row followed by different letters are significantly different (Student's *t-test*, $p < 0.05$)
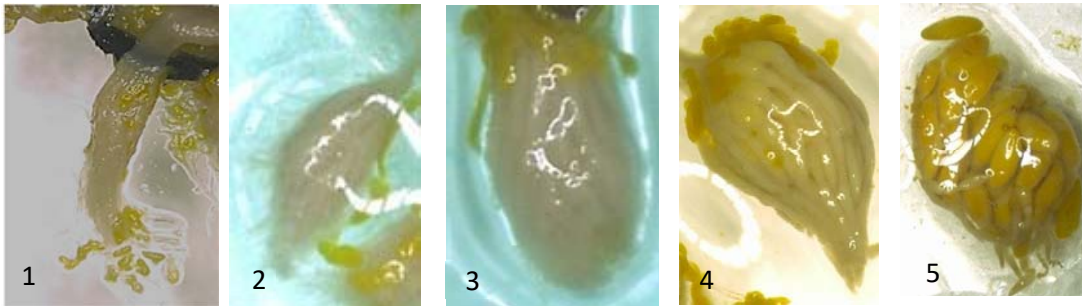
Figure 5-3 The different oogenic stages of *C. septempunctata bruckii* ovarioles. Stage 1 is

undeveloped follicles, stage 2 is growing follicles, stage 3 is pre-vitellogenic, stage 4

is mid-vitellogenic and stage 5 is post-vitellogenic and mature eggs

Figure 5-4 Percentage of *C. septempunctata bruckii* females that have ovarioles at various oogenic stages; (1) undeveloped follicles, (2) growing follicles, (3) pre-vitellogenic stage, and (5) post-vitellogenic and mature eggs sampled from vetch or Solidago patches

Chapter 6 General discussion and conclusion

The predatory lady beetles prey on widely varied aphid species. These prey aphid species suitability and availability affect markedly the lady beetles life history parameters (Kalushkov and Hodek 2004; Seagraves 2009; Zhang et al. 2012; Kumar et al. 20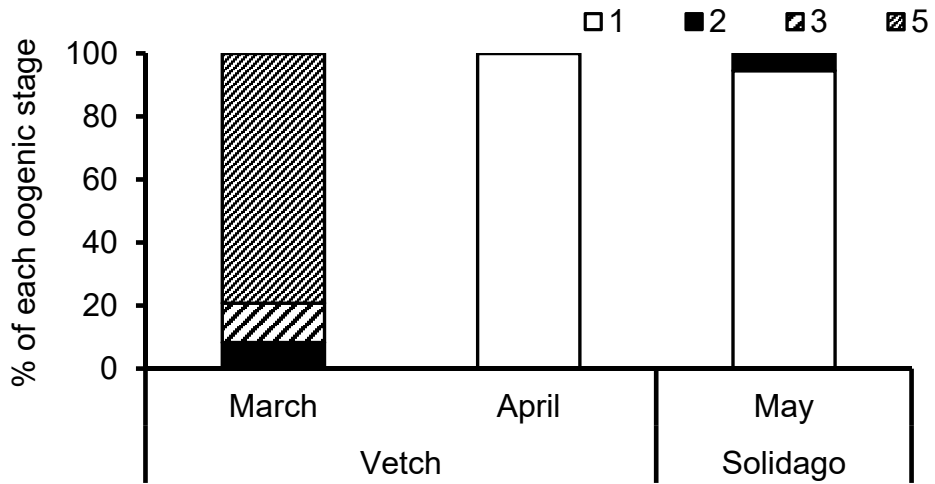13) and their population dynamics (Honěk 2012). The prey aphid species composition and variation are associated with the natural habitat in the agroecosystem. Therefore, the biological control service that lady beetles provide depends strongly on the diversity and complexity of the natural habitat around the arable lands (Landis et al. 2000; Tscharntke et al. 2007; Gardiner et al. 2009). In Japan, Solidago is considered as a main established exotic plant which has replaced some native herbaceous plants (Mito and Uesugi 2004). Solidago patches are prey aphid rich habitats for the native aphidophagous lady beetles in late spring to early summer because they are heavily infested with the goldenrod aphid. Although this prey aphid has been reported noxious to *Harmonia axyridis* (Kamo and Tokuoka 2011), my study is the first on the effects of goldenrod aphid on life parameters and the reproductive performance of both *C. septempunctata bruckii* and *P. japonica* (Chapter 2). The larval development time was significantly prolonged, survival rate of the larvae was dramatically reduced, and adults' weight and fecundity were drastically affected. However, since the occurrence of this prey aphid coincides temporarily with a period of scarcity of suitable prey aphids, it may have positive impacts on the overall conservation of these lady beetles in the Japanese agroecosystems. As the lady beetle adults find abundant alternative prey aphid in Solidago patches from late April into mid-June, my results (Chapter 2) suggest that the adults of both lady beetles can be maintained on this prey aphid, even though *C. septempunctata bruckii* cannot reproduce

successfully. This prey aphid also may provide *C. septempunctata bruckii* adults with necessary energy for estivation.

The overwintered lady beetles reproduce from March to late April in vetch patches infested with vetch aphid. Since the colonies of vetch aphid decline upon the offsprings of lady beetles are reaching adult stage, the newly emerged adults must immigrate into Solidago patches infested with the goldenrod aphid. Therefore, Solidago patches are a bridge that links the period of prey scarce in early summer with aphid outbreaks in the crop fields and other habitats in late summer and early autumn. Because the immigrant populations of lady beetles from depleted vetch patches into Solidago patches contain gravid females, larval populations are often present in Solidago patches, even though they are sinking in this hostile habitat due to the high larval mortality (Chapter 2). Particularly, there is no realized reproduction for *C. septempunctata bruckii* in Solidago patches, whereas *P. japonica* is less affected and can reproduce with low outcome in Solidago patches.

In chapter (3) of this dissertation, I reported evidences on the deadly effects of the defensive response of the goldenrod aphid by secreting red gluing bulbs from its cornicles upon being attacked by the lady beetles. Most of the dead larvae of both *C. septempunctata bruckii* and *P. japonica* were smeared with the gluing secretions from aphid cornicles or even stuck with the prey aphid. However, despite alarm signaling effects of cornicle secretions reported widely in the literature (Verheggen et al. 2010; Vandermoten et al. 2012; Moayeri et al. 2014), only few reports are available about the physical or mechanical effects of cornicle secretions (Edwards 1966; Butler and O'Neil 2006). Because the aphids neighboring to the attacked and cornicle droplets secreting aphid did not respond in the same fashion described in previous studies that aphid alarm pheromones induce the escape response, I considered these secretions were not carrying alarm

pheromones. Consequently, the main functions of the goldenrod aphid's red gluing droplet secretions are as active and physical defense.

Smearing the predatory larvae with the sticky secretion causes gumming up the mouthparts, sensory organs or sticking the prey with its predators. These situations hampered the predator's predatory potential and could increase the inclusive fitness among the aphid clone-mates as Wu et al.(2010) have reported that *Sitobion avenae* (Homoptera: Aphididae) increased their inclusive fitness by smearing the assailing parasitoid, *Aphidius rhopalosiphi* with cornicle secretions. The younger predatory larvae suffered more from the aphid defensive secretions. In contrast, as much the lady beetles larvae are growing, their mortality associated with the effects of the cornicle secretions decreased. It can be because killing the advanced larval stages requires larger amount of cornicle secretions, and on the other hand, the old larvae may become more efficient in grooming their body and thus clean themselves off the gluing secretions before they dry and have effects.

Although in the field conditions the interaction between aphid and its predator becomes more complicated, I have observed smearing of cornicle secretions on the larvae of *C. septempunctata bruckii*. My field data strongly supported the laboratory data on the effects of cornicle secretions on the lady beetle larvae and I could deduce that more than 30% of each of 1st and 2nd instar larvae of *C. septempunctata bruckii* foraging in Solidago patches will not molt to the next instar because of the effects of cornicle secretions. Therefore, the goldenrod aphid deploys actively the cornicle secretions as active defense against the attacked enemies rather than passive arm by alerting the clone-mates. Even though the aphid which secreted the cornicle secretions was not saved from the predators' attacks, such secretion may lead to an increased inclusive fitness.

The prey preference and foraging behavior of predatory lady beetles are affected by the suitability (Hammill and Beckerman 2010; Hodek and Evans 2012) and defensive response of its prey aphid (Provost et al. 2006; Uematsu et al. 2013). As I reported in chapter (4) of this dissertation, the larvae of *C. septempunctata bruckii* selected *U. nigrotuberculatum* in the early feeding trial but switched their preference of the noxious and defended prey aphid, *U. nigrotuberculatum* with time passes to the more suitable and defenseless aphid, *M. crassicauda*. The foraging behavior of *C. septempunctata bruckii* larvae on the goldenrod aphid were altered compared with that on the more suitable aphid prey *M. crassicauda*, their main foraging bouts (searching, feeding, resting and grooming) were significantly prolonged. Consequently, the number of the goldenrod aphid consumed by the fourth instar larvae of *C. septempunctata bruckii* was drastically lower than that of vetch aphid. Interestingly, however, the larvae of *P. japonica* were not selective, they foraged randomly and consumed indifferently both prey aphids in a mixed diet of the goldenrod aphid and vetch aphid. The Foraging behavior of *P. japonica* larvae on *U. nigrotuberculatum* was not much different to that on *M. crassicauda*. The main foraging bouts had similar time length except of the feeding time, which affected the total number of prey aphid, *U. nigrotuberculatum* consumed compared with that of the more suitable prey aphid, *M. crassicauda*.

These lady beetle aphidophagous have short evolutionary history with the goldenrod aphid, *U. nigrotuberculatum* thus presence of such noxious prey in abundance and in a period of scarcity of suitable prey aphids can threaten their conservation as well as their function as aphid population regulators in the agroecosystem. This aphid can be an ecological trap for these lady beetles in the Japanese agroecosystem. Therefore, it is necessary to take in account the potential trap effects of the goldenrod aphid in any conservation biological control program which is based on the aphidophagous lady beetles.

Population dynamics of the lady beetles during the 2 years survey in 2013 and 2014 in Solidago patches and in both vetch and Solidago patches in 2014 suggested a fluctuation in the presence of *C. septempunctata bruckii* and *P. japonica* adults and larvae on these habitats. *Coccinella septempunctata bruckii* adults occur first in Solidago but after around 1-2 weeks later *P. japonica* becomes the dominant species. The body size and weight of *C. septempunctata bruckii* females depend on the suitability and abundance of their prey aphid (Hauge et al. 1998; Simelane et al. 2008). Concordantly, the size and body weight of *C. septempunctata bruckii* females were significantly different between the two habitats of vetch plants infested with vetch aphid, *M. crassicauda* and Solidago infested with the goldenrod aphid. The ovarioles of *C. septempunctata bruckii* were matured in early spring in vetch patches while in mid-April they were undeveloped. This suggests that these females were the newly emerged adults of the 1st generation. However, astonishingly, the females sampled from Solidago patches in late May were still having undeveloped ovarioles. Since the pre-oviposition period of *C. septempunctata bruckii* is about 2 weeks under a plenty suitable prey condition, the delay in the lady beetles' ovarioles development in Solidago patches could be attributed to the prey aphid quality (i. e., the goldenrod aphid is non-essential prey). Therefore I concluded that the prey aphid, *U. nigrotuberculatum* is not an essential prey defined as the prey aphid which can maintain adults but does not support their reproduction (Hodek and Evans 2012).

In conclusion, the prey aphid, *U. nigrotuberculatum* is a noxious prey for both of the larvae of *C. septempunctata bruckii* and *P. japonica* through its active defense by secreting the gluing red bulbs which are physically fatal but this aphid can maintain the lady beetle adults during when the suitable prey aphids are very scarce. Since it supports low reproduction rate of *P. japonica*, this lady beetle might be able to realize 3 generations per year. Because this aphid infestation in

Solidago patches precedes the estivation period of *C. septempunctata bruckii*, it must have a positive impact in its overall population dynamics. This prey aphid may improve the survival rate of estivating adults by providing enough energy for estivation. However, it hinders *C. septempunctata bruckii* adults to realize one more generation from late-April to June before its estivation period starts in Japanese agroecosystem (Kawauchi 1997).

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