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Impacts of Two Introduced Ladybeetles, *Coccinella septempunctata* and *Harmonia axyridis* (Coleoptera: Coccinellidae), on Native Coccinellid Species at Mount St. Helens, Washington and in Southwestern Virginia

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To the Graduate Council:

I am submitting herewith a thesis written by Catherine Marie Sheehy entitled "Impacts of Two Introduced Ladybeetles, *Coccinella septempunctata* and *Harmonia axyridis* (Coleoptera: Coccinellidae), on Native Coccinellid Species at Mount St. Helens, Washington and in Southwestern Virginia." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Ecology and Evolutionary Biology.

Dan Simberloff, Major Professor

We have read this thesis and recommend its acceptance:

Paris Lambdin, Nathan Sanders, James Fordyce

Accepted for the Council:

Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

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Coccinella septempunctata and *Harmonia axyridis*
(Coleoptera: Coccinellidae), on native coccinellid species
at Mount St. Helens, Washington and in southwestern Virginia**

A Thesis Presented for the
Master of Science Degree
The University of Tennessee, Knoxville

Catherine Marie Sheehy
December 2007

Abstract

To assess changes in coccinellid communities subsequent to the introduction of two non-native species, *Coccinella septempunctata* and *Harmonia axyridis*, ladybird numbers were monitored over a 20 year period at Mt. Saint Helens, Washington and in three years between 1996 and 2004 in southwestern Virginia. Surveys conducted at Mt. Saint Helens included pre- and post- invasion years for both non-natives. At Mt. Saint Helens, *C. septempunctata* and *H. axyridis* both increased in abundance, accompanied by declining numbers of the native ladybird *Adalia bipunctata* at one of the two Washington sites and of the native ladybird *Coccinella transversoguttata* at both sites. Declines in these two native ladybirds are strongly correlated with increases in the two introduced species. Given the long study period at Mt. Saint Helens, the declines in these two natives appear to be real trends. Further research is required to determine if the introduced species have indeed been the direct cause of the declines, and to find the mechanisms of interactions. The arrival of *C. septempunctata* to Virginia predated the first year of this study likely by several years. *Harmonia axyridis* was detected in Virginia by 1993, three years prior to the first study year in this state. In Virginia, only one introduced species, *H. axyridis*, showed an increase during the study and this was at one site only. This increase was not accompanied by declines in native species. In fact, the native *Coleomegilla maculata* also increased at the site during this period. Lack of evidence for declines in native species in Virginia may simply indicate that native species were not affected by the two introduced species, or it may reflect lack of power in the data to detect changes. Alternatively, any changes to the ladybird community may have occurred prior to the start of this study.

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I. Literature Review

Use of ladybirds in biological control

Ladybird beetles are generally regarded as beneficial, frequently playing key roles in controlling pest insects in many habitats, particularly agricultural ecosystems (e.g., Hodek and Honek 1996, Gordon 1985, Obrycki and Kring 1998). Humans have long been aware of this valuable role. In medieval Europe, believed sent from heaven to save crops, lady beetles were called “Our Lady’s beetle,” referring to the Virgin Mary. In ephemeral agroecosystems, predatory insects play a critical role in pest suppression, with ladybirds among the most important of such groups. As natural enemies of whiteflies, aphids, scales, mealybugs, and mites, they have been extensively used in many biological control strategies for over a century, more often than any other predator taxon (Obrycki and Kring 1998, Hagen 1962). These strategies include natural or passive biological control, in which the control agent is a native organism at normal densities controlling a native or exotic pest; augmentative biological control, in which populations of a native agent are directly increased to control a native or exotic pest; classical biological control, in which an exotic agent is imported and released to control an exotic and presumed coevolved pest; and neoclassical biological control, a controversial approach in which an exotic agent is used to control a native pest (Lockwood 1993).

The phenomenal success of the imported Vedalia beetle, *Rodolia cardinalis* (Mulsant), in controlling cottony cushion scale in California in the late 1800s became a classical example for biological control (Obrycki and Kring 1998, De Bach and Rosen 1991, Caltagirone *et al.* 1989) and sparked what has been described as the “ladybird fantasy” period during the early 1900s. During this time, over forty ladybird beetle species were introduced to the United States, only four of which became established. So far, approximately 180 coccinellid species have been deliberately or inadvertently introduced to the United States, 27 of which have established populations (Gordon 1985). The efficacy of coccinellids in suppressing pests in natural or managed ecosystems can

be difficult to assess given their typically highly mobile nature and catholic feeding habits (Obrycki and Kring 1998). Population dynamics of prey such as aphids can be complex; many processes can contribute to regulation, making it difficult to determine relative importance (Strong *et al.* 1984). High rates of population increase combined with delayed predator responses or density-dependent feedback loops can lead to large oscillations in population densities (May 1972). Initial numbers of aphids and environmental conditions can therefore greatly influence the success or failure of ladybirds in controlling population outbreaks (Strong *et al.* 1984).

Historically, many have viewed biological control as a cost-effective and environmentally friendly strategy for pest control (Thomas and Willis 1998, De Bach and Rosen 1991). Recently, however, several ecologists have called attention to potential pitfalls of biological control (e.g., Howarth 1991, Lockwood 1993, Simberloff and Stiling 1996). Introduced biological control agents can threaten the components and dynamics of ecological communities through competitive suppression or displacement of similar native species and through depression, even extinction of non-target prey species (e.g., Wheeler and Hoebeke 1995, Elliott *et al.* 1996, Louda *et al.* 2003). Current regulations regarding evaluation, importation, and release of biological control agents of insects are insufficient to assess agent efficacy and potential non-target impacts (Howarth 1991, Simberloff and Stiling 1996).

One of the biggest concerns about biological control is the potential for unintended and unforeseen consequences (Lockwood 1993, Simberloff and Stiling 1996). Biological species might undergo evolutionary adaptation to new hosts, they may become pests themselves, and, as is especially true of ladybirds, they might disperse to new areas and new habitats. Furthermore, once a biological control agent has been released and become established, there is no turning back. So, not only are the consequences often unpredicted, they are also generally irreversible, raising the stakes in biological control.

There are other important caveats inherent to biological control. Polyphagous species generally offer the greatest promise as biological control agents to become established and control insect pests of ephemeral crops; however, they also pose a substantially greater risk to native species than do oligophagous or monophagous species

(Samways 1988). The character traits often key to biological control (i.e., high dispersal ability and capability to colonize new habitats, rapid population increase, lack of dependence on the presence of the target pest species to persist, and opportunistic or polyphagous feeding habits) in fact increase the odds for nontarget impacts and unanticipated ecological consequences (e.g., Elliott *et al.* 1996). The aims of biological control and species conservation can therefore easily be at cross purposes.

***Coccinella septempunctata*: Taxonomy and Description**

Coccinella septempunctata L. 1758, also referred to as *Coccinella septempunctata septempunctata* L. or *Coccinella 7-punctata* L. belongs to the tribe Coccinellini, subfamily Coccinellinae in the family Coccinellidae. It is commonly referred to as C7, seven-spot, or seven-spotted lady beetle, ladybird, or ladybird beetle. This beetle is ovate and has red or reddish-orange elytra with seven black spots, including one common scutellar spot and three spots on each elytron, one lateral before middle, one middle, and one lateral and subapical (Downie and Arnett 1996, Gordon 1985, Hoebeke and Wheeler 1980).

C. septempunctata has a black head with two broadly spaced pale spots. The anterior margin of the pronotum is black at the middle. The North American description of *C. septempunctata* gives body size as 6.5-7.8 mm in length; however Sasaji (1971), based on specimens from the Oriental region, describes this beetle as relatively small, 5.0-6.7 mm long and 3.9-5.4 mm wide. It should be noted that *Coccinella septempunctata* L. has previously been confused with *Coccinella septempunctata brucki* (Hodek and Honek 1996). This confounding has been accounted for to the extent possible in the treatment here, although complete certainty as to species identity may not be possible in a small portion of the literature.

C. septempunctata beetles lay clusters of 10 to 30 spindle-shaped or oblong eggs, yellow to orange-red in color and approximately 1 mm. long. The larvae are dark and alligator-like with three pairs of prominent legs, growing to 7 – 8 mm. long (Brown 1962). Pupal color is highly influenced by environmental conditions and can vary from

light orange to dark brown (Hodek and Honek 1996). Newly emerged adults have soft elytra, which are pale in color and without spots. The hind wings remain unfolded, protruding beneath the elytra until hardening. The normal spot pattern develops over the course of a few hours. The red elytra, however, generally maintain a lighter shade for weeks to months. *Coccinella septempunctata* is found in a broad range of climatic environments throughout the Palearctic and Oriental regions (e.g., Sasaji 1971, Angalet *et al.* 1979, Phoofolo and Obrycki 2000) and has been introduced to the Nearctic region.

***C. septempunctata*: Biology**

Developmental Stages

As are other coccinellids, *Coccinella septempunctata* is holometabolous (exhibits complete metamorphosis) and undergoes eight developmental stages: egg, four larval instars, prepupa, pupa, and adult (e.g., Katsarou *et al.* 2005). Larval instars develop over a period of 13 to 72 days and the pupal stage from three to twenty days, depending on ambient temperature and the supply of aphids (Omkar and Srivastava 2003, Katsarou *et al.* 2005). Total development time from egg to adult may take as little as two to three weeks. *C. septempunctata* adults generally live for a span of weeks to months, depending on location, availability of prey, and time of year (Kalushkov and Hodek 2004, Katsarou *et al.* 2005, Omkar and Srivastava 2003).

Among populations of ecologically plastic *C. septempunctata* beetles there are no geographic trends in temperature requirements (Hodek and Honek 1996). Katsarou *et al.* (2005) reported a lower development threshold (LDT) or temperature at which development ceases of 10.7°C and sum of effective temperatures (SET) or number of required days above LDT to complete development of 281.5 days for *C. septempunctata* raised on tobacco aphid, *Myzus persicae nicotianae* Blackman. Obrycki and Tauber (1981) reported a LDT of 12.1°C and SET of 197 days. Ladybirds in general develop more rapidly and consume fewer aphids as temperatures increase (Michels and Behle 1991).

Fecundity and Oviposition

Coccinella septempunctata exhibits tremendous variation in mean daily parity per female, with ranges from zero to >59 eggs per day (Phoofolo and Obrycki 2000, Frazer and McGregor 1992, Stewart *et al.* 1991). Phoofolo and Obrycki (2000) demonstrated a positive relationship between food consumption and number of eggs laid, thus explaining part of the variation. In addition to prey availability, the number of eggs per batch deposited by *C. septempunctata* is constrained by the beetle's ovariole number. At a given moment, roughly half a female's ovarioles are producing eggs, while the other half are preparing for new oviposition (Hodek and Honek 1996). This alternation results in smaller individual batch sizes but allows for more continuous egg production (Stewart *et al.* 1991). In a landscape of temporally and spatially fluctuating resources, such a strategy could potentially increase the chances for at least some of the larvae to emerge at optimum times.

As with clutch size, the ovariole number of *C. septempunctata* also varies. Phoofolo and Obrycki (2000) reported the range of ovariole numbers to be 80 to 94. A broader range of 26 to 82 ovarioles per female was reported by Rhamhalinghan (1985 from Hodek and Honek 1996), the lower range obtained under limited food availability and the upper under a surplus of food. Ovariole number also varies seasonally, perhaps as a result of seasonal changes in body size. In India, fecundity was greater in the large, winter-born females than in the small, summer-born females. The mean number of ovarioles was 55 to 62 in the winter and 41 to 45 in spring and summer, though this difference was slightly dampened by smaller egg per ovariole production in the winter-born individuals.

Several authors have suggested that females might have evolved behaviors that promote stochastic oviposition rates in response to their constantly changing environments (Godfray and Ives 1988, Phoofolo and Obrycki 2000). Phenotypic variation in reproductive parameters could possibly be an important, bet-hedging strategy for survival in novel or temporally and spatially unpredictable environments, especially when optimal reproduction conditions are fleeting (Godfray and Ives 1988, Phoofolo and Obrycki 2000, Hemptinne *et al.* 1992, Evans *et al.* 1999).

Adult *Coccinella septempunctata* beetles are highly mobile in their search for suitable oviposition sites near aphid colonies or other prey (Evans and Richards 1997, Evans *et al.* 1999) and choose sites based on microclimatic preferences. *C. septempunctata* is positively geotropic with a preference for rough surfaces and insolated spaces (Hodek and Honek 1996). Females typically lay their eggs in small clusters on insolated pieces of soil or insolated upper surfaces of leaves (Coderre and Tourneau 1986, Hodek and Honek 1996). Temperature preferences influence location of oviposition. Subsequently, oviposition sites may vary with seasonal changes (Hodek 1983).

The size and age (i.e., quality) of aphid colonies is also an important determinant of the timing and location of oviposition. Ladybirds will avoid aphid colonies that are too old, either because the colonies are perceived as an unreliable future source of food or perhaps owing to an overabundance of honeydew. At times of food scarcity, females will briefly delay oviposition. In seeking oviposition sites, *C. septempunctata* females likely respond to chemical cues from the host plants (Shands *et al.* 1970, Hodek and Honek 1996). Smirnof (1958) found that laboratory-raised females laid eggs only in cages containing parts of plants, and Shah (1983 in Hodek and Honek 1996) demonstrated that *C. septempunctata* lay more eggs on a particular plant species, even when aphids were more abundant on other available species.

***C. septempunctata*: Life cycle and diapause**

Life cycle

Coccinella septempunctata has a very plastic life cycle with several types of voltinism (e.g., Angalet *et al.* 1979, Hodek and Honek 1996). It is typically univoltine at cool, northern latitudes (Schellhorn *et al.* 2005) and multivoltine in warmer climes. Intrapopulation variation in voltinism of the phenotypically plastic *C. septempunctata* has been frequently observed in several parts of Europe (Hodek and Honek 1996, Phoofolo

and Obrycki 2000), North America (Angalet *et al.* 1979), and Japan (Ohashi *et al.* 2003, 2005).

C. septempunctata has also been observed using artificial substrates, such as discarded metal cans, wooden materials, and papers to complete additional generations (Ohashi *et al.* 2005). In central Honshu, Japan, where ambient winter air temperatures are not high enough for the beetle to complete development, Ohashi *et al.* (2005) reported preferential selection and use of artificial substrates warmed by solar radiation, enabling completion of a winter generation. Survival of eggs and pupae was not possible on live plant leaves, the summer site choice for oviposition. In more natural environments, *C. septempunctata* lay eggs in thermal microhabitats such as dead leaves during early spring. This behavior may have served as a preadaptation for using artificial substrates for the same purpose during winter. By engaging in winter reproduction, *C. septempunctata* benefited from fewer competitors and natural enemies and avoidance of mortality risk during hibernation (Ohashi *et al.* 2005).

Diapause

Coccinella septempunctata, as do most other coccinellids, spends the winter months in diapause, with a possible quiescence or second diapause in some populations in summer. In this manner, the beetle escapes less favorable climatic and resource conditions and synchronizes its life cycle with the seasonal cycle of the local climate (Ohashi *et al.* 2003).

C. septempunctata ladybirds do not make long distance migrations to hibernation sites but remain near the breeding and feeding area (Hodek 1973), selecting protected microhabitats near the ground, such as under stones, in litter, or near the base of plants or, if available, higher areas, often at forest edges, isolated shrubs, trees, or posts in a flat landscape (Angalet *et al.* 1979, Hodek and Honek 1996). *C. septempunctata* also uses artificial substrates as hibernation sites (Ohashi *et al.* 2005). The beetles form only small or medium sized aggregations, not exceeding tens of beetles, possibly reducing risk of mycosis (Hodek and Honek 1996). Angalet *et al.* (1979) reported higher numbers of aggregated individuals in New Jersey, ranging from 5 to over 500 beetles per cluster.

During dormancy, supercooling occurs and development of ovaries stops (Hodek and Honek 1996). Frequency of diapause and timing of the stages in this cycle vary between regions and even within populations. Hodek and Honek (1996) reported that while some aggregations of dormant adults were in their hibernation quarters from August onwards, actively feeding adults were still found on aphid-infested vegetation throughout September and early October. Telenga (1948 from Hodek and Honek 1996) likewise found both dormant and active, feeding adults during the same season in a population of *C. septempunctata* in the Ukraine. Emergence from dormancy and dispersal from hibernation sites coincide with increasing photoperiod, light intensity, and ambient temperature. An extended period of time with average temperatures over 10° C generally induces emergence (Hodek and Honek 1996).

Altitudinal and latitudinal clines in diapause expression have been observed in *C. septempunctata*. In Central Honshu, Japan, Ohashi *et al.* (2003) discovered that beetles underwent diapause in summer and quiescence in winter, but in Hokkaido, northern Japan, beetles did not enter summer diapause. In the intermediate area of northern Honshu, as well as in the mountain population of central Honshu, diapausing and non-diapausing adults were observed simultaneously, suggestive of a possible genetic basis underlying this variation. Ohashi *et al.* (2003) identified daily mean air temperature in July to be the most important factor influencing frequency of diapause expression. They found no significant effect of aphid abundance, in contrast to Nijima and Kawashita (1982 from Ohashi *et al.* 2003), who suggested that food scarcity is the main factor in diapause induction. These ideas are not entirely at odds; temperature could simply be the selective force by which escape from periods of low food availability is ensured. Ohashi *et al.* (2003) further concluded that diapause induction is either not controlled by photoperiodic response in these populations or the populations have a plastic photoperiodic response affected by ambient thermal and/or food conditions.

As in nearly all other aspects of this beetle's life cycle, the duration or intensity of diapause too shows considerable phenotypic plasticity. Pando (1995) interpreted this as a bet-hedging strategy. Phoofolo and Obrycki (2000) found univoltine individuals to be characterized by long preoviposition periods, in other words a long diapause, and

multivoltine individuals by short preoviposition periods (i.e., no diapause). Altitudinal variation in diapause duration has also been reported. In France, intensity of diapause, as measured by duration of oviposition delay, was greater in individuals from “montane” dormancy sites than in beetles from “lowland” dormancy sites (Hodek *et al.* 1977). Honek (1989) proposed that larger individuals, which develop earlier in the season, are better suited for long-range flight and thus occupy montane dormancy sites, where they experience a longer diapause.

***C. septempunctata*: Habitat, prey, and foraging behavior**

C. septempunctata inhabits various habitat types, including arboreal and herbaceous settings, with higher abundances generally associated with herbaceous habitats and areas of high aphid abundance (e.g., Angalet *et al.* 1979). Adults are very mobile and switch habitats to track resources. *C. septempunctata* adults and, to an extent, larvae exhibit microclimatic preferences, likely in response to plant, air, and soil temperatures and humidity (Honek 1985, Evans and Dixon 1986). An affinity for particular strata of different plants at different times of the year has been reported for this coccinellid, but it generally prefers sparse and well-insolated stands with a warm microclimate (Honek 1985). *C. septempunctata* is one of several species that increase their body temperature by basking in the sun (Honek 1985).

Coccinella septempunctata is foremost an aphidophagous coccinellid but has a catholic diet and will feed on other homopterans, mites, small nematoceros Diptera, young instars of Lepidoptera, Coleoptera, and Hymenoptera, minute larvae of Thysanoptera, pollen, mildew, honeydew, nectar, and fruit, such as pears and peaches (Schellhorn *et al.* 2005, Majerus 1994, Hodek and Honek 1996, Koch 2003). Important life history parameters of the predatory beetle vary tremendously depending upon the prey (Blackman 1967, Evans *et al.* 1999, Hauge *et al.* 1998, Kalushkov and Hodek 2004, Nielsen *et al.* 2002, Obrycki and Orr 1990, Omkar and Srivastava 2003, Ozder and Saglam 2003). “Lower quality” prey can slow larval development, increase

mortality at all life stages, and produce smaller adult sizes; they may not support complete development or reproduction; or they may even be toxic to the predator (Hauge *et al.* 1998).

Hodek and Honek (1996) make a fundamental distinction between essential prey (those that support complete development and reproduction) and acceptable prey (those that may provide varying amounts of energy and nutrients but do not support development or reproduction). By this definition, though 64 aphid species have been identified as acceptable food for *C. septempunctata*, current estimates of essential aphid prey of *C. septempunctata* fall just under 30 species (Hodek and Honek 1996, Kalushkov and Hodek 2004). It is also noteworthy that, though a prey item may not support full development and therefore be classified as “acceptable” or “alternative” prey, it may still increase the predator’s chance at survival and enhance the predator’s fecundity when augmenting a limited essential prey (Evans *et al.* 1999). Likewise, the effect of the predator on the alternative prey need not be insignificant.

Cannibalism by fourth instars and adults on larvae, pupae, and at times, freshly molted adults appears to offer an important safeguard for population survival during times of temporary food shortage and may serve as a self-regulating mechanism to prevent exponential growth (Koide 1962, Hodek and Honek 1996). Though development rate decreased 38% and larvae were 15 - 25% smaller, Koide (1962) got *C. septempunctata* to complete larval development on an exclusive diet of eggs of their own species.

Several studies on prey searching mechanisms in *C. septempunctata* and other coccinellids (e.g., Storch 1976, Harmon *et al.* 1998, Evans and Dixon 1986) have produced varying results. Earlier studies found that neither visual nor olfactory sensory organs played a role in prey searching behavior, suggesting random search. However, researchers may not have examined distance on an appropriate scale and may not have considered alternate explanations for lack of response to prey, such as satiation, searching for mates by males, or females seeking oviposition sites (Hodek and Honek 1996).

Findings in some later studies did indeed point to the importance of both visual and olfactory cues in prey searching (Harmon *et al.* 1998, Evans and Dixon 1986, Hodek

and Honek 1996). Harmon *et al.* (1998) found that the beetles consumed more aphids in light than in dark and Lambin *et al.* (1996) demonstrated sight detection of aphids by *C. septempunctata* adults from approximately 7 mm. Evans and Dixon (1986) found that both *C. septempunctata* larvae and adults respond to odors of aphid prey. Adults arrive at stands of agricultural crops rapidly after aphid establishment (Evans 2004). Results by Ninkovic and Pettersson (2003) supported not only the general importance of olfactory cues, but also indicated that plant stands of greater diversity and therefore of a more complex mixture of plant volatiles act as greater attractant to *C. septempunctata* than less complex mixtures.

In prey searching, coccinellids exhibit positive phototaxis and negative geotaxis. Not surprisingly, aphids have the same tactic responses (Hodek and Honek 1996). Plant morphological characteristics, such as trichome density and leaf vein prominence, can be important to foraging efficiency and success (Carter *et al.* 1984, Legrand and Barbosa 2003, Kareiva and Sahakian 1990). Upon encountering prey, *C. septempunctata* switches from extensive search to intensive search, marked by slow, winding movements. This behavior develops only after some initial experience with prey capture, suggesting that it is a learned response (Ettifouri and Ferran 1993). Coincident with individuals switching from extensive to intensive search, aggregations may result in patches of high prey density.

***C. septempunctata*: Natural enemies**

For defense, *Coccinella septempunctata* employs both aposematic (warning) coloration and toxic secretions. When disturbed, *C. septempunctata* enters thanatosis (a stupor, during which the antennae and legs are pressed into abdominal depressions, and secretes a toxic, yellowish fluid from the femorotibial articulations (Hodek and Honek 1996, Rothschild 1961, Holloway *et al.* 1991). The toxicity of this “reflex bleeding” is derived mainly from coccinelline and precoccinelline, alkaloid substances recorded by Pasteels *et al.* (1973 from Hodek and Honek 1996). This defense appears very effective

against vertebrate and invertebrate predators; nonetheless, *C. septempunctata* is not without predators. These include several bird and spider species (Hodek and Honek 1996, Majerus 1994, Angalet *et al.* 1979), as well as pentatomids and centipedes (Angalet *et al.* 1979). Several parasitoids of *Coccinella septempunctata* have been recorded, including species of phorid and tachinid flies, parasitic wasps, podaplipid mites, and nematodes (Angalet *et al.* 1979, Disney *et al.* 1994, Hodek 1973, Hodek and Honek 1996, Schaefer and Semyanov 1992). A few fungal pathogens, including *Nosema sp.*, *Beauveria bassiana*, *Cephalosporium sp.*, *Paecilomyces farinosus*, and *Paecilomyces fumosoroseus*, have been reported (Hodek and Honek 1996).

***Harmonia axyridis*: Taxonomy and description**

Harmonia axyridis belongs to the tribe Coccinellini, subfamily Coccinellinae in the family Coccinellidae (Kovar 1996). This species has, however, undergone several taxonomic transfers since its original description in 1773 as *Coccinella axyridis* Pallus (Sasaji 1971, Chapin 1965). Proposed junior synonyms include *Coccinella bisex-notata* Herbst 1793, *Coccinella 19-sinata* Faldermann 1835, *Coccinella aulica* Faldermann 1835, *Coccinella conspicua* Faldermann 1835, *Harmonia spectabilis* Falderman 1835, *Coccinella succinea* Hop 1845, *Anatis circe* Mulsant 1850, and *Ptychanatis yedoensis* Takizawa 1917. The original generic placement in *Coccinella* was changed to *Leis* by Mulsant in 1850, to *Ptychanatis* by Crotch in 1874, and then to the subgenus *Harmonia* in *Coccinella* in 1885. Jacobson (1915) and Timberlake (1943) subsequently raised *Harmonia* to generic status. *H. axyridis* is commonly referred to as the multicolored Asian lady beetle, ladybird, or ladybird beetle or as the Japanese lady beetle. It is also sometimes referred to as the Halloween beetle, owing to massive fall migrations begun in late October in North America.

Eggs of *H. axyridis* are yellow, oval shaped, and about 1.2 mm long (El-Sebaey and El-Gantiry, 1999). Larvae are dark and covered with scoli (Savoiskaya and Klausnitzer, 1973) and grow from approximately 2 mm in the first instar to 7.5 to 10.7

mm in the fourth instar (Sasaji 1977). Coloration and markings of *H. axyridis* adults are highly variable (e.g., Korchevsky 1932). Elytra can range from yellow-orange to red with zero to 19 black spots or can be black with red spots. The pronotum is yellowish with black central markings in the form of a solid trapezoid, an M-shape, four spots, or two curved lines (Chapin and Brou 1991) and yellowish oval-shaped spots at the lateral edges (Chapin 1965). The head is typically black, but can also be yellow or black with yellow markings (Sasaji 1971). The ventral surface can be black or yellow-orange (Chapin and Brou 1991). Adults are oval and moderately convex and reach 4.9 to 8.2 mm in length and 4.0 to 6.6 mm in width (Sasaji 1971).

There appears to be a genetic basis for the color polymorphism of *H. axyridis* (Honek 1996, Komai 1956). Phenotypic variability may also be determined by larval diet (Grill *et al.* 1997) and temperatures during pupal development (Sakai *et al.* 1974). Osawa and Nishida (1992) demonstrated seasonal variation in proportion of dark and light forms. In three seasons, the proportion of light phenotypes increased during the spring and early summer because of the higher mating frequency of this phenotype. Females (regardless of their morph) consistently preferred light males in the spring and dark males in the summer. Geographic variation in color and maculation has also been noted (Dobzhansky 1933). In parts of Asia, dark morphs may form up to 85% of populations, whereas in North America, dark morphs are rare (LaMana and Miller 1996).

***H. axyridis*: Biology**

Developmental Stages

H. axyridis is holometabolous (exhibits complete metamorphosis) and passes through egg, four larval instars, prepupa, pupa, and adult stages (Hodek 1973). Larval instars develop over ca. 11 days and the pupal stage over ca. 5 days, depending on ambient temperature, prey species, and even host plant species of the aphids (LaMana and Miller 1996). Food scarcity or low quality prey causes increased development time and decreased growth. With increased temperatures, development time is shorter and

adult weight lowered (Kawauchi 1997). Total development time from egg to adult takes approximately 17 days (LaMana and Miller 1996). *H. axyridis* adults typically live for one to three months but may live up to three years, depending on location, availability of prey, and time of year (El-Sebaey and El-Gantiry, 1999).

LaMana and Miller (1998) reported a lower development threshold (LDT) or temperature at which development ceases of 11.2° C and sum of effective temperatures (SET) or number of required days above LDT to complete development of 267.3 days for *H. axyridis* in the United States. Ladybirds in general develop more rapidly and consume fewer aphids as temperatures increase (Michels and Behle 1991).

Fecundity and Oviposition

H. axyridis females typically oviposit 23 to 41 eggs (Takahashi 1987, Hodek and Honek 1996); however fecundity can vary depending upon food resources. The number of eggs deposited is constrained by the beetle's ovariole number in addition to prey availability. At a given moment, roughly half a female's ovarioles are producing eggs, while the other half are preparing for new oviposition (Hodek and Honek 1996). This alternation results in smaller individual batch sizes but allows for more continuous egg production (Stewart *et al.* 1991). This strategy might yield higher fitness given the unpredictable environment ladybirds typically encounter. Under the influence of type and quantity of prey, preoviposition and oviposition periods in *H. axyridis* can vary greatly. Preoviposition periods can range from ca. 7 to 30 days. Oviposition and postoviposition periods range from ca. 35 to 155 days and 2 to 37 days, respectively (Abdel-Salam *et al.* 1997, Abdel-Salam and Abdel-Baky 2001, McClure 1987, Hodek and Honek 1996). These periods may be reduced with higher temperatures (Stathas *et al.* 2001). Total fecundity of *H. axyridis* has been as high as 3,819 eggs under laboratory conditions (Hodek and Honek 1996). However, Stathas *et al.* (2001) reported a maximum total fecundity of 1,642 eggs. Total fecundity depends partly on prey and has been reported as low as 257 total eggs per female (Abdel-Salam *et al.* 1997).

H. axyridis females tend to select oviposition sites apart from aphid colonies (Osawa 1989). Osawa (1989) suggested that this behavior may have evolved in response to the intensification of non-sibling cannibalism associated with increasing proximity to

aphid colonies. If oviposition occurs when an aphid colony is waning, the resulting offspring will face potential starvation (Dixon 2000). In seeking oviposition sites, females avoid colonies that are too old, likely using semiochemicals as cues. Yasuda *et al.* (2000) reported inhibition of oviposition by the presence of conspecific larval tracks and Agarwala *et al.* (2003) reported the same effect from conspecific feces.

***H. axyridis*: Life cycle and diapause**

H. axyridis is typically bivoltine in North America (LaMana and Miller 1996) as well as in Europe and Asia (Osawa 2000, Sakurai *et al.* 1992); however, three to five generations per year have also been observed (LaMana and Miller 1996, Katsoyannos *et al.* 1997). *H. axyridis* spends winter in diapause, with a possible quiescence in some populations in summer, thereby avoiding periods unfavorable for growth and reproduction (Sakurai *et al.* 1992). Beetles enter diapause with an enlarged fat body and empty digestive tract (Iperti and Bertrand 2001, Hodek and Honek 1996). Most females enter diapause unmated (Nalepa *et al.* 1996). During diapause, *H. axyridis* lowers its supercooling point and lower lethal temperature (Watanabe, 2002).

Timing of diapause varies between regions; however, in North America, migration to overwintering sites typically begins in late October (Kidd *et al.* 1995, LaMana and Miller, 1996) and continues on warm, sunny days through mid-November (McCutcheon and Scott unpubl.). Ambient temperature may serve as a signal; migrations appear to begin on the first sunny day with temperatures exceeding ca. 18° C after previously dropping to near freezing (Koch 2003). *H. axyridis* makes lengthy migratory flights and exhibits hypostatic behavior, orienting towards prominent features of the landscape, such as hillcrests, large rocks, or buildings. This appears to be an active and visual response in particular to light-colored surfaces (Obata 1986). No evidence has been found for use of chemical cues in forming aggregations (Nalepa *et al.* 2000). In the beetle's native range in Asia, mass aggregations frequently form on mountainsides in crevices of south facing rocks (e.g., Kidd *et al.* 1995, Ohashi *et al.* 2003). In the U.S.,

aggregations are often formed on light-colored buildings or houses on southern or western-facing walls (Kidd *et al.* 1995). In spring, in concert with increasing photoperiod, light intensity, and ambient temperature, *H. axyridis* adults mate and disperse from overwintering sites (LaMana and Miller 1996, Hodek and Honek 1996).

***H. axyridis*: Population dynamics**

Male-killing bacteria

Some populations of *H. axyridis* harbor a maternally-inherited bacterium of the genus *Spiroplasma* that causes female-biased sex ratios by killing males early in embryogenesis (Majerus *et al.* 1998, Majerus *et al.* 1999, Hurst *et al.* 1992). Inviability of male eggs can actually confer benefits to ladybird populations. Consumption of killed male eggs by neonate siblings reduces the likelihood of females being cannibalized and lessens competition for resources, thereby increasing female sibling longevity (Majerus 1994, Hurst *et al.* 1992). Elimination of sibling brothers can also decrease likelihood of close inbreeding, increasing population fitness (Hurst *et al.* 1992). Though there is a high rate of infection by *Spiroplasma sp.* in some Asian populations of *H. axyridis*, no evidence has been found for female-biased sex ratios in North America (Heimpel and Lundgren 2000).

Cannibalism

Another important factor shaping population dynamics of *H. axyridis* is intraguild and intraspecific cannibalism (Osawa 1993). *H. axyridis* displays kin recognition and is less likely to cannibalize a sibling than a non-sibling (Joseph *et al.* 1999, Michaud 2003). Intensity of cannibalism on sibling eggs is typically density-independent and cannibalism on non-siblings, density-dependent (Michaud 2003). Degree of cannibalistic tendency, which is heritable, varies between lineages (Wagner *et al.* 1999); however, intensity of cannibalism generally increases with lower aphid density and closer proximity to aphid colonies (Burgio *et al.* 2002, Hironori and Katsuhiko 1997, Osawa 1989). Cannibalism is

clearly beneficial when prey are scarce, nutrient-poor, or toxic (Wagner *et al.* 1999, Snyder *et al.* 2000). At high aphid density, intense sibling cannibalism appears maladaptive. However, *H. axyridis* females frequently oviposit during or after peaks in aphid abundance, producing offspring that develop under extreme food scarcity (Osawa 1992). Sibling cannibalism appears to be of great benefit under such conditions and a highly adaptive strategy in an environment of constantly fluctuating resources.

***H. axyridis*: Habitat, prey, and foraging behavior**

H. axyridis is considered primarily arboreal but inhabits old-field vegetation and many agricultural crop fields in addition to orchards and forests (Chapin and Brou 1991, LaMana and Miller 1996, Tedders and Schaefer 1994). More polyphagous than *Coccinella septempunctata*, *H. axyridis* feeds on many aphid species (Lucas *et al.* 1997, Tedders and Schaefer 1994) in addition to Psyllidae (Michaud 2001), Coccoidea (McClure 1987), Tetranychidae (Lucas *et al.* 1997), young instars of Coleoptera, Lepidoptera (Koch *et al.* 2003, Hoogendoorn and Heimpel 2002), Chrysomelidae, Curculionidae (Kalaskar and Evans 2001), and pollen and nectar (LaMana and Miller 1996). Life history parameters, including developmental time, growth, longevity, and reproductive success, vary depending upon prey and ambient temperature (Lucas *et al.* 1997, Abdel-Salam and Abdel-Baky 2001).

H. axyridis is very mobile and closely tracks aphid populations across the landscape (Osawa 2000). In searching for prey, *H. axyridis* larvae exhibit positive phototaxis and negative geotaxis. Unsurprisingly, aphids have the same tactic responses (Hodek and Honek 1996). Upon encountering prey, *H. axyridis* switches from extensive search to intensive search, marked by slow, winding movements (Ettifouri and Ferran 1993). This switch can lead to aggregations in patches of high prey density (Kawai 1976). Earlier research described prey searching as strictly random; however, roles for both visual and olfactory perception have since been identified (e.g., Obata 1986, Harmon *et al.* 1998). Obata (1986) found that adults were attracted to gauze and

polyethylene bags with aphid-infested leaves. Harmon *et al.* (1998) found that beetles consumed more aphids in light than in dark and Lambin *et al.* (1996) demonstrated short and long distance visual perception. Mondor and Warren (2000) demonstrated both conditioned and unconditioned responses by *H. axyridis* to color. Adults were attracted to yellow over green. If conditioned to receiving food with one of the colors, however, females spent more time on the color associated with food.

***H. axyridis*: Natural enemies**

H. axyridis defends itself with aposematic coloration (Hodek and Honek 1996). When disturbed, it enters thanatosis and exudes toxic, alkaloid secretions from the femorotibial articulations (Holloway *et al.* 1991). Several bird species and at least one pentatomid species prey on *H. axyridis* (De Clercq *et al.* 2003, Hodek and Honek 1996). Other ladybird species sometimes prey upon *H. axyridis*, though usually only if they are larger (e.g., Cottrell and Yeargan 1998). Several parasitoids attack *H. axyridis*, including tachinid and phorid flies (Nalepa and Kidd 2002, Nalepa *et al.* 1996, Park *et al.* 1996), and a braconid wasp (Hoogendoorn and Heimpel 2002, Park *et al.* 1996).

Introduction history: *Coccinella septempunctata*

Coccinella septempunctata, native to Eurasia, was imported and released at several locations in the United States between 1956 and 1990 (e.g., Angalet *et al.* 1979, Schellhorn *et al.* 2005). The first importation of *C. septempunctata*, made by the USDA, came from India in 1956. Between 1958 and 1973, additional shipments arrived from India, France, Italy, Norway, and Sweden. Early release attempts, presumed unsuccessful, were made in the Middle Atlantic states to target aphid pests of agriculture (Angalet *et al.* 1979, Hodek and Honek 1996, Schellhorn *et al.* 2005). Twenty-seven F1 adults were recovered from Ohio in 1966 and twelve in New Jersey in 1970, but

establishment was not confirmed in the release areas. The first confirmed records of establishment were in 1973 in the Hackensack Meadowlands in New Jersey and in Quebec in the same year.

In the late 1980s the species was abundant enough to make conspicuous mass appearances on beaches of Delaware (Hoebeke and Wheeler 1980, Tedders and Angalet 1981, Schaefer *et al.* 1987). By this time, *C. septempunctata* had established populations throughout most of the continental United States and southeast Canada (Krafsur *et al.* 1992, Schaefer *et al.* 1987). By the mid-1990s, populations were found in every state in the continental U.S. (Phoofolo and Obrycki 1995). This range expansion likely originated from both natural dispersion and human-assisted redistribution (Angalet *et al.* 1979, Krafsur *et al.* 1992, Phoofolo and Obrycki 1995, 2000). Between 1974 and 1978, redistribution efforts were made primarily against the Russian wheat aphid, *Diuraphis noxia* Mordvilko (Phoofolo and Obrycki 2000), with approximately 500,000 adult *C. septempunctata* collected and released in Delaware, D.C., Florida, Georgia, Illinois, Maine, Maryland, Nebraska, Nevada, New Jersey, New Mexico, New York, Ohio, Oklahoma, Pennsylvania, South Dakota, Texas, Virginia, Washington, Wisconsin, and Utah (Angalet *et al.* 1979, Cartwright *et al.* 1979). Redistribution continued in subsequent years and included the additional destinations of Arizona, Connecticut, the Dominican Republic and the Virgin Islands .

It is not entirely clear if the distribution of cultured beetles throughout much of North America was responsible for its subsequent spread (Schaefer *et al.* 1987). Schaefer *et al.* (1987) pointed out that the Quebec and New Jersey populations were near major waterways where transoceanic freighters are common and that *C. septempunctata* may have been introduced there accidentally. Despite the multiple intentional releases, many have generally accepted a scenario of inadvertent introduction (e.g., Day *et al.* 1994). In an effort to address these questions, genetic diversity in 14 *C. septempunctata* populations in North America and in three western Palearctic populations was assessed by Krafsur *et al.* (1992). No evidence for genetic differentiation in the introduced range was found; genetic diversity was found to be essentially the same in North American populations and in Eurasia. Heterogeneity was large at the population level. Sixteen of

28 loci were polymorphic, with expected heterozygosities of 0.03-0.71. Each of the Nearctic and Palearctic populations showed more variance within subpopulations than variance among subpopulations. These findings would accord with multiple deliberate introductions; however, accidental colonization via transoceanic transportation remains a valid hypothesis because of the tendency of overwintering adults to cluster (Hodek 1973). Dispersal of a large cluster of overwintering *C. septempunctata* adults probably would preserve such variation as existed in the original population because bottleneck effects rapidly diminish when effective population size exceeds 10 (Nei *et al.* 1975).

Supporting the findings of Krafur *et al.* (1992), Phoofolo and Obrycki (1995) examined four North American and Eurasian populations of *C. septempunctata* and found no differences in several life-history parameters, including preoviposition and interoviposition, or number of days on which eggs were laid. In addition, preimaginal development time, sex ratio, and life table parameters, including intrinsic rates of increase and fecundity, did not differ significantly among the four populations. The populations also appeared not to differ from other Eurasian (Czech and Finnish) populations.

It has also been suggested that the establishment of *C. septempunctata* may have been related to trash disposal sites in the Meadowlands, where trash from Kennedy International Airport and other transportation services was dumped (Angalet and Jacques 1975). First records of establishment in the U.S. were made in the Hackensack Meadowlands region of Bergen County, New Jersey in 1973. The following year, *C. septempunctata* was found in high densities at several sites in the Meadowlands and along the New Jersey Turnpike. The largest concentration in the Meadowlands was found in an area located between the turnpike and the landfill. *C. septempunctata* abundance quickly dropped off away from this source, but many small clusters were found up to 20 km away. In Japan, *C. septempunctata* made use of artificial substrates (e.g., metal cans) as microhabitats sufficiently warmed by the sun to complete an extra winter generation (Ohashi *et al.* 2005). Perhaps similar behavior at the New Jersey landfill helped speed establishment. *C. septempunctata* continued as the dominant aphid predator in the Meadowlands in subsequent years.

In many regions of North America, *Coccinella septempunctata* became one of the dominant coccinellid species within three years of detection, accompanied by declines in native coccinellid species (Day *et al.* 1994, Elliott *et al.* 1996, Hoebeke and Wheeler 1980, Obrycki and Kring 1998, Tedders and Angalet 1981, Gordon 1985, Schaefer *et al.* 1987). The native *Coccinella novemnotata* Herbst was once common in the northeastern United States and Canada. Despite extensive fieldwork and coccinellid surveys, very few collections of this species have been made in the Northeast since the mid-1980s (Schaefer *et al.* 1998). *C. septempunctata* and its rapid expansion have been invoked as a possible factor in this apparent population crash (Wheeler and Hoebeke 1995).

In potato crops in northern Maine, prior to 1980, ladybird communities comprised almost exclusively two native species, *H. tredecimpunctata* and *C. transversoguttata* (Alyokhin and Sewell 2004). Despite extensive releases of *C. septempunctata* eggs and larvae between 1964 and 1969 (Shands *et al.* 1970), this non-native was virtually absent from experimental plots until 1980. Starting in 1980, however, *C. septempunctata* became permanently established, and its relative abundance climbed from 6.1% in 1980 to 100% in 1994 (Alyokhin and Sewell 2004). Relative abundances and population density of *H. tredecimpunctata* and *C. transversoguttata* plummeted, with no record of *C. transversoguttata* for spans of several years during this time.

Prior to the arrival of *C. septempunctata* in Manitoba in 1988, Turnock *et al.* (2003) found the most abundant coccinellid species to be *Hippodamia tredecimpunctata tibialis* (Say), followed by *Coccinella transversoguttata richardsonii* Brown, *Hippodamia convergens* Guerin, *H. parenthesis* (Say), then *C. trifasciata perplexa* Mulsant. In the subsequent years of 1989 to 2001, *C. septempunctata* increased rapidly to become the second most abundant species after *H. tredecimpunctata* and briefly became the dominant species. Since the establishment of *C. septempunctata*, *C. transversoguttata*, *H. convergens*, and *H. parenthesis* have declined in relative abundance and *C. trifasciata* has become sufficiently rare to escape detection. Densities also show a downward trend; however it is unclear how many of these changes may be attributed to natural variation. Though Turnock *et al.* (2003) give relative abundance data for 1983-2001, they provide data on densities for only 1987 (one year prior to *C. septempunctata*

arrival)-2001. Two natives, *C. transversoguttata* and *H. convergens*, increase, then decrease during 1987-2001. *C. septempunctata* and *H. tredecimpunctata* together accounted for 75% of coccinellids in all habitats and over 90% in canola and perennial herbaceous plants and shrubs. Turnock *et al.* (2003) identified body size as a possible factor in the differential impact on different species, suggesting that the smaller size of *H. tredecimpunctata* might have permitted coexistence with *C. septempunctata*. Similarly, in a study examining the impact of *C. septempunctata* on abundances of native coccinellids in southern Manitoba from 1988 to 2001, Wise *et al.* (2001) found that along with *Hippodamia tredecimpunctata tibialis* (Say), the European ladybird had become a dominant species among the region's coccinellid fauna.

In apple orchards in eastern West Virginia, *C. septempunctata* became the dominant coccinellid species in 1989, six years after arrival, and remained dominant through 1994, when *Harmonia axyridis* arrived on the scene (Brown and Miller 1998). In southern Michigan, *C. septempunctata* was the dominant coccinellid in a variety of habitats during the study period of 1989-1990 (Colunga-Garcia *et al.* 1997). *C. septempunctata* and *C. maculata* were the most abundant ladybirds in southern Michigan agricultural crops, including alfalfa, maize, soybean, and triticale in 1989 and 1990 (Maredia *et al.* 1992). However, in deciduous habitats, such as apple orchards, native species *Adalia bipunctata* and *Cycloneda munda* were the most abundant. In corn ecosystems in South Dakota, invasion by *C. septempunctata* was associated with annual abundance of *Coccinella transversoguttata* Brown in some crops 20-32 times lower than pre-invasion years, and 20 times lower for *Adalia bipunctata* L. abundance in corn (Elliott *et al.* 1996). By 1984, *C. septempunctata* arrived in Nova Scotia, and by the 1990s it was the most common coccinellid in disturbed habitats on Cape Breton Island (McCorquodale 1998). From 1990 to 1998 two natives, *Adalia bipunctata* and *Coccinella trifasciata*, experienced large declines on Cape Breton. Between 1990 and 1996 these two natives together comprised approximately 25% of all coccinellids collected, with the far greater contribution coming from *A. bipunctata*. In 1998, only two individuals of *A. bipunctata* were found, and native species combined contributed less than 4% to the total coccinellids collected (Cormier *et al.* 2000).

Given the broad diet and high mobility of *C. septempunctata*, the negative effects of this introduced predator likely expand beyond aphidophagous coccinellids. In fact, Horn (1991) found that *C. septempunctata* will eat eggs of the lycaenid *Everes conyntas* in the lab. Further, in Minnesota and Wisconsin, *C. septempunctata* co-occurred spatially and temporally with eggs, larvae, and adults of the endangered Karner blue butterfly *Lycaeides melissa samuelis* (Schellhorn *et al.* 2005). These two species were also observed together on *Lupinus perennis*, the butterfly's sole host plant, and adult *C. septempunctata* were observed consuming second instar larvae of the butterfly. There may be more such stories; however, until the 1990s, quantifiable evidence of harm to native species as a result of the introduction of an arthropod biological control agent was not available (Samways 1988, Howarth 1991, Louda *et al.* 1997). Generally very little attention has been given to potential nontarget impacts and post-release monitoring has been scant (Simberloff and Stiling 1996). The federally-endangered Karner blue butterfly disappeared from Ohio in 1988, approximately when *C. septempunctata* became the most abundant ladybird in the butterfly's habitat. The butterfly had been declining owing to habitat alteration long before *C. septempunctata*'s spread to the area. However, there is a strong possibility that *C. septempunctata* sped the demise of this tiny remnant butterfly population.

Introduction history: *Harmonia axyridis*

The Asian ladybird, *Harmonia axyridis*, has a presumed native distribution extending from southern China to as far north as southern Siberia and from the Altai Mountains east to the Pacific coast (Chapin 1965, Dobzhansky 1933, Sasaji 1971). It was released as a biological control agent for pear psylla, pecan aphid, and other arboreal Homoptera in North America as early as 1916 in California and later in large numbers between 1978 and 1982, mainly in Georgia and Alabama (Kidd *et al.* 1995). *H. axyridis* was first recorded as established in the U.S. in 1988 in Louisiana (Chapin and Brou 1991)

and now occurs throughout much of the continental United States, except for Montana, Wyoming, and parts of the Southwest (Koch 2003).

Day *et al.* (1994) suggested that *H. axyridis* may owe its foothold here to accidental introduction, as the infestation site in Louisiana is near ports used for international shipping, including cargo from Asia. Gene flow analysis by Krawfsur *et al.* (1997) supports a single-source scenario to account for North American populations but cannot determine whether that source was a USDA culture or accidental seaport invasion. After its initial recovery in Louisiana in 1988, this coccinellid underwent rapid population explosions, at times occurring in different regions of the country simultaneously.

Intentional introductions include California (1916, 1964-1965); Washington state (1978-1982); Connecticut (1978-1981, 1985-1986); North Carolina (1978-1981, 1983); Nova Scotia, Georgia, Louisiana, Maryland, Washington D.C., Delaware, Maine, Mississippi, Ohio, and Pennsylvania (1978-1981) (Gordon 1985, Krawfsur *et al.* 1997, McClure 1987, Michaud 2002, Tedders and Schaefer 1994). *H. axyridis* was released for biological control in pecans (Tedders and Schaefer, 1994) and red pines (McClure, 1987). *H. axyridis* has also been documented in alfalfa (Colunga-Garcia and Gage 1998), cotton (Wells *et al.*, 2001), tobacco (Wells and McPherson 1999), and winter wheat (Colunga-Garcia and Gage 1998), where it may be contributing to biological control.

First appearances of established *Harmonia axyridis* populations in North America include Louisiana (1988); Georgia and Mississippi (1990); Washington state (1991); North Carolina, northern Florida, eastern South Carolina, and Arkansas (1992); Virginia, Pennsylvania, and Oregon (1993); West Virginia, Iowa, Illinois, and Quebec (1994), and Nova Scotia (1995) (Brown and Miller 1998, Coderre *et al.* 1995, Colunga-Garcia and Gage 1998, Kidd *et al.* 1995, Krawfsur *et al.* 1997, McCorquodale 1998, Tedders and Schaefer 1994).

Harmonia axyridis not only appeared rapidly throughout the United States, but also quickly became the dominant coccinellid in many settings. *H. axyridis*, first collected in apple orchards in eastern West Virginia in 1994, became dominant in 1995, (replacing *Coccinella septempunctata*), and still dominated at the end of the study in

1996 (Brown and Miller 1998). Brown and Miller (1998) did not provide details of changes in relative or total abundances. Interestingly, Brown (2003) suggested that *H. axyridis* may be suppressing *C. septempunctata*, allowing native ladybirds to increase in abundance. Surveys conducted during 1993-1996 in Pennsylvania documented explosive colonization, in addition to declines in numbers of *Coccinella novemnotata* and *Hippodamia convergens* (Wheeler and Stoops 1996).

In southwestern Michigan, coccinellid species diversity and abundance in an agricultural landscape was documented before and after *H. axyridis* became established. The Asian beetle became dominant in the landscape four years after its arrival. During this four-year period, indigenous populations of *Brachiacantha ursina*, *Cycloneda munda*, and *Chilocorus stigma* declined (Colunga-Garcia and Gage 1998).

LaMana and Miller (1996) conducted field surveys in Oregon and found 70% of individual lady beetles and 82% of live adult coccinellid mass in arboreal habitats to consist of *H. axyridis*. The Asian beetle represented only 4% of coccinellids in alfalfa, clover, and peppermint. As this study captures just a “snapshot” in time, no conclusions can be drawn regarding changes in the ladybird community. Over a five-year period in Florida citrus, Michaud (2002) found that the relative abundance of *Coccinella sanguinea* declined significantly in all five counties surveyed during the study and the relative abundance of *H. axyridis* increased significantly in three of the five counties. However, no information was provided on population densities or changes in absolute abundance.

For a species with such a broad diet, it is entirely reasonable to expect a whole suite of potentially detrimental impacts on other organisms, including nontarget prey and other predatory insects. Nonetheless, very few such evaluations have been conducted and little to this effect is known. Koch *et al.* (2003) demonstrated that *H. axyridis* adults and larvae both consumed significant quantities of the eggs and larvae of the monarch butterfly, *Danaus plexippus*, in both laboratory and field-cage studies. *H. axyridis* may also inadvertently feed on parasitoids of aphids, and the presence of *H. axyridis* near an aphid colony may decrease the oviposition rate of parasitoids (Takizawa *et al.* 2000). In addition to nontarget impacts on native ladybirds and other arthropods, *H. axyridis* has become a pest to humans. In the native range of *H. axyridis*, individuals form

aggregations in the fall and migrate to overwintering sites, usually cliffs, where they seek warmth and protection in small cracks in the rocks. In North America, overwintering *H. axyridis* adults often select homes or other buildings (Kidd *et al.* 1995), resulting at times in heavy infestations as high as 20,000 (Mizell 2001) per building. With warming temperatures in the spring, the ladybirds become active, crawling and flying, as well as emitting a foul odor (“reflex bleeding”) if alarmed or, say, trod upon accidentally. Aside from being a nuisance, large infestations under house siding can promote moisture damage (McCutcheon and Scott Unpubl.). Some people experience allergic rhinoconjunctivitis in response to *H. axyridis* (Koch 2003). And, reflecting its polyphagous nature, this species also bites humans occasionally, likely testing for suitable prey (Koch 2003). *H. axyridis* also sometimes overwinters in beehives. This practice apparently does not harm the bees but is a nuisance to the beekeepers (Caron 1996). Its pest status does not end there; *H. axyridis* has also been wreaking havoc with fruit production and processing. This ladybird aggregates and sometimes feeds on pears, apples, and grapes and can be a real pest in vineyards, where it gets mixed in with the grapes during processing. Alkaloids present in *H. axyridis* alter the flavor and have been reported to ruin entire batches of wine (Ejbich 2003).

Research Objectives

Coccinella septempunctata and *Harmonia axyridis*, both of Old World origin, are two polyphagous species introduced widely in the United States for biological control (Angalet *et al.* 1979, Kidd *et al.* 1995). They are currently very common throughout North America and have been associated with declines in native populations (Schaefer *et al.* 1987, Brown and Miller 1998). The objectives of this study were 1) to assess changes in ladybird beetle communities at Mt. St. Helens, Washington over a 20-year period (1985-2004), with emphasis on dynamics subsequent to the arrival to the area by *C. septempunctata* and *H. axyridis*, and 2) to assess changes in ladybird beetle communities

in southwestern Virginia from 1996 to 2004 following invasion by *C. septempunctata* and *H. axyridis*.

II. Impacts of two introduced ladybeetles, *Coccinella septempunctata* and *Harmonia axyridis*, (Coleoptera: Coccinellidae), on native ladybeetles at Mount St. Helens in Washington

Introduction

Exotic natural enemies introduced to control alien pest species can have unforeseen and irreversible consequences (Louda *et al.* 2003, Simberloff and Stiling 1996). Risks associated with biological control include host-switching by the introduced agent, displacement or extinction of native species, and dispersal into nonagricultural habitats. Until the 1990s, quantifiable evidence of harm to native species as a result of the introduction of an arthropod biological control agent was unavailable (Samways 1988, Howarth 1991, Louda *et al.* 1997). Better understanding of both natural enemy fate following introduction and impacts on nontarget species is greatly needed.

Ladybeetles are one of the most important groups that prey on pest insects and have been repeatedly used in biological control programs (Gordon 1985, De Bach and Rosen 1991). For example, *Coccinella septempunctata*, native to Eurasia, was imported and released at multiple locations (in 23 states) in the United States between 1956 and 1990 to target several aphid pests of agriculture (Angalet *et al.* 1979, Hodek and Honek 1996, Schellhorn *et al.* 2005). *Coccinella septempunctata* was first reported as established in 1973 in New Jersey and by the late 1980s had spread throughout most of the continental U.S. and southeast Canada (Krafsur *et al.* 1992, Schaefer *et al.* 1987). In many regions of North America, *Coccinella septempunctata* rapidly became one of the most common coccinellid species. In corn ecosystems in South Dakota, invasion by *C. septempunctata* was associated with decline in annual abundance of *Coccinella transversoguttata* and *Adalia bipunctata* (Elliott *et al.* 1996). In Maine, establishment by *C. septempunctata* was associated with declines in relative abundance and population density of *H. tredecimpunctata* and *C. transversoguttata* (Alyokhin and Sewell 2004). *Coccinella septempunctata* has also been implicated in the population crash of *Coccinella*

novemnotata Herbst in the northeastern U.S. (Wheeler and Hoebeke 1995). Little attention has been given to other possible nontarget species; however, two lycaenid species, including the federally-endangered Karner blue butterfly, have been identified as of potential concern with regard to *C. septempunctata* (Horn 1991, Schellhorn *et al.* 2005).

The Asian ladybird, *Harmonia axyridis*, was released as a biological control agent in 13 states for pear psylla, pecan aphid, and other arboreal Homoptera as early as 1916 in California and later in large numbers between 1978 and 1982, mainly in Georgia and Alabama (Kidd *et al.* 1995, McClure 1987, Michaud 2002, Tedders and Schaefer 1994). *Harmonia axyridis* was first recorded as established in the U.S. in 1988 in Louisiana (Chapin and Brou 1991) and by 1991 had established populations in Washington state (Krafsur *et al.* 1997). After its initial recovery in 1988, *H. axyridis* underwent rapid population explosions, at times occurring in different regions of the country simultaneously. *Harmonia axyridis* not only appeared rapidly throughout the United States but also quickly became the dominant coccinellid in many settings. *Harmonia axyridis*, first collected in apple orchards in eastern West Virginia in 1994, became dominant in 1995 (replacing *Coccinella septempunctata*) and still dominated at the end of the study in 1996 (Brown and Miller 1998). Surveys conducted during 1993-1996 in Pennsylvania documented explosive colonization, in addition to declines in numbers of *Coccinella novemnotata* and *Hippodamia convergens* (Wheeler and Stoops 1996). In southwestern Michigan, native populations of *Brachiacantha ursina*, *Cycloneda munda*, and *Chilocorus stigma* declined after establishment by *H. axyridis* (Colunga-Garcia and Gage 1998). Very few evaluations of other nontarget organisms have been conducted, although Koch *et al.* (2003) demonstrated that *H. axyridis* adults and larvae both consumed significant quantities of the eggs and larvae of the monarch butterfly, *Danaus plexippus*, in both laboratory and field-cage studies.

The full extent of impacts on native ladybird species by these two introduced coccinellids is poorly understood. Given the extreme degree of spatial and temporal variation characteristic of ladybird populations (Kieckhefer and Elliott 1990), long-term studies can be critical to extract trends and patterns in population dynamics (Elliott *et al.*

1996); very few such studies have been conducted in this context (Elliott et al. 1996, Brown and Miller 1998, Alyokhin and Sewell 2004). The purpose of this research was to assess changes in native ladybird beetle communities at Mt. St. Helens, Washington over a 20-year period (1985-2004), with emphasis on dynamics subsequent to the arrival in the area of *C. septempunctata* and *H. axyridis*.

Methods

Study Sites

Two sites in Washington were located at Mount St. Helens, a volcano in the Cascades, ca. 100 km northeast of Portland, OR. In May 1980, Mount St. Helens erupted with catastrophic effects. Large tracts of vegetation were buried by landslides or blown down by molten rock hurled at speeds up to 1000 km/h (Weyerhaeuser 2006). The eruption essentially destroyed all aboveground life within ca. 600 sq km surrounding the summit (Lawrence and Ripple 1998). Our study was conducted in September of each year beginning in 1985, five years after the eruption, through 2004. The Pumice Plains site is in the heart of the blast zone, near the base of Mount St. Helens. The Green River site is within the outer reaches of the blast zone and therefore is experiencing a faster vegetation recovery. Regeneration of vegetation is slow at both areas owing in part to high ash levels (Weyerhaeuser 2006).

Sampling Methods and Data Analysis

At the Pumice Plains and Green River sites, we haphazardly chose and thoroughly searched 100 individuals of fireweed (*Epilobium angustifolium*) and recorded numbers and species of ladybirds. We compared numbers of ladybirds per site for different time periods (pre-invasion to post-invasion years) with Wilcoxon two-sample tests with normal approximation with a continuity correction of 0.5 for z (SAS Institute 2003). Interactions between ladybird species were analyzed by Spearman's rank correlations (PROC CORR; SAS Institute 2003). An alternate approach would be to apply diffusion approximation methods designed to handle high sampling error and yearly variation (e.g.

Holmes 2001); however, this method would have required more observation points, particularly from years preceding the arrival of *H. axyridis* and *C. septempunctata*. Despite these challenges, the length of this study does permit us to draw some conclusions on population trends.

Results

We found five coccinellid species routinely at both Mount St. Helens sites. These are *C. septempunctata* and *H. axyridis*, both introduced to North America, and the natives *Adalia bipunctata*, *Hippodamia convergens*, and *Coccinella transversoguttata*.

At Pumice Plains, we first detected *C. septempunctata* in 1990. From then through 2004, we found this species during all but two years, 1992 and 1994. From 1985-1994 to 1995-2004, numbers of *C. septempunctata* adults increased considerably (Wilcoxon 2-sample test: $z = -3.1152$, $p = 0.0009$). We first detected *H. axyridis* at Pumice Plains in 1992. From then through 2004, we found this beetle in ten of thirteen years. *H. axyridis* numbers increased at Pumice Plains from 1985-1994 to 1995-2004 (Wilcoxon 2-sample test: $z = -0.2649$, $p = 0.004$).

We found the native *Adalia bipunctata* four of the first six years, 1985 to 1990, at Pumice Plains (Fig. 1). After *C. septempunctata* first appeared there in 1990 (and *H. axyridis* in 1992), *A. bipunctata* was found in only two of the remaining 14 years. Despite the appearance of a decline, *A. bipunctata* was never found in sufficient numbers to confirm such a trend. However, a Wilcoxon 2-sample test showed more individuals of *A. bipunctata* in pre-*Harmonia* years 1985-1991 than in post-*Harmonia* years 1992-2004: ($z = 2.0155$, $p = 0.0219$). *Hippodamia convergens* was found approximately every other year during the initial eight years. It was then not detected from 1993 to 1999, then resurfaced in 2000 and occurred every year from 2002 to 2004. At Pumice Plains, a decline from pre-*Harmonia* years 1985-1991 to post-*Harmonia* years 1992-2004 was revealed by a Wilcoxon 2-sample test ($z = 2.4404$, $p = 0.0073$). *Coccinella transversoguttata* was the only species found at Pumice Plains in 1985. It was last

spotted in 1993, the only year it was detected after the first appearances of *C. septempunctata* and *H. axyridis* in 1990 and 1992, respectively (Fig. 1). At Pumice Plains, a decline from pre-*Harmonia* years 1985-1991 to post-*Harmonia* years 1992-2004 was revealed by a Wilcoxon 2-sample test ($z = 2.4404$, $p = 0.0073$). There was a negative correlation between *C. transversoguttata* and *H. axyridis* ($r_s = -0.53412$, $p = 0.0153$) and between *C. transversoguttata* and total exotic ladybirds ($r_s = -0.56917$, $p = 0.0088$). *Coccinella transversoguttata* had a marginally significant, negative correlation with *C. septempunctata* ($r_s = -0.38788$, $p = 0.0911$). We detected the remaining species found at Pumice Plains sporadically. *Coccinella novemnotata* was found only in 1988 and in 1991, *Coccinella polita* in 1987, 1989, and 1997. One individual of *Hippodamia glacialis glacialis* and four individuals of *Hippodamia quinquesignata* were found only in 2003.

There was a significant decline in total native ladybirds at Pumice Plains from 1985-1991 (pre-*Harmonia axyridis* arrival) to 1992-2004 (post-*H. axyridis* years) (Wilcoxon 2-sample test: $z = 2.0234$, $p = 0.0215$). Inherent variation makes these results sensitive to the lead and cutoff dates, such that a comparison of the periods 1985-1994 and 1995-2004 reveals no significant change. Total native ladybirds were negatively correlated with *H. axyridis* ($r_s = -0.50026$, $p = 0.0247$).

At Green River, we first detected *C. septempunctata* in 1991 and found it every year thereafter. The increase in numbers between 1985-1994 and 1995-2004 was also significant by a Wilcoxon 2-sample test ($z = 3.0805$, $p = 0.001$). We first found *H. axyridis* in 1993, two years after the first discovery of *C. septempunctata* and one year after *H. axyridis* first appeared at Pumice Plains. *Harmonia axyridis* was not found the following two years, but was found almost every year from 1996 to 2004. It increased in total abundance from 1985-1994 to 1995-2004 (Wilcoxon 2-sample test: $z = 2.9668$, $p = 0.0015$). We found *C. transversoguttata* at Green River during the first eight years of the study and, as at Pumice Plains, this was the only coccinellid encountered in 1985. We then found this species in only two of the remaining twelve years (Fig. 2).

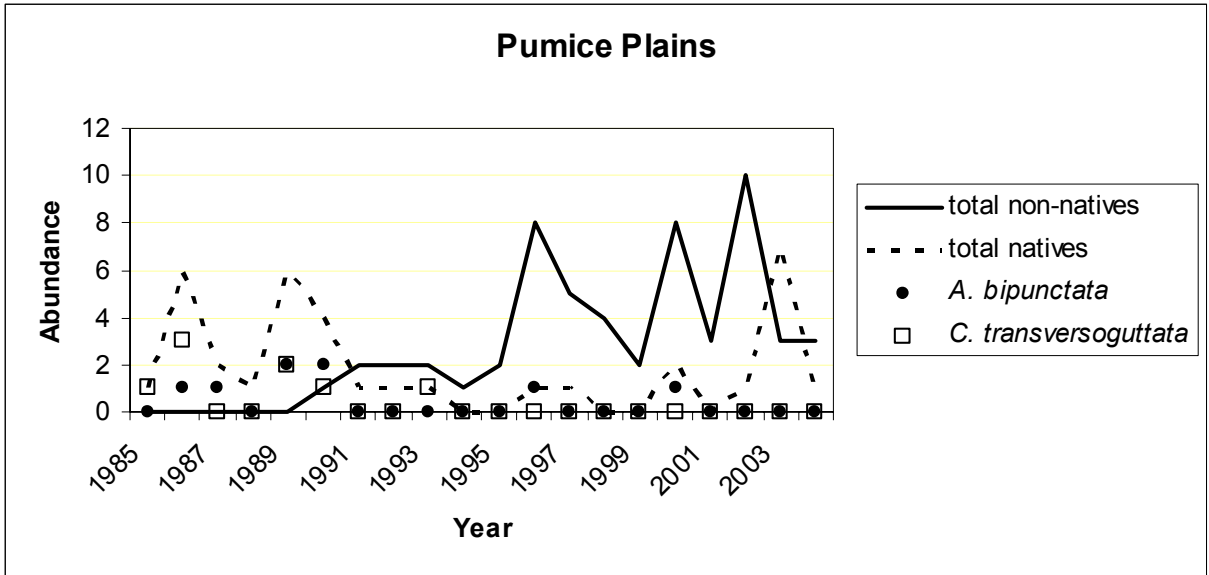


Fig. 1 Population trends for the total non-natives, total natives, and the two most commonly found native species at Pumice Plains.

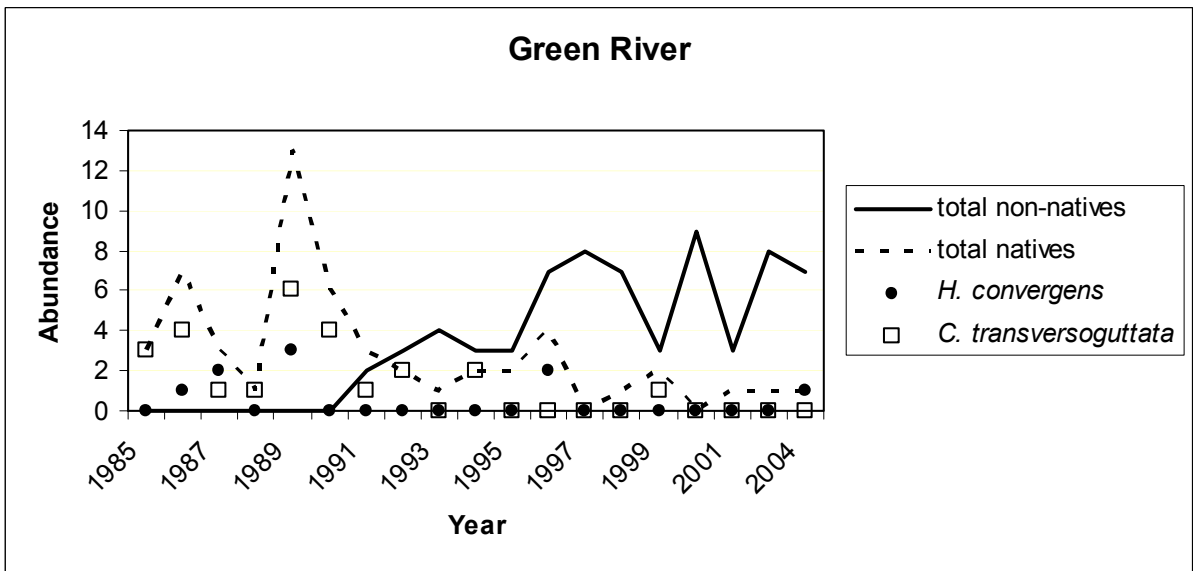


Fig. 2 Population trends for total non-natives, total natives, and the two most commonly found native species at the Green River site.

Negative correlations were found with *H. axyridis* ($r_s = -0.79266$, $p < .0001$), with *C. septempunctata* ($r_s = -0.74729$, $p = 0.0002$), and with total exotics ($r_s = -0.76168$, $p = 0.0002$). A Wilcoxon 2-sample test revealed a significant decline by *C. transversoguttata* from 1985-1994 to 1995-2004 ($z = -3.2996$, $p = 0.0005$). *Hippodamia convergens* appeared sporadically, with only slightly greater frequency in the initial years. There were no significant changes between 1985-1994 and 1995-2004 at either site. The remaining species found at Green River – *C. polita*, *C. novemnotata*, *A. bipunctata* and *C. californica* – made only occasional appearances with no detectable patterns or trends.

At Green River, there was a significant decline in total native individuals from 1985-1994 to 1995-2004 (Wilcoxon 2-sample test: $z = -2.4816$, $p = 0.0065$). There were negative correlations between total native ladybirds and *H. axyridis* ($r_s = -0.55169$, $p = 0.0143$), total natives and *C. septempunctata* ($r_s = -0.72070$, $p = 0.0005$), and total natives and total introduced ladybirds ($r_s = -0.63918$, $p = 0.0032$).

Discussion

The strong decline of *Coccinella transversoguttata* following invasion by *C. septempunctata* and *H. axyridis* at both Green River and Pumice Plains is in keeping with several other studies (Elliott et al. 1996, Evans 2004, Snyder et al. 2004, Turnock et al. 2003, Alyokhin and Sewell 2004). *Coccinella septempunctata* spread to eastern Washington by the early 1990s. By the close of the decade, it had become common and the previously dominant *C. transversoguttata* had become rare (White and Eigenbrode 2000, Snyder et al. 2004). Decline of *C. transversoguttata*, previously the second most abundant ladybird, followed establishment of *C. septempunctata* in Manitoba in 1998 (Turnock et al. 2003). In addition to displacement through intraguild predation, *C. transversoguttata* and *C. septempunctata* are of similar size and may well compete more directly for resources (Turnock et al. 2003). In South Dakota, invasion by *C. septempunctata* was associated with annual abundance of *C. transversoguttata* in some crops 20-32 times lower than in pre-invasion years (Elliott et al. 1996). Prior to

establishment of *C. septempunctata* in northern Maine in 1980, ladybird communities comprised almost exclusively two native species, *H. tredecimpunctata* and *C. transversoguttata* (Alyokhin and Sewell 2004). Over the next 14 years, *C. septempunctata* increased from a relative abundance of ca. 6% to 100% while populations of both native species plummeted.

Coccinella transversoguttata larval interactions with *H. axyridis* heavily favor the latter (Snyder et al. 2004, Yasuda et al. 2004). Addition of aphid prey surprisingly did not alter the frequency of intraguild predation, suggesting the potential for negative impacts on the native coccinellid regardless of prey resources (Snyder et al. 2004). Increased frequency of intraguild predation following invasion by *C. septempunctata* and *H. axyridis* may well have contributed to the rapid displacement of *C. transversoguttata* at Mt. Saint Helens.

Even though there was not sufficient information to obtain statistical significance, *Adalia bipunctata*, present in four of the first six sampling years but only two of the following fourteen, appeared to decline in abundance at Pumice Plains, coinciding with an increase in *H. axyridis* and *C. septempunctata*. In a study of larval interactions between *C. septempunctata* and *A. bipunctata* and between *H. axyridis* and *A. bipunctata*, the introduced species more often acted as predator and *A. bipunctata* as prey (Kajita et al. 2000). Under conditions of low food availability, intraguild predation was common. With surplus food resources, *A. bipunctata* experienced slower larval development when *H. axyridis* larvae were present. *Harmonia axyridis* was a more aggressive intraguild predator of *A. bipunctata* than was *C. septempunctata* (Kajita et al. 2000).

Life history parameters of these individual species may also be a factor in their relative success rates. *Coccinella septempunctata* has a lower K value, or thermal constant, than *A. bipunctata* (Obrycki and Tauber 1981). As a result, at high temperatures consistently above threshold values, as would be the case later in the season when the Pumice Plains and Green River sites were sampled, *C. septempunctata* completes development more quickly than *A. bipunctata* (Obrycki and Tauber 1981). Under limited resources, these asymmetrical developmental rates could help the introduced species edge out *A. bipunctata*.

Our data do not show clear trends for *Hippodamia convergens* at Mt. St. Helens. Tests of larval interactions reveal clear advantages of both introduced species over *H. convergens* (Snyder et al. 2004, Yasuda et al. 2004). Evans (1991) reported no difference when pairing *H. convergens* larvae with a conspecific larva versus a *C. septempunctata* larva, but he studied these interactions at high prey levels.

Both *Harmonia axyridis* and *Coccinella septempunctata* were mass-reared by the USDA and released to control specific crop pests. We and several others have found these generalized predators widely distributed and common in communities and vegetation far from any cropping system that was their intended target. Mt. St. Helens is located in a remote area of southwestern Washington, yet even at Pumice Plains, scoured clear of all biota by the heat and force of the 1980 blast and still today resembling a moonscape, these two non-native species have dominated the coccinellid fauna since shortly after their arrival. Generalized predators with general habitat affinities should be expected to spread, and these two ladybird beetles have done just that. We found *Harmonia axyridis* at Mt. St. Helens merely a year after its first detection in Washington State. Although our data do not allow us to convict either of these species of the local extinction or even local decline of native species, our data do show a striking coincidence with the decline of the native coccinellid community at Mt. St. Helens. It would be especially interesting to ask whether the success of these introduced beetles has any implications for aphid populations or for interactions between aphids and predators, outside of the agroecosystems for which they were intended.

III. Impacts of *Coccinella septempunctata* and *Harmonia axyridis*, (Coleoptera: Coccinellidae), on native ladybeetles in southwestern Virginia

Introduction

Exotic natural enemies introduced to control alien pest species can have unforeseen and irreversible consequences (Louda *et al.* 2003, Simberloff and Stiling 1996). Risks associated with biological control include host-switching by the introduced agent, displacement or extinction of native species, and dispersal into nonagricultural habitats. Until the 1990s, quantifiable evidence of harm to native species as a result of the introduction of an arthropod biological control agent was unavailable (Samways 1988, Howarth 1991, Louda *et al.* 1997). Better understanding of both natural enemy fate following introduction and impacts on nontarget species is greatly needed.

Ladybeetles are one of the most important groups that prey on pest insects and have been repeatedly used in biological control programs (Gordon 1985, De Bach and Rosen 1991). For example, *Coccinella septempunctata*, native to Eurasia, was imported and released at multiple locations (in 23 states) in the United States between 1956 and 1990 to target several aphid pests of agriculture (Angalet *et al.* 1979, Hodek and Honek 1996, Schellhorn *et al.* 2005). *Coccinella septempunctata* was first reported as established in 1973 in New Jersey and by the late 1980s had spread throughout most of the continental U.S. and southeast Canada (Krafsur *et al.* 1992, Schaefer *et al.* 1987). In many regions of North America, *Coccinella septempunctata* rapidly became one of the most common coccinellid species. In corn ecosystems in South Dakota, invasion by *C. septempunctata* was associated with decline in annual abundance of *Coccinella transversoguttata* and *Adalia bipunctata* (Elliott *et al.* 1996). In Maine, establishment by *C. septempunctata* was associated with declines in relative abundance and population density of *H. tredecimpunctata* and *C. transversoguttata* (Alyokhin and Sewell 2004). *Coccinella septempunctata* has also been implicated in the population crash of *Coccinella novemnotata* Herbst in the northeastern U.S. (Wheeler and Hoebeke 1995). Little

attention has been given to other possible nontarget species; however, two lycaenid species, including the federally-endangered Karner blue butterfly, have been identified as of potential concern with regard to *C. septempunctata* (Horn 1991, Schellhorn *et al.* 2005).

The Asian ladybird, *Harmonia axyridis*, was released as a biological control agent in 13 states for pear psylla, pecan aphid, and other arboreal Homoptera as early as 1916 in California and later in large numbers between 1978 and 1982, mainly in Georgia and Alabama (Kidd *et al.* 1995, McClure 1987, Tedders and Schaefer 1994, Michaud 2002). *Harmonia axyridis* was first recorded as established in the U.S. in 1988 in Louisiana (Chapin and Brou 1991) and by 1993 had established populations throughout most regions of Virginia (Nault and Kennedy 2003). After its initial recovery in 1988, *H. axyridis* underwent rapid population explosions, at times occurring in different regions of the country simultaneously. *Harmonia axyridis* not only appeared rapidly throughout the United States but also quickly became the dominant coccinellid in many settings. *Harmonia axyridis*, first collected in apple orchards in eastern West Virginia in 1994, became dominant in 1995 (replacing *Coccinella septempunctata*) and still dominated at the end of the study in 1996 (Brown and Miller 1998). Surveys conducted during 1993-1996 in Pennsylvania documented explosive colonization, in addition to declines in numbers of *Coccinella novemnotata* and *Hippodamia convergens* (Wheeler and Stoops 1996). In southwestern Michigan, native populations of *Brachiacantha ursina*, *Cycloneda munda*, and *Chilocorus stigma* declined after *H. axyridis* established (Colunga-Garcia and Gage 1998). Very few evaluations of other nontarget organisms have been conducted, although Koch *et al.* (2003) demonstrated that *H. axyridis* adults and larvae both consumed significant quantities of eggs and larvae of the monarch butterfly, *Danaus plexippus*, in both laboratory and field-cage studies. The full extent of impacts on native ladybird species by these two introduced coccinellids is poorly understood. The purpose of this research was to assess changes in native ladybird beetle communities in southwestern Virginia from 1996 to 2004 following invasion by *C. septempunctata* and *H. axyridis*.

Methods

Study Sites

Sites in Virginia, sampled during the summers of 1996, 2003, and 2004, were located near Mountain Lake Biological Station, near Blacksburg. They included a corn field and corn field margin at Virginia Polytechnic Institute and State University (Virginia Tech); an old goldenrod-dominated field (Bob's field) in an isolated forest clearing off the old Appalachian Trail in Jefferson National Forest and separated from other fields by >6 km; a hillside field dominated by blueberry shrubs and goldenrod adjacent to the Mountain Lake Hotel in Pembroke; and, in Eggleston, an old field of mixed herbaceous species bordered by the New River on one side and a railway line on the other.

Sampling Methods and Data Analysis

We sampled each site by visual survey using 25-meter long transects and 0.25 m² quadrats. We searched a meter-wide swath of vegetation along eight haphazardly chosen transects per site and recorded ladybird species present. In addition, we established quadrats at 5-meter intervals along four of the 25-meter transects, for a total of 24 quadrats per site. For each quadrat, we recorded ladybird species present.

Results

Five coccinellid species consistently appeared at the Virginia sites: the introduced *C. septempunctata* and *H. axyridis* and natives *Cycloneda munda*, *Coleomegilla maculata*, and *Psyllobora vigintimaculata*. Other species encountered were *Brachiacanthus* sp., *Subcoccinella vigintiquattuor punctata* L., and *Hippodamia convergens*.

At Bob's Field, we first found *C. septempunctata* when we first sampled, in June, 1996, and first found *H. axyridis* in July, 2003. But at no sampling period did we find

more than 3 individuals of either introduced species or of *C. maculata*, while *C. munda* numbers fluctuated between 0 to 5 individuals and those of *P. vigintimaculata* between 0 and 5 except for 108 individuals in August 2003. At Eggleston, no *C. septempunctata* were ever seen, nor *P. vigintimaculata* or *C. maculata* among the natives. We first found *H. axyridis* in August, 1996, and numbers of *H. axyridis* and *C. munda* are depicted in Fig. 3. At Mountain Lake, there were never more than two *C. septempunctata* (first found in August 1996) or six *H. axyridis* (first found in July 2003) and, among the natives, more than four *C. munda* or one *C. maculata*. However, there were 11 individuals of *P. vigintimaculata* in July 2003, 73 individuals in August 2003, and 10 individuals in August 2004. The corn field was the only site to have substantial numbers of both introduced species (from our earliest sampling in June, 2006) as well as all three natives (Fig. 4).

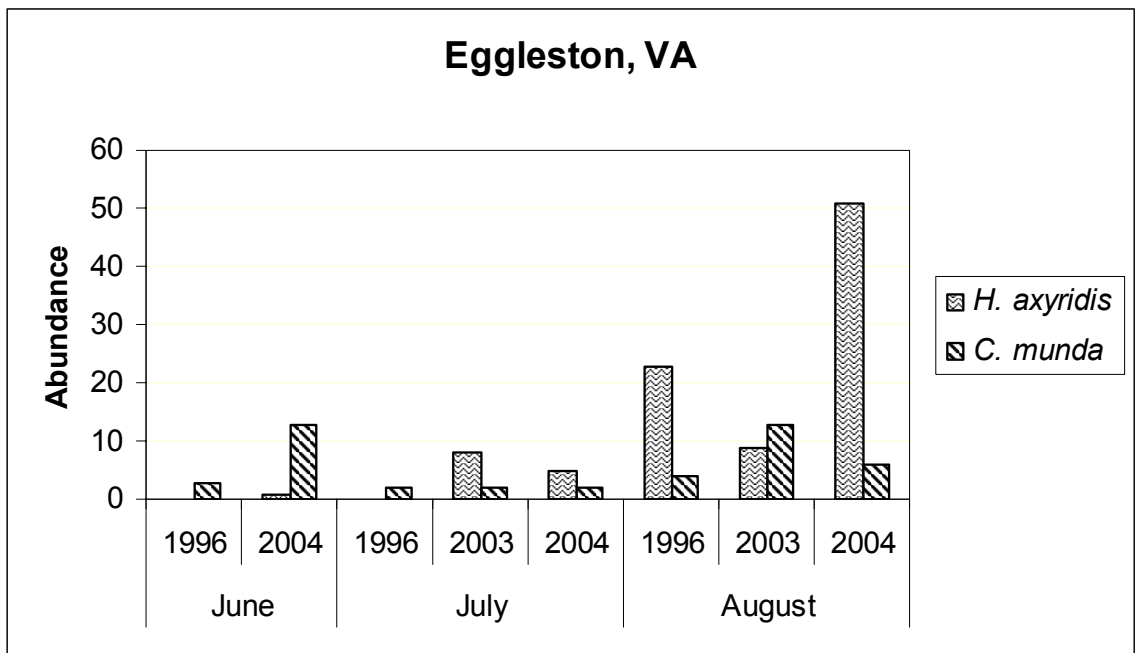


Fig. 3 Population numbers of *H. axyridis* and *C. munda* at Eggleston site.

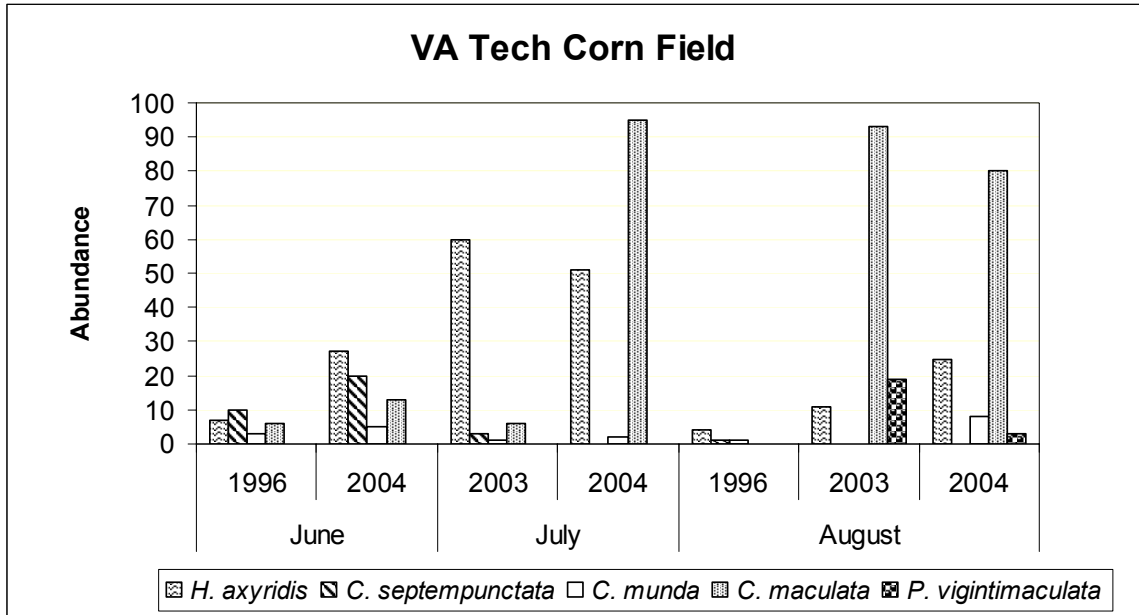


Fig. 4 Coccinellid population numbers at corn field site.

Discussion

Harmonia axyridis was the most ubiquitous coccinellid in our samples, which is noteworthy because it has probably been established in North America for only 19 years. By contrast, *Coccinella septempunctata* appears to have declined in abundance. No increase in numbers or relative abundance of *C. septempunctata* was observed at any site between 1996 and 2004. In some instances, *C. septempunctata* even appeared to have declined. *Harmonia axyridis* may have played a role in the decline of *C. septempunctata*, perhaps even prior to this study. In several regions of North America, *C. septempunctata* became the dominant ladybird species shortly after arrival, only to be replaced by the subsequent spread of *H. axyridis* (e.g., Brown and Miller 1998). The dominance of *H. axyridis*, including over *C. septempunctata*, may be attributed to its aggressiveness as intraguild predator, in addition to its catholic eating habits (e.g., Shinya 1997, Yasuda *et al.* 2001, Nault and Kennedy 2003, Brown 2003). Yasuda and Ohnuma (1999) found that, when offered *H. axyridis* larvae as food, *C. septempunctata* larvae survived at significantly lower rates. Larvae of *H. axyridis*, which are more polyphagous than *C.*

septempunctata (Lucas *et al.* 1997, Yasuda and Ohnuma 1999), experienced equivalent growth and survival rates regardless of food, including heterospecific larvae. In its native range, *H. axyridis* is also a very successful intraguild predator, including against *C. septempunctata*, with which it is frequently sympatric (Kajita *et al.* 2000; Yasuda and Ohnuma 1999; Yasuda *et al.* 2001).

Brown (2003) suggested that *C. septempunctata* might pose a greater threat than *H. axyridis* to indigenous ladybird species. Yasuda *et al.* (2004) argued the reverse. Whichever, the question remains as to why we detected no measurable impact on native species in Virginia in spite of several studies elsewhere reporting such effects for *C. septempunctata* (Wheeler and Hoebeke 1995, Elliott *et al.* 1996, Brown and Miller 1998, Cormier *et al.* 2000, Turnock *et al.* 2003, Alyokhin and Sewell 2004) or *H. axyridis* (Wheeler and Stoops 1996, Colunga-Garcia and Gage 1998). The most obvious explanation is that there were not, in fact, any changes experienced in these ladybird communities. Given the dynamic nature of ladybird populations across temporal and spatial dimensions (e.g., Kieckhefer and Elliott 1990), it is also possible that significant changes in one or more of the sites went undetected. Only one “early” year (1996) was sampled, which may not have sufficed to represent the “before” picture. Also, not all months were sampled at all sites every year. As a striking monthly effect was present, this paucity of sampling dates could further cloud results.

Another possibility is that any changes that occurred in the ladybird community had already occurred by the first year of sampling. The exact timing of the spread of these two introduced species into the study area is not entirely clear. However, *C. septempunctata* had reached Delaware and Georgia by 1979 and most of the continental U.S. by the late 1980s (Schaefer *et al.* 1987, Angalet *et al.* 1979). *H. axyridis* had been detected in Virginia by 1993. In some areas, *H. axyridis* and *C. septempunctata* have been reported as reaching dominant status within three years of initial detection. As the arrival of both *C. septempunctata* and *H. axyridis* preceded the beginning of this study, one or both species might already have affected indigenous ladybird species.

Despite ability to use a variety of different habitats, at least some aphidophagous lady beetle species prefer certain environments (Honek 1985). Therefore, it has been

argued that arrival of the invaders might simply cause “habitat compression” for native lady beetles, pushing them back to ancestral habitats (Evans 2004). In fact, coccinellids were not dominant in the aphid predator guild in North America prior to the introduction of *C. septempunctata* (Brown 2003). This hypothesis might explain why native populations did not decline at some sites; but the results at the corn field are unexpected under this scenario. In fact, the indigenous *Coleomegilla maculata* thrived at this site. *Coleomegilla maculata* is able to complete development on corn pollen without aphids and also feeds on eggs of corn earworm and European corn borers (Park and Obrycki 2004). Musser and Shelton (2003) attributed *H. axyridis* and *C. maculata* coexistence in New York corn fields to different seasonal timing and within-plant distributions. *H. axyridis* adults were generally found later and higher on plants than *C. maculata*. The two were most likely to encounter one another during high aphid abundance and pollen shed, both factors that should minimize intraguild predation.

Coccinella septempunctata can negatively affect *C. maculata* through intraguild predation (Obrycki *et al.* 1998). Obrycki *et al.* (1998) reported that survival of *C. maculata* larvae was significantly reduced when they were reared with a *C. septempunctata* larva. Survival of *C. septempunctata* larvae, on the other hand, significantly increased when reared with a *C. maculata* larva. When prey were not limiting, no negative interactions were measured between larval *C. maculata* and *C. septempunctata*. Aphids might have been sufficiently abundant at the corn field site to deter predation by *C. septempunctata* on *C. maculata*, or the two may simply experience distinct seasonal timing or spatial distribution. It is also possible that *H. axyridis* may help maintain this coexistence by competing with *C. septempunctata*. Hoogendoorn and Heimpel (2002) examined an indirect interaction between *H. axyridis* and *C. maculata* mediated by the parasitoid, *Dinocampus coccinella*. The presence of *H. axyridis* benefited *C. maculata* by diverting some of the parasitoid eggs away from the *C. maculata* population.

Laboratory studies on intraguild predation in coccinellids do not necessarily predict field interactions. Natural habitat complexity accommodates key behavioral modifications, such as avoidance and emigration, that are impossible in the lab at

comparable spatial scales (e.g., Phoofolo and Obrycki 1998, Dixon 2000, Sato *et al.* 2003, Yasuda *et al.* 2004). Interspecific interactions in natural settings will be dictated by these behaviors, as well as temporal and spatial distributions of individual species, such as degree of microhabitat overlap and timing of arrival and larval development (e.g., Musser and Shelton 2003). *Harmonia axyridis*, for example, tends to arrive at habitats and oviposit later than other coccinellids (Yasuda and Shinya 1997, Musser and Shelton 2003, Nault and Kennedy 2003). Other species, including *C. septempunctata*, may derive protection by co-occurring as older larvae with younger larvae of *H. axyridis* (e.g., Sato *et al.* 2003.) Whatever the explanation for our failure to detect impacts to the native coccinellid community in southwestern Virginia, we cannot ignore the fact that *H. axyridis* is the most ubiquitous ladybeetle across a wide range of open habitats.

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