

Intraguild Interactions of Native and Introduced Coccinellids: The Decline of a Flagship Species

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ABSTRACT The decline of *Coccinella novemnotata* Herbst, the ninespotted lady beetle, across North America has been attributed to the introduction of *Coccinella septempunctata* L. It has been suggested that *C. septempunctata* negatively impacted *C. novemnotata* through a combination of mechanisms. We investigated the effects of scramble competition and intraguild predation between groups of *C. septempunctata* and *C. novemnotata*. A novel aspect of these experiments for this species combination was that we provided beetles the option to cannibalize conspecifics or predate on heterospecifics (i.e. intraguild predation); thus, we were able to compare interspecific versus intraspecific competition. Increasing prey density resulted in significantly lower rates of intraguild predation on *C. novemnotata* by *C. septempunctata*. Percentage survival of *C. novemnotata* grouped with *C. septempunctata* at low and high aphid densities was 6 and 61%, respectively. For our second study, we increased the spatial complexity and volume of the assay system, and provided prey ad libitum. *C. novemnotata* survival from first-instar to adult was significantly lower than *C. septempunctata* survival when grouped heterospecifically (43 vs 61% survival, respectively). Finally, we conducted a study to determine if hungry larvae discriminate conspecific versus heterospecific larvae by testing whether they predated selectively on the basis of species, which they did not appear to do. We conclude that *C. novemnotata* larvae suffer greater rates of intraguild predation from *C. septempunctata* compared with cannibalism, that this difference appears to be due to size asymmetry between the two species, and that local conditions impact the severity of intraguild predation by *C. septempunctata*.

KEY WORDS intraguild predation, *Coccinella novemnotata*, *Coccinella septempunctata*, competition, invasive species

Introduction

It has been widely reported that the accidental and intentional introduction of nonnative species can negatively impact native flora and fauna (Manchester and Bullock 2000, McNeely 2001, Sax et al. 2008). The introduction of nonnative species as agents of pest control to protect agricultural crops has driven shifts in local species complexes (Vitousek et al. 1997). The ninespotted lady beetle, *Coccinella novemnotata* Herbst, was historically one of the most prevalent species of lady beetle in the United States (Harmon et al. 2007), and its range spanned most of North America (Gordon 1985). It was an economically important biological control agent that helped to manage aphid populations on farms and in gardens. New York State officially recognized its importance and declared it their state insect in 1989. Since then, substantial decreases in its range, population density, and body size have been reported (Losey et al. 2012). For over a decade *C. novemnotata* is not known to have been

collected in the eastern United States until a single dead adult *C. novemnotata* was found in Virginia in 2006 (Losey et al. 2007), followed by the discovery of a small population on Long Island in 2011 (Losey et al. 2014).

Coinciding with the decline of *C. novemnotata* was the establishment, spread, and population increase of *Coccinella septempunctata* L., which the U.S. Department of Agriculture had been trying to introduce since 1956 (Gordon 1985). Although many attempts at establishment were unsuccessful, in 1973–1974 the first *C. septempunctata* populations were discovered in New Jersey (Gordon 1985). This species has since become cosmopolitan, and its current range completely overlaps and now extends beyond the former range of *C. novemnotata* (Gordon 1985, Lost LadyBug Project Data: www.lostladybug.org). Several reports have suggested that *C. septempunctata* may have served as the driver of the decline of native coccinellids including *C. novemnotata* (Staines et al. 1990; Elliott et al. 1990, 1996; Wheeler and Hoebeke 1995; Simberloff and Stiling 1996; Aloykhin and Sewell 2004; Snyder et al. 2004; Harmon et al. 2007; Losey et al. 2012). It is important to note that while the release of *Harmonia axyridis* (Pallas) began as early as 1964 (Brown et al. 2011), the first report of an established population was not made

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until 1988 (Chapin and Brou 1991), and that was well after *C. novemnotata* had declined from a very common species to an extremely rare one (Wheeler and Hoebeke 1995, Harmon et al. 2007). Two of several proposed mechanisms that may be behind the widespread decline of native coccinellids (Losey et al. 2012) are resource competition with and intraguild predation by invasive coccinellids (Evans 1991, Cottrell and Yeargan 1998, Obycki et al. 1998, Kajita et al. 2000, Michaud 2002).

Published studies on the intraguild interactions of introduced and native coccinellids have had mixed results. Hoogendoorn and Heimple (2004) reported that there was no effect on the larval weight and survival of *Coleomegilla maculata* De Geer larvae when they were grouped together with the nonnative *H. axyridis* on corn in field cages for 5 d. Evans (1991) and Obycki et al. (1998) report no significant effect of introduced coccinellids on native species fitness, and Smith and Gardiner (2013) report that the consumption of lady beetle eggs was largely performed by extraguild predators. On the other hand, Kajita et al. (2000) reported that both *C. septempunctata* and *H. axyridis* were strong intraguild predators of *Adalia bipunctata* L., and that the invasive species caused the development time of *A. bipunctata* to become extended. Kajita et al. (2006) and Soares and Serpa (2007) showed that invasive lady beetles can reduce native species' fecundity. In one of the first studies with a congeneric pair, Turnipseed et al. (2014) showed that *C. septempunctata* exerts a strong negative effect on *C. novemnotata* in the form of intraguild predation, and attributes the effect, in part, to the relative size difference between the two species.

Resource availability is known to affect the intensity of intraguild interactions between coccinellids. *C. septempunctata* and *H. axyridis* have been shown to exhibit varying levels of intraguild predation of native coccinellids as a function of changing aphid density (Hironori and Katsushiro 1997; Schellhorn and Andow 1999; Michaud 2002; Musser and Shelton 2003; Snyder et al. 2004; Cottrell 2005; Thomas et al. 2013; Turnipseed et al. 2014). It has also been demonstrated that some coccinellid species choose to predate heterospecifically rather than consume conspecifics. For example, Cottrell and Yeargan (1998) showed that nonnative larvae are more likely to predate on eggs of native species. Agarwala and Dixon (1992), Snyder et al. (2004), and Pervez et al. (2006) reported that the larvae of several nonnative lady beetle species predate more often and successfully on the larvae of native species.

Turnipseed et al. (2014) made direct observations of competitive interactions between pairs of larval *C. novemnotata* and *C. septempunctata*. They reported significantly greater rates of *C. septempunctata* survival when they were paired with a same-instar or smaller *C. novemnotata* larva, and that *C. novemnotata* survival was greater only when paired with an earlier-instar *C. septempunctata*. To gain additional insight into possible mechanisms behind the decline of native species, especially *C. novemnotata*, and to begin to

understand what limits their recovery and how to protect extant populations, we extended Turnipseed et al.'s (2014) research on intraguild predation between *C. septempunctata* and *C. novemnotata*. We did this by adding the dimension of prey choice (conspecific vs heterospecific) to the experimental design, and we performed our experiments across a wider range of conditions. We conducted a series of experiments to evaluate competitive interactions between groups of *C. novemnotata* and *C. septempunctata* at a range of aphid prey densities. We also conducted an experiment to determine whether either of these species actively choose to predate heterospecifically versus conspecifically as a species-related effect.

Materials and Methods

Insects. Adult ninespotted lady beetles and sevenspotted lady beetles were collected from field populations in Oregon, South Dakota, and New York in 2013, and used to generate laboratory colonies. Colony beetles were maintained in 44-ml plastic cups containing a single piece of paper towel (2.5 by 7 cm²), and they were provided an ad libitum diet of mixed-aged pea aphids (*Acyrtosiphon pisum* Harris), which were reared on fava beans (*Vicia faba* L.). Old and dead aphids were removed from cups and freshly collected aphids were added daily. Eggs were collected from cups containing a single mated female on a daily basis. Lady beetle and aphid colonies were maintained at 22–25°C and a photoperiod of 16:8 (L:D) h for several generations prior to being used for experimental purposes.

Experiment 1: The Effect of Aphid Density on Survival of *C. novemnotata* Grouped with *C. septempunctata*. We conducted a laboratory experiment (3 × 3 factorial) to determine the effect of competition for prey on rates of intraguild predation between *C. septempunctata* and *C. novemnotata*. Clutches of *C. novemnotata* and *C. septempunctata* eggs were collected every 24 h from 5–10 cups, each containing a single mated female. The date of oviposition was recorded, and eggs were monitored daily for larval emergence. On the day of egg hatch, larvae from all of the cups within a species that contained viable eggs were pooled and then arbitrarily split amongst our experimental treatment and the controls. Our three-species combinations consisted of groups of 16 newly hatched (<24 h old) first-instar *C. septempunctata*, 16 newly hatched first-instar *C. novemnotata*, and equal numbers ($n = 8$ per species) of newly hatched first-instar *C. novemnotata* and *C. septempunctata*. Each of these species combinations was made at a low (0.3 ± 0.01 g/d), medium (0.45 ± 0.01 g/d), and high aphid density (ad libitum), for a total of nine treatments, which we selected based on the results of preliminary trials that showed that they produced varying levels of survival within the treatment and the controls (data not shown). Groups of larvae and aphids were placed in 473-ml clear plastic containers that were lined with a coffee filter (24 cm in diameter). Coffee filters were replaced every 1–3 d, and old and dead

aphids were removed and replaced with freshly collected aphids daily. All of the experimental containers were maintained in a growth chamber at $25 \pm 1^\circ\text{C}$ and a photoperiod of 16:8 (L:D) h. Ladybeetles were monitored until adult eclosion. We recorded the date of egg hatch, the date of eclosion, which allowed us to calculate the development time from first-instar to adult, and the percentage survival for each species within a treatment. We also recorded the weight and sex of emerged adults within 24 h posteclosion. The experiment was then repeated two more times for a total of nine replicates per treatment ($n = 3$ containers per treatment per date).

Experiment 2: Survival of *C. novemnotata* and *C. septempunctata* Grouped Conspecifically or Heterospecifically Within Cages Containing Fava Bean Plants. A second experiment was conducted to determine the rate of intraguild predation of *C. novemnotata* by *C. septempunctata* under less controlled conditions (i.e. increased complexity within the test system). We used a randomized block design, with experimental dates as blocks. The three-species combinations (one treatment and two controls) and the number of newly eclosed lady beetle larvae introduced into each cage were the same as in Experiment 1. Fava bean seeds were germinated over a 7- to 8-d period, and groups of three seedlings were then transplanted into 12.5-cm plastic pots such that the stems of the plants would grow through the base of a plastic funnel (1.9 cm in length and 19 cm in diameter), which had been coated with Insect-a-slip (Bioquip Products Inc. Rancho Dominguez, CA). The funnels were then filled up to the bottleneck with peat-based LM-series professional growing media (Lambert Peat Moss Inc., Quebec City, Canada). The funnel was used to increase the likelihood that small lady beetle larvae that fell off of plants would be able to relocate the plant. The entire funnel and the plants were enclosed in a mesh bag (30 by 60 cm²) that was supported by a flask clamp, which was attached to a ring stand (Fig. 1). After two additional days of plant growth, 5 g of mixed-aged pea aphids were added to each enclosure and allowed to establish themselves on the plants for 3 d prior to the addition of lady beetles. Newly hatched lady beetle larvae (<24 h old) were introduced into each cage, and the bags were sealed using Velcro. Additional aphids were added to cages as needed to maintain an ad libitum diet, and plants were watered as needed. Lady beetles remained in these enclosures until pupation. Enclosures were kept in a greenhouse at $20 \pm 3^\circ\text{C}$. After all of the larvae pupated, the mesh bags containing the pupae were removed from the ring stand and placed into an incubator maintained at $25 \pm 2^\circ\text{C}$ with a photoperiod of 16:8 (L:D) h until eclosion. The date of egg hatch and adult eclosion was recorded to allow us to generate relative estimates of development time and percentage survival of the two species. Lady beetle sex and the weight of emerged adults were determined within 24 h posteclosion. The entire experiment was conducted on seven independent dates with 1–2 replicates of each treatment per test date, for a total of 9–10 replicates per treatment.

Experiment 3: The Effect of Prey Species on Rates of Intraguild Predation by *C. novemnotata* and *C. septempunctata*. An experiment was conducted to determine whether *C. novemnotata* and *C. septempunctata* make intraguild predation decisions based on species recognition. Independent cohorts of lady beetle larvae were reared on an ad libitum diet of pea aphids to either the second- or the fourth-instar. A subset of ten 24- to 72-h-old fourth-instar *C. novemnotata* and *C. septempunctata* larvae ($n = 10$ per species) were removed from their rearing containers and placed singly in 44-ml cups and starved for 24 h. Hereafter, starved fourth-instar beetles will be referred to as the “focal” individuals, as it was their response we were interested in measuring. After the starvation period, focal individuals were provided two same-sized larvae (intraguild prey) that had been fed pea aphids ad libitum—one *C. novemnotata* and one *C. septempunctata*. Intraguild prey treatments consisted of either two second-instar or two fourth-instar larvae. Intraguild prey within a cup were selected to be as close in size as possible to minimize the risk of introducing a size bias into predation decisions by the focal individual. For each focal individual, we recorded the species of intraguild prey that it first contacted, and we recorded the species of intraguild prey that was first consumed. The experiment was conducted on two independent occasions for a total of 20 replicates of each of the four treatments.

Statistical Methods. All statistical analyses were performed with JMP Pro version 9 (SAS Institute, Cary, NC). Lady beetle survival to the adult stage in the first and second experiments was analyzed using nominal logistic regression. Because we were not interested in all pair-wise comparisons of survival, we analyzed a subset of relevant pairs and determined significant differences via the sequential Bonferroni method of Holm (1979; see also Sokal and Rohlf 1995, Shaffer 1995). Mixed-model analysis of variance was used to examine the development times of lady beetles from newly emerged first-instar to newly eclosed adult, and the weight of newly eclosed adults; data were untransformed. Because the survival of individual beetles within any given experimental unit (473-ml container or mesh bag) was correlated, we conducted our analyses on the average development time and weight of beetles within each experimental unit. Separate mean development times and weights for *C. septempunctata* and *C. novemnotata* within the heterospecific treatment were generated, and because we had only three experimental treatments, one treatment containing two species, we nested lady beetle species within treatment when necessary. Assays were blocked over time (dates), date was coded as a random variable, and block interactions were not tested due to the possibility of restriction error (Sokal and Rohlf 1995). Post hoc analyses were conducted using Tukey's HSD test at $\alpha = 0.05$. Nominal logistic regression was used to determine whether focal (starved for 24 h) fourth-instar *C. novemnotata* and *C. septempunctata* discriminate

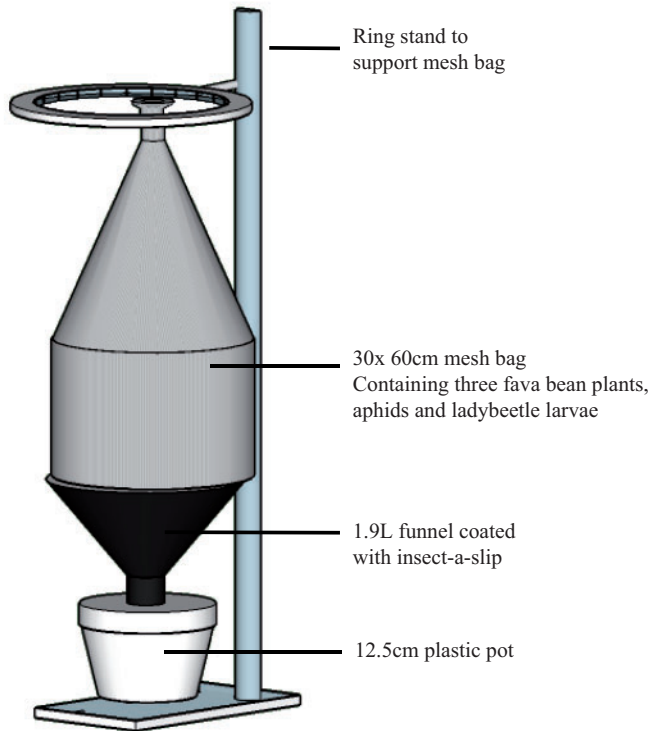


Fig. 1. Experimental unit for testing the survival of conspecific and heterospecific groups of *C. novemnotata* and *C. septempunctata* on fava bean plants in mesh bags.

conspecific larvae versus heterospecific larvae and whether this influences their choice of intraguild prey (satiated second-instar or fourth-instar larvae). Because we observed that the first-attacked intraguild prey sometimes escaped the focal individual, we also analyzed whether prey were more likely to escape from *C. novemnotata* versus *C. septempunctata*.

Results

Experiment 1: The Effect of Aphid Density on Survival of *C. novemnotata* Grouped Interspecific With *C. septempunctata*. Overall, the survival of *C. septempunctata* and *C. novemnotata* from first-instar to newly eclosed adults in heterospecific groups was significantly affected by the interaction of lady beetle species and aphid density ($\chi^2 = 36.4$, $df = 2$, $P < 0.0001$). Increasing aphid density resulted in higher rates of survival of *C. novemnotata* (55.6% higher), but rates of survival of *C. septempunctata* decreased numerically, although not significantly ($\chi^2 = 1.1$, $df = 2$, $P = 0.58$), by 6.9% as aphid density increased. The difference in percentage survival of *C. novemnotata* and *C. septempunctata* at the low and high aphid densities was 76.4 and 13.9%, respectively (Fig. 2). The survival of *C. novemnotata* in the heterospecific treatment, as compared with the conspecific *C. novemnotata* treatment, was significantly affected by the aphid density by

treatment interaction ($\chi^2 = 19.6$, $df = 2$, $P < 0.0001$). When *C. novemnotata* were grouped conspecifically, their survival was greater at every aphid density than when they were grouped heterospecifically (Fig. 2), and the rate of increase in survival with increasing aphid density was higher in the heterospecific treatment. The survival of *C. septempunctata* in the conspecific treatment versus the heterospecific treatment was significantly affected by the aphid density by treatment interaction ($\chi^2 = 14.6$, $df = 2$, $P = 0.0007$). *C. septempunctata* survival increased (29.2 %) with increasing aphid density in the conspecific treatment. Survival of *C. novemnotata* and *C. septempunctata* in the two conspecific groupings did not differ significantly as a function of the aphid density by species interaction ($\chi^2 = 2.6$, $df = 2$, $P = 0.27$). There was, however, a significant main effect of aphid density on survival across species ($\chi^2 = 45.3$, $df = 2$, $P < 0.0001$), but there was not an effect of species on survival ($\chi^2 = 1.1$, $df = 1$, $P = 0.30$).

There was not a significant effect of the three-way species by aphid density nested in treatment interaction on the development times of *C. novemnotata* and *C. septempunctata* from first-instar to newly eclosed adult ($F_{2,78,2} = 1.3$, $P = 0.27$). There was a significant effect of lady beetle species nested within treatment ($F_{1,84,1} = 52.6$, $P < 0.0001$) on development times (Fig. 3a). The development times of *C. novemnotata* in

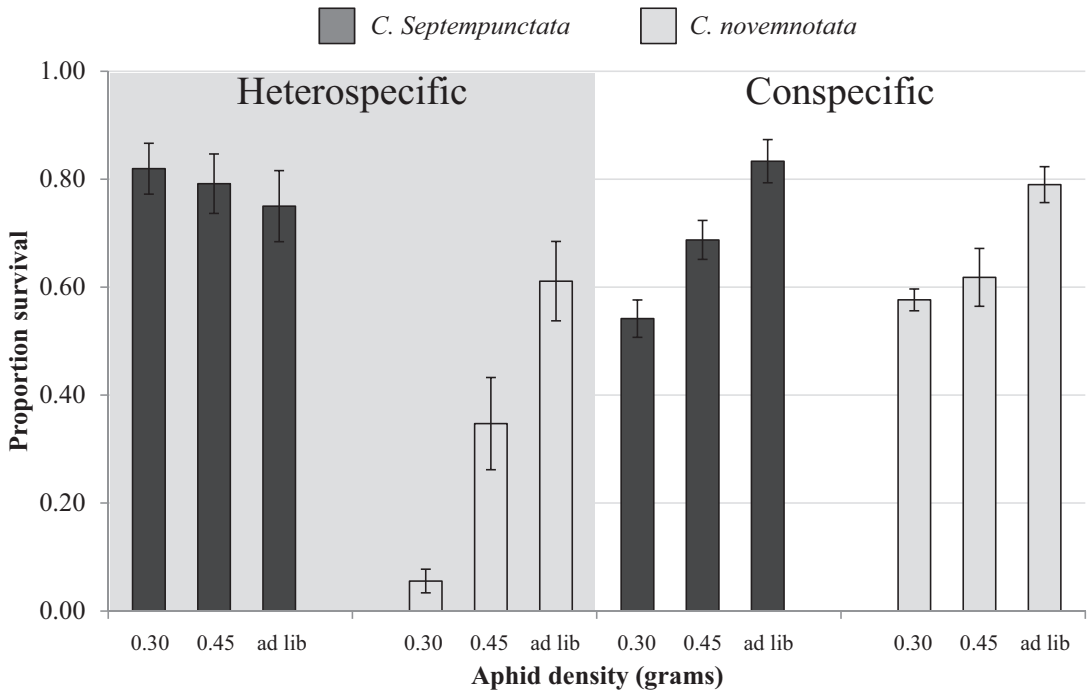


Fig. 2. Mean (\pm SE) proportion survival of *C. septempunctata* and *C. novemnotata* from newly hatched first-instar to newly eclosed adult at three aphid densities when paired heterospecifically versus conspecifically in 473-ml plastic containers.

the conspecific and heterospecific treatments did not differ from each other; however, the development times of *C. septempunctata* was lower in the heterospecific treatment than in the conspecific treatment. Across treatment *C. septempunctata* had lower development times than *C. novemnotata* (Fig. 3a). There was not a significant effect of increasing aphid density on the development time of lady beetles ($F_{1,24,6} = 2.4$, $P = 0.11$), and there was no effect of beetle sex on development times ($F_{1,84} = 0.08$, $P = 0.37$).

Newly eclosed adult lady beetle weight was significantly affected by species nested within treatment ($F_{1,88,9} = 215.7$, $P < 0.0001$). The mean weight of *C. novemnotata* in the conspecific and heterospecific treatments did not differ from each other; however, they weighed significantly less than *C. septempunctata* when grouped conspecifically and heterospecifically. *C. septempunctata* in the heterospecific treatment weighed significantly more than *C. septempunctata* in the conspecific treatment (Fig. 3b). There were also significant effects of both beetle sex and aphid density on adult lady beetle weights ($F_{1,87,8} = 151.5$, $P < 0.0001$; $F_{1,18,2} = 5.1$, $P = 0.02$, respectively). Female lady beetles weighed an average of 32.5 ± 0.6 mg and males weighed 28.1 ± 0.5 mg, and lady beetle weight increased with increasing aphid density.

Experiment 2: Survival of *C. novemnotata* and *C. septempunctata* Grouped Conspecifically or Heterospecifically Within Cages Containing Fava Bean Plants. Survival of lady beetles from the first-instar to newly eclosed adult in greenhouse cages

with fava bean plants was significantly affected by the species by treatment interaction ($\chi^2 = 6.0$, $df = 1$, $P = 0.01$). Within the heterospecific treatment, significantly more *C. septempunctata* (18.8% more; Table 1) survived to adulthood than *C. novemnotata* ($\chi^2 = 6.7$, $df = 1$, $P = 0.01$). There was not a significant difference in the number of individuals surviving as a function of species in the conspecific control treatments ($\chi^2 = 0.23$, $df = 1$, $P = 0.63$). *C. septempunctata* survival in the heterospecific treatment as compared with *C. septempunctata* in the conspecific treatment was not significantly different ($\chi^2 = 4.5$, $df = 1$, $P = 0.03$, sequential Bonferroni-corrected critical alpha = 0.017). Survival of *C. novemnotata* in conspecific versus heterospecific treatments was not significantly different ($\chi^2 = 2.2$, $df = 1$, $P = 0.14$).

Development times from first-instar to newly eclosed adults were significantly affected by the species by treatment interaction ($F_{1,29,0} = 30.2$, $P < 0.0001$; Table 1). The mean development times of *C. novemnotata* in the conspecific and heterospecific treatments did not differ from each other; however, the mean development time of *C. novemnotata* in the heterospecific treatment was significantly slower than *C. septempunctata* in both the conspecific and heterospecific treatments. Adult beetle weights were significantly affected by the species by treatment interaction ($F_{1,61,1} = 31.7$, $P < 0.0001$; Table 1). *C. novemnotata* weighed significantly less than *C. septempunctata* in all treatments, and there was no effect of treatment on beetle weight within a species.

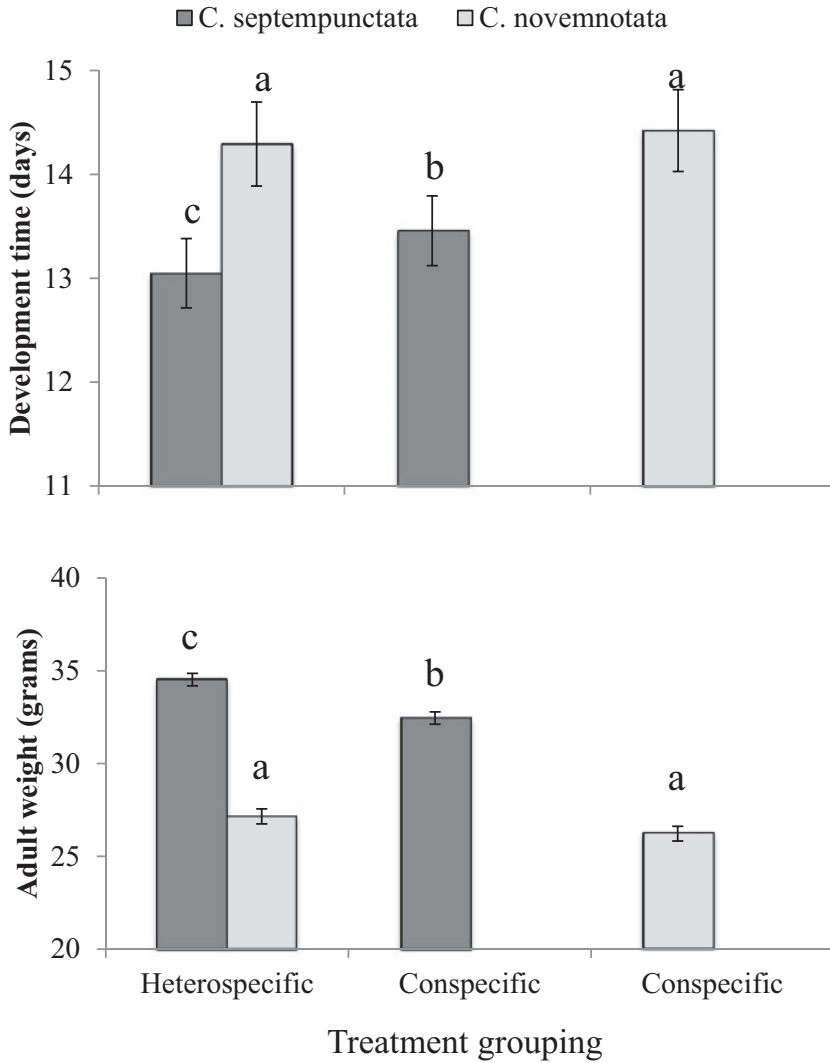


Fig. 3. The main effect of lady beetle species and treatment (conspecific versus heterospecific grouping) on (a) the mean (\pm SE) development time from first-instar to newly eclosed adult, and (b) the mean (\pm SE) weight of adult beetles at eclosion, when reared in groups in 473-ml plastic containers.

Table 1. Mean (\pm SE) percentage survival, development time from first-instar to newly eclosed adult, and weight of newly eclosed adult *C. novemnotata* and *C. septempunctata* reared in conspecific or heterospecific groups, within small mesh cages in a greenhouse

Species	Treatment	N	Mean \pm SE percentage survival	Mean \pm SE development time (d)	Mean \pm SE weight (mg)
<i>C. novemnotata</i>	Heterospecific	10	42.5 \pm 0.08a ^a	23.1 \pm 0.86a ^b	24.8 \pm 0.64b ^b
<i>C. novemnotata</i>	Conspecific	9	49.3 \pm 0.06a	22.5 \pm 1.01ab	23.6 \pm 0.96b
<i>C. septempunctata</i>	Heterospecific	10	61.3 \pm 0.07b	21.2 \pm 1.01c	32.5 \pm 0.78a
<i>C. septempunctata</i>	Conspecific	10	47.5 \pm 0.06a	21.4 \pm 1.0bc	30.7 \pm 1.15a

^a Mean (\pm SE) percentage survivals that are followed by the same letter are not significantly different (sequential Bonferroni-corrected pairwise comparisons).

^b Mean (\pm SE) development times and adult beetle weights that are followed by the same letter are not significantly different (Tukey's HSD, alpha = 0.05).

Experiment 3: The Effect of Intraguild Prey Species on Rates of Intraguild Predation by *C. novemnotata* and *C. septempunctata*. All predation events occurred within the first 10 min of the start

of a test. In only one instance was the focal individual predated on by an intraguild prey; this occurred in the treatment with fourth-instar intraguild prey. There was not a significant effect of focal species (i.e. intraguild

Table 2. The mean percentage of second- and fourth-instar larvae contacted and consumed when maintained with a starved con- or heterospecific fourth-instar in 44-ml plastic cups

Focal species	Intraguild prey instar	N	First contact <i>C. novemnotata</i> (percentage)	First contact <i>C. septempunctata</i> (percentage)	First consumed <i>C. novemnotata</i> (percentage)	First consumed <i>C. septempunctata</i> (percentage)
<i>C. novemnotata</i>	Second-instar	20	55a ^a	45a	50a	50a
<i>C. septempunctata</i>	Second-instar	20	45a	55a	40a	60a
<i>C. novemnotata</i>	Fourth-instar	20	55a	45a	70a	30a
<i>C. septempunctata</i>	Fourth-instar	20	40a	60a	45a	55a

^a Percentage first contact and first consumed within a row followed by the same letter are not significantly different ($\chi^2 > 0.05$).

predator) on which second-instar or fourth-instar intraguild prey the focal individual contacted first ($\chi^2 = 0.4$, $df = 1$, $P = 0.53$; $\chi^2 = 0.9$, $df = 1$, $P = 0.34$, respectively), or on which second-instar or fourth-instar individual was consumed first ($\chi^2 = 0.4$, $df = 1$, $P = 0.52$; $\chi^2 = 2.6$, $df = 1$, $P = 0.11$, respectively; Table 2). There was no significant difference between the two prey species' ability to escape the first predation attempt ($\chi^2 = 0.80$, $df = 1$, $P = 0.37$; $\chi^2 = 1.6$, $df = 1$, $P = 0.20$, respectively). Second-instar intraguild prey escaped focal *C. novemnotata* and *C. septempunctata* on 10 and 20% of first encounters, respectively, and fourth-instar intraguild prey escaped focal *C. novemnotata* and *C. septempunctata* on 60 and 40% of first encounters, respectively.

Discussion

When groups of first-instar *C. novemnotata* larvae were maintained together with groups of first-instar *C. septempunctata* for the duration of their development to the adult stage, *C. novemnotata* suffered high rates of mortality (20–94%). This was true when larvae were maintained together in 473-ml plastic containers and when they were maintained together in larger mesh cages containing fava bean plants. The survival of heterospecifically grouped *C. novemnotata* was lowest in the first experiment at the low and intermediate aphid densities; only 6 and 35% of *C. novemnotata* survived to adulthood, respectively. *C. novemnotata* paired with *C. septempunctata* always suffered greater rates of mortality compared with when they were maintained in conspecific groups, which lends support to the hypothesis that *C. septempunctata* has a negative influence on *C. novemnotata*.

In the first experiment, we observed a strong positive linear relationship of aphid density on rates of survival of *C. novemnotata* and *C. septempunctata*, with the exception of *C. septempunctata* in the heterospecific grouping. Percentage survival of *C. novemnotata* increased at a greater rate with increasing aphid density in the heterospecific grouping (from 6 to 61%) compared with the conspecific grouping, where survival increased from 58–79%. It is interesting to note that we did not have >83% survival of either species in any of our experimental treatments across the first two experiments. This is despite our providing larvae a surfeit of prey in several of our treatments. This observation could be an inherent characteristic of our experimental designs or it might suggest that aphid-based

resource competition is not the only factor involved in intraguild interactions like predation.

In the heterospecific grouping of the second experiment, which represents the combination of species after the introduction of *C. septempunctata*, the rate of survival of *C. novemnotata* (42.5%) was significantly lower than the survival of *C. septempunctata* (61.3%). Thus, in the most realistic experiment yet conducted on these two species, based on highest system complexity, we confirmed that *C. septempunctata* outcompetes *C. novemnotata*. Our findings of a significant competitive advantage for *C. septempunctata* over *C. novemnotata* are similar to other published reports (Obrycki et al. 1998, Michaud 2002, Snyder et al. 2004, Turnipseed et al. 2014). Turnipseed et al. (2014) reported reduced rates of survival of various *C. novemnotata* larval instars when they were paired heterospecifically with larval *C. septempunctata* versus conspecifically, and that when the two species were reared together *C. septempunctata* had higher survival. We expanded on the inference space of Turnipseed et al. (2014) by maintaining larvae in groups, thus allowing for both intra- and interspecific predation, and increasing complexity with greater arena size and the addition of plants. Although our experiments are still highly controlled small-scale studies, they are closer to field level than previous studies and they demonstrate that even under a wider set of conditions, *C. septempunctata* outcompetes *C. novemnotata*.

The trends in development times of the two species were similar to the patterns of survival. In experiments one and two of this study, we did not observe a significant increase in the mean development time of *C. novemnotata* maintained in conspecific versus heterospecific groupings. However, *C. septempunctata* developed significantly faster when reared in the same container with *C. novemnotata*, but not in the less-controlled experiment conducted with fava bean plants in mesh bags. Turnipseed et al. (2014) reported a significant increase in the development time of *C. novemnotata* to the adult stage when reared together with *C. septempunctata*. It is not clear why Turnipseed et al. (2014) saw a difference in development times between *C. novemnotata* reared conspecifically and we did not. It may be related to the number of larvae interacting within an assay system, the volume of the assay system, or a number of other, as yet, undetermined factors.

Our third experiment provided insight into the mechanism underlying the survivorship patterns we

observed in the first and second experiments. This free-choice predation experiment demonstrated that focal *C. novemnotata* and *C. septempunctata* larvae did not distinguish between intraguild species when prey size was held constant. These data, as well as the results obtained by Turnipseed et al. (2014), demonstrate that body size, and more specifically, the difference in body size among two intraguild individuals, will strongly influence which individual is the predator and which is the prey. An interesting observation of this experiment was that the fourth-instar intraguild prey escaped more frequently than the second-instar intraguild prey, presumably because of their larger size.

Hoki et al. (2014) showed that *C. septempunctata* consumes significantly more aphids per day than *C. novemnotata*. Ugine and Losey (2014) showed that *C. septempunctata* develops significantly faster from egg to adult compared with *C. novemnotata*, and Turnipseed et al. (2014) showed a significant effect of larval size (paired different instars of *C. novemnotata* with *C. septempunctata*) on rates of intraguild predation. The faster development times of *C. septempunctata* coupled with its higher rate of attack of aphids compared with *C. novemnotata* may be increasing the effect of resource competition exerted on *C. novemnotata*. This would serve to exacerbate the effect of resource competition on *C. novemnotata* by exaggerating the difference in the development times and sizes of the two species. This would in turn favor increased rates of intraguild predation of *C. novemnotata* by *C. septempunctata* by depriving *C. novemnotata* of food and making them less vigorous. Other studies investigating interactions between native and invasive coccinellids have also reported higher performance of nonnative lady beetles (Hoogendoorn and Heimpel 2004, Leppanen et al. 2012, Smith and Gardiner, 2013).

Our findings support the hypothesis that the displacement of *C. novemnotata* from its native range was likely driven by *C. septempunctata*. Although this is not a new theory (Wheeler and Hoebeke 1995), our data clearly demonstrate the potential for antagonistic interactions favoring *C. septempunctata*. These results add to a growing body of literature (Losey et al. 2012, Turnipseed et al. 2014) that indicate that competition by means of intraguild predation or exploitative competition favors the nonnative *C. septempunctata* asymmetrically, and we now show that this asymmetrical effect functions on a group level and across different aphid densities. Through fuller integration of the competitive impacts we measured with other mechanisms such as competition for nonprey resources, hybridization, and the roles of native pathogens and parasitoids that may have led to the decline of *C. novemnotata*, we may be able to conserve this and other declining native species and be better equipped to prevent the negative impacts from species introductions that will occur in the future.

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