Effect of Prey Limitation on Competitive Interactions Between a Native Lady Beetle, *Coccinella novemnotata*, and an Invasive Lady Beetle, *Coccinella septempunctata* (Coleoptera: Coccinellidae)

RAKIM K. TURNIPSEED,[1,2,3] TODD A. UGINE,[2] AND JOHN E. LOSEY[2]

ABSTRACT   The size and geographic distribution of *Coccinella novemnotata* Herbst populations have been decreasing rapidly across North America closely following the establishment, spread, and population growth of the invasive seven-spotted lady beetle, *Coccinella septempunctata* L. To determine whether intraguild predation and competition for prey may be partially responsible for the decline, we paired first-instar larvae of two populations of *C. novemnotata* (eastern and western) with first-instar *C. septempunctata* at low or high aphid densities. Survival of both *C. novemnotata* populations was significantly lower when larvae were paired with *C. septempunctata*, and western *C. novemnotata* exhibited significantly lower survival compared with the eastern population. This relationship depended on aphid density with the greatest survival of both *C. novemnotata* populations occurring at the high aphid density. Both male and female *C. novemnotata* weighed less on the day of eclosion when paired with *C. septempunctata* as compared with pairings with conspecifics. In a second test, *C. septempunctata* and *C. novemnotata* instars were varied at the start of the trial and *C. novemnotata* survival to adulthood in the presence of *C. septempunctata* was dependent of the instar of *C. novemnotata* used to initiate the experiment. *C. novemnotata* exhibited higher rates of survival and weighed significantly more on the day of eclosion when *C. novemnotata* was older than its *C. septempunctata* partner. These results suggest that interspecific competition including intraguild predation by *C. septempunctata* may contribute to *C. novemnotata* population declines, but that the intensity of this impact may vary across *C. novemnotata* populations.

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

Introduced coccinellid species can provide effective control of pest populations (Debach and Rosen 1991); however, some introduced lady beetle species have been shown to have a significant negative impact on native species and are considered invasive (Stiling and Simberloff 2000, Evans 2004, Yasuda et al. 2004). Before the mid-1980s, *Coccinella novemnotata* Herbst was among the most abundant coccinellids in the continental United States and portions of Canada (Gordon 1985). It was the dominant coccinellid species in the northeastern region of the United States (Gordon 1985). However, following the establishment of *Coccinella septempunctata* L. in the mid-1980s (Hoebeke and Wheeler 1980), populations of *C. novemnotata* began declining and now appear to be locally extinct in many areas of North America. The only sightings of *C. novemnotata* east of Minnesota come from a single record in the vicinity of the District of Columbia (Losey et al. 2007) and a viable population in Long Island, NY (Losey et al. 2014). Several studies have suggested that the es-

tablishment and spread of *C. septempunctata* was an important cause of *C. novemnotata* decline (Wheeler and Hoebeke 1995, Losey et al. 2012).

Research on the impact of introduced coccinellids on native lady beetle species has yielded mixed results. Evans (1991), Obrycki et al. (1998), Yasuda et al. (2004), and Hoogendoorn and Heimpel (2004) found no impact of invasive coccinellids on the fitness of native lady beetles. In contrast, Kajita et al. (2000) demonstrated that introduced coccinellids can prolong the developmental time of native coccinellids, and both Soares and Serpa (2007) and Kajita et al. (2006) showed that invasive lady beetles can reduce native species' fecundity. Possible mechanisms underlying the negative interactions between introduced and native coccinellids include intraguild predation, scramble competition for prey, competition for non-prey resources, the introduction of parasitoids or pathogens, and hybridization (Losey et al. 2012). Within this range of possible mechanisms, we focused our research on competition and intraguild predation based on evidence presented in Losey et al. (2012).

Body size is one factor that can influence the magnitude of competition for prey (Obrycki et al. 1998). Field-collected *C. septempunctata* are known to be

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larger than *C. novemnotata* (Losey et al. 2012), and it has been documented that the mean body size of adult *C. novemnotata* has decreased since the introduction of *C. septempunctata* (Losey et al. 2012). Hoki et al. (2014) showed that *C. septempunctata* has a higher aphid attack rate and lower aphid handling time compared with *C. novemnotata*. The authors suggest that these relationships are consistent with the expectations arising from prey limitation for *C. novemnotata* by *C. septempunctata*, and prey limitation can lead to the competitive exclusion of the weaker species within a guild (Gakkhar et al. 2007). Body size differential is also known to influence intraguild predation (Roy et al. 2002, Yasuda et al. 2004, Evans et al. 2011), and this would add to the potential for exclusion of *C. novemnotata* by *C. septempunctata.*

A preliminary laboratory study in which same-aged *C. novemnotata* and *C. septempunctata* larvae were reared in pairs suggested that *C. novemnotata* survival was reduced in the presence of *C. septempunctata* versus survival in conspecific pairings (J. Lai and J.E.L., unpublished). Other laboratory studies demonstrated that the strength of interactions between larvae of native and exotic species could depend on prey availability and the developmental stage of the larvae (Polis et al. 1989, Lucas et al. 1998, Yasuda et al. 2004). Based on the results from these laboratory studies, our goal was to assess the interaction between *C. novemnotata* and *C. septempunctata* across a range of prey availability and larval stages. Specifically, we assessed at low and high aphid densities 1) the survival of *C. novemnotata* reared from first instar to adult in the presence of a same-aged *C. septempunctata* larvae, and 2) the survival of *C. novemnotata* larvae when paired with either an earlier or later instar *C. septempunctata.*

Materials and Methods

Insects. Adult *C. septempunctata* and *C. novemnotata* were collected from Oregon, South Dakota, and New York during 2012 and were used to generate laboratory colonies. Adult *C. novemnotata* that were collected from western states (Oregon and South Dakota) were pooled and were designated "*C. novemnotata*-west." Because *C. novemnotata* collected from Long Island, NY, appear slightly different in their elytral-spotting pattern and likely represent a genetically distinct population, they were maintained as a separate colony and were designated "*C. novemnotata*-east." Colony beetles were maintained singly in 44-ml plastic cups containing a single 2 by 7 cm piece of paper towel, and were provided an ad libitum diet of pea aphids, *Acyrthosiphon pisum* Harris, and green peach aphid, *Myzus persicae* Sulzer (Hemiptera: Aphididae). Aphids were reared on fava bean plants, *Vicia faba* L. (Fabales: Fabaceae). All insect colonies were maintained at 25 ± 2°C and a photoperiod of 16:8 (L:D) h for several generations before their being used for experimental purposes.

Experiment 1: The Effect on *C. novemnotata* of Being Paired Intraspecifically Versus Interspecifically With Same-Aged *C. septempunctata*. To determine whether *C. septempunctata* affects *C. novemnotata* via intraguild predation, competition for prey, or a combination of the two, we paired newly emerged (<24 h old) first instar *C. novemnotata* (eastern or western) with a first-instar *C. septempunctata* (<24 h old) in a 44-ml plastic cup containing a 2 by 7 cm piece of paper towel. Our control treatments consisted of conspecific pairs of *C. novemnotata* with both larvae in the pair from the same geographic origin (either eastern or western populations). All species pairings were made at two aphid densities, "low" (0.035 ± 0.001 g of aphids) and "high" (0.07 ± 0.001 g of aphids) for a total of eight treatment combinations. The low aphid density was selected based on research by Losey et al. (2012) that demonstrated that *C. novemnotata* fitness, on a per beetle basis, was maximized at 21 third- to fourth-instar pea aphids per day. The weight of these 21 third- to fourth-instar aphids was approximately equivalent to the weight in grams of the "low" aphid density. Cups were opened once daily and the old aphids were removed and replaced with fresh aphids at the appropriate treatment level, up to the day that both larvae pupated and for 1-d postadult eclosion. For each individual within a cup, we recorded larval survival and stadium at 24-h intervals, and the weight of emerged adults 24 h posteclosion. These data allowed us to calculate the development time from newly emerged first instar to adult stages, percentage survival of each instar, and total survival. Intraguild predation in heterospecific pairings and cannibalism in conspecific pairings was determined by tallying the number of cups containing only a single beetle or when the partial remains of an individual were found per cup. Given the 24-h interval between cup checks, it was not possible to differentiate events of intraguild predation from the scavenging of dead or dying individuals within a cup. Therefore, all instances in which a partner was missing were scored as intraguild predation events. Experimental arenas were maintained in an environmental incubator at 25 ± 2°C and a photoperiod of 16:8 (L:D) h. The experiment was conducted on two occasions (completely randomized block design with dates as blocks) and a total of 30 replicates for each of the four conspecific pairings (i.e., eastern or western populations at high or low aphid densities; n = 120) and 60 replicates for each of the four interspecific pairings (i.e., eastern or western *C. novemnotata* vs. *C. septempunctata* at high or low aphid densities; n = 240) were monitored.

Experiment 2: The Influence of Larval Instar on Survival of *C. novemnotata* in Mixed-Instar Interspecific Pairings With *C. septempunctata*. To determine how differences in larval coccinellid instar affected rates of intraguild predation and competition, we conducted an experiment that paired *C. novemnotata* with same-instar or younger *C. septempunctata* larvae. Our treatment pairings consisted of first-, second-, or third-instar *C. novemnotata* placed in an arena with a first- or second-instar *C. septempunctata* (see Table 2). To obtain a baseline estimate of the survival of *C. novemnotata* in the absence of intraguild predation, we also monitored pairs of first instar *C. novemnotata* for con-

trols. We chose to use first-instar larvae to obtain baseline data because this maximized the opportunity for antagonistic interactions between pairs within a cup. All six species × instar combinations and the controls were conducted at the low (0.035 ± 0.001 g aphids) and high (0.07 ± 0.001 g aphids) aphid densities referred above, for a total of 14 treatments (Table 2). Because we observed the strongest negative effects of *C. septempunctata* on the western population of *C. novemnotata* (c.f. results), we chose to use only the western population of *C. novemnotata* in this study. Pairs of larvae were maintained in 44-ml plastic cups with a piece of paper towel as described above. Cups were opened once daily and the old aphids were removed and replaced with fresh aphids up to the day that both larvae pupated and for 1-d postadult eclosion. For each individual beetle within a cup, we recorded larval survival and instar every 24 h. The weight of emerged adults was recorded 24 h posteclosion. The experiment was conducted on three independent occasions (completely randomized block design with dates as blocks) and the total number of replicates of each treatment across the three experimental dates ranged from 20 to 35 pairs of larvae. Experimental arenas were maintained in an environmental incubator at 25 ± 2°C and a photoperiod of 16:8 (L:D) h.

Statistical Analyses. All statistical analyses were conducted using SAS (SAS Institute, Cary, NC) or JMP Pro 9 (SAS Institute, Cary, NC). Lady beetle survival to the adult stage in experiment one was analyzed via nominal logistic regression using PROC GENMOD. To simplify analyses, we created a category we called "group" ($n = 3$), which combined lady beetle species and point of origin for *C. novemnotata*. Experimental block (date), treatment, aphid density, and group nested within treatment were modeled as fixed effects, as were the treatment × aphid density, group × aphid density, and treatment × group × aphid density interactions. To account for the correlation among individuals within a bioassay cup (dyadic data), we included a "cup" variable as a repeated subject. Insignificant interactions were sequentially dropped from the model (and reported in the text), and the model was rerun. Planned two-way comparisons were made using *t*-tests and the family-wise error rate was maintained at an overall value of $P = 0.05$ using the Bonferroni correction.

C. novemnotata survival to the adult stage in the second experiment was analyzed via nominal logistic regression (PROC GENMOD) using two separate models. The first model included the fixed effects: experimental block (date), *C. septempunctata* instar used to initiate the experiment, *C. novemnotata* instar used to initiate the experiment and aphid density. To control for the fact that the survival of *C. novemnotata* was correlated with the survival of the within-cup *C. septempunctata* partner, we included the binomial effect "*C. septempunctata* survival." In addition, we included all two- and three-way interactions, which were evaluated for significance (reported in the text), and dropped from the model if insignificant. Because

the effect of the survival of the within-cup *C. septempunctata* partner has the potential to subsume the *C. septempunctata* instar effect, we ran a second model, as above, that excluded *C. septempunctata* survival and all its associated interactions.

For the first experiment, the development time (in days) from newly emerged first instar to adult eclosion was analyzed using mixed model analysis of variance. Development times were transformed by taking the natural log to correct for skew and to meet the assumption of equal variance. For experiment 1, "cup" was included as a random variable, and treatment, aphid density, and species nested within treatment were modeled as fixed effects as were all two- and three-way interactions. Preplanned comparisons were made using contrasts, and the family-wise error rate was maintained at of alpha = 0.05 using the Bonferroni correction.

The weight of newly emerged adults was analyzed using mixed models for experiments 1 and 2. Adult weights were transformed by taking the natural log to correct for skew and to meet the assumption of equal variance. Analysis of adult weight in experiment 1 included cup as a random variable, and date, treatment, aphid density, and species nested within treatment were modeled as fixed effects. Development time of the within-cup partner was included in the model as a covariate. For experiment 2, we only analyzed the weights of adult *C. novemnotata*. The initial model included a random block (date) effect, and the fixed effects *C. septempunctata* instar at the start of the trial, *C. novemnotata* instar at the start of the trial, aphid density, and all two-way interactions. The final model excluded all insignificant interactions. Post hoc comparisons for main effects were made using Tukey's honestly significant difference test.

Results

The likelihood that lady beetles survived to adulthood did not depend on the treatment × aphid density, aphid density × group, or the treatment × aphid density × group interactions ($P > 0.32$), and there was no significant effect of experimental block ($\chi^2 = 0.00$; df = 1; $P = 0.99$). There was a significant effect of lady beetle group nested within treatment ($\chi^2 = 73.9$; df = 2; $P < 0.0001$) on the likelihood of survival to the adult stage. Specifically, we found that across aphid densities, more eastern *C. novemnotata* survived when paired with another eastern *C. novemnotata* versus a *C. septempunctata* (66 vs. 43% survival, respectively; $P = 0.0008$); the same was true for western *C. novemnotata* (76 vs. 21% survival, respectively; $P < 0.0001$; Table 1). In addition, significantly more eastern *C. novemnotata* survived in the presence of *C. septempunctata* as compared with western *C. novemnotata* in the presence of *C. septempunctata* (43 vs. 21% survival, respectively; $P = 0.002$). Aphid density had a significant positive effect on the survival of *C. novemnotata* from both locations ($\chi^2 = 29.6$; df = 1; $P < 0.0001$). The percentage survival of eastern and western *C. novemnotata* maintained with a *C. septempunctata* at the high

Table 1. Mean (±SE) percentage survival, development time (days) from first-instar to newly eclosed adult, and weight (grams) of newly eclosed adult *C. novemnotata* reared interspecifically with a *C. septempunctata* or intraspecifically at one of two aphid densities

Species	Treatment	Aphid density	Mean ± SE percentage survival[a]	Mean ± SE development time in days[b] (n)[c]	Mean ± SE wt (g) of newly eclosed adults
Eastern *C. novemnotata*	Interspecific	Low	30.0 ± 3.3	13.1 ± 0.3 (18)	0.0218 ± 0.0004
Eastern *C. novemnotata*	Interspecific	High	55.0 ± 1.7	12.4 ± 0.2 (33)	0.0249 ± 0.0006
Eastern *C. novemnotata*	Intraspecific	Low	58.3 ± 1.7	11.6 ± 0.2 (35)	0.0253 ± 0.0006
Eastern *C. novemnotata*	Intraspecific	High	73.3 ± 3.3	11.4 ± 0.1 (44)	0.0288 ± 0.0005
Western *C. novemnotata*	Interspecific	Low	15.0 ± 1.7	16.6 ± 0.5 (9)	0.0215 ± 0.0008
Western *C. novemnotata*	Interspecific	High	26.7 ± 0.0	15.3 ± 0.4 (16)	0.0216 ± 0.0004
Western *C. novemnotata*	Intraspecific	Low	65.0 ± 1.7	12.9 ± 0.2 (39)	0.0210 ± 0.0003
Western *C. novemnotata*	Intraspecific	High	86.7 ± 3.3	12.7 ± 0.2 (52)	0.0259 ± 0.0005
C. septempunctata	Eastern *C. novemnotata*	Low	66.7 ± 6.7	12.0 ± 0.2 (40)	0.0314 ± 0.0007
C. septempunctata	Eastern *C. novemnotata*	High	71.7 ± 5.0	11.9 ± 0.1 (43)	0.0375 ± 0.0007
C. septempunctata	Western *C. novemnotata*	Low	78.3 ± 1.7	11.2 ± 0.1 (47)	0.0371 ± 0.0006
C. septempunctata	Western *C. novemnotata*	High	90.0 ± 0.0	10.9 ± 0.1 (54)	0.0381 ± 0.0006

Comparisons among treatments were tested using contrasts and the results are presented in the text.

[a] The percentage survival of each species within a treatment and aphid density was calculated for each of the two experimental dates and the grand mean ± SE across the experimental dates was calculated and is presented above.

[b] Mean development time of lady beetles reared from newly hatched (<24 h old) first instars to newly eclosed (<24 h old) adults. Beetles were reared to adulthood in pairs (treatment) or singly after the death of a partner.

[c] n, the number of individuals included in the calculations of mean development time and mean weight of newly eclosed adults.

aphid and low aphid densities were 55 and 30%, and 27 and 15%, respectively (Fig. 1; Table 1).

The total development time of individuals that survived to the adult stage was significantly affected by the group × treatment × aphid density interaction ($F_{(2, 358.3)} = 3.5$; $P = 0.03$; Table 1). The development time of western *C. novemnotata* increased significantly by 3.7 and 2.6 d at the low and high aphid densities, respectively, when reared with a *C. septempunctata* compared with when they were reared with another western *C. novemnotata* ($t = 12.2$; $P < 0.0001$). Similarly, eastern *C. novemnotata* took longer to develop to adulthood (1.5 and 1.0 d longer at the low and high aphid densities, respectively) when reared with a *C. septempunctata* compared with when they were reared with a conspecific ($t = 6.2$; $P < 0.0001$; Table 1). Western *C. novemnotata* developed significantly slower (10% slower at both aphid densities) than eastern *C. novemnotata* in conspecific pairings ($t = -7.7$; $P < 0.0001$), and they developed slower (21 and 19% slower at the low and high aphid densities, respectively) than eastern *C. novemnotata* did when each was paired with *C. septempunctata* ($t = -11.5$; $P < 0.0001$).

The weight of individuals that survived to the adult stage did not depend on the four-way group × aphid density × treatment × development time (covariate) interaction ($F_{(2, 403.5)} = 0.01$; $P = 0.98$), any of three-way interactions ($P > 0.25$), or the two-way interactions of development time × aphid density ($F_{(1, 402.2)} = 0.20$; $P = 0.65$) and development time × treatment ($F_{(3, 407.1)} = 1.04$; $P = 0.37$). There was a significant two-way group × treatment interaction ($F_{(2, 382.7)} = 226.4$; $P < 0.0001$). Planned comparisons using contrasts (across aphid densities) revealed that the weight of newly emerged eastern *C. novemnotata* was significantly larger than that of western *C. novemnotata* ($t = 7.5$; $P < 0.0001$; Table 1) when each group was reared conspecifically. Similarly, when eastern and western *C. novemnotata* were reared with a *C. septempunctata*, both groups emerged significantly smaller than when

reared conspecifically ($t = 6.7$, $P < 0.0001$; $t = 3.0$, $P = 0.003$, respectively), and eastern *C. novemnotata* emerged significantly larger than western *C. novemnotata* when reared with *C. septempunctata.*

Experiment 2. The survival of *C. novemnotata* larvae that were reared in same-aged or mixed-instar pairs with *C. septempunctata* was significantly affected by the instar of the *C. novemnotata* used to initiate the trial ($\chi^2 = 53.9$; df = 2; $P < 0.0001$; Table 2). Across *C. septempunctata* instars and aphid densities, 20% of first-instar, 71% of second-instar, and 90% of third-instar *C. novemnotata* survived to adulthood. *C. novemnotata* survival was significantly affected by the density of aphids provided to the pair of lady beetle larvae for food ($\chi^2 = 13.2$; df = 1; $P = 0.0003$). At the high aphid density, 63.6% of *C. novemnotata* survived to adulthood compared with 49.3% survival at the low aphid density. There was a highly significant effect of the survival of *C. septempunctata* through the larval instars on the survivorship of larval *C. novemnotata* ($\chi^2 = 22.4$; df = 1; $P < 0.0001$). Across the treatment combinations, 75 of 280 *C. septempunctata* died before pupating. Within the cups where the *C. septempunctata* died, 72 *C. novemnotata* (96%) survived to adulthood. Conversely, 205 of 280 *C. septempunctata* lived to adulthood, and only 86 *C. novemnotata* (42%) in those cups survived to adulthood. There was not a significant effect of *C. septempunctata* instar when it was included as a fixed effect along with *C. septempunctata* survival ($\chi^2 = 1.9$; df = 1; $P = 0.17$). When the survival of *C. septempunctata* was left out of the logistic regression, there was a significant effect of the instar of *C. septempunctata* used to initiate the experiment ($\chi^2 = 119.1$; df = 2; $P < 0.0001$; Table 2). Survival of *C. novemnotata* was 58.8 ± 4.1 and 46.0 ± 9.2 when paired with a first- or second-instar *C. septempunctata*, respectively. There were no significant two- or three-way interactions ($P > 0.50$).

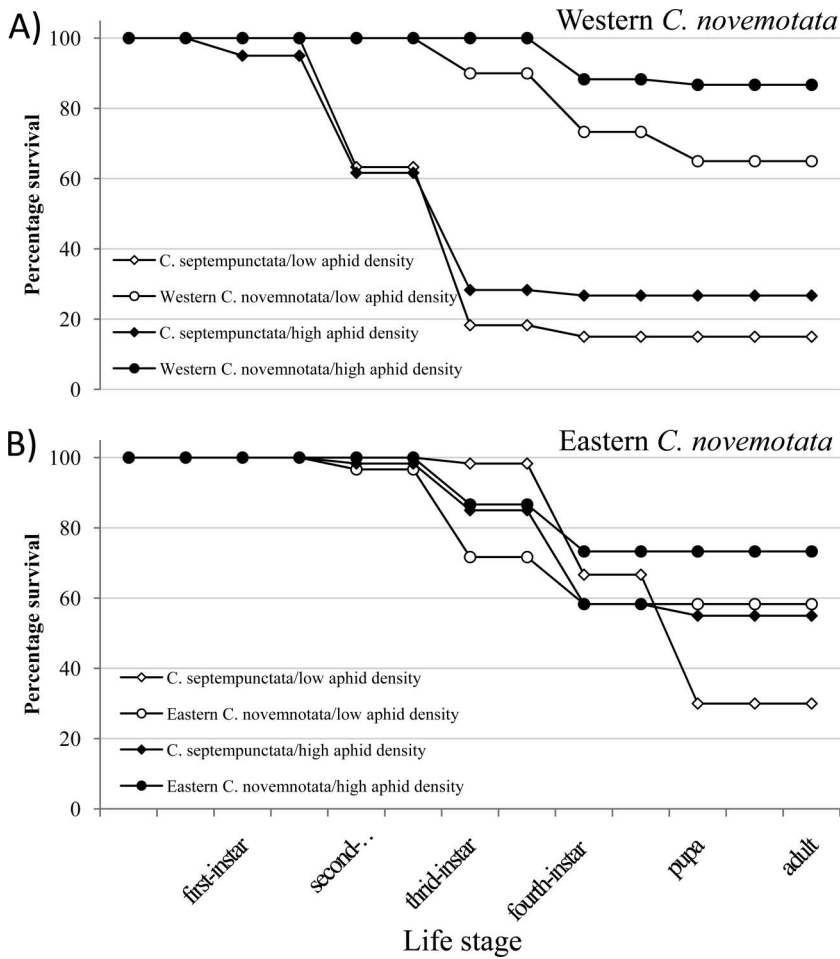The instar of *C. septempunctata* that was paired with a *C. novemnotata* at the start of the experiment had a

Fig. 1. Percentage survival over time of Western (A) and Eastern (B) populations of *C. novemnotata* reared in pairs from first instar to adulthood either intraspecifically or interspecifically with a same-aged *C. septempunctata* larvae at a low (open markers) or high density (filled markers) of pea aphids. Diamond-marked or circle-marked lines represent the partner species being *C. septempunctata* or a conspecific (*C. novemnotata*), respectively.

significant negative effect on adult *C. novemnotata* weight ($F_{1, 151.7} = 83.9$; $P < 0.0001$; i.e., *C. novemnotata* weighed significantly less when paired with a second-instar compared with a first-instar *C. septempunctata*). As expected, aphid density had a significant positive effect on the weight of newly eclosed adult *C. novemnotata* ($F_{1, 152.3} = 16.5$; $P < 0.0001$), the mean weight of adults emerging from the low and high aphid densities differed by an average of 1 mg (0.024 ± 0.0004 vs. 0.025 ± 0.0004 g, respectively) or ≈4% of the total body weight. There was a significant positive effect of the *C. novemnotata* instar used to initiate the experiment on adult *C. novemnotata* weight ($F_{1, 120.8} = 31.6$; $P < 0.0001$), as the instar of the *C. novemnotata* used to start the experiment increased, so to did their adult weight. There was not a significant *C. septempunctata* instar × *C. novemnotata* instar, *C. septempunctata* instar × aphid density, or a *C. novemnotata* instar × aphid density interaction ($P > 0.15$).

Discussion

The most important finding of this study was that *C. novemnotata* larvae were more likely to survive to become adults when reared with a conspecific larvae (the condition that would have existed before the introduction of *C. septempunctata*) than they were to survive when reared with *C. septempunctata* larvae. Similar results were found in studies involving other coccinellid species (Obrycki et al. 1998, Michaud 2002, Sato et al. 2003, Snyder et al. 2004) including *Coccinella transversoguttata* Brown, a native species that has exhibited declines in North America following the establishment of *Harmonia axyridis* Pallas and *C. septempunctata*. However, there have not been adequate interactive studies between the two species *C. novemnotata* and *C. septempunctata* largely because of the rarity of *C. novemnotata* populations in the field. Our findings have the potential to at least partially explain the decline of *C. novemnotata* and other native lady

Table 2. Mean percentage survival and weight (±SE) of newly eclosed adult western *C. novemnotata* reared in same-aged or mixed-instar pairs with *C. septempunctata* at low and high aphid densities

Western *C. novemnotata* instar	*C. septempunctata* instar	Aphid density	No. of replicates	No. surviving to adulthood[a]	Percentage survival to adulthood[b]	Mean wt (g)
First instar	First instar	Low	35	5	14.3	0.0208 ± 0.0003
First instar	First instar	High	35	13	37.1	0.0237 ± 0.0007
First instar	Second instar	Low	20	1	5.0	0.0208
First instar	Second instar	High	20	3	15.0	0.0201 ± 0.0001
Second instar	First instar	Low	24	19	79.2	0.0242 ± 0.0007
Second instar	First instar	High	24	19	79.2	0.0272 ± 0.0008
Second instar	Second instar	Low	20	10	50.0	0.0220 ± 0.0008
Second instar	Second instar	High	20	14	70.0	0.0229 ± 0.0006
Third instar	First instar	Low	20	19	95.0	0.0283 ± 0.0004
Third instar	First instar	High	20	20	100.0	0.0284 ± 0.0005
Third instar	Second instar	Low	21	15	71.4	0.0221 ± 0.0007
Third instar	Second instar	High	21	20	95.2	0.0243 ± 0.0007
Controls: pair of first instars	None	Low	66	54	81.8	0.0207 ± 0.0003
Controls: pair of first instars	None	High	62	57	91.9	0.0280 ± 0.0004

Comparisons among treatments were tested using contrasts and the results are presented in the text.

[a] The number of individuals included in the calculations of mean development time and mean weight of newly eclosed adults.

[b] The percentage survival of *C. novemnotata* within a treatment and aphid density was calculated across the three experimental dates to avoid the creation and presentation of percentages based on small numbers.

beetle species in the United States following the introduction and rapid spread of *C. septempunctata* and other introduced coccinellids.

While the main finding is clear, a closer examination of the competitive mechanism behind this asymmetric interaction might facilitate predictions of the results of interactions between native and introduced species that have not yet been tested. Specifically, our findings can potentially elucidate two modes of competitive interaction between native and introduced species in the same guild, exploitation competition and intraguild predation, which we considered an extreme form of interference competition (Polis et al. 1989). In the first study, we observed that pairing young *C. novemnotata* with a conspecific at the low aphid prey density and pairing it with *C. septempunctata* at either prey density resulted in an increase in the development time and a decrease in the adult weight of *C. novemnotata*, both being measures of exploitation competition. These results suggest that interspecific competition is stronger than intraspecific competition for prey in this species. These results corroborate the findings of Yasuda et al. (2004), who concluded that greater voracity can give an introduced species a competitive advantage over native species. In addition, Hoki et al. (2014) reported that adult *C. septempunctata* were more voracious than adult *C. novemnotata* across a range of prey densities, and it stands to reason that their increased voracity would extend to the larval stages and result in a competitive advantage through greater exploitation of shared prey.

Our data support a hypothesis that exploitation competition for prey and intraguild predation could be mechanisms contributing to the decline of *C. novemnotata*. The level and direction of any interaction between these factors has yet to be determined. Mortality of *C. novemnotata* reared with *C. septempunctata* could be partially attributed to intraguild predation given observations of half consumed or missing individuals. While it was not possible to differentiate incidence of intraguild predation from the scavenging of larvae dying from nonintraguild predation causes (e.g., loss of vigor or starvation due to exploitation competition), we observed numerous instances of *C. septempunctata* in the process of consuming live *C. novemnotata*. It is also noteworthy that first- through fourth-instar *C. septempunctata* and *C. novemnotata* survive for >24 h without food (T.A.U., unpublished data). This suggests that outright starvation and subsequent scavenging due to exploitation competition was not likely to have occurred. Although the lack of direct observation does not allow us to quantify the levels of exploitation competition for prey versus intraguild predation versus scavenging, especially given that coccinellids are known to exhibit the latter behavior (Majerus 1994), it seems likely that both modes of interaction are having an impact on the native species. This is supported by differences in the development time and adult weight of *C. novemnotata* (indicating exploitation), and observations of live individuals in the process of being consumed (demonstrating intraguild predation).

Our first study explored the interactions of *C. novemnotata* and *C. septempunctata* when they were maintained together for the duration of their development from the first instar to adulthood. However, as there is no complete phenological synchrony among or within species in the field, it is inevitable that there will be encounters between individuals of different ages and developmental stages.

In the second study, we observed that survival of *C. novemnotata* larvae increased as it was paired with increasingly "younger" (earlier instar) *C. septempunctata* larvae. Thus, *C. novemnotata* larvae are at a competitive disadvantage when they encounter a *C. septempunctata* larva of the same or more advanced developmental stage, but not when they encounter a less developmentally mature *C. septempunctata* larvae. A similar trend was found for the native species *C. transversoguttata* and *Hippodamia convergens* Guérin-

Méneville when they were paired with the introduced species, *C. septempunctata* and *H. axyridis* (Yasuda et al. 2004). These results can allow a more accurate prediction of the impact of an introduced species on a native one as they make it possible to estimate the proportion of encounters that will be positive, negative, or neutral.

While we have discussed the potential overall impact of *C. septempunctata* on *C. novemnotata*, we are convinced that there is substantial variation among populations of *C. novemnotata* based on their spatial separation and morphological differences, and this variation could contribute to a variable interaction with *C. septempunctata.* Examining the eastern and western *C. novemnotata* separately, the larvae from the eastern population develop more rapidly and are more likely to survive an encounter with *C. septempunctata* compared with their western counterparts. Although *C. novemnotata* is much rarer in the western United States than they were historically, the decline in the west has not been as extreme as the decline in the eastern United States. It is possible that the greater decline in the east has lead to stronger selective pressure that has, in turn, produced a strain of *C. novemnotata* that is more competitive with *C. septempunctata.*

Our results support the hypothesis that *C. septempunctata* played a central role in the decline of *C. novemnotata* as first suggested in Wheeler and Hoebeke (1995) and followed other studies based primarily on the synchrony of the timing *C. septempunctata* establishment and *C. novemnotata* decline (Harmon et al. 2007). Other studies have reported on the occurrence of competition and intraguild predation between introduced coccinellids such as *C. septempunctata* and *H. axyridis* and native species (Evans 1991, Agarwala and Dixon 1992, Elliot et al. 1996, Cottrell and Yeargan 1998, Obrycki et al. 1998, Sato and Dixon 2004, Snyder and Evans 2006, Hautier et al. 2011, Thomas et al. 2013), and Losey et al. (2012) reported a field-level morphological change in *C. novemnotata* following the establishment of *C. septempunctata.* We believe this is the first study to quantify direct interaction between these two species. While it is thought that *C. septempunctata* may have been the initial cause of the decline of *C. novemnotata*, *H. axyridis* has also been shown to be aggressive and strongly competitive against several species of native coccinellids (Leppanen et al. 2012), and thus could potentially be playing a role in preventing *C. novemnotata* populations from recovering. Additional field and smaller scale studies including other combinations of species could guide the conservation of natives species that have declined and help prevent future nontarget impacts from biological control releases.

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