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Interactions of the Asian Lady Beetle, *Harmonia axyridis* (Coleoptera: Coccinellidae), and the North American Native Lady Beetle, *Coccinella novemnotata* (Coleoptera: Coccinellidae): Prospects for Recovery Post-Decline

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Abstract

The decline of the North American native lady beetle, *Coccinella novemnotata* Herbst, is strongly correlated with the introduction of *Coccinella septempunctata* L., and *C. novemnotata* are locally extirpated across much of the United States. Since *C. novemnotata*'s decline, the invasive *Harmonia axyridis* Pallas has become dominant in North America. This study investigated whether *H. axyridis* has the potential to impede the recovery of *C. novemnotata* populations. To determine how *H. axyridis* interacts with *C. novemnotata* via intraguild predation and competition for prey, we paired first-instar *C. novemnotata* with first-instar *H. axyridis* at low and high densities of pea aphid. *Coccinella novemnotata* survival when paired interspecifically was significantly lower than *H. axyridis* survival at both aphid densities. Both species had similar weights at eclosion across aphid densities; however, *H. axyridis* developed faster than *C. novemnotata*. To examine the effect of larval size on intraguild interactions, we conducted a second experiment where we varied the *C. novemnotata* and *H. axyridis* instar in our pairings. *Coccinella novemnotata* survival and final weight increased when paired with younger *H. axyridis* larvae. The percentage survival of *C. novemnotata* in interspecific treatments, at the low aphid density, was lower than for same-aged *C. novemnotata* reared conspecifically, except for pairs initiated with *C. novemnotata* larvae that were two instars more advanced than *H. axyridis* larvae. These results suggest that intraguild predation and competition for prey by *H. axyridis* have the potential to affect the recovery of *C. novemnotata* populations negatively.

Key words: Intraguild predation, cannibalism, competition, invasive species, coccinellid

Coccinellids have been used for classical and augmentative biological control of soft-bodied prey, mainly aphids (Burgio et al. 2002), and are a well-established means of pest suppression in many agroecosystems. They aid in reducing aphid control costs (DeBach and Rosen 1991) and provide yield savings. During the past century, more than 179 non-native coccinellids were introduced into the United States in an effort to help suppress crop-damaging populations of aphids and other soft-bodied insects (Harmon et al. 2007).

Prior to the mid-1980s, lady beetle assemblages around the United States were composed almost exclusively of native species (Harmon et al. 2007), with *Coccinella novemnotata* Herbst ranking among the most abundant and widely distributed species of coccinellid in North America (Gordon 1985). During the mid-1980s and following the introduction and establishment of the European native

Coccinella septempunctata L., there was a large decrease in the abundance of native coccinellids, including documented declines in *C. novemnotata* and *Coccinella transversoguttata* Faldermann (Elliott et al. 1996, Harmon et al. 2007, Gardiner et al. 2011, Losey et al. 2012). Harmon et al. (2007) reported that between the years 1987 and 2001, native lady beetles represented less than 67.5% of the total number of individuals captured, although this decrease in native abundance may be the result of an increase in the absolute abundance of coccinellids, native and non-native, as a whole.

The decline in abundance of native lady beetles in general, and *C. novemnotata* specifically, which has been extirpated from much of its range, is thought to have been the result of a combination of factors related to the introduction and establishment of *C. septempunctata* (Hoebeke and Wheeler 1980, Harmon et al. 2007, Losey

et al. 2012, Hoki et al. 2014, Turnipseed et al. 2014, Tumminello et al. 2015). These authors posit several mechanisms that may have caused these declines, including: 1) the destruction of lady beetle and aphid habitats (farmland succession); 2) intraguild predation, which provides the intraguild predator direct nutritional benefits and indirect benefits through reducing competition (Dixon 2000); and 3) decreased aphid availability due to scramble competition. Several manuscripts have shown a strong negative interaction between these two species, mainly intraguild predation and scramble competition, favoring *C. septempunctata* over *C. novemnotata* (Hoki et al. 2014, Turnipseed et al. 2014, Tumminello et al. 2015). Turnipseed et al. (2014) studied the interactions of larval *C. novemnotata* and *C. septempunctata* maintained in pairs, and reported that *C. novemnotata* suffered high rates of mortality when paired with same-aged or older *C. septempunctata*, and that *C. novemnotata* survival rates were only greater than *C. septempunctata* when it was paired with an earlier-instar *C. septempunctata*. Tumminello et al. (2015), extending the work of Turnipseed et al. (2014), studied intraguild predation between *C. septempunctata* and *C. novemnotata* in larger groups of larvae and found that intraguild predation was heavily dependent on larval size asymmetry.

Another non-native North America coccinellid, *Harmonia axyridis*, the multicolored Asian lady beetle, is also a voracious predator of a multitude of pest species (Hodek and Honek 1996). Many unsuccessful attempts, dating back to 1916 (Gordon 1985), were made to introduce this species for biological control of aphids, and in 1988, an established population was detected in New Orleans (Chapin and Brou 1991), although this is believed to have occurred inadvertently via international commerce (Day and Tatman 2006). This species has since been collected and redistributed to multiple locations throughout the United States (Koch et al. 2006), and can now be found throughout the contiguous United States (www.lostladybug.org, accessed 10 November 2016).

Harmonia axyridis has been reported to contribute to the decreased abundance of both native and non-native coccinellids, including *Cycloneda munda* (Say), *Olla v-nigrum* Mulsant, *Adalia bipunctata* L., and *Coccinella septempunctata* (Brown 1999, Brown 2003, Yasuda et al. 2004, Sato et al. 2005). Negative impacts of *H. axyridis* have been of particular concern, and several studies have investigated the role of intraguild predation on the recovery of native species (Brown 2003, Pell et al. 2008, Roy and Wajnberg 2008). It is believed that the high rate of intraguild predation by *H. axyridis* is the main factor that contributed to its rapid establishment and spread (Rosenheim et al. 1995).

Based on the findings of Turnipseed et al. (2014) and Tumminello et al. (2015), the reported strength of *H. axyridis* as an intraguild predator, and the direct overlap of these species' ranges, we wanted to determine whether *H. axyridis* was also an intraguild predator of *C. novemnotata*, and if this species has the potential to impede the reestablishment of *C. novemnotata* in North America. We analyzed the interactions of *H. axyridis* and *C. novemnotata* using the same experimental designs used by Turnipseed et al. (2014). Specifically, we assessed the interactions of same-instar and mixed-instar pairings of larval *C. novemnotata* and *H. axyridis* at low and high aphid densities.

Materials and Methods

Insects

Adult *C. novemnotata* collected from Long Island, New York, during the summers of 2014 and 2015 and adult *H. axyridis* collected from the Cornell University Campus during 2015 were used to generate laboratory colonies. Colony beetles were kept singly in lidded 44-ml plastic cups containing a single 2.5 by 7-cm² piece of paper towel. All beetles were provided an ad libitum diet of mixed-aged pea aphids (*Acyrthosiphon pisum* Harris), which were reared on fava bean plants (*Vicia faba* L.). Cups were opened daily for the removal of old and dead aphids, and for the addition of freshly collected pea aphids (no plant foliage). Mated females were moved to fresh cups daily, and the eggs that they had laid were held until hatch. Colonies of lady beetles, eggs, and aphids were maintained at $25 \pm 2°C$ and a photoperiod of 16:8 (L:D) h.

Experiment 1: The Effects of Aphid Density on Intraguild Predation and Cannibalism of *C. novemnotata* and *H. axyridis*

We conducted a hierarchical experiment (lady beetle species nested within treatment pairings) to determine whether *H. axyridis* affects *C. novemnotata* via intraguild predation, competition for prey, or a combination of the two mechanisms. Lady beetle eggs were collected every 24 h and 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 arbitrarily divided among our experimental treatments and controls. Our experimental treatment consisted of interspecific pairings of newly hatched (< 24-h-old) first-instar *C. novemnotata* and *H. axyridis*, and our control treatments consisted of conspecific pairings of newly hatched (<24-h-old) first-instar *C. novemnotata* and *H. axyridis*. All pairings were maintained in 44-ml plastic cups containing a single 2.5 by 7-cm² piece of paper towel and were provided one of two aphid densities, "low" (0.035 ± 0.001 g/cup/day) and "high" (0.07 ± 0.001 g/cup/day), for a total of eight treatment combinations. The aphid densities used were based on the protocols described in the work by Turnipseed et al. (2014). All cups were opened once daily for the removal of old and dead aphids, and the addition of freshly collected aphids at the appropriate treatment level until the day that both larvae had pupated. Every day we recorded larval survival and larval instar for each individual within a cup, which allowed us to calculate the development time from newly emerged first instar to the adult stage, the percentage survival of each instar, and total survival. Upon adult eclosion, lady beetles were weighed and sexed. Cups were checked at 24-h intervals, and all occasions in which a partner was missing were scored as intraguild predation/cannibalism for the interspecific and conspecific treatments, respectively. The experiment was conducted on three independent occasions (completely randomized block design with dates as blocks) for a total of 30 replicate cups for each of the treatments, 10 replicate cups per treatment per block. Experimental arenas were maintained in an environmental incubator at $25 \pm 2°C$ and a photoperiod of 16:8 (L:D) h.

Experiment 2: The effects of larval instar and aphid density on intraguild predation and cannibalism of *C. novemnotata* and *H. axyridis*

An experiment was conducted to determine whether differences in larval coccinellid instar affected rates of intraguild predation. Based on the very low rates of survival for *C. novemnotata* reared with same-aged *H. axyridis*, we concluded there would be very little data to be obtained by initiating trials in which the *H. axyridis* larvae were older than the *C. novemnotata* larvae. In a preliminary experiment, we fed neonate *H. axyridis* and *C. novemnotata* held singly an *ad libitum* diet of pea aphids and recorded their instar and weight daily. We determined for both species that the mean larval weight increased significantly as a function of larval instar, and that the weights of *C. novemnotata* and *H. axyridis* were not significantly different from each other until they reached the third instar (data not shown; $P = 0.006$). Thus, this experiment paired *C. novemnotata* with same-instar or younger *H. axyridis* larvae. We placed a

single first-, second-, or third-instar *C. novemnotata* in a 44-ml cup containing a first-, second-, or third instar *H. axyridis* larva. We also reared pairs of first-instar *C. novemnotata* as a control treatment to obtain a baseline estimate of the survival of *C. novemnotata* in the absence of intraguild predation. All experimental treatments and controls were conducted at "low" and "high" aphid densities, as in the first experiment, for a total of 12 treatments. All pairings were maintained in 44-ml plastic cups with a single 2.5 by 7-cm^2 piece of paper towel. Cups were opened once daily for the removal of old and dead aphids and the addition of freshly harvested aphids at the appropriate treatment level until pupation. Larval survival and the instar of each individual within a cup were recorded every 24 h. The weight and sex of emerged adults were recorded within 24 h post-eclosion. The experiment was conducted on three independent occasions (completely randomized block design with dates as blocks) for a total of 25 replicate cups (10, 10, and 5 replicates per block, respectively) of each treatment across the three experimental blocks. Experimental arenas were maintained in an environmental incubator at $25 \pm 2°C$ and a photoperiod of 16:8 (L:D) h.

Statistical Analyses

All statistical analyses were performed using SAS (SAS Institute, Cary, NC) or JMP Pro 12 (SAS Institute, Cary, NC). Lady beetle survival to the adult stage in the first experiment was analyzed via nominal logistic regression using PROC GENMOD. Experimental block, treatment (species of lady beetles within a pair), aphid density, and individuals nested within treatments were modeled as fixed effects, as were the treatment $\times$ aphid density, individual $\times$ aphid density, and treatment $\times$ aphid density $\times$ individual interactions. To account for the correlation among individuals within a bioassay cup (dyadic data), we included "cup" as a repeated subject; "individual" was used to code each animal separately within a cup. Insignificant interactions were sequentially dropped from the model, and the model was re-run. Pre-planned two-way comparisons were made using t-tests, and the family-wise error rate was maintained at an overall value of alpha = 0.05 using the Bonferroni correction.

The survival of *C. novemnotata* and *H. axyridis* in the second experiment was analyzed via nominal logistic regression (JMP Pro 12), with block, aphid density, and treatment as fixed effects. Pairwise comparisons of survival as a function of aphid density by treatment and block were conducted using nominal logistic regression (JMP Pro 12). The controls were analyzed having block, aphid density, treatment, and the aphid density $\times$ treatment interaction as fixed effects.

The developmental time (in days) from newly emerged first-instar to adult eclosion was analyzed using mixed-model analysis of variance. The development times were transformed by taking the natural log to correct for skew. For Experiment 1, cup and block were included as random variables, and treatment, aphid density, individual nested within treatment, treatment $\times$ aphid density, and individual $\times$ aphid density $\times$ treatment were modeled as fixed effects. Multiple comparisons were made using Tukey's HSD (alpha = 0.05).

The untransformed weight of newly emerged adult beetles in the first experiment was analyzed using mixed-model analysis of variance. Analysis of adult weight included cup and block as random variables, and treatment, aphid density, individual nested within the treatment, treatment $\times$ aphid density, and individual $\times$ aphid density $\times$ treatment were modeled as fixed effects. For the second experiment, the weight was also analyzed using mixed-model analysis of variance, with block and cup as random effects and treatment

and aphid density as fixed effects. Multiple comparisons were made using Tukey's HSD (alpha = 0.05). We also analyzed the weight of the treatments as a whole, i.e., categorized together, versus the controls, and included aphid density and the aphid density by treatment interaction in the model. Insignificant interactions were dropped from the model (reported in text) and the reduced model was rerun.

Nominal logistic regression (JMP) was used to analyze differences in rates of mortality as a function of our treatment and developmental stage. The analysis of mortality as a function of treatment and developmental stage included individual, treatment, aphid density, developmental stage, treatment $\times$ aphid density, treatment $\times$ developmental stage, individual $\times$ developmental stage, individual $\times$ aphid density, aphid density $\times$ developmental stage, individual $\times$ aphid density $\times$ developmental stage, and treatment $\times$ aphid density $\times$ developmental stage as fixed effects. Our developmental stage referred to the instar in which larvae predation or larvae death occurred. Insignificant interactions were sequentially dropped from the model (not reported in text), and the model was rerun. We did not include in our statistical analyses, the single individual that failed to pupate even though it was still alive by the end of the 26th day of the experiment.

Results

Experiment 1

We found that *C. novemnotata* experienced significantly lower survival when paired with *H. axyridis* and that this effect was greater in the low versus the high aphid density treatment (Table 1), demonstrating that competition for aphids increased the incidence of intraguild predation. Overall, we found that *C. novemnotata* survival to adulthood increased approximately threefold when reared interspecifically with *H. axyridis* at the high versus low aphid density (Table 2), and that *H. axyridis* survival did not change as a function of aphid density for the interspecific treatment. The difference in the percentage survival of *C. novemnotata* versus *H. axyridis* when reared interspecifically at the low and high aphid density was 76.6 and 63.3%, respectively (Table 2). *Coccinella novemnotata* reared conspecifically at the high aphid density experienced a 15% decrease in the survival to adulthood compared with the low aphid density (Table 2), although post hoc analyses (lsmeans) indicated that this was not significantly different ($z = 1.8$, $P = 0.08$). *Harmonia axyridis* reared conspecifically were significantly affected by aphid density, having a percentage survival 28.3% higher when reared in the high aphid density. Generally, *C. novemnotata* in conspecific pairs survived at a higher rate compared with *C. novemnotata* in interspecific pairs, indicating strong negative intraguild interactions. *Harmonia axyridis* survival in all treatments was greater than 80% over the course of the study, with the exception of the low aphid/conspecific treatment, in which survival was only ~60%. The bulk of *H. axyridis* mortality occurred during the first 9 d of the test, after which the percentage survival plateaued (Fig. 1) owing to the pupation of *H. axyridis* at this time. *Coccinella novemnotata* mortality followed a similar pattern over the first 9 d; however, additional morality accrued in those treatments in which *C. novemnotata* were paired conspecifically (Fig. 1).

We found that larval mortality was significantly affected by the treatment $\times$ aphid density interaction (Table 1). *Harmonia axyridis*, in the conspecific high aphid density treatment, had a mortality of only 7 out of 60 individuals (11.6%), whereas the mortality of *C. novemnotata* was of 59 out of 120 individuals (49.1%) at both high and low aphid density. For the significant individual $\times$

Table 1. F-statistics, chi-square, and *P* values for analyses of lady beetle survival to the adult stage, larval mortality, development time, and adult beetle weight for Experiment 1

Survival to the adult stage	Degrees of freedom	Chi-square	*P*-value
Treatment	2	30.4	<0.0001
Aphid density	1	7.3	0.007
Individual	1	32.7	<0.0001
Treatment × aphid density	2	19.7	<0.0001
Larval mortality	Degrees of freedom	Chi-square	*P*-value
Developmental stage	4	122.1	<0.0001
Treatment	2	60.8	<0.0001
Individual	1	141	<0.0001
Treatment × aphid density	2	29	<0.0001
Individual × development stage	4	19.5	0.0006
Treatment × development stage	8	41.2	<0.0001
Aphid density × developmental stage	7	4	0.13
Individual × aphid density	1	1.2	0.28
Treatment × aphid density × developmental stage	8	9.1	0.33
Individual × aphid density × developmental stage	4	5.2	0.27
Development time	Degrees of freedom	*F*-value	*P*-value
Treatment	2, 153.3	19.7	<0.0001
Aphid density	1, 172.2	19.7	<0.0001
Treatment(individual)[a]	1, 198.2	15.9	<0.0001
Treatment × aphid density	2, 153	6.16	0.003
Treatment(individual) × aphid density	1, 198.2	4.8	0.03
Adult beetle weight	Degrees of freedom	Chi-square	*P*-value
Treatment	2, 162.1	18.4	<0.0001
Aphid density	1, 179.5	4.7	0.03
Treatment(individual)	1, 199.8	5.2	0.02
Treatment × aphid density	2, 162.5	5.7	0.004
treatment(individual) × aphid density	1, 199.8	4.2	0.04

[a] Parentheses indicate a nested variable, and "individual" refers to a single individual within a pair of beetles.

Table 2. Mean (±SE) percentage survival, development time (days) from first instar to newly eclosed adult, and weight (grams) of newly eclosed adult *C. novemnotata* and *H. axyridis* reared interspecifically or conspecifically at high and low aphid densities

Species	Treatment (n)[a]	Aphid density	Mean ± SE percentage survival[b]	Mean ± SE development time in days[c] (n)[d]	Mean ± SE wt (g) of newly eclosed adults[e]
C. novemnotata	Conspecific (60)	Low	58.3 ± 6.7	16.9 ± 0.5 (35)	0.0242 ± 0.0008c
C. novemnotata	Conspecific (60)	High	43.3 ± 4.4	16.9 ± 0.2 (26)	0.0264 ± 0.0009bc
C. novemnotata	Interspecific (30)	Low	6.7 ± 6.7	18.5 (2)	0.0223 ± 0.0029abc
C. novemnotata	Interspecific (30)	High	20.0 ± 15.3	16.7 ± 1.3 (6)	0.0305 ± 0.0017ab
H. axyridis	Conspecific (60)	Low	60.0 ± 5.0	16.2 ± 0.5 (36)	0.0302 ± 0.0007a
H. axyridis	Conspecific (60)	High	88.3 ± 4.4	15.5 ± 0.4 (53)	0.0288 ± 0.0006ab
H. axyridis	Interspecific (30)	Low	83.3 ± 3.3	15.5 ± 0.4 (25)	0.0302 ± 0.0009a
H. axyridis	Interspecific (30)	High	83.3 ± 12.0	14.7 ± 0.2 (25)	0.0309 ± 0.0009a

Comparisons of survival and development times were tested using contrast and the results are presented in the text.

[a] Number of individuals used to start the experiment.

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

[c] 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 when the death of a partner occurred.

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

[e] Mean (±SE) weight of newly eclosed adults that are followed by the same letter are not significantly different (Tukey's HSD, alpha 0.05%).

developmental stage interaction, we found that *C. novemnotata* died at a rate 5.14 and 10.4 times greater than *H. axyridis* during the first and third instars (Fig. 2), respectively. The percentage mortality of each *H. axyridis* instar (Fig. 2) shows that *H. axyridis* experienced the greatest rates of mortality during the fourth instar in the conspecific low aphid density treatment, and that *C. novemnotata*

suffered the largest percentage mortality during the third instar when paired with *H. axyridis*, especially at the low aphid density where competition for aphids would be the most fierce.

The total development time of *H. axyridis* reared conspecifically was 0.7 and 1.4 d faster than that of *C. novemnotata* reared conspecifically at the low and high aphid density, respectively (Table 2).
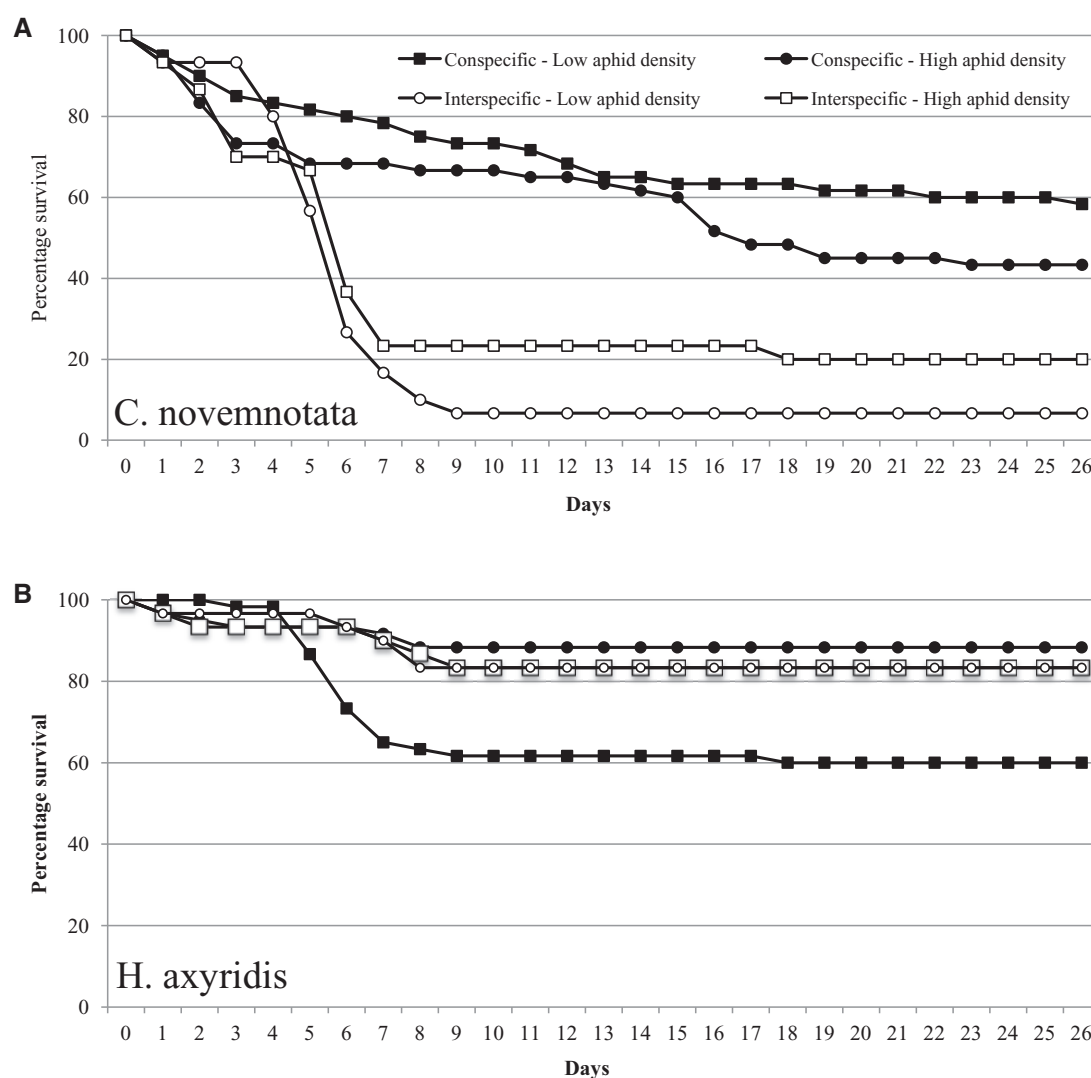
Fig. 1. Percentage survival over time of *C. novemnotata* (A) and *H. axyridis* (B) reared in pairs from first instar to adulthood either inter- or conspecifically at a low or high level of pea aphids.

When reared interspecifically, *H. axyridis* developed 3.0 and 2.0 d faster than *C. novemnotata* in the low and high aphid density treatments, respectively. *Harmonia axyridis* in the interspecific high aphid density treatment developed the fastest; however, this was not significantly faster than *H. axyridis* in the interspecific low aphid density or the *C. novemnotata* interspecific high aphid density treatments (Table 2, Fig. 3). There was no difference among the development times of *C. novemnotata* in the conspecific low aphid density, *C. novemnotata* conspecific high aphid density, *C. novemnotata* interspecific low aphid density, and the *H. axyridis* conspecific low aphid density treatments (Table 2, Fig. 3). The random effects (cup and block) accounted for 36% of all the variation in the development time of the lady beetles.

The weights of *C. novemnotata* individuals from the interspecific treatments were not significantly different from the weights of *H. axyridis* (Tables 1 and 2); however, we had only small number of *C. novemnotata* that survived in the interspecific low aphid ($n = 2$) and interspecific high aphid ($n = 6$) treatments on which to base our estimates of adult weight. *Coccinella novemnotata* from the conspecific treatments at the low and high aphid densities were significantly smaller than *H. axyridis* from all treatments, with the

exception of *C. novemnotata* and *H. axyridis* from the conspecific high aphid density treatments, which did not differ from one another (Table 2). The random effects accounted for 10% of all the variation in the weight of adult beetles.

Experiment 2
The survival of *C. novemnotata* larvae that were reared in same-instar or mixed-instar pairs with *H. axyridis* was significantly affected by the interaction between the instar of *C. novemnotata* and *H. axyridis* larvae used to initiate the trial and the aphid density ($\chi^2 = 26.2$, df $= 6$, $P = 0.0002$). The probability of *C. novemnotata* surviving to adulthood only increased appreciably when there was a two-instar difference between the larvae used to initiate the trial (third instar vs first instar; Table 3). The highest survival rates (in the third-instar *C. novemnotata*–first-instar *H. axyridis* treatment) were numerically higher (64 and 76% survival) than the conspecific rates from Experiment 1 (58 and 43% survival). *Harmonia axyridis* survival to adulthood across instars and aphid densities was of 88, 92, and 96% for the first, second, and third instars, as compared with 6, 22, and 34% for *C. novemnotata*, respectively. *Coccinella novemnotata* survival to adulthood was significantly affected by the
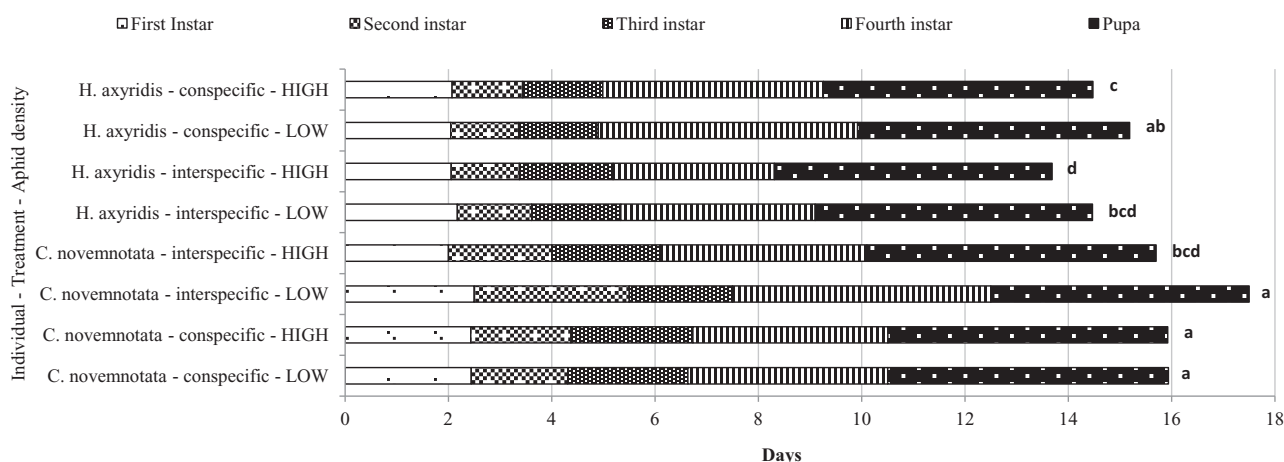
Fig. 2. Stage-specific percentage mortality (bars) and cumulative percentage mortality of individuals dying (lines) for *C. novemnotata* (A) and *H. axyridis* (B) reared from first instar to adulthood either inter- or conspecifically at a low or high density of pea aphids.
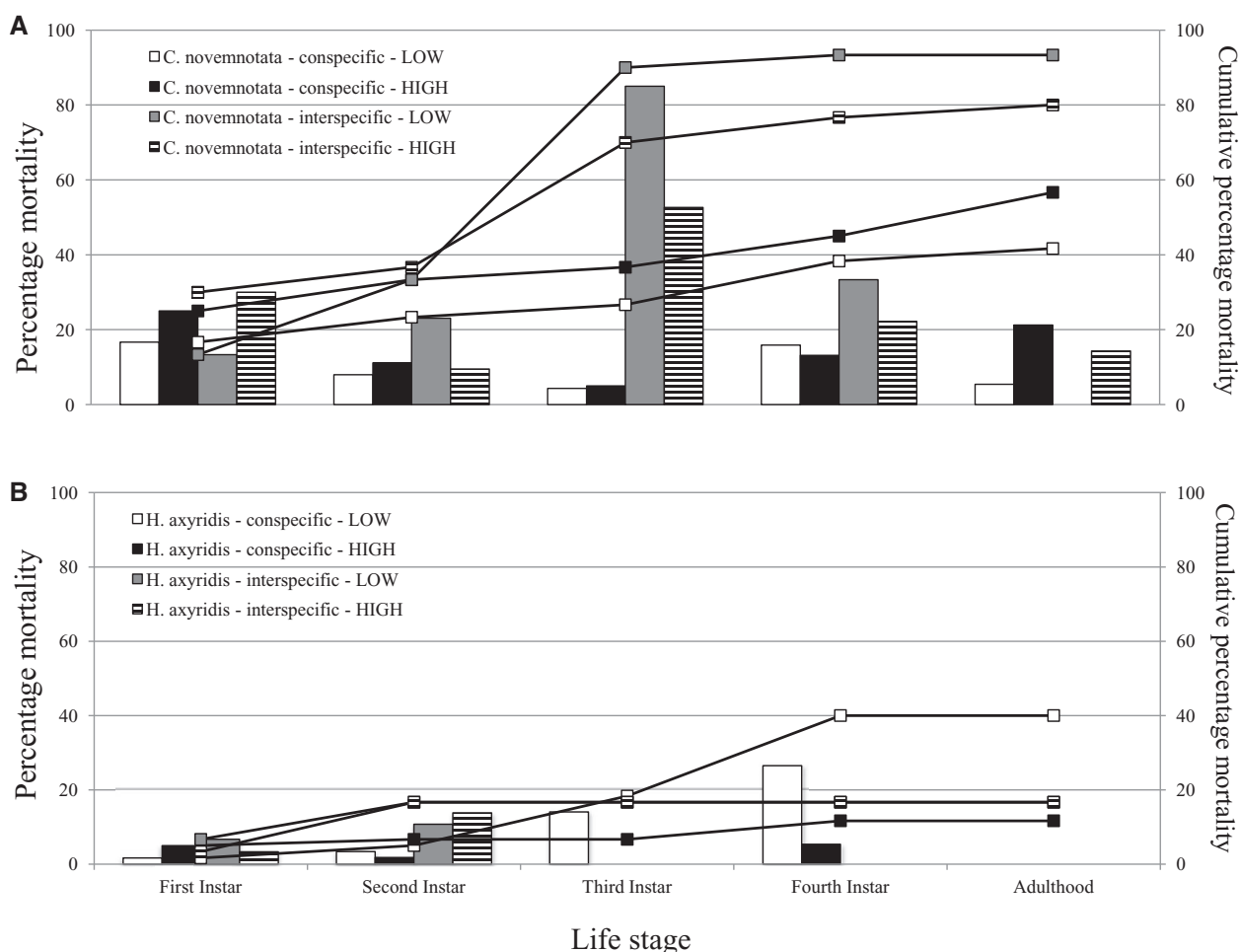

Fig. 3. Length (in days) of larval and pupal stadia of *C. novemnotata* and *H. axyridis* reared from first instar to adulthood either inter- or conspecifically at a low or high level of pea aphids. Total development times that are followed by the same letter are not significantly different (Tukey's HSD, alpha = 0.05).

density of aphids provided to the pair of lady beetle larvae for food ($\chi^2 = 32.0$, df = 1, $P < 0.0001$). At the high aphid density, 41.3% of *C. novemnotata* survived to adulthood, compared with 16% survival at the low aphid density. *Harmonia axyridis* survival to adulthood was significantly affected by the aphid density × treatment

two-way interaction ($\chi^2 = 17.0$, df = 5, $P = 0.0045$). The "3 vs 1" treatment was the only treatment that did not have a significant effect of aphid density on the survival of *C. novemnotata* (Table 3).

The instar of *H. axyridis* that was paired with *C. novemnotata* at the start of the trials had a significant effect on the adult *C.*

Table 3. Mean percentage survival, statistical results, and weight (±SE) of newly eclosed adult *C. novemnotata* reared in same-aged or mixed-instar pairs with *H. axyridis* at a low and high pea aphid density

Treatment (Coccinella vs Harmonia)	No. of replicates across aphid densities[a]	No. surviving to adulthood across aphid densities[b]	Percentage survival to adulthood[c]		χ^2, df, P[d]	Mean Weight (g) ± SE[e]
			Low aphid density	High aphid density		
1 vs 1	50	3	0	12	$\chi^2 = 4.4$, df = 1, P = 0.04	0.027 ± 0.003abc
2 vs 1	50	8	4	28	$\chi^2 = 6.0$, df = 1, P = 0.01	0.019 ± 0.002c
2 vs 2	50	14	16	40	$\chi^2 = 4.9$, df = 1, P = 0.03	0.027 ± 0.001ab
3 vs 1	50	35	64	76	$\chi^2 = 0.5$, df = 1, P = 0.46	0.029 ± 0.001a
3 vs 2	50	9	0	36	$\chi^2 = 14.5$, df = 1, P = 0.0001	0.024 ± 0.002bc
3 vs 3	50	16	8	56	$\chi^2 = 14.8$, df = 1, P = 0.0001	0.026 ± 0.001ab
Control	100	63	66	60	$\chi^2 = 0.4$, df = 1, P = 0.51	0.025 ± 0.001b

[a] The number of replicates is a sum of the replicates used for the low and high aphid density (25 replicates for each density of aphids).

[b] The number of individuals surviving to adulthood is a sum of individuals that made to the adulthood stage at the low and high aphid density.

[c] The percentage survival to adulthood for the low and high aphid density is based on 25 individuals.

[d] The statistical analyses presented in this table represent the pairwise comparisons (logistic) of survival of *C. novemnotata* as a function of aphid density within each treatment.

[e] Mean weight was obtained from all *C. novemnotata* individuals that reached adulthood at both aphid densities. Mean (±SE) weight of newly eclosed adults that are followed by the same letter within a column are not significantly different (Tukey's HSD, alpha 0.05%).

novemnotata weight ($F_{5, 69.1} = 10.4$; $P < 0.0001$), as did aphid density ($F_{1, 46.2} = 4.6$; $P = 0.0372$). As expected, the weight of *C. novemnotata* in the "3 vs 1" treatment was higher than all the other treatments. Aphid density had a positive effect on the mean weight of the lady beetles, in which the mean weight of adults emerging from the low and high aphid densities differed by an average of 1 mg (0.0236 ± 0.0009 vs 0.0258 ± 0.0004, respectively) or ~4% of the total body weight. We used aphid density, treatment versus control, and aphid density × treatment versus control as fixed effects and found no significant interaction between the aphid density × treatment versus control two-way interaction ($F_{1, 88.2} = 3.7$; $P = 0.055$); however, after dropping the two-way interaction, we found a significant effect of the aphid density ($F_{1, 106.2} = 4.2$; $P = 0.043$) on the weight of newly eclosed lady beetles, but no significant effect on the weight of lady beetles reared in conspecific or interspecific pairs (treatment vs control; $F_{1, 92} = 3.5$; $P = 0.064$).

Discussion

The results of our studies showed that *C. novemnotata* reared singly with *H. axyridis* (Experiment 1) in small arenas suffered high rates of mortality (80–93%) and were much less likely to survive to adulthood compared with pairs of *C. novemnotata* that were reared conspecifically (42–57% survival), and that survival was dependent on the level of competition for aphids. We also observed high rates of survival of *H. axyridis* (60–88%) reared both con- and interspecifically at the low and high aphid densities. Given that the distribution of *H. axyridis* completely overlaps that of *C. novemnotata*, and that *H. axyridis* are often found inhabiting the same plants as *C. novemnotata* in those locations in North America where they can still be found (TAU, personal observations), these findings suggest that *H. axyridis* has the potential to exert negative pressure, via intraguild predation and scramble competition, on *C. novemnotata* population growth.

These findings are similar to those reported by multiple other authors who investigated the interactions of native and invasive lady beetle species (Obrycki et al. 1998, Michaud 2002, Sato et al. 2003, Snyder et al. 2004, Turnipseed et al. 2014). Turnipseed et al. (2014), in a study of the interactions of *C. novemnotata* and its invasive congener *C. septempunctata*, and whose protocols and aphid densities

we replicated, reported an identical level of survival of Long Island, NY, *C. novemnotata* at the low aphid density (58%). The survival of *C. novemnotata* at high aphid density in this study, however, was much lower in comparison with that reported in the work by Turnipseed et al. (2014; 43 vs 73% survival, respectively). Interestingly, we observed a negative relationship between *C. novemnotata* survival and increasing aphid density (Table 2) when *C. novemnotata* was paired conspecifically, which is similar to the results published by Losey et al. (2012), who found that *C. novemnotata* survival from newly emerged larvae to adult decreased from 75 to 60% at low and high aphid densities, respectively; the same 15% decrease that we observed.

In our first experiment, *H. axyridis* developed faster than *C. novemnotata* in both the conspecific and interspecific treatments at both aphid densities, although our sample sizes for *C. novemnotata* reared interspecifically were low ($n = 2$ and 6 individuals at the low and high aphid densities, respectively) owing to high rates of mortality from intraguild predation. Under optimal field conditions, the faster development times of *H. axyridis* would allow newly developed adult beetles a longer window of opportunity to locate aphid populations in space (Osawa 2000, With et al. 2002). *Harmonia axyridis* are also reported to be more voracious aphid predators than some other native coccinellids (Michaud 2002), which is likely tied to their faster developmental rates. Jarosik et al. (2015) showed that invasive species are more likely establish in regions that are warmer than their native range in part because they are able to develop at lower temperature, resulting in faster overall development times. The development times of *H. axyridis* from neonate larvae to newly emerged adult that we obtained were similar to those reported by Auad et al. (2014), who reared *H. axyridis* on *Rhopalosiphum padi* L. at different temperatures to determine the optimal temperature for development and survival. Similar development times were obtained by Phoofolo and Obrycki (1995) and LaMana and Miller (1998), who reared *H. axyridis* on *A. pisum* at 26 °C, and Lanzoni et al. (2004), who reared *H. axyridis* on *Myzus persicae* (Sulzer) at 25 °C. On the other hand, the development times of *C. novemnotata* were slightly shorter than those obtained by Tumminello et al. (2015) and longer than those obtained by Turnipseed et al. (2014), although the patterns in length observed

among treatments and aphid densities were the same. Ugine and Losey (2014) had similar results as to the development times of *C. novemnotata* when rearing them on *A. pisum* at 25 °C. One large difference in the methodology of Tumminello et al. (2015) and our study was that we reared our larvae in pairs, whereas Tumminello et al. (2015) reared their larvae in larger groups of 16 per container, which could have affected the development times in unforeseen ways.

We observed an increase in the rate of mortality of *H. axyridis* reared conspecifically at the low versus high aphid density (40% vs 12%, respectively). This finding is consistent with the results of Burgio et al. (2002), who reported that rates of cannibalism were inversely related to aphid density, indicating a relationship between cannibalism and competition for aphids. Similarly, Osawa (1992), in a study of sibling and non-sibling cannibalism of *H. axyridis*, found that mortality within the fourth instar (93.3%) was higher than those from any other instars, probably owing to food shortages caused by adult oviposition timing synchronized with seasonal change in aphid abundance. Hence, cannibalism seems to play an important role in the population dynamics of *H. axyridis*, providing nutritional benefits when prey are scarce (Wagner et al. 1999).

Our second experiment was designed to determine the effect of size asymmetry in interspecific pairs on *C. novemnotata* survival as a function of aphid density. Overall, *C. novemnotata* survival increased when provided more aphids no matter what instar of *H. axyridis* they were paired with. The effect of size asymmetry on *C. novemnotata* survival was apparent under two different scenarios in our experimental design. First, *C. novemnotata* survival increased significantly from the first through third instars when paired with first-instar *H. axyridis* at both aphid densities. Second, survival of third-instar *C. novemnotata* decreased as the instar of *H. axyridis* increased from the first to the third instar, although the effect was not as strong in pairings of second instars. It is noteworthy that Turnipseed et al. (2014), who used slightly different instar pairings of *C. novemnotata* and *C. septempunctata*, reported higher rates of survival of *C. novemnotata* than we observed in this study, and that they used a population of *C. novemnotata* that they considered more vulnerable to intraguild predation than the Long Island population that we used. Although the results of these studies are not directly comparable, the data suggest that *C. novemnotata* is more susceptible to intraguild predation by *H. axyridis* than *C. septempunctata*, and additional studies investigating the intraguild predation interactions of all three species might yield additional useful information on the abundance and distribution of all three species

Analysis of data collected from 1983–1996 in apple orchards of eastern West Virginia showed that *H. axyridis* has the potential to displace native and non-native species of lady beetles (Brown and Miller 1998). These authors found that between 1992–1993, *C. septempunctata* represented more than 93% of total coccinellids present in apple orchards. Three years after *H. axyridis* became established, *C. septempunctata* represented approximately 10% of all coccinellids, whereas *H. axyridis* comprised 88%. Colunga-Garcia and Gage (1998) studied the interaction of *H. axyridis* and the 11 species of resident lady beetles in Michigan for the five years following its establishment. They found that *H. axyridis* became the dominant species four years after its arrival and appeared to have caused a reduction in the abundance of *Cycloneda munda* (Say), *Brachiacantha ursina* (Fabricius), and *Chilocorus stigma* (Say). Michaud (2002) also reported a decline in abundance of a native North American coccinellid, *Cycloneda sanguinea* L., subsequent to the introduction of *H. axyridis* in the Florida citrus ecosystem; Sato et al. (2005) documented intraguild predation of the native *A.*

bipunctata by *H. axyridis*, and Honek et al. (2016) reported declines in the abundance of several native European lady beetles following the introduction of *H. axyridis*.

Our studies demonstrate that *C. novemnotata* survival to adulthood is strongly affected by prey availability, size of competitor, and intraguild predation by *H. axyridis*. Since its introduction and establishment in North America, *H. axyridis* has displaced native and non-native species of coccinellids, including the "problematic" *C. septempunctata*, owing to its voracity and resource competition (Brown and Miller 1998).

It has been widely reported that *C. septempunctata* may be responsible, at least in part, for the decline of the native lady beetle, *C. novemnotata*, which is at near-extinction levels in much of North America (Wheeler and Hoebeke 1995, Elliott et al. 1996, Harmon et al. 2007, Losey et al. 2012). Brown and Miller (1998) found that two years after the introduction of *H. axyridis* in apple orchards, in West Virginia, the native lady beetles *A. bipunctata*, *C. munda*, *Anatis labiculata* (Say), and *Olla v-nigrum* and the non-native *C. septempunctata* were not seen anymore.

There are very few studies published that investigate interactions between *C. novemnotata* and *H. axyridis*, presumably because of the rarity of *C. novemnotata* field populations. Our results support the hypothesis that *H. axyridis* has the potential to prevent the natural recovery of *C. novemnotata* populations. Other studies have shown the capacity of *H. axyridis* on the decline of other coccinellid species through either intraguild predation or competition for prey (Osawa 1992, Elliott et al. 1996, Brown and Miller 1998, Colunga-Garcia and Gage 1998, Osawa 2000, Burgio et al. 2002, Michaud 2002, With et al. 2002, Sato et al. 2003).

We believe this is the first study on the ecology and interaction between these two species, and although *C. septempunctata* may have initially caused the decline of *C. novemnotata* populations, *H. axyridis* may be preventing their recovery. Additional field studies to elucidate the interactions between these two species using or additional species combinations, such as *C. septempunctata* and *H. axyridis*, would help us better understand the mechanisms that underlie the recovery of *C. novemnotata* and provide guidance in preventing additional declines of native species owing to nontarget impacts caused by the use of biological controls.

Acknowledgments

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