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PHYSIOLOGICAL ECOLOGY - ORIGINAL PAPER

Intraspecific alkaloid variation in ladybird eggs and its effects on con- and hetero-specific intraguild predators

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Abstract Egg predation and cannibalism are common phenomena in predatory ladybirds despite the presence of defensive alkaloids. Consumption of heterospecific eggs negatively affects survivorship and development; however, intraspecific variation in quantities of alkaloids and post-ingestion responses to con- and hetero-specific alkaloids, are not well understood. We examined variation in the quantity of alkaloids in eggs of *Harmonia axyridis* (Pallas), *Coccinella septempunctata* L., and *Hippodamia convergens* (Guérin) using gas chromatography-mass spectrometry, and show a link between heterospecific alkaloids and their toxicity and/or costs by feeding high and low alkaloid eggs to first instar *H. axyridis* and *C. septempunctata*. The repeatability of alkaloid measurements in eggs in an egg cluster was high; however, the amount of alkaloids varied significantly between egg clutches within and among females. This variation affected egg consumption by *C. septempunctata* when fed *H. axyridis* eggs. *Harmonia axyridis* accumulated their own alkaloid by cannibalism and synthesized it de novo, but *C. septempunctata* lost some portion of the consumed conspecific alkaloids. Both species lost most of the consumed heterospecific alkaloids, but *C. septempunctata* died within 3 days. Most *H. axyridis* survived to the second instar, but *C. septempunctata* alkaloids led to a significant reduction in weight gain compared to an aphid control. In addition, ingestion of high alkaloid *C. septempunctata* extended development of *H. axyridis* compared to the aphid control or conspecific eggs. *Harmonia axyridis* had greater abilities to process ingested con- and hetero-specific alkaloids compared with *C. septempunctata*, which may, in part, explain their interspecific interactions in nature.

Keywords Defensive alkaloids · Predatory ladybirds · Intraguild predation · *Harmonia axyridis* · *Coccinella septempunctata*

Introduction

Many insects sequester defensive chemicals, including alkaloids, from their diet, or synthesize these compounds de novo. Generally, long-term exposure to heterospecific alkaloids results in deleterious effects on survival and fecundity (Aniszewski 2007). Many studies have focused on plant alkaloids and their effects on insect herbivores (Rosenthal and Berenbaum 1992), and herbivores and predatory insects interactions (Eisner and Eisner 1991; Hare and Eisner 1993; Eisner et al. 2000; Pasteels 2007). An understanding of the effects of ingested alkaloids is still lacking among generalist arthropod predators. Because cannibalism and intraguild predation are common phenomena among generalist arthropod predators (Fox 1975; Polis 1981; Rosenheim et al. 1995; Rosenheim 1998), how generalist predators respond to ingested conspecific and heterospecific

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alkaloids, and how these compounds affect development and survivorship are intriguing topics relative to their life history strategies.

Identification of alkaloids in predatory ladybirds (Coleoptera: Coccinellidae) has been investigated extensively (King and Meinwald 1996). Alkaloids of ladybirds are synthesized in fat bodies (Laurent et al. 2002), and secreted as defensive fluids (reflex bleeding) in larvae and adults. Alkaloids are also found in eggs (Daloze et al. 1995; Sloggett et al. 2009a, b). Despite the presence of alkaloids, cannibalism and intraguild predation are common phenomena among predatory ladybirds (Takahashi 1989; Agarwala and Dixon 1992; Yasuda and Shinya 1997). Eggs are especially vulnerable to predation in fields (Koide 1962; Hodek 1973; Osawa 1989, 1992a, b). Previous studies show that consumption of heterospecific eggs negatively affects feeding behavior of adults and larvae, and survivorship and development during the larval stages, but the toxic levels of alkaloids in eggs and the ability to tolerate heterospecific alkaloids is likely to vary among species (Agarwala and Dixon 1992; Agarwala et al. 1998; Sato and Dixon 2004; Cottrell 2004, 2005, 2007; Rieder et al. 2008; Sloggett et al. 2009a). A more thorough understanding of the defensive roles of alkaloids requires quantification of levels of alkaloids in eggs, intraspecific variation in these quantities, and the predators' response to ingested con- and heterospecific alkaloids. Together these factors may influence the outcome of interactions among predatory ladybirds.

Harmonia axyridis (Pallas) and *Coccinella septempunctata* L. are invasive species in North America, but they are coevolved species in native ranges. *Harmonia axyridis* is dominant (or more abundant) relative to *C. septempunctata* when in sympatry in their native range (Yasuda and Shinya 1997; Sato et al. 2003, 2008), and in selected orchard habitats in an invaded range (Brown and Miller 1998; Brown 2003). Larvae of *H. axyridis* are more aggressive and consume a wider range of prey and non-prey food items compared with *C. septempunctata* (Yasuda and Ohnuma 1999). In addition, consumption of *H. axyridis* eggs lowers survivorship of *C. septempunctata*, but not vice versa (Sato and Dixon 2004; Rieder et al. 2008). Based on these studies, we hypothesized that *H. axyridis* larvae have a superior ability to process ingested con- and heterospecific defense chemicals compared with *C. septempunctata*, which may, in part, explain the characteristics of *H. axyridis* as an intraguild predator, and interspecific interactions with *C. septempunctata*.

We investigated variation in the amount of alkaloids in eggs of *H. axyridis*, *C. septempunctata* and *Hippodamia convergens* (Guérin). We also quantified the effect of variable amounts of alkaloids in eggs on first instar *H. axyridis* and *C. septempunctata*. We measured feeding activity, development, and survivorship of first instar *H. axyridis* and *C. septempunctata*, and compared responses to ingested con- and heterospecific alkaloids between the two species.

Materials and methods

Insect materials

Adults of *Harmonia axyridis*, *Coccinella septempunctata*, and *Hippodamia convergens* were collected from alfalfa and strawberry fields at the Research Farms of the University of Kentucky (Lexington, KY, USA), during September 2008. In the laboratory, females and males were paired and provided with pea aphids (*Acyrthosiphon pisum* Harris) ad libitum daily. Pea aphids were reared on fava bean, *Vicia faba* L. Each pair was placed in a Petri dish (9 cm in diameter and 1.5 cm in height), maintained in an incubator at 22°C (17L:7D), and transferred to a new Petri dish daily to reduce potential egg cannibalism. A strip of folded filter paper (1 × 4 cm) provided oviposition sites. Eggs from individual females were transferred daily to a 1.5 ml tube with the records of the female and the date, and stored at −80°C for 2–3 months.

Variation in alkaloid content in eggs

Alkaloids within individual eggs laid by five females of *H. axyridis*, and four females of *C. septempunctata* and *H. convergens* were quantified by gas chromatography-mass spectrometry (GC–MS). Egg clutches were collected daily at three time periods in 2008: 24–28 September, 4–9 October, and 13–16 October. Four eggs were randomly selected from one egg clutch produced by each female. Alkaloids of the three species have previously been identified (Tursch et al. 1971a, b, 1974; Braconnier et al. 1985a, b; Alam et al. 2002; Sloggett et al. 2009b): harmonine and 3-hydroxypiperidine-2-one in *H. axyridis*, precoccinelline and coccinelline in *C. septempunctata*, and hippodamine, convergine, harmonine and *n*-octylamine in *H. convergens*. 3-hydroxypiperidine-2-one in *H. axyridis* and *n*-octylamine in *H. convergens* are not detectable using the GC–MS method used here (Sloggett et al. 2009b). The wet weight of each egg was measured to the nearest 0.01 mg using a Mettler AE163 microbalance before GC–MS analysis.

GC–MS methods

Each egg was homogenized in 200 µl methanol containing 2,000 ng (*Z*)-13-octadecenol in a conical reaction glass vial (1 ml). After homogenization, 400 µl distilled hexane was added and mixed vigorously. The methanol phase was transferred by 100 µl glass pipette and filtered through a

glass tube packed with glass-wool. The glass tube was rinsed with 120 µl methanol twice. The filtered sample was split into two glass vials (200 µl for each vial transferred by a glass pipette), which allowed us to duplicate the sample for the further derivatization treatment for harmonine [e.g., to detect harmonine using GC–MS, derivatization with MBTFA (*N*-methyl-*bis*, trifluoroacetamide) was used (Sloggett et al. 2009b)]. The repeatability (R) of the split-sample technique was high ($R = 0.95$), based on the results from *C. septempunctata* by using Proc VARCOMP (SAS 9.1): R = [Var (measurements)/(Var (measurements) + Var (error)]. The sample was dried under a stream of nitrogen gas. For the analysis of alkaloids of *C. septempunctata*, and hippodamine and convergine of *H. convergens*, the dried sample was dissolved in an additional internal standard solution [100 µl of methylene chloride with (*Z*)-13-octadecenyl acetate (20 ng/µl)] and transferred to an auto-sample vial. For detection of harmonine from *H. axyridis* and *H. convergens*, the dried sample was dissolved in 100 µl methylene chloride with 10 µl MBTFA for derivatization. The solution was placed in an oven at 60°C for 1 h (Sloggett et al. 2009b), dried under a stream of nitrogen gas, and dissolved in 100 µl methylene chloride with (*Z*)-13-octadecenyl acetate (20 ng/µl), and transferred to an auto-sample vial.

GC–MS analyses were conducted using the methods of Sloggett et al. (2009b) on a Hewlett-Packard HP 6890 gas chromatograph with a HP 7683 auto-sampler coupled to a HP 5973 mass spectrometer. A split-splitless injector at 200°C and a DB5 GC column (0.25 mm diameter, 30 m length, and 0.25 µm film thickness) was used. The carrier gas was helium with a flow rate of 1 ml/min. Mass spectra were obtained using electron ionization mode at 70 eV (scan range m/z 35–500). The GC temperature program was 60°C for 2 min, followed by an increase of 10°C/min up to 325°C and holding at this final temperature for 15 min. For the analysis, 2 µl sample (out of 100 µl) in methylene chloride was injected. The quantity of each alkaloid was estimated using an area comparison to (*Z*)-13-octadecenol (2,000 ng). Because authentic standards of the alkaloids were not available, we could not correct for the differential sensitivity of the instrument to the alkaloids and the internal standards. However, relative differences in the quantities of alkaloids among treatments should be accurate and reproducible. An additional internal standard of (*Z*)-13-octadecenyl acetate was used as a further reference [for comparison with the (*Z*)-13-octadecenol]. The estimation of hippodamine and convergine between the treatment with derivatization and no derivatization by MBTFA did not differ significantly ($P = 0.86$ by t test); however, the estimated values from the non-derivatization were used for the analyses.

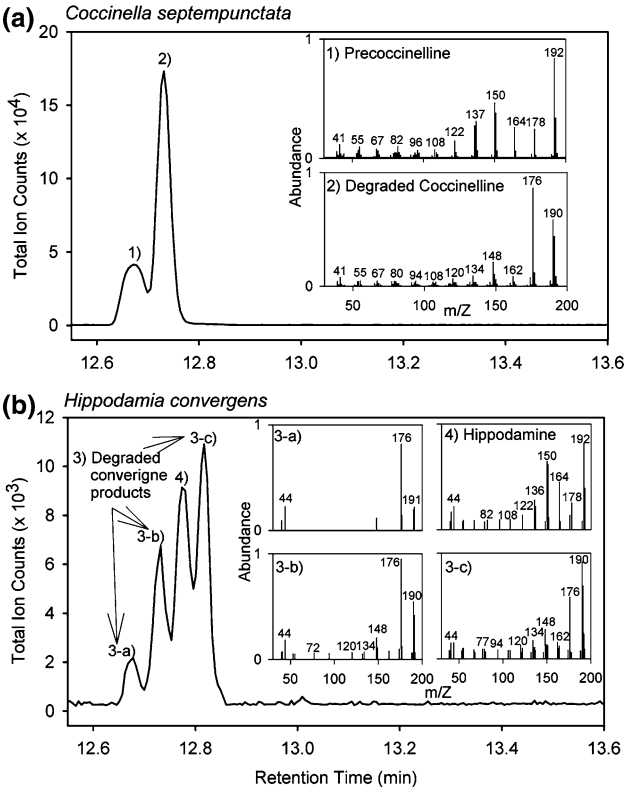

Fig. 1 Total ion chromatogram and mass spectrum of precoccinelline and degraded coccinelline products from *Coccinella septempunctata* (**a**), and hippodamine and degraded convergine products from *Hippodamia convergens* (**b**). Coccinelline and convergine, which are N-oxide alkaloids, were thermally degraded by the injector during gas chromatography-mass spectrometry (GC–MS); however, it is possible to trace their degraded products by searching the ions of m/z 192, 191, 190, 176, 162, 148 (see Sloggett et al. 2009b)

Detection of alkaloids was based on the occurrence of a mass spectrum containing the key ions; for example, harmonine *bis*-trifluoracetyl derivative: m/z 140, 361, 405, 474 (Sloggett et al. 2009b), precoccinelline: m/z 150, 164, 178, 192, degraded coccinelline products: m/z 148, 162, 176, 190 (Fig. 1a), hippodamine: m/z 150, 164, 178, 192, and degraded convergine: m/z 148, 162, 176, 190 (Fig. 1b, also see Sloggett et al. 2009b). Coccinelline and convergine, which are *N*-oxide alkaloids, are thermally degraded by GC–MS; however, their degraded products are detectable by searching for ions as described above at the appropriate retention times. The amount of free-base alkaloids (precoccinelline and hippodamine) and degraded N-oxide alkaloids (coccinelline and convergine) are highly correlated in our data (*C. septempunctata*: $R^2 = 0.834$, $P < 0.0002$, *H. convergens*: $R^2 = 0.830$, $P < 0.0001$). For data analyses, both free-base and N-oxide alkaloids are combined for *C. septempunctata* and *H. convergens*.

Larval performance after feeding on eggs with high or low alkaloid levels

Levels of alkaloid (high vs low) in an individual egg from an egg clutch of *H. axyridis* and *C. septempunctata* were categorized by the results of GC–MS analyses as described above. For each category, data from all females and three time periods were pooled for each species, which allowed us to obtain a sufficient number of eggs for the feeding experiment. The average amounts of alkaloids of four eggs from an egg cluster produced by each female during the three time periods were grouped as high and low levels. Because the repeatability of our measurements of alkaloids in eggs in an egg cluster was high ($R = 0.99$, see Results), we assumed that the remaining eggs in an egg clutch contain similar amounts of alkaloids. Therefore, the eggs in an egg clutch were pooled with eggs from other clutches, categorized as high or low, and used in the feeding experiment. Eggs used for the feeding experiment were selected randomly.

Harmonia axyridis and *C. septempunctata* first instars (<4 h after emergence from an egg) were used as the predators of eggs in this experiment. A larva (<1 h old) was immediately removed from an egg clutch to avoid sibling egg cannibalism (Osawa 1989). Wet weight of each individual (in mg) was measured before the start of the experiment. Each individual was placed in a cell of a 24-well cell culture plate (15.6 mm in diameter). The inside of each cell was coated with Fluon to prevent escape. A piece of filter paper was placed in the bottom of each cell, and one drop of distilled water was added to provide moisture in the cell.

The five treatments designed to measure the effects of variation in alkaloid levels on biological parameters were high alkaloid eggs from con- and hetero-specifics, low alkaloid eggs from con-and hetero-specifics, and a pea aphid control. One egg with high or low level alkaloids from *H. axyridis* or *C. septempunctata* was placed on the filter paper in each cell. As a control, frozen immature pea aphids (2nd–3rd instar) were given ad libitum to each individual larva. Observations were made every 4 h to determine survival and developmental time. An additional egg was added when a larva nearly finished consuming a given egg. Egg consumption was estimated by measuring the proportion of an egg consumed, i.e., 0.25, 0.5, 0.75 and 1. The number of eggs consumed by a larva was recorded every 12-h period, and summed for the total egg consumption. Wet weight was measured when each individual became 2nd instar (within 4 h of ecdysis) to calculate individual weight gain. Each individual was transferred into a 1.5 ml tube and stored in a −20°C freezer when they became a 2nd instar or died. Alkaloids from five individual larvae in each treatment, including the aphid control, were quantified by the methods described above to examine the amount of conspecific and/or heterospecific alkaloids in each individual *H. axyridis* and *C. septempunctata* larva.

Analyses

The repeatability of measurements of alkaloids from four eggs in an egg clutch laid by each female was calculated by Proc VARCOMP (SAS 9.1) [R = Var (4 eggs)/Var (4 eggs) + Var (error)] for each species. The amount of alkaloids in eggs of *H. axyridis*, *C. septempunctata*, and *H. convergens* during the three-oviposition time periods were analyzed by two-way ANOVA (female and time effects) for each species. Correlation and regression analyses were conducted to examine the relationship between egg clutch size or individual egg weight and amount of alkaloids within eggs of each species.

T-tests were conducted to analyze differences between high and low levels of alkaloids in individual eggs and the egg weight of *H. axyridis* and *C. septempunctata*. One-way ANOVA was also conducted to examine the variances of the egg weight (combined eggs of both high and low alkaloid contents) between the two species. Because there were positive relationships between egg weight and alkaloid amount in individual eggs for each species (but no differences in the variation of eggs weight between the two species in our egg samples for the feeding experiment), egg weight was considered when estimating the cumulative amount of alkaloids consumed by a larva (see "Results" and Supplemental Materials). Therefore, cumulative weight of eggs consumed by a larva was calculated by the total number of eggs consumed by a larva × the mean egg weight with high or low level of alkaloids for each species. These data were analyzed by one-way ANOVA.

The amount of conspecific alkaloid within *H. axyridis* and *C. septempunctata* larvae fed *H. axyridis* and *C. septempunctata* eggs with high or low levels of alkaloids, or an aphid diet were analyzed by one-way ANOVA and a Tukey post-hoc test. Relative percentages of alkaloid content in larvae fed eggs compared with the aphid diet were also calculated. The amount of alkaloid consumed by *H. axyridis* and *C. septempunctata* larvae was estimated by the total number of eggs consumed × the mean amount of alkaloids in an egg as described below (see "Results"). Estimated amount of alkaloids consumed by a larva and the observed amount of alkaloids in a larva were compared by paired-*t* test for each treatment.

Larval survival among treatments was analyzed by a Chi-squared test for each species. Effects on first instars (weight gain and developmental time) following consumption of eggs with high or low alkaloid levels and an aphid control were analyzed by one-way ANOVA and a Tukey post-hoc test. The time to death of *C. septempunctata* first instars fed on high or low level *H. axyridis* eggs was

analyzed by a *t* test. All analyses were conducted using SAS 9.1 (SAS Institute 2002).

Results

Variation in egg alkaloid content

The repeatability of alkaloid measurements of four eggs from an egg clutch produced by an individual female was high ($R = 0.99$ for each of the three species), which indicates low variation in the amount of alkaloids in individual eggs within an egg cluster. The amount of alkaloids in an egg from each species varied significantly among females and three oviposition periods (time; $P < 0.01$ for female, time, and the interaction of female and time; see Electronic Supplementary Material, Table A1 and Fig. A1). The ranges in the amount of alkaloids [mean of four eggs (±SE) in an egg clutch, data from all females and three-time periods were pooled] were 62.8(±11.1) to 451.2(±43.9) ng in *H. axyridis* (harmonine *bis*-trifluoracetyl derivative), 770(±33.3) to 1,568.9(±62.8) ng in *C. septempunctata* (precoccinelline + degraded coccinelline products), and 113.8(±13.8) to 390.5(±42.6) ng in *H. convergens* (hippodamine + degraded convergine products). Harmonine *bis*-trifluoracetyl derivative was not detected in *H. convergens* eggs, probably due to its low concentration in this species. There was no correlation between egg clutch size and the amount of alkaloids in eggs of the three species ($P = 0.62$ in *H. axyridis*, $P = 0.95$ in *C. septempunctata*, $P = 0.31$ in *H. convergens*). However, there was a positive linear relationship between egg weight (mg) and the amount of alkaloid (ng) [*H. axyridis*: alkaloid = 3,694.5 (egg weight) − 562.1, $R^2 = 0.25$, $P < 0.0001$, *C. septempunctata*: alkaloid = 12,386.0 (egg weight) − 1,501.8, $R^2 = 0.12$, $P = 0.02$, *H. convergens*: alkaloid = 2,034.6 (egg weight) − 177.6, $R^2 = 0.16$, $P = 0.001$, see Electronic Supplementary Materials, Fig. A2]. The correlations (low value of R^2) explain little variation in alkaloid content, indicating that size is a relatively minor contributor to variation in alkaloid quantity.

Larval performance following feeding on eggs with high or low alkaloid levels

The mean amount (±SE) of alkaloids in eggs selected from high and low categories were 388.8(±22.7) ng and 85.7(±6.2) ng in *H. axyridis* ($t_{12} = 13.04$, $P < 0.0001$), and 1,418.4 (±43.3) ng and 804.8 (±21.5) ng in *C. septempunctata* ($t_{14} = 12.14$, $P < 0.0001$). Weight of individual eggs from the high and low categories of alkaloids differed significantly (*H. axyridis*: "High" 0.235 (±0.005) mg and "Low" 0.201 (±0.004) mg, $t_{15} = 5.25$, $P < 0.0001$, *C. septempunctata*: "High" 0.215 (±0.002) mg and "Low" 0.204 (± 0.002) mg, $t_{15} = 4.04$, $P = 0.0003$). There was no significant difference in egg weight variations between the two species ($F_{1,62} = 3.50$, $P = 0.07$).

The total egg consumption by *H. axyridis* first instars did not differ significantly among treatments ($F_{3,35} = 0.11$, $P = 0.95$, Fig. 2a); however, there was a significant effect on egg weight consumption in *C. septempunctata* first instars among treatments ($F_{3,36} = 24.51$, $P < 0.0001$, Fig. 2b). First instar *C. septempunctata* consumed significantly fewer *H. axyridis* eggs with high levels of alkaloids compared to consumption of *H. axyridis* eggs with low alkaloid levels and conspecific eggs.

Quantified conspecific alkaloids in *H. axyridis* and *C. septempunctata* larvae differed significantly among treatments (one-way ANOVA: *H. axyridis* $F_{4,18} = 58.51$, $P < 0.0001$, *C. septempunctata* $F_{4,18} = 152.53$, $P < 0.0001$, Fig. 3). Both *H. axyridis* and *C. septempunctata* larvae had more of their own alkaloids (*H. axyridis* high: +215%, low: +140%, *C. septempunctata* high: +498%, low: +391%) when they fed on high and low conspecific eggs compared

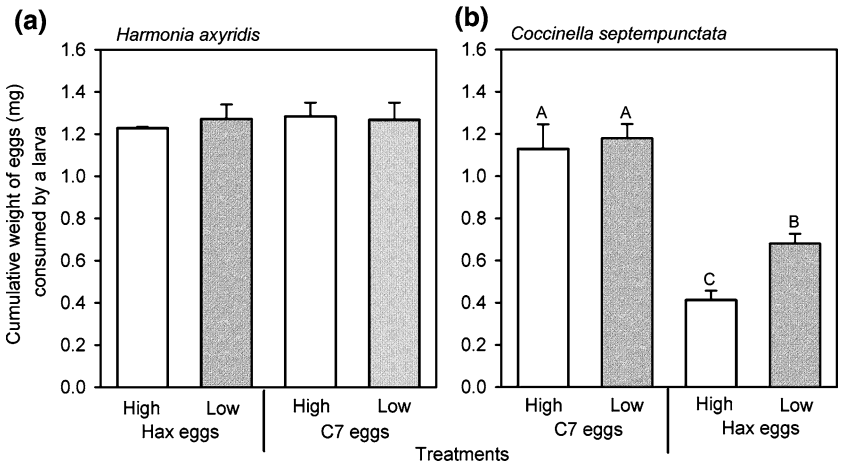

Fig. 2 Total cumulative weight of eggs consumed by **a** *Harmonia axyridis* (Hax) and **b** *Coccinella septempunctata* (C7) first instars, when fed eggs containing high or low levels of alkaloids. Differences in means among treatments as determined by Tukey ($P < 0.05$) are indicated by *different upper case letters*

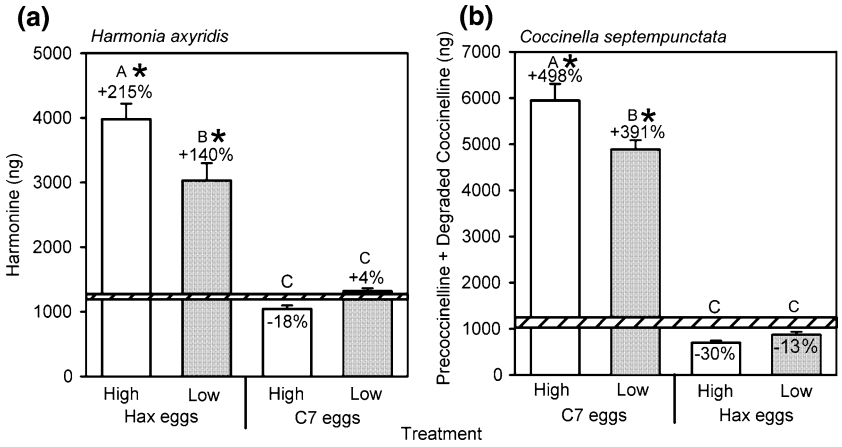

Fig. 3 Comparison of the observed amount of conspecific alkaloids (SE) in larvae of **a** *Harmonia axyridis* (Hax) and **b** *Coccinella septempunctata* (C7) among treatments. *Horizontal bar* Mean with ±SE for aphid diet. The percentage increase or decrease in the amount of conspecific alkaloids, compared with the mean aphid control, is shown for each treatment. Differences in means among treatments as determined by Tukey ($P < 0.05$) are indicated by *different upper case letters*. *Significant difference compared with aphid control

with an aphid control (Fig. 3). The quantities of conspecific alkaloids were significantly greater in larvae fed eggs with high vs low alkaloid content (Tukey: $P < 0.05$, Fig. 3). The amount of alkaloids in these larvae was significantly greater compared with larvae fed aphids and heterospecific eggs (Tukey: $P < 0.05$). For both *H. axyridis* and *C. septempunctata* larvae, however, the amount of their own alkaloid production was similar among larvae fed heterospecific eggs with either high or low alkaloid levels or an aphid control (Fig. 3).

The estimated amount of alkaloids consumed by a larva based on egg consumption was compared with the observed amount of alkaloids in larvae for both species (Fig. 4). There was significantly more conspecific alkaloid in *H. axyridis* larvae than expected based on dietary input alone, which suggests that they also synthesize their own alkaloids in these treatments (Fig. 4a: paired *t* test; high alkaloid level $t_4 = 8.48$, $P = 0.0034$, low alkaloid level $t_4 = 9.82$, $P = 0.0006$). On the other hand, there was a significant decrease compared to estimated intake in the amount of alkaloids in *C. septempunctata* larvae when they consumed conspecific eggs with the high level alkaloids (paired *t*-test: $t_4 = 3.18$, $P = 0.03$, Fig. 4b). Estimated and observed amounts of alkaloids were similar in *C. septempunctata* larvae fed conspecific eggs with low level of alkaloids (paired *t* test: $t_4 = 0.57$, $P = 0.60$, Fig. 4b). When *H. axyridis* and *C. septempunctata* first instars consumed either high or low levels of heterospecific alkaloids, they significantly reduced the amount of alkaloids (paired *t* test: $P < 0.01$ in all cases, Fig. 4c, d). *Harmonia axyridis* first instars lost (e.g., defecated or metabolized) 74.2 and 71.8% when they consumed high and low levels of *C. septempunctata* alkaloids, respectively. *Coccinella septempunctata* first instar lost 95.5 and 91.0% of ingested alkaloids when they consumed high and low levels *H. axyridis* alkaloids, respectively, but nonetheless died.

All *H. axyridis* larvae survived in the treatments of high and low levels of alkaloid in *H. axyridis* eggs, low level of alkaloids in *C. septempunctata* eggs and an aphid control, and 90% survived in the treatment of high level of alkaloids in *C. septempunctata* eggs ($x^2_4 = 4.08$, $P = 0.39$). However, there was a significant difference among treatments in the survival of *C. septempunctata* first instars ($x^2_4 = 46.31$, $P < 0.0001$). All *C. septempunctata* first instars survived when they consumed conspecific eggs and 90% of larvae survived on pea aphids; however, all individuals died when they consumed *H. axyridis* eggs. The average time (±SE) to death of *C. septempunctata* when they consumed high alkaloid level *H. axyridis* eggs was 60.9 (±8.3) h, and 56.8 (±3.1) h at the low alkaloid level.

Weight gain in *H. axyridis* differed among the treatments (one-way ANOVA: $F_{4,44} = 7.00$, $P = 0.0002$, Fig. 5a). Consumption of heterospecific eggs lowered the weight gain of the first instar *H. axyridis*, compared with the aphid control (Tukey: $P < 0.05$). However, there was no significant difference in weight gain between high and low alkaloid levels (Tukey: $P > 0.05$). Weight gain of *C. septempunctata* first instars was similar among the conspecific egg treatments and the aphid control (one-way ANOVA: $F_{2,26} = 0.31$, $P = 0.73$, Fig. 5b).

The developmental time of *H. axyridis* first instars differed significantly among treatments (one-way ANOVA: $F_{4,44} = 6.44$, $P = 0.0004$, Fig. 5c), and was prolonged when larvae consumed a high level of heterospecific alkaloids compared with conspecific egg treatments (Tukey: $P < 0.05$). There was no significant difference in the

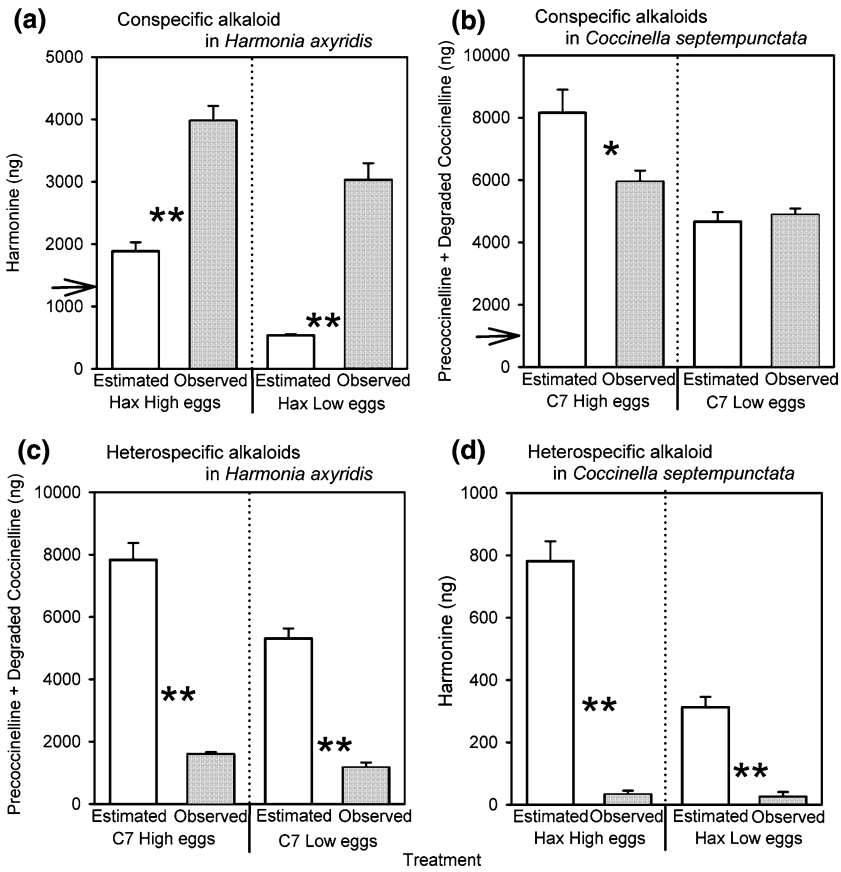

Fig. 4 Comparison between the estimated amount of alkaloids consumed (±SE) from eggs with the observed amount of conspecific (**a**, **b**) and heterospecific (**c**, **d**) alkaloids (±SE) in *Harmonia axyridis* (Hax; **a**, **c**) and *Coccinella septempunctata* (C7; **b**, **d**) larvae, when fed eggs with high or low level of alkaloids. Observed amount of conspecific and heterospecific alkaloids were quantified by GC–MS, from 2nd instar *H. axyridis*. Because all *C. septempunctata* died when fed on *H. axyridis* eggs, heterospecific alkaloid was quantified from dead 1st instar *C. septempunctata*. Conspecific alkaloids were quantified from 2nd instar *C. septempunctata*. *, **Significant difference determined by paired *t* test at $P < 0.05$ and $P < 0.01$, respectively. *Arrow* Mean for aphid control

developmental time of *C. septempunctata* first instars fed on *C. septempunctata* eggs and pea aphids (one-way ANOVA: alkaloid levels $F_{1,18} = 1.48$, $P = 0.24$, Fig. 5d).

Discussion

We documented variation in the amount of alkaloids in eggs of *Harmonia axyridis*, *Coccinella septempunctata*, and *Hippodamia convergens*, and its effect on the feeding behavior, development, survivorship, and con- and heterospecific alkaloid amount in first instar *H. axyridis* and *C. septempunctata*. The repeatability of our measurements of alkaloids in eggs in an egg cluster was high ($R = 0.99$), which suggests that females invest similar amounts of alkaloids when they produce eggs within an individual cluster. The amount of alkaloids in eggs differed significantly among females, and between different oviposition periods. Such intraspecific variations in amount of alkaloids in eggs was also reported in Lepidoptera (Eisner et al. 2000), but this is the first report of such variation in coccinellid eggs. Because of a positive correlation between egg weight and amount of alkaloids, some of this variation may be explained by differences in egg sizes. However, correlations with egg size explain relatively little of the variation in alkaloid content ($R^2 = 0.25$ for *H. axyridis*, 0.12 for *C. septempunctata*, and 0.16 for *H. convergens*). Generally, egg size among ladybird species shows low intraspecific variation (Stewart et al. 1991; Dixon and Guo 1993). Previously, de Jong et al. (1991) and Holloway et al. (1991) reported intraspecific variation in the amount of alkaloids and its concentration in reflex blood in adult ladybirds. In the herbivorous ladybird, *Epilachna paenulata*, male alkaloids are transferred to eggs (Camarano et al. 2009), as observed in other insects (Dussourd et al. 1988; González et al. 1999; Schroeder et al. 2000). Therefore, further studies are needed to explain the variation in the amount of alkaloids in eggs, considering female and male contributions and other factors such as age, and genetic and environmental variations.

We observed interspecific differences in response to ingested conspecific alkaloids by cannibalism. *Harmonia axyridis* in both high and low conspecific alkaloids treatments synthesized additional alkaloid, because the observed amount of conspecific alkaloids was greater than the estimated consumption of conspecific alkaloids within a larva. On the other hand, *C. septempunctata* larvae lost some consumed conspecific alkaloids in the high alkaloid egg treatment. The amounts of estimated and observed conspecific alkaloids were similar in the low alkaloid treatment

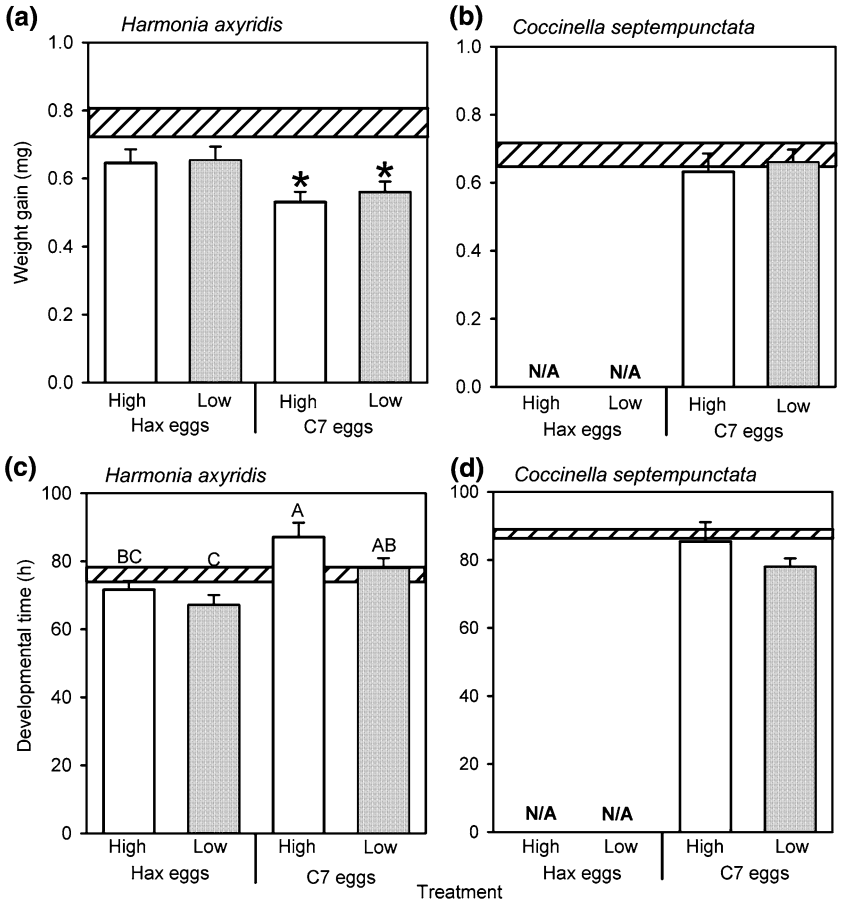

Fig. 5 Weight gain (**a**, **b**; mg ± SE) and developmental time (**c**, **d**; h ± SE) of *Harmonia axyridis* (Hax; **a**, **c**) and *Coccinella septempunctata* (C7; **b**, **d**) from 1st to 2nd instar, when fed pea aphids (control) and eggs with high or low levels of conspecific or heterospecific alkaloids. *Horizontal rectangular bar* Mean ± SE from the aphid control. Differences among treatments as determined by Tukey ($P < 0.05$) are indicated by *different upper case letters*. *Significant difference compared with aphid control. *N/A* No *C. septempunctata* survived to the 2nd instar on *H. axyridis* eggs

in *C. septempunctata* larvae. It is uncertain how much alkaloid *C. septempunctata* larvae retained and synthesized in these treatments; however, such interspecific differences in response to conspecific alkaloids ingested by egg cannibalism is an interesting species-specific characteristic. For example, *H. axyridis* may synthesize their own alkaloid actively, which may be related to their aggressive activity compared with *C. septempunctata* (Yasuda and Shinya 1997; Yasuda and Ohnuma 1999; Kajita et al. 2000; Yasuda et al. 2004). The accumulated alkaloid amount was much greater than in individuals fed aphids. Interestingly, accumulation of ingested conspecific alkaloids did not affect the developmental time or weight gain in either species. Larvae may simply store and use ingested conspecific alkaloids for their future defense.

Consumed heterospecific alkaloids were extensively lost (i.e., defecated or metabolized) by first instars in both species. *Coccinella septempunctata* lost over 95% of ingested *H. axyridis* alkaloid, but nonetheless died within 3 days. *Harmonia axyridis* lost over 74% of ingested *C. septempunctata* alkaloids. All first instar *H. axyridis* survived to the second instar, but consumption of *C. septempunctata* eggs led to a significant reduction in weight gain compared to the aphid control. In addition, feeding on *C. septempunctata* eggs with high levels of alkaloids extended development of *H. axyridis* by about 10% compared to a diet of aphids, or conspecific eggs. There are many reports that consumption of eggs negatively affects survivorship and development of larval stages of predatory coccinellids (Agarwala and Dixon 1992; Sato and Dixon 2004; Cottrell 2004, 2005; Rieder et al. 2008; Sloggett et al. 2009a); however, this is the first report to show a link between heterospecific alkaloids and their toxicity and/or costs by feeding high and low alkaloid eggs to predatory ladybirds.

Based on our studies, *H. axyridis* larvae have a greater ability to process ingested con- and hetero-specific defense chemicals compared with *C. septempunctata*, which may, in part, be linked to the aggressive behavior and wide food habit of *H. axyridis* as an intraguild predator, and interspecific interactions with *C. septempunctata*. For example, *C. septempunctata* leave overwintering sites and lay eggs earlier in the spring than *H. axyridis* when they are in sympatry, and larvae of *C. septempunctata* emigrate to escape predation by *H. axyridis* (Yasuda and Shinya 1997; Sato et al. 2003). The invasive *C. septempunctata* had dominated the coccinellid fauna in orchards in West Virginia in the United States from 1983 to 1996; however, the second invader, *H. axyridis*, replaced *C. septempunctata* in 2 years after *H. axyridis* invaded the habitat (Brown and Miller 1998; Brown 2003).

The ability to tolerate heterospecific alkaloids might vary in later instars and adults, however. Larger individuals of *C. septempunctata* might be able to tolerate higher levels of heterospecific alkaloids. We fed high and low alkaloid eggs to ladybird larvae; however, egg-choice studies would be also important to examine the response of predators to different alkaloid eggs. In addition, other factors, such as species-specific seasonal phenology (Snyder and Hurd 1995) and life history strategies (Schellhorn and Andow 1999) also influence the relative abundance of predators that share prey in the same habitat. Further studies are needed to understand the role of variation in defensive chemicals and its processes to provide a basis for understanding interspecific interactions.

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References

Agarwala BK, Dixon AFG (1992) Laboratory study of cannibalism and interspecific predation in ladybirds. Ecol Entomol 17:303–309

Agarwala BK, Bhattacharya S, Bardhanroy P (1998) Who eats whose eggs? Intra- versus inter-specific interactions in starving ladybird beetles predaceous on aphids. Ethol Ecol Evol 10:361–368

Alam N, Choi IS, Song KS, Hong J, Lee CO, Jung JH (2002) A new alkaloid from two coccinellid beetles *Harmonia axyridis* and *Aiolocaria hexaspilota*. Bull Kor Chem Soc 23:497

Aniszewski T (2007) Alkaloids—secrets of life, alkaloid chemistry, biological significance, applications and ecological role. Elsevier, Amsterdam

Braconnier MF, Braekman JC, Daloze D (1985a) Synthesis of the racemic form of (*Z*)-1, 17-diaminooctadec-9-ene, an aliphatic diamine from Coccinellidae: determination of the absolute configuration of the (+)-naturally-occurring antipode. Bull Soc Chim Belg 94:605–613

Braconnier MF, Braekman JC, Daloze D, Pasteels JM (1985b) (*Z*)-1, 17-diaminooctadec-9-ene, a novel aliphatic diamine from Coccinellidae. Cell Mol Life Sci 41:519–520

Brown MW (2003) Intraguild responses of aphid predators on apple to the invasion of an exotic species, *Harmonia axyridis*. Biocontrol 48:141–153

Brown MW, Miller SS (1998) Coccinellidae (Coleoptera) in apple orchards of eastern West Virginia and the impact of invasion by *Harmonia axyridis*. Entomol News 109:143–151

Camarano S, González A, Rossini C (2009) Biparental endowment of endogenous defensive alkaloids in *Epilachna paenulata*. J Chem Ecol 35:1–7

Cottrell TE (2004) Suitability of exotic and native lady beetle eggs (Coleoptera: Coccinellidae) for development of lady beetle larvae. Biol Control 31:362–371

Cottrell TE (2005) Predation and cannibalism of lady beetle eggs by adult lady beetles. Biol Control 34:159–164

Cottrell TE (2007) Predation by adult and larval lady beetles (Coleoptera: Coccinellidae) on initial contact with lady beetle eggs. Environ Entomol 36:390–401

Daloze D, Braekman J-C, Pasteels JM (1995) Ladybird defense alkaloids: structural, chemotaxonomic and biosynthetic aspects (Col.: Coccinellidae). Chemoecology 5(6):173–183

de Jong PW, Holloway GJ, Brakefield PM, de Vos H (1991) Chemical defense in ladybird beetles (Coccinellidae). II: amount of reflex fluid, the alkaloid adaline and individual variation in defence in 2-spot ladybirds (*Adalia bipunctata*). Chemoecology 2:15–19

Dixon AFG, Guo Y (1993) Egg and cluster size in ladybird beetles (Coleoptera: Coccinellidae): the direct and indirect effects of aphid abundance. Eur J Entomol 90:457–463

Dussourd DE, Ubik K, Harvis CA, Resch J, Meinwald J (1988) Biparental defensive endowment of eggs with acquired plant alkaloid in the moth *Utetheisa ornatrix*. Proc Natl Acad Sci USA 85:5992–5996

Eisner T, Eisner M (1991) Unpalatability of the pyrrolizidine alkaloid-containing moth *Utetheisa ornatrix*, and its larva, to wolf spiders. Psyche 98:111–118

Eisner T, Eisner M, Rossini C, Iyengar VK (2000) Chemical defense against predation in an insect egg. Proc Natl Acad Sci USA 97:1634–1639

Fox LR (1975) Cannibalism in natural populations. Annu Rev Ecol Syst 6:87–106

González A, Rossini C, Eisner M, Eisner T (1999) Sexually transmitted chemical defense in a moth (*Utetheisa ornatrix*). Proc Natl Acad Sci USA 96:5570–5574

Hare JF, Eisner T (1993) Pyrrolizidine alkaloid deters ant predators of *Utetheisa ornatrix* eggs: effects of alkaloid concentration, oxidation state, and prior exposure of ants to alkaloid-laden prey. Oecologia 96:9–18

Hodek I (1973) Biology of Coccinellidae. Academia, Prague

Holloway G, de Jong P, Brakefield P, de Vos H (1991) Chemical defence in ladybird beetles (Coccinellidae). I: distribution of coccinelline and individual variation in defence in 7-spot ladybirds (*Coccinella septempunctata*). Chemoecology 2:7–14

Kajita Y, Takano F, Yasuda H, Agarwala BK (2000) Effects of indigenous ladybird species (Coleoptera: Coccinellidae) on the survival of an exotic species in relation to prey abundance. Appl Entomol Zool 4:437–479

King AG, Meinwald J (1996) Review of the defensive chemistry of coccinellids. Chem Rev 96:1105–1122

Koide T (1962) Observations on the feeding habit of the larva of *Coccinella septempunctata* bruckii Mulsant: the feeding behaviour and number of prey fed under different temperatures. Kontyû 30:236–241

Laurent P, Braekman JC, Daloze D, Pasteels JM (2002) In vitro production of adaline and coccinelline, two defensive alkaloids from ladybird beetles (Coleoptera: Coccinellidae). Insect Biochem Mol Biol 32:1017–1023

Osawa N (1989) Sibling and non-sibling cannibalism by larvae of a lady beetle *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae) in the field. Res Popul Ecol 31:153–160

Osawa N (1992a) A life table of the ladybird beetle *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae) in relation to the aphid abundance. Jpn J Entomol 60:575–579

Osawa N (1992b) Sibling cannibalism in the ladybird beetle *Harmonia axyridis*: fitness consequences for mother and offspring. Res Popul Ecol 34:45–55

Pasteels JM (2007) Chemical defense, offence and alliance in ants-aphids-ladybirds relationships. Popul Ecol 49:5–14

Polis GA (1981) The evolution and dynamics of intraspecific predation. Annu Rev Ecol Syst 12:225–251

Rieder JP, Newbold TAS, Sato S, Yasuda H, Evans EW (2008) Intraguild predation and variation in egg defence between sympatric

and allopatric populations of two species of ladybird beetles. Ecol Entomol 33:53–58
Rosenheim JA (1998) Higher-order predators and the regulation of insect herbivore populations. Annu Rev Entomol 43:421–447
Rosenheim JA, Kaya HK, Ehler LE, Marois JJ, Jaffee BA (1995) Intraguild predation among biological-control agents: theory and evidence. Biol Control 5:303–335
Rosenthal GA, Berenbaum M (1992) Herbivores: their interactions with secondary plant metabolites. Academic, San Diego
SAS 9.1 (2002) SAS Institute, Cary
Sato S, Dixon AFG (2004) Effect of intraguild predation on the survival and development of three species of aphidophagous ladybirds. Agric For Entomol 6:21–24
Sato S, Dixon AFG, Yasuda H (2003) Effect of emigration on cannibalism and intraguild predation in aphidophagous ladybirds. Ecol Entomol 28:628–633
Sato S, Jimbo R, Yasuda H, Dixon AFG (2008) Cost of being an intraguild predator in predatory ladybirds. Appl Entomol Zool 43:143–147
Schellhorn NA, Andow DA (1999) Cannibalism and interspecific predation: role of oviposition behavior. Ecol Appl 9:418–428
Schroeder FC, González A, Meinwald J, Eisner T (2000) Chemical defense: bestowal of a nuptial alkaloidal garment by a male moth on its mate. Proc Natl Acad Sci USA 97:14406–14411
Sloggett JJ, Haynes KF, Obrycki JJ (2009a) Hidden costs to an invasive intraguild predator from chemically defended native prey. Oikos 118:1396–1404
Sloggett JJ, Obrycki JJ, Haynes KF (2009b) Identification and quantification of predation: novel use of gas chromatography-mass spectrometric analysis of prey alkaloid markers. Funct Ecol 23:416–426
Snyder WE, Hurd LE (1995) Egg-hatch phenology and intraguild predation between two mantid species. Oecologia 104:496–500
Stewart LA, Dixon AFG, Ruzicka Z, Iperti G (1991) Clutch and egg size in ladybird beetles. Entomophaga 36:329–333
Takahashi K (1989) Intra- and inter-specific predations of lady beetles in spring alfalfa fields. Jpn J Appl Entomol Zool 57:199–203
Tursch B, Daloze D, Dupont M, Pasteels JM, Tricot M-C (1971a) A defensive alkaloid in a carnivorous beetle. Experientia 27:1380–1381
Tursch B, Daloze D, Dupont M, Hootele C, Kaisin M, Pasteels JM, Zimmermann D (1971b) Coccinelline, the defensive alkaloids of the beetle *Coccinella septempunctata*. Chimia 25:307–308
Tursch B, Daloze D, Braekman JC, Hootele C, Cravador A, Losman D, Karlsson R (1974) Chemical ecology of arthropods. IX: structure and absolute configuration of hippodamine and convergine, two novel alkaloids from the American ladybug *Hippodamia convergens* (Coleoptera: Coccinellidae). Tetrahedron Lett 5:409–412
Yasuda H, Ohnuma N (1999) Effect of cannibalism and predation on the larval performance of two ladybird beetles. Entomol Exp Appl 93:63–67
Yasuda H, Shinya K (1997) Cannibalism and interspecific predation in two predatory ladybirds in relation to prey abundance in the field. Entomophaga 42:153–163
Yasuda H, Evans EW, Kajita Y, Urakawa K (2004) Asymmetric larval interactions between introduced and indigenous ladybirds in North America. Oecologia 141:722–731