

# Susceptibility of immature monarch butterfly, *Danaus plexippus* (Lepidoptera: Nymphalidae: Danainae), to predation by *Harmonia axyridis* (Coleoptera: Coccinellidae)

R.L. Koch,\* W.D. Hutchison, R.C. Venette, and G.E. Heimpel

Department of Entomology, 219 Hodson Hall, 1980 Folwell Avenue, University of Minnesota, St. Paul, MN 55108, USA

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## Abstract

The potential for a classical biological control agent, *Harmonia axyridis* (Pallas), to have non-target effects on populations of the monarch butterfly, *Danaus plexippus* (L.), was examined using no-choice predation studies under laboratory and caged field conditions. Three separate laboratory functional response experiments were conducted with adult and larval *H. axyridis* preying on immature *D. plexippus*. Nonlinear functional responses for third instar *H. axyridis* preying on *D. plexippus* eggs and first instars reached plateaus at about 25 and 15 prey consumed per day, respectively. A linear response resulted for adult *H. axyridis* preying on *D. plexippus* eggs. Potted *Asclepias syriaca* L. placed into large field-cages were infested with first instar *D. plexippus* and third instar *H. axyridis*. Survival of first instar *D. plexippus* was significantly lower in cages with *H. axyridis* larvae than in cages without *H. axyridis* larvae. The present experiments identify *H. axyridis* as a potential stressor to populations of *D. plexippus*. Further research is needed to assess the likelihood of *D. plexippus* exposure to *H. axyridis* in the field and to quantify the likelihood of *H. axyridis* having an adverse effect on *D. plexippus* in the presence of other prey, particularly aphids. These data could be used to develop an ecological risk assessment.

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## 1. Introduction

The multicolored Asian lady beetle, *Harmonia axyridis* (Pallas), has spread rapidly across much of the US (Chapin and Brou, 1991; Colunga-Garcia and Gage, 1998; Dreistadt et al., 1995; Hesler et al., 2001; Smith et al., 1996; Tedders and Schaefer, 1994). Intentional releases of *H. axyridis* for biological control in the US date back to 1916 (Gordon, 1985), but some populations may stem from accidental seaport introductions (Day et al., 1994). *H. axyridis* is primarily aphidophagous, but also feeds on pollen (Hukusima and Itoh, 1976) and other small soft-bodied insects, such as Psyllidae, various Coccoidea, and immature stages of Chrysomelidae and Lepidoptera (reviewed by Tedders and Schaefer, 1994). *H. axyridis* has proven to be an effective biolog-

ical control agent of rose aphid, *Macrosiphum rosae* (L.) (Ferran et al., 1996), the pecan aphid complex, including *Monellia caryella* (Fitch), *Moneliopsis pecanis* Bissel, and *Melanocallis caryaefoliae* (Davis) (LaRock and Elington, 1996; Tedders and Schaefer, 1994), and other homopteran pests, such as red pine scale, *Matsucoccus matsumurae* (Kuwana) (McClure, 1986).

Common milkweed, *Asclepias syriaca* L., a host plant of the monarch butterfly, *Danaus plexippus* (L.), is a ubiquitous weed in agricultural systems (Hartzler and Buhler, 2000). The majority of the *D. plexippus* population in the central US reproduces on *A. syriaca* (Wassenaar and Hobson, 1998). In Minnesota and Wisconsin, Oberhauser et al. (2001) estimated that up to 73% more *D. plexippus* are produced in agricultural habitats than in non-agricultural habitats. *H. axyridis* has become a dominant generalist predator in many of these same agricultural systems across the central US (e.g., Colunga-Garcia and Gage, 1998; Cottrell and

\* Corresponding author. Fax: 1-612-625-5299.

E-mail address: [koch0125@tc.umn.edu](mailto:koch0125@tc.umn.edu) (R.L. Koch).

Yeargan, 1998; Wold et al., 2001). The co-occurrence of these two species in agricultural habitats makes it theoretically possible for *H. axyridis* to encounter immature stages of *D. plexippus*, which may be susceptible to predation by coccinellids (e.g., Zalucki and Kitching, 1982). We conducted both laboratory and field-cage predation experiments to evaluate the susceptibility of immature *D. plexippus* to predation by the exotic predator *H. axyridis*.

## 2. Materials and methods

### 2.1. Insects

*Harmonia axyridis* used in these experiments were reared from eggs obtained from a greenhouse colony maintained by the Minnesota Department of Agriculture. Batches of *H. axyridis* eggs were placed into 6 × 1.5 cm petri dishes and held in a Percival growth chamber at 22 °C and 70% relative humidity with a 16:8 (L:D) h cycle. At 24 h after eclosion, first instar *H. axyridis* were transferred to separate 6 × 1.5 cm petri dishes. Larvae were reared to the desired predator stage (i.e., third instar or adult) at the aforementioned environmental conditions. *H. axyridis* were reared on an ad libitum supply of pulverized, freeze-dried drone honeybees, *Apis mellifera* L. (Okada and Matsuka, 1973). *A. mellifera* were obtained from colonies maintained by the University of Minnesota. Water was supplied by a 0.5 ml micro-centrifuge tube filled with water and plugged with a small piece of cotton.

*Danaus plexippus* eggs were obtained from laboratory colonies maintained at the University of Minnesota and the University of Kansas. Greenhouse grown *Asclepias curassavica* L. was used as the host plant for oviposition and rearing of *D. plexippus*. *D. plexippus* eggs and larvae were reared at 25 °C and 70% relative humidity with a 16:8 (L:D) h cycle.

### 2.2. Laboratory experiments

Three separate laboratory functional response experiments were conducted: third instar *H. axyridis* feeding on 24–48 h old *D. plexippus* eggs, third instar *H. axyridis* feeding on 12–24 h old first instar *D. plexippus*, and adult female *H. axyridis* feeding on *D. plexippus* 36–48 h old eggs. In the experiments with *D. plexippus* eggs as prey, the adhesive attaching *D. plexippus* eggs to *A. curassavica* leaves was wetted using a small camel-hair brush dipped in distilled water. The brush was used to lift eggs from leaves, and transfer eggs to 9 cm, white filter paper disks. When the moistened adhesive on the base of the eggs was set on the filter paper and allowed to dry, the eggs became firmly attached to the filter paper. Filter paper disks were set-up

with densities of 1, 5, 10, 20, 30, 40, and 50 *D. plexippus* eggs per disk or with 1, 5, 10, 20, 30, and 50 eggs per disk for experiments with *H. axyridis* larvae and adult *H. axyridis*, respectively. The filter paper disks containing eggs were then placed into 10 × 1.5 cm petri dishes.

In the experiment with *D. plexippus* larvae used as prey, first instar *D. plexippus* were transferred, using a small camel-hair brush, from *A. curassavica* to moistened filter paper disks in 10 × 1.5 cm petri dishes. Before being transferred to the dishes, all larvae were allowed to feed on *A. curassavica* for 12–24 h after eclosion. Dishes were set up with densities of 1, 5, 10, 20, 30, 40, and 50 first instar *D. plexippus* per dish.

All *H. axyridis* used in these experiments had been starved for 24 h. Larval predators were 24 h-old third instars, and adult predators were 2-week-old unmated females. A single predator was placed into each petri dish, containing a filter paper disk with one of the various densities of *D. plexippus* eggs or first instars. The petri dishes were then held at 22 °C and 70% relative humidity with a 16:8 (L:D) h cycle. After 24 h, the number of *D. plexippus* killed was recorded. For *D. plexippus* eggs, if an egg was missing or if the chorion was damaged, the egg was counted as killed by *H. axyridis*. For *D. plexippus* larvae, if a larva was missing or if a larva was dead with apparent traumatic injuries, the larva was counted as killed by *H. axyridis*. Predators were monitored for molting for 24 h after completion of each trial to ensure that a pre-molt period had not altered our results.

These experiments were blocked over time, with uneven replication of prey densities per block, due to limited prey availability. In the experiment with third instar *H. axyridis* preying on *D. plexippus* eggs, prey densities of 1, 5, 10, 20, 30, 40, and 50 eggs per dish were replicated 5, 5, 6, 5, 6, 3, and 5 times, respectively. Initial prey densities of 20, 40, and 50 eggs per dish had 1, 3, and 1 observations, respectively, with a predator that did not feed. The observations with third instar *H. axyridis* that did not feed were omitted, leaving the aforementioned replication. In the experiment with third instar *H. axyridis* preying on first instar *D. plexippus*, prey densities of 1, 5, 10, 20, 30, 40, and 50 eggs per dish were replicated 5, 5, 5, 5, 5, 5, and 4 times, respectively. In the experiment with adult *H. axyridis* preying on *D. plexippus* eggs, prey densities of 1, 5, 10, 20, 30, and 50 eggs per dish were replicated 6, 7, 7, 7, 7, and 6 times, respectively.

These data were used to fit functional response models of *H. axyridis* predation on various densities of immature *D. plexippus*. Using non-linear least squares regression (proc NLIN, SAS, 1995), Rogers' Type II random predator model was fit to describe the functional response for the experiments (Rogers, 1972). The form of Rogers' Type II random predator model can be expressed as follows:

$$N_c = N_0 \{1 - \exp[-a(T - T_h N_c)]\}, \quad (1)$$

where  $N_0$  represents initial prey density,  $N_c$  represents the number of prey consumed,  $T$  represents total time, and  $a$  and  $T_h$  are the attack coefficient and handling time (in days), respectively. Unlike Holling's disk equation (Holling, 1959), Roger's random predator model was appropriate for these data, because prey were not replaced after being killed or consumed.

### 2.3. Field experiment

The field predation experiment was conducted at the Rosemount Research and Outreach Center, University of Minnesota, Rosemount, MN. On 5 May 2001, *A. syriaca* seed from a wild population at Rosemount was planted into an open field, not treated with herbicide. When the plants reached a height of 10–15 cm they were transplanted into 11.4-liter pots, with one plant in each pot. In a fallow field, cages ( $1.82 \times 1.82 \times 2.74$  m,  $32 \times 32$  cm Lumite screen, BioQuip, Gardena, CA) were set-up in a randomized complete block design, for three replications of three treatments. Two trials of this experiment were conducted, beginning 25 August and 17 September 2001. Four potted *A. syriaca* were placed into each cage. At initiation of the experiment, plants exhibited normal latex production and were 15–25 cm tall. Before the pots were placed into the cages, each plant and pot was thoroughly inspected, and all arthropods were removed.

A small camel-hair brush was used to place two 12–24 h old first instar *D. plexippus* onto the second pair of leaves from the top of each plant in each cage. The cages were then assigned to one of three third instar *H. axyridis* densities. The treatments were: zero *H. axyridis* per cage, four *H. axyridis* per cage (one per plant), or 16 *H. axyridis* per cage (four per plant). The aforementioned densities of *H. axyridis* lie within the range of observed *H. axyridis* densities on *A. syriaca* growing in and near corn fields (RLK unpublished data). *H. axyridis* were placed onto the fourth pair of leaves from the top of the plants. At 1, 3, and 7 days, the number of *D. plexippus* remaining on the plants was recorded. Analysis of variance by date and blocked on trial, along with the Ryan–Einot–Gabriel–Welsch multiple range test were used to test for differences among treatments in the number of *D. plexippus* remaining (SAS, 1995).

## 3. Results

### 3.1. Laboratory experiments

Under laboratory conditions, third instar *H. axyridis* consumed *D. plexippus* eggs and first instars, and adult *H. axyridis* consumed *D. plexippus* eggs (Figs. 1A and B).

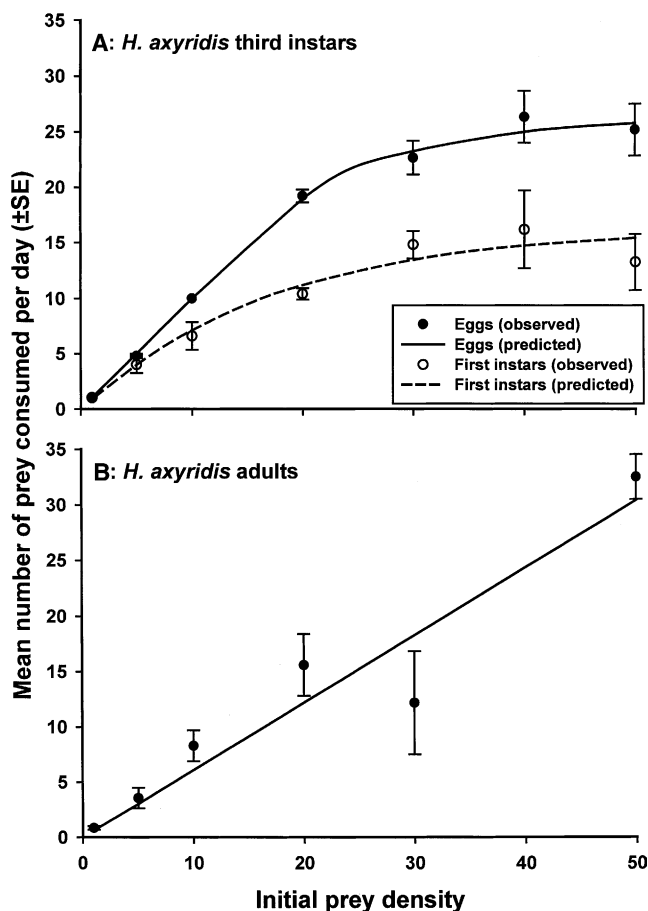


Fig. 1. Functional response of individual *H. axyridis* on various densities of *D. plexippus* for 24 h under laboratory conditions. (A) Third instar *H. axyridis* preying on *D. plexippus* eggs and first instars. (B) Adult *H. axyridis* preying on *D. plexippus* eggs. Predicted lines come from Rogers' Type II random predator model (see text).

Both larvae and adults of *H. axyridis* consumed nearly all of the prey at low prey densities (Figs. 1A and B). The functional response of third instar *H. axyridis* preying on *D. plexippus* eggs reached an asymptote of about 25 eggs per day, with an attack coefficient of 9.05 ( $\pm 4.626$ ) and a handling time of 0.04 ( $\pm 0.003$ ) days (Fig. 1A; Table 1). The functional response of third

Table 1  
Regression output from Rogers' Type II random predator model for three functional response experiments

$N$	$r^2$	$a$ ( $\pm$ SE)	$T_h$ ( $\pm$ SE) (days)
Third instar <i>H. axyridis</i> preying on <i>D. plexippus</i> eggs			
35	0.98	9.05 ( $\pm 4.626$ )	0.04 ( $\pm 0.003$ )
Third instar <i>H. axyridis</i> preying on first instar <i>D. plexippus</i>			
34	0.89	2.01 ( $\pm 0.950$ )	0.05 ( $\pm 0.010$ )
Adult <i>H. axyridis</i> preying on <i>D. plexippus</i> eggs			
40	0.83	0.94 ( $\pm 0.374$ )	N/A <sup>a</sup>

$a$ , attack coefficient;  $T_h$ , handling time.

<sup>a</sup> Parameter estimate was not significantly different from 0.

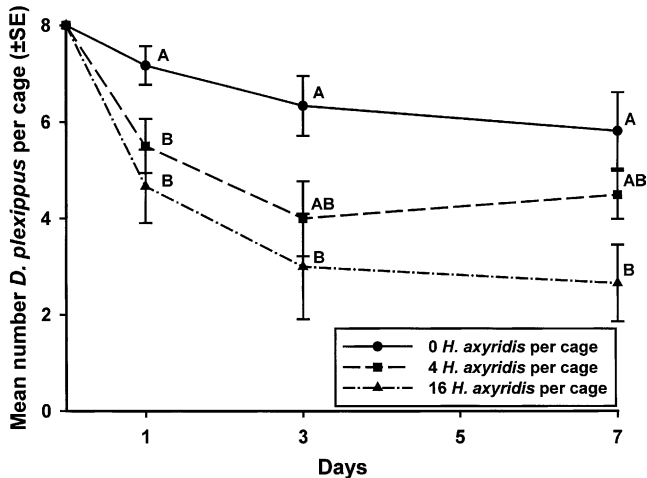


Fig. 2. Survival of first instar *D. plexippus* on *A. syriaca* (four potted plants per cage) under caged field conditions with different densities of third instar *H. axyridis* per cage. Initial *D. plexippus* densities were eight larvae per cage (two larvae per plant).

instar *H. axyridis* preying upon first instar *D. plexippus* reached an asymptote of about 15 larvae per day, with an attack coefficient of  $2.01 (\pm 0.950)$  and a handling time of  $0.05 (\pm 0.010)$  days (Fig. 1A; Table 1). An asymptote was not seen for the functional response of adult *H. axyridis* preying on *D. plexippus* eggs. The response increased linearly across prey densities, with an attack coefficient of  $0.94 (\pm 0.374)$ . A meaningful estimate of handling time was not available from this analysis (i.e.,  $T_h$  was not significantly different from 0) (Fig. 1B; Table 1).

### 3.2. Field experiment

Third instar *H. axyridis* caused significant reductions of *D. plexippus* densities under field conditions (Fig. 2). Analysis of variance indicated statistically significant differences in densities of first instar *D. plexippus* among treatments (i.e., a function of *H. axyridis* density) for 1, 3, and 7 days post infestation (Table 2). At 1 day post

Table 2  
ANOVA table by date, blocked on trial for field experiment with three densities of *H. axyridis* as treatments (0, 4, and 16 *H. axyridis* per cage)

Source	DF	SS	F	P
<i>1 day post infestation</i>				
Trial	1	10.88	7.34	0.0170
<i>H. axyridis</i> density	2	19.44	6.55	0.0098
<i>3 day post infestation</i>				
Trial	1	10.88	2.80	0.1165
<i>H. axyridis</i> density	2	35.11	4.51	0.0307
<i>7 day post infestation</i>				
Trial	1	14.22	6.33	0.0247
<i>H. axyridis</i> density	2	30.33	6.75	0.0089

infestation, *D. plexippus* densities were significantly lower in cages with 1 and 4 *H. axyridis* per plant compared to cages with 0 *H. axyridis* (Fig. 2). At 3 and 7 days post infestation, *D. plexippus* densities were significantly lower in cages with 4 *H. axyridis* per plant compared to cages with 0 *H. axyridis* (Fig. 2).

## 4. Discussion

*Harmonia axyridis*, like most other aphidophagous coccinellids, is not a specialist predator (Hodek and Honěk, 1996). The lack of dietary specificity allows for potential non-target effects. For example, *H. axyridis* co-occurs in corn fields with *Coleomegilla maculata* (De Geer), and has been observed preying on *C. maculata* in corn and in laboratory studies (Cottrell and Yeargan, 1998). Boettner et al. (2000) suggest that *H. axyridis* may be impacting native, non-pest aphid species. Exotic coccinellids may also impact non-target Lepidoptera. *Coccinella septempunctata* L. has been found to prey on endangered Lycaenidae (Horn, 1991). *H. axyridis* is also capable of preying on Lepidoptera, including *Hyphantria cunea* (Drury) (Kim et al., 1968; Shu and Yu, 1985) and European corn borer, *Ostrinia nubilalis* (Hübner) (Hoogendoorn and Heimpel, 2003). Lepidopteran eggs, including *Sitotroga cerealella* (Olivier) (Abdel-Salam and Abdel-Baky, 2001) and *Ephesia kuehniella* Zeller (Ferran et al., 1997), have also been used as diets for rearing *H. axyridis*. The present study confirms that *H. axyridis* is capable of feeding on *D. plexippus*, an aesthetically pleasing, non-pest species under laboratory and field-cage conditions.

The functional response of *H. axyridis* to various aphids has been characterized as Type I (Lou, 1987), Type II (He et al., 1994), and Type III (Hu et al., 1989). The present studies are the first to report the functional response of *H. axyridis* to non-aphid prey. *D. plexippus* eggs appear to be more susceptible than first instars to predation by larval *H. axyridis*. Third instar *H. axyridis* consumed about 10 more *D. plexippus* eggs than first instars in 24 h. The linearity of the functional response of adult *H. axyridis* on *D. plexippus* eggs was likely due to the predators not being exposed to high enough prey densities to induce satiation. There is a peculiar dip in observed predation by adult *H. axyridis* on *D. plexippus* eggs at the initial prey density of 30 eggs per dish. Heimpel and Hough-Goldstein (1994a) found a similar response for the stink bug *Perillus bioculatus* (F.) preying on neonate *Leptinotarsus decimlineata* (Say), and attributed the response to successful area-restricted search following subsatiation feeding at high prey densities (Heimpel and Hough-Goldstein, 1994b), but not at low densities.

Our caged field study indicated that, in the absence of other prey, *H. axyridis* could significantly reduce den-

sities of *D. plexippus* larvae in a more complex environment. Thus, the phenomenon of *H. axyridis* preying on *D. plexippus* is not restricted to laboratory microcosm studies. A quadrupling of the per plant predator density (i.e., from 1 to 4 *H. axyridis* per plant) only resulted in a 58% further reduction in the number of *D. plexippus* remaining. The number of predators per plant may have been reduced during the experiment by intraspecific competition. *H. axyridis* larvae are known cannibals (Snyder et al., 2000). The presence of multiple *H. axyridis* larvae on each plant may have also increased the departure rate of predators from the plants. Samu et al. (1996) showed that the probability of the spider *Lepthyphantes tenius* (Blackwall) leaving a web site increased with increasing conspecific spider density. The resulting lower density of effective predators may have caused predation rates to be lower than expected in the cages.

The present studies identify a possible stressor upon which an ecological risk assessment for *D. plexippus* can be built. In the context of an ecological risk assessment, risk can be defined as the joint probability of *D. plexippus* being exposed to *H. axyridis* and resultant mortality occurring (Environmental Protection Agency, 1998). Future work is needed to more fully quantify the likelihood of *H. axyridis* having an adverse effect on *D. plexippus*, especially in the presence of other prey, particularly aphids. The likelihood of *D. plexippus* exposure to *H. axyridis* must also be determined. Moreover, in a full risk analysis (e.g., Lonsdale et al., 2001), where the total benefits of *H. axyridis* would be weighed against the total negative impacts, we would also consider effects of *H. axyridis* on other “non-pest” insects, crop processing/marketability, and human nuisances (Ratcliffe, 2002). Potential benefits could include reduced pest levels and pesticide use. The outcome of such a risk analysis would help to determine whether control of this biological control agent/potential pest is warranted.

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