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Predicted impact of an exotic generalist predator on monarch butterfly (Lepidoptera: Nymphalidae) populations: A quantitative risk assessment

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Abstract

The multicolored Asian lady beetle, *Harmonia axyridis* (Pallas), was recently identified as a potential hazard to monarch butterflies, *Danaus plexippus* (L.). A quantitative risk assessment for the potential impact of *H. axyridis* on *D. plexippus* in Minnesota corn and soybean fields was developed using risk analysis software. This assessment considered a potential worst-case scenario for the impact of *H. axyridis* on *D. plexippus*. Habitat-specific recruitment of *D. plexippus* eggs was determined empirically. Subsequently, simulated abundance of *D. plexippus* in each habitat was reduced by two classes of stage-specific mortality: predation by *H. axyridis* and causes other than *H. axyridis*. Predation was modeled as a function of *D. plexippus* exposure to *H. axyridis*, and predation rate of *D. plexippus* by *H. axyridis*. Exposure and subsequent risk varied considerably by habitat, with a low risk of *H. axyridis* adversely affecting *D. plexippus* populations developing in corn fields, but a moderate to high risk in soybean fields. Predicted rates of *D. plexippus* mortality attributable to *H. axyridis* were greater in soybean compared to corn fields, possibly due to the numerical response of *H. axyridis* to soybean aphid, *Aphis glycines* Matsumura. This study demonstrates that *H. axyridis* has the potential to have a strong adverse impact on *D. plexippus* populations. However, the likelihood of occurrence for this worst-case scenario remains uncertain. To evaluate the landscape-level risk of *H. axyridis* impacting *D. plexippus*, further data on recruitment of *D. plexippus* in other habitats and exposure estimates specific to additional habitats are needed.

Introduction

Classical biological control is the use of exotic natural enemies to control exotic pests (Van Driesche and Bellows 1996). The history of classical biological control contains many success stories (e.g., Caltagiron and Doutt 1989; Debach and Rosen 1991; Radcliffe and Flanders 1998), and this tactic continues to be an important component of management programs for emerging pests (e.g., Heimpel et al. 2004). However, classical biological control may not be as environmentally benign as was once thought (Howarth 1991, 2000; Simberloff and Stiling 1996; Follet and Duan 1999; Louda et al. 2003; Stiling 2004). Many of the natural enemies released in previous biological control programs had generalist feeding behaviors (Stiling 2004). Generalist species have been favored by some biological control practitioners on the tenet that these natural enemies could become established prior to the targeted prey, and that the natural enemies could persist on alternate prey when target prey populations are low (De Clercq 2002).

However, a generalist feeding behavior makes a biological control agent candidate prone to having impacts on non-target species (e.g., Boettner et al. 2000). Because of concerns over the non-target impacts of generalist natural enemies, the tendency of biological control practitioners to release these species is decreasing, and more practitioners are pursuing highly specific natural enemies (Strong and Pemberton 2000; Heimpel et al. 2004).

Despite these recent improvements, some previously released exotic organisms have become permanent fixtures in the environment. A recent example is the multicolored Asian lady beetle, *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae), which was introduced repeatedly to North America from Asia as a classical biological control agent of aphids (Gordon 1985). However, Day et al. (1994) argue that current populations of *H. axyridis* in North America may have stemmed from unintentional seaport introductions. A gene flow analysis conducted by Krafsur et al. (1997) was unable to determine the source (i.e., intentional vs unintentional) of current *H. axyridis* populations. Regardless of its mode of entry, *H. axyridis* became an abundant member of the generalist predatory guild in many agricultural systems (e.g., Brown and Miller 1998; Colunga-Garcia and Gage 1998; Michaud 2002b; Musser et al. 2004). This coccinellid may be contributing to the biological control of insect pests in crops such as pecans (Tedders and Schaefer 1994), citrus (Michaud 2002a), apples (Brown and Miller 1998), sweet corn (Musser and Shelton 2003), and soybean (Fox et al. 2004; Rutledge et al. 2004). However, *H. axyridis* is probably having adverse impacts on native, non-pest species (Koch 2003). Long-term monitoring projects have shown declines in populations of existing coccinellids after the establishment of *H. axyridis* (Brown and Miller 1998; Colunga-Garcia and Gage 1998; Michaud 2002b; Alyokhin and Sewell 2004). Aside from the guild of generalist predators, little work has been done to assess the impacts of *H. axyridis* on other non-target insects. However, Koch et al. (2003a) recently identified *H. axyridis* as a potential hazard to immature monarch butterflies, *Danaus plexippus* (L.) (Lepidoptera: Nymphalidae).

Danaus plexippus is an aesthetically-pleasing, non-pest insect, with value as a model system for research on migration and utilization of plant-derived toxins (Oberhauser and Solensky 2004). Approximately 50% of the population of *D. plexippus* overwintering in Mexico develops in the Midwestern U.S. (Wassenaar and Hobson 1998). Throughout this region, common milkweed, *Asclepias syriaca* L., is the primary host plant of *D. plexippus* larvae (Malcolm and Zalucki 1993). Recent research examining the impact of transgenic insecticidal corn on *D. plexippus* suggested that a large portion of the summer populations of *D. plexippus* develop on *A. syriaca* growing in agricultural systems, such as corn and soybeans (Oberhauser et al., 2001). *Harmonia axyridis* occurs in many of the same agricultural systems (Wold et al. 2001; Koch and Hutchison 2003), and coccinellids, in general, are reported among the generalist predators preying on immature *D. plexippus* (Zalucki and Kitching 1982; Zangerl et al. 2001). Theoretically, this co-occurrence of *D. plexippus* and *H. axyridis* puts *D. plexippus* at risk of being preyed upon by *H. axyridis*.

In this paper we develop a model, under the framework of an ecological risk assessment (EPA 1998), to evaluate the impact of an invasive, generalist natural enemy on a non-target prey, using the *D. plexippus*–*H. axyridis* system as a case study. Here, risk is defined as the joint probability of exposure and effect. Exposure is the probability of *D. plexippus* co-occurring temporally and spatially with *H. axyridis*. Effect is the magnitude of impact on *D. plexippus* populations by *H. axyridis* when they co-occur. This assessment is focused on an examination of a worst-case scenario for the impact of *H. axyridis* on *D. plexippus*. The objectives of this study were to: (1) quantify the temporal and spatial co-occurrence of *D. plexippus* with *H. axyridis* on *A. syriaca* in corn and soybean fields; and (2) quantitatively estimate the overall risk of *D. plexippus* populations developing in Minnesota corn and soybean fields being impacted by *H. axyridis*.

Materials and methods

Model framework

This risk assessment focuses on the potential impact of *H. axyridis* on the overlapping second and third generation of Minnesota *D. plexippus* developing in corn, *Zea mays* L., and soybean, *Glycine max* L., fields. First generation Minnesota *D. plexippus* were not included for two reasons. First, *H. axyridis* was relatively rare in corn and soybean fields during the first generation of *D. plexippus* (RLK personal observation). Second, it is the second and third generations of Minnesota *D. plexippus* that contribute to the overwintering population and hence to the population the following year (Oberhauser and Solensky 2004). For the present assessment, we assume that second instar to adult *H. axyridis* can prey on eggs and first and second instars of *D. plexippus* (Koch et al. 2003a; RLK unpublished data). Figure 1 depicts a conceptual model used to evaluate the risk of *H. axyridis* impacting *D. plexippus*. In general, the recruitment of *D. plexippus* eggs in corn and soybean fields is estimated from our empirical observations of *A. syriaca* densities and egg densities per plant (see *Habitat Productivity* below). Then, the abundance of *D. plexippus* in a given habitat is reduced by stage-specific mortality rates. Baseline mortality rates (i.e., without *H. axyridis* in the system) were obtained from the literature (Borkin 1982). In addition, mortality induced by *H. axyridis* was estimated from research presented herein to examine the temporal and spatial co-occurrence of *D. plexippus* with *H. axyridis*, and the propensity of *H. axyridis* to consume *D. plexippus* when they co-occur (see *Exposure assessment* and *Effect assessment* below).

To incorporate stochasticity into the model, risk analysis software (@RISK Pro ver. 4.5, Palisade Corp., Newfield, NY) was used to model the probability distribution of outcomes by sampling values for each input variable from their respective probability distributions, and calculating the model outcomes through many iterations. The spreadsheet model was run using 100,000 iterations with Latin hypercube sampling. Probability distributions for terms in the model were obtained by fitting theoretical distributions to observed data (BestFit, @RISK), and by defining hypothetical distributions. Below, we describe how the terms within the model were derived.

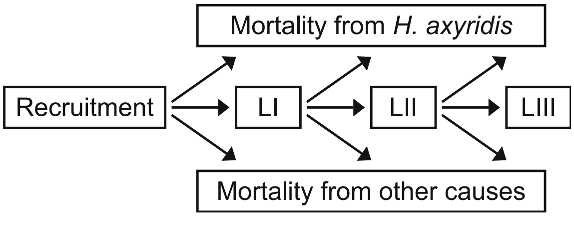

Figure 1. Conceptual model used to evaluate the impact of *Harmonia axyridis* on *Danaus plexippus*. The model begins with recruitment of *D. plexippus* eggs in a given habitat. The abundance of subsequent developmental stages (LI, LII, and LIII represent first, second, and third instars, respectively) of *D. plexippus* are reduced by stage-specific mortalities due to *H. axyridis* and other causes.

Habitat productivity

Asclepias syriaca densities in corn and soybean fields were obtained from the Minnesota Department of Agriculture (MDA), St. Paul, MN. During late-July through mid-August of 2003 and 2004, MDA conducted weed surveys in corn and soybean fields throughout Minnesota, except for the northeast portion of the state where agriculture is minimal. In 2003, 257 corn fields and 260 soybean fields were inspected. In 2004, 229 corn fields and 223 soybean fields were inspected. In the interior of each field, ten 6.1 × 0.6-m plots ($37.16\ \mathrm{m}^2$ in total per field), with 25 m separating plots, were inspected along a transect. The number of *A. syriaca* occurring in each plot was recorded. Densities of *D. plexippus* eggs per *A. syriaca* in each habitat were obtained from the season long monitoring project described below for the *Exposure assessment*.

The number of *D. plexippus* produced per hectare in Minnesota corn and soybean fields was calculated as the product of the *A. syriaca* densities per hectare and *D. plexippus* densities per *A. syriaca*. The risk analysis software was used to characterize the distributions of *A. syriaca* and *D. plexippus* egg densities pooled across years. We characterized each distribution using a two-stage distribution, with a binomial distribution characterizing the frequency of zero vs non-zero events, and a separate distribution to characterize the non-zero events. The overall distributions were calculated as the product of the two distributions. For the binomial distributions of *A. syriaca* and *D. plexippus* egg densities, the probability of obtaining a non-zero event was estimated from the sampling data (Table 1). A

Table 1. Characterization of the densities of *Asclepias syriaca* per ha and *Danaus plexippus* eggs per ramet of *A. syriaca* in corn and soybean fields.

Habitat	Plants/ha	Eggs/plant
Corn fields	Bin (1, 0.049)	Bin (1, 0.19)
	Uni (1, 3768)	Exp (0.81)
Soybean fields	Bin (1, 0.0083)	Bin (1, 0.22)
	Uni (1, 1884)	Exp (1.12)

A two-stage distribution was used to describe the densities of plants and eggs: a binomial[a] distribution for zero vs non-zero values, and then a uniform[b] or exponential[c] distribution for the non-zero values.

[a] Binomial distributions are represented by Bin (*n*, *p*), where *n* is the number of trials and *p* is the probability of a non-zero observation.

[b] Uniform distributions are represented by Uni (m_1, m_2), where m_1 and m_2 are the minimum and maximum, respectively.

[c] Exponential distributions are represented by Exp (β), where β is the decay coefficient. The exponential distributions were right shifted to begin at one instead of zero.

uniform distribution was used to characterize non-zero *A. syriaca* densities, with the maximum value obtained from the sampling data and the minimum value set to one (Table 1). An exponential distribution was used to characterize the non-zero *D. plexippus* egg densities, with the decay constant estimated from the sampling data using the risk analysis software (Table 1). Despite the fitted distributions not fitting some of the data sets statistically ($P < 0.05$, chi-square goodness-of-fit) and other data sets not having enough non-zero observations to test the fit, the two-stage distributions provided a qualitatively satisfactory fit (Figures 2, 3).

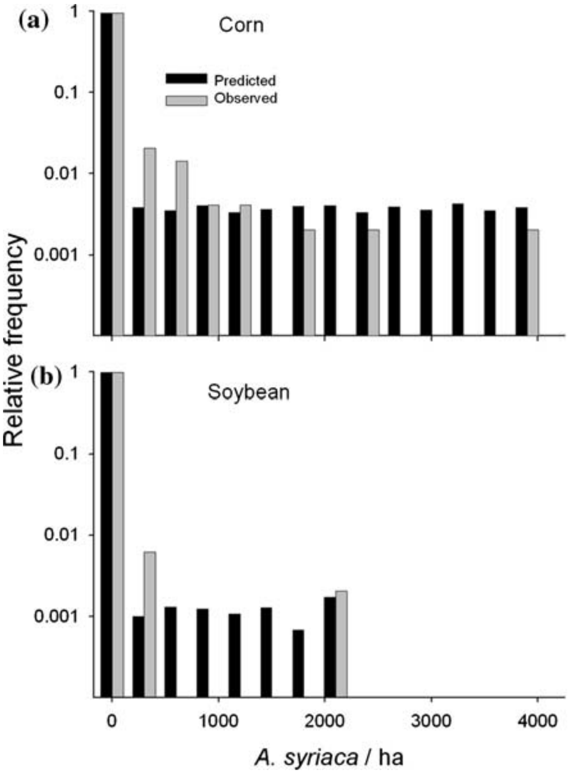

Figure 2. Observed and predicted distribution of *Asclepias syriaca* in Minnesota corn and soybean fields. Distributions used to characterize each predicted distribution are displayed in Table 1.

Exposure assessment

We monitored *D. plexippus* and *H. axyridis* populations on *A. syriaca* in commercial corn and soybean fields at the University of Minnesota Outreach, Research and Education (UMORE) Park, Rosemount, MN. In 2001, 3 corn fields and 1 soybean field containing *A. syriaca* were selected; in 2002, 3 corn fields; and in 2003, 2 corn fields and 2 soybean fields. Within each habitat, 14–41 *A. syriaca* were randomly selected. We monitored the selected *A. syriaca* approximately weekly from mid-June to late-August. A sampling interval of approximately 1 week was chosen to minimize the likelihood of encountering an individual in the same developmental stage on two consecutive sample dates (Oberhauser et al. 2001). On each sample date, *A. syriaca* were sampled using whole-plant visual inspection, with counts and developmental stages of *D. plexippus* and *H. axyridis* being recorded. Immature stages of *D. plexippus* were identified to instar using a field guide (Oberhauser and Kuda 1997). Adult and immature stages of *H. axyridis* were identified using a diagnostic guide (Schellhorn 2003) and stage-specific descriptions from Koch (2003).

We conducted an exposure analysis to simultaneously examine the temporal and spatial co-occurrence of *D. plexippus* and *H. axyridis* on an individual plant scale. For each observation date, the ratio of *H. axyridis* vs *D. plexippus* was calculated for each pairwise combination of predator and prey developmental stages on each plant. The ratio was then multiplied by the duration of time, in days, between mid-points between sample dates to provide an estimate of exposure measured in *H. axyridis*-days experienced per

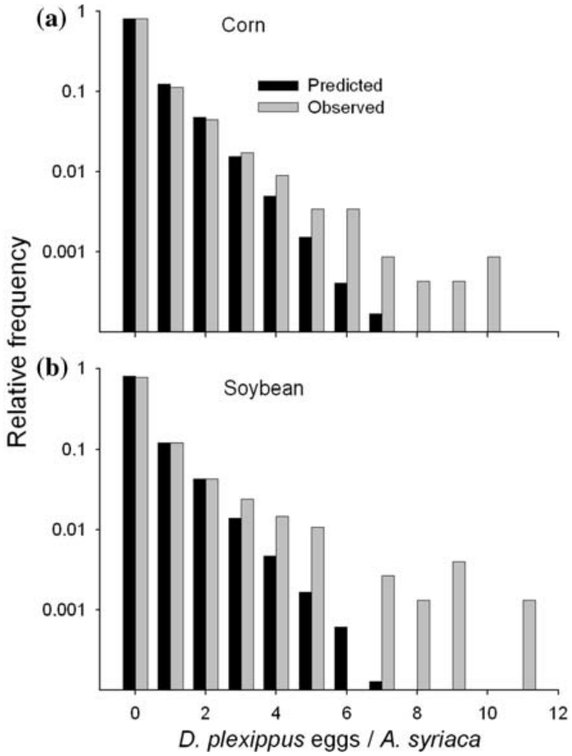

Figure 3. Observed and predicted distributions of *Danaus plexippus* eggs on *Asclepias syriaca* in Minnesota corn and soybean fields. Distributions used to characterize each predicted distribution are displayed in Table 1.

D. plexippus for each pairwise combination of predator and prey developmental stages. The risk analysis software was used to characterize the distributions of the measures of exposure. We characterized each distribution using a two-stage distribution, with a binomial distribution for zero vs non-zero values, and a uniform distribution for the non-zero values. The overall distribution was then calculated as the product of the binomial and uniform distributions. For the binomial distribution, the probability of obtaining a non-zero value was estimated from the monitoring data (Table 2). For the uniform distribution, the maximum value was set as the maximum observed number of *H. axyridis*-days per *D. plexippus* for a given combination of predator and prey developmental stages; the minimum was assumed to be 0.02 *H. axyridis*-days (*ca.* 30 min) per *D. plexippus* (Table 2). Despite the fitted distributions not fitting some of the data sets statistically ($P < 0.05$, chi-square goodness-of-fit) and other data sets having too many zero observations (i.e., instances when *D. plexippus* occurred on plants without *H. axyridis*) to test the fit, the two-stage distributions provided a qualitatively satisfactory fit (Figures 4, 5).

Effect assessment

A predation model developed by Koch et al. (2005) was used as the basis for the effect assessment. This model uses multiple linear regression to predict decreasing *D. plexippus* survival with increasing *H. axyridis* density, and greater *D. plexippus* survival on plants with alternate prey (i.e., the oleander aphid, *Aphis nerii* Boyer de Fonscolombe) than on plants without. For this assessment, the effect of alternate prey was omitted to provide an assessment of a worst-case scenario. The form of the model used here is: $N = 1.00 + (P \times -0.20)$, where N represents the proportionate survival of first instar *D. plexippus*. The model focused on the survival of first instar *D. plexippus* over 1 day exposure to third instar *H. axyridis*. Therefore, P represents the measure of exposure of *D. plexippus* to *H. axyridis* measured in *H. axyridis*-days per *D. plexippus*. To expand the use of this model to other pairwise combinations of developmental stages of *H. axyridis* and *D. plexippus*, we created adjustment factors for differences in voracity among pairwise combinations of predator and prey developmental stages. The adjustment factors (a), presented in Table 3, were then multiplied by the predation rate as follows, $N = 1.00 + (P \times (-0.20 \times a))$. The relative voracity among developmental stages of *H. axyridis* was accounted for by creating an adjustment factor based on the mass of *Aphis gossypii* Glover consumed per day by various developmental stages of *H. axyridis* (Lee and Kang 2004). These consumption rates were relativized to 1 for third instar *H. axyridis* predation on first instar *D. plexippus*, creating adjustment factors of 0.27, 1.00, 3.24 and 2.80 for second, third and fourth instars and adults (Table 3). Therefore, the predation rate for third instar *H. axyridis* on first instar *D. plexippus* (i.e., the combination of predator and prey developmental stages tested in Koch et al. (2005)) remains unchanged when multiplied by the adjustment factor of 1.00, whereas the predation rates for

Table 2. Characterization of the exposure of *Danaus plexippus* to *Harmonia axyridis* in corn and soybean fields.

H. axyridis	*D. plexippus*		
	Egg	LI	LII
Corn fields			
LII	Bin (1, 0) N.A.	Bin (1, 0) N.A.	Bin (1, 0.011) Uni (0.02, 9.00)
LIII	Bin (1, 0.0067) Uni (0.02, 9.00)	Bin (1, 0.033) Uni (0.02, 9.00)	Bin (1, 0.021) Uni (0.02, 11.00)
LIV	Bin (1, 0.02) Uni (0.02, 11.00)	Bin (1, 0.0082) Uni (0.02, 16.50)	Bin (1, 0.021) Uni (0.02, 11.00)
Adult	Bin (1, 0.009) Uni (0.02, 16.00)	Bin (1, 0) N.A.	Bin (1, 0.011) Uni (0.02, 7)
Soybean fields			
LII	Bin (1, 0.012) Uni (0.02, 8.00)	Bin (1, 0) N.A.	Bin (1, 0) N.A.
LIII	Bin (1, 0.036) Uni (0.02, 9.00)	Bin (1, 0.016) Uni (0.02, 9.00)	Bin (1, 0.054) Uni (0.02, 9.00)
LIV	Bin (1, 0.078) Uni (0.02, 9.00)	Bin (1, 0.032) Uni (0.02, 7.00)	Bin (1, 0.18) Uni (0.02, 23.00)
Adult	Bin (1, 0.078) Uni (0.02, 21.00)	Bin (1, 0.065) Uni (0.02, 7.00)	Bin (1, 0.13) Uni (0.02, 18.00)

Exposure was measured as *H. axyridis*-days per *D. plexippus*. A two-stage distribution was used to describe the exposure for each combination of life stages: a binomial distribution[a] for zero vs non-zero values, and then a uniform distribution[b] for the non-zero values.

[a] Binomial distributions are represented by Bin (n, p), where n is the number of trials and p is the probability of a non-zero observation.

[b] Uniform distributions are represented by Uni (m_1,m_2), where m_1 and m_2 are the minimum and maximum, respectively.

N.A. = Not applicable.

other stages of *H. axyridis* on first instar *D. plexippus* are increased or decreased when multiplied by their respective adjustment factors (Table 3). For example, multiplying the predation rate of fourth instar *H. axyridis* by 3.24 accounts for fourth instars consuming approximately three times more prey per unit time than third instars (Table 3). For predation on second instar *D. plexippus*, we assumed that the predation rate would be inversely proportional to prey mass, and multiplied the adjustment factors for first instar *D. plexippus* by 0.06 (i.e., the ratio of the masses of first vs second instar *D. plexippus*) (Table 3). The mass of first instar *D. plexippus* was assumed to be 0.9 mg (Stanley-Horn et al. 2001), and the mass of second instars was assumed to be 14.9 mg (i.e., the average weight of second instars across treatments of high and low nitrogen host plants from Lavoie and Oberhauser (2004)). For predation of *D. plexippus* eggs by various stages of *H. axyridis*, the aforementioned adjustment factors for predation on first instar *D. plexippus* were multiplied by 1.67 to account for greater rates of predation on *D. plexippus* eggs compared to first instars (Koch et al. 2003a) (Table 3). This means that among developmental stages of *H. axyridis*, the ratio of predation rates on first and second instar *D. plexippus* is fixed *ca.* 0.06 and that of *D. plexippus* eggs and first instars is fixed at *ca.* 1.67. The use of fixed ratios is supported by results of a study examining the predation of a *Leptinotarsa decemlineata* (Say) eggs and larvae by adults and larvae of *Coleomegilla maculata* DeGeer (Giroux et al. 1995). To account for uncertainty around estimated mortality rates, the distribution of these estimates were characterized as normal distributions defined by the mean parameter estimate and its standard error, as reported in Koch et al. (2005).

Overall risk

To calculate the overall risk of *D. plexippus* being impacted by *H. axyridis*, the exposure, in *H. axyridis*-days per *D. plexippus*, for each pairwise combination of developmental stages was inserted as the exposure variable (i.e., P) in the adjusted predation model from Koch et al.

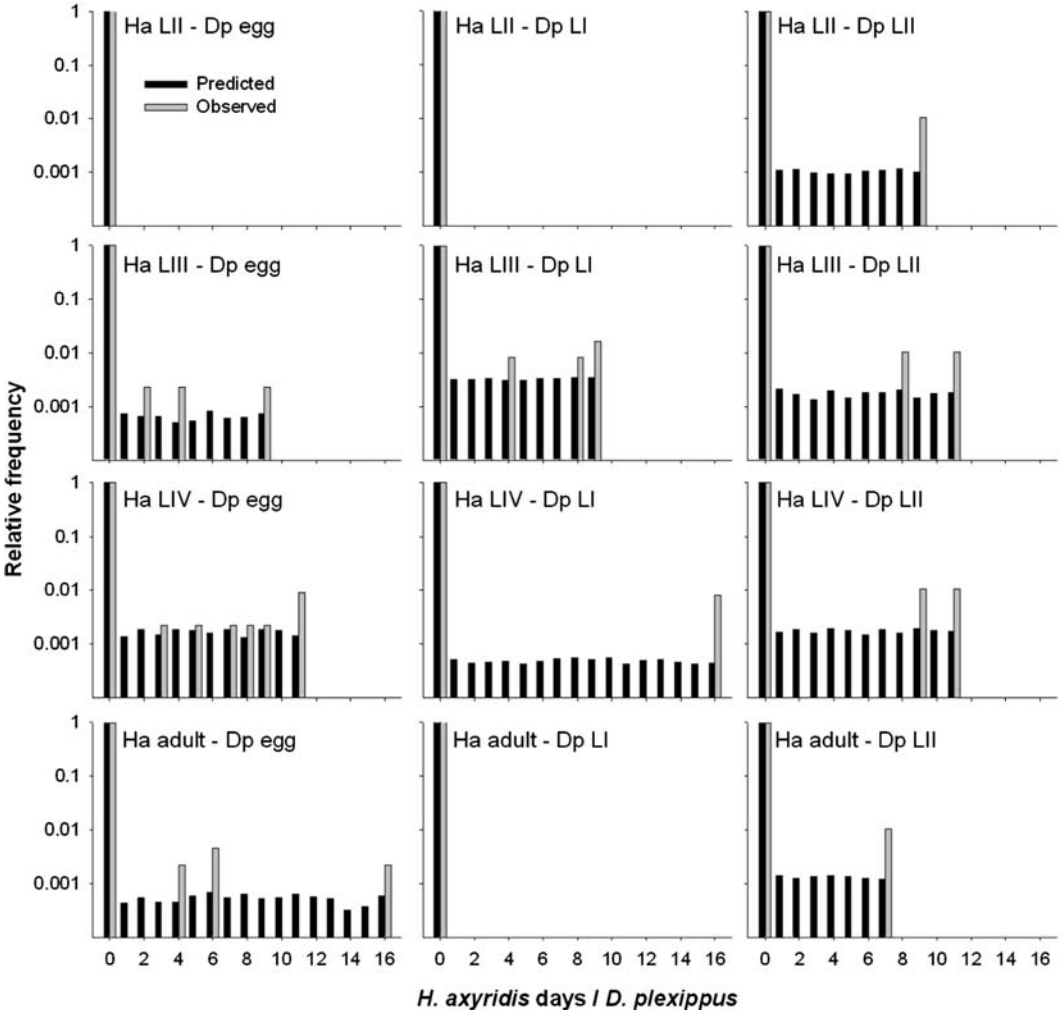

Figure 4. Observed and predicted distributions of exposure, measured in *Harmonia axyridis* days per *Danaus plexippus*, for each pairwise combination of predator and prey developmental stages in Minnesota corn fields. Distributions used to characterize each predicted distribution are displayed in Table 2. In the graph titles, Ha represents *H. axyridis*, Dp represents *D. plexippus*, and LI, LII, LIII, LIV represent the first, second, third and fourth instars, respectively.

(2005). The resulting estimates of stage-specific *D. plexippus* mortality induced by various stages of *H. axyridis* were summed within each stage of *D. plexippus* to give proportionate mortality rates for each stage of *D. plexippus*. Due to the linearity of the predation model, predicted proportionate mortality may exceed 1.0 if the number of *H. axyridis*-days per *D. plexippus* is great enough. In these cases, predicted mortality was set to 1.0. Mortality rates due to causes other than *H. axyridis* were obtained from a life table study conducted in Wisconsin for monarchs developing on *A. syriaca* (Borkin 1982). From these proportions and the number of individuals reported by Borkin (1982), we calculated the standard error for each proportionate mortality rate. The stage-specific density independent mortality rates were 0.68 ± 0.029, 0.38 ± 0.052, and 0.74 ± 0.061 for eggs and first and second instars, respectively. To account for uncertainty around these estimates, the distribution of these mortality rates was characterized as a triangular distribution, with the most likely observations set as the proportionate mortality and the minima and maxima set as

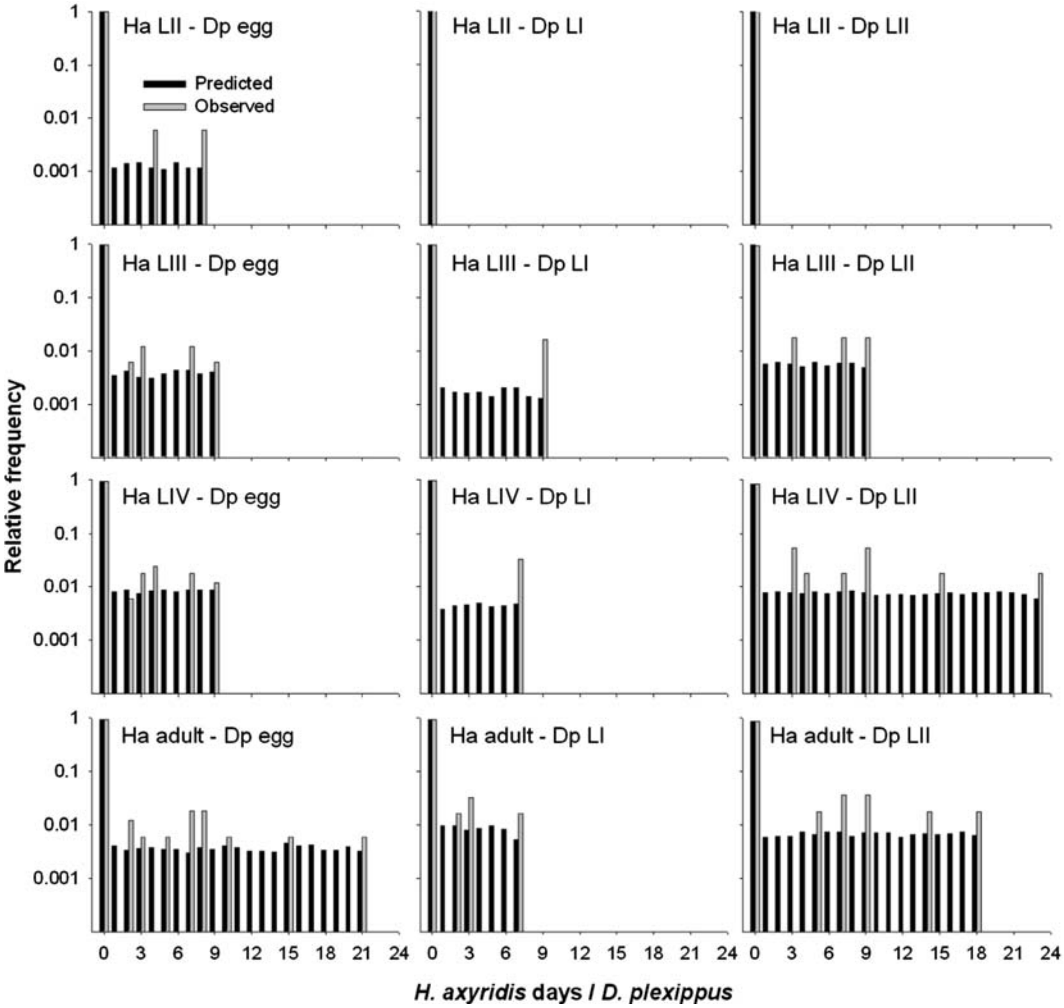

Figure 5. Observed and predicted distributions of exposure, measured in *Harmonia axyridis* days per *Danaus plexippus*, for each pairwise combination of predator and prey developmental stages in Minnesota soybean fields. Distributions used to characterize each predicted distribution are displayed in Table 2. In the graph titles, Ha represents *H. axyridis*, Dp represents *D. plexippus*, and LI, LII, LIII, LIV represent the first, second, third and fourth instars, respectively.

twice the standard error. Stage-specific *D. plexippus* survival was calculated as one minus the stage-specific proportionate mortality (i.e., natural mortality alone or natural plus *H. axyridis* induced mortality). These stage-specific survival rates were used sequentially to calculate the number of first, second, and third instar *D. plexippus* surviving per ha from the initial recruitment of eggs per ha. To determine the relative impact of *H. axyridis* on populations of *D. plexippus*, the ratio of the count of third instar *D. plexippus* produced per ha with *H. axyridis* in the system (i.e., after mortality from *H. axyridis* and other causes) vs the count of third instar *D. plexippus* produced per ha without *H. axyridis* in the system (i.e., after mortality from causes other than *H. axyridis*) was calculated. This value estimates the relative production of third instar *D. plexippus* in a system with *H. axyridis* compared to one without *H. axyridis*.

With many highly skewed distributions as model inputs, model outputs were also highly skewed. Throughout this text we refer to means among outputs, because median and mode values

Table 3. Adjustment factors used to account for differences in relative voracity among pairwise combinations of predator and prey developmental stages.

D. plexippus	*H. axyridis*			
	LII	LIII	LIV	Adult
Egg	0.46	1.67	5.39	4.67
LI	0.27	1.00	3.24	2.80
LII	0.02	0.06	0.20	0.17

The adjustment factors (a) were multiplied by the predation rate (i.e., −0.20) in the predation model, $N = 1.00 + (P\times\ (-0.20 \times a))$. Explanation of how these adjustments were derived can be found in the text.

were zero in all cases. However, probability distributions are presented for the outputs to indicate the likelihood of a given result occurring.

Results and discussion

Simberloff and Stiling (1996) suggest that biological control could benefit from post-release evaluations of non-target impacts of exotic biological control agents. Ecological risk assessment can provide a framework for evaluating such a risk. In recent years, advances have been made toward evaluating the impact of introduced natural enemies on non-target prey. The depth of these studies has ranged from examinations of the likelihood of an effect occurring (i.e., laboratory and field predation/parasitism studies) (Boettner et al. 2000; Kajita et al. 2000; Mochizuki and Mitsunga 2004; Yasuda et al. 2004) to more complete evaluations of risk where both the likelihoods of effect and exposure are examined, as in the series of papers examining the non-target impacts of *Trichogramma brassicae* Bezdenko (Babendreier et al. 2003a, 2003b, 2003c; Kuske et al. 2003). In addition, Andow et al. (1995) developed a framework to evaluate the impact of inundative releases of *Trichogramma nubilale* Ertle and Davis on an endangered lepidopteran. However, we are unaware of any assessments that have combined exposure and effect assessments to provide an overall quantitative estimate of the risk of a non-target prey being impacted by an introduced predator. Previously, the risk of *D. plexippus* being impacted by *H. axyridis* was qualitatively ranked as moderate (Koch et al. 2004). Our work presented herein represents the first quantitative risk assessment of an introduced predator impacting a non-target prey.

The probability of *A. syriaca* occurring in soybean fields was an order of magnitude lower than for its occurrence in corn fields (Table 1 and Figure 2). Across years, *A. syriaca* densities in corn and soybean fields were 36.54 ± 10.73 and 5.57 ± 4.01 plants per ha, respectively. Hartzler and Buhler (2000) also reported lower densities of *A. syriaca* in soybean compared to corn fields. With similar per plant densities of eggs in both habitats, the low density of *A. syriaca* in soybean fields contributed to the lower recruitment of *D. plexippus* eggs in soybean fields (4.39 eggs per ha) compared to corn fields (33.67 eggs per ha) (Table 4). In Ontario, a similar level of recruitment of *D. plexippus* eggs was observed in corn fields (34.00 eggs per ha) (Oberhauser et al. 2001).

Table 4. Model predictions for the impact of *Harmonia axyridis* on *Danaus plexippus* developing in Minnesota corn and soybean fields.

D. plexippus	Corn			Soybean		
	Without *H. axyridis*	With *H. axyridis*		Without *H. axyridis*	With *H. axyridis*	
	Count/ha	Count/ha	aq_{ix}[a]	Count/ha	Count/ha	aq_{ix}[a]
Egg	33.67 (0, 20554)	33.67 (0, 20554)	0.033 (0, 1)	4.39 (0, 9507)	4.39 (0, 9507)	0.174 (0, 1)
LI	10.79 (0, 6695)	10.45 (0, 6695)	0.031 (0, 1)	1.40 (0, 3069)	1.17 (0, 3069)	0.094 (0, 1)
LII	6.69 (0, 4301)	6.21 (0, 4301)	0.007 (0, 0.63)	0.86 (0, 1994)	0.66 (0, 1994)	0.104 (0, 1)
LIII	1.74 (0, 1227)	1.57 (0, 1227)		0.22 (0, 490)	0.12 (0, 490)	
Relative production[b]	0.899 (0, 1)			0.531 (0, 1)		

[a] Stage-specific proportionate mortality of *D. plexippus* induced by *H. axyridis*.

[b] Calculated as the ratio of the count of third instar *D. plexippus* produced per ha with *H. axyridis* in the system (i.e., after natural mortality plus *H. axyridis* induced mortality) vs the count of third instar *D. plexippus* produced per hectare without *H. axyridis* in the system (i.e., after natural mortality alone).

The phenologies of *H. axyridis* and *D. plexippus* overlapped in corn and soybean fields. However, when looked at on an individual plant scale, the likelihood of *D. plexippus* co-occurring with *H. axyridis* was relatively low compared to the duration of time both species were present in the field (Figures 4, 5). Across combinations of predator and prey developmental stages, mean levels of exposure ranged from 0 to 1.19 and 0 to 1.61 *H. axyridis*-days per *D. plexippus* in corn and soybean fields, respectively. More importantly, there was generally a higher probability of *D. plexippus* occurring on *A. syriaca* with *H. axyridis* in soybean compared to corn fields. The presence of the soybean aphid, *Aphis glycines* Matsumura, in soybean (RLK unpublished data) may have made soybean fields a favorable habitat for *H. axyridis* population increase; therefore, increasing the probability of *D. plexippus* encountering *H. axyridis* on *A. syriaca* in that system. Days provided a better temporal scale than degree days for use in this study, because there is no common lower developmental threshold for both species.

Under the assumptions of a worst-case scenario, the mean stage-specific *D. plexippus* mortality rates attributable to *H. axyridis* (i.e., aq_{ix} in standard multiple decrement life tables (Carey 1993)) ranged from 0.007 to 0.033 and 0.094 to 0.174 among stages in corn and soybean fields, respectively. *Harmonia axyridis* induced mortality rates in corn fields were 81.03%, 67.02% and 93.27% lower than those in soybean fields for *D. plexippus* eggs, and first and second instars, respectively (Table 4). Across both habitats, all stage-specific mortality rates ranged from 0 to 1, except for second instars developing in corn fields, were the maximum mortality was 0.63 (Table 4 and Figure 6). For eggs and first instars of *D. plexippus* in both systems, the relative frequency of observations at a proportionate mortality of 1.0 was greater than the frequency of other non-zero mortality predictions (Figure 6). This phenomenon is likely an artifact of the linearity of the predation model, and the imposed upper limit of 1.0 even if the model predicts mortality greater than 1.0. The production of third instar *D. plexippus* with *H. axyridis* in the system was 89.9% and 53.1% of the production without *H. axyridis* in the system for corn and soybean fields, respectively (Table 4 and Figure 7). More specifically, in corn fields, there was an 87.33% chance of *H. axyridis* having no effect on the production of third instar *D. plexippus* (i.e., relative productivity = 1), and a 7.93% chance of *H. axyridis* killing all *D. plexippus* (i.e., relative productivity = 0) (Figure 7). In soybean fields, *H. axyridis* showed a much greater impact, with a 48.13% chance of *H. axyridis* having no effect on the production of third instar *D. plexippus*, and

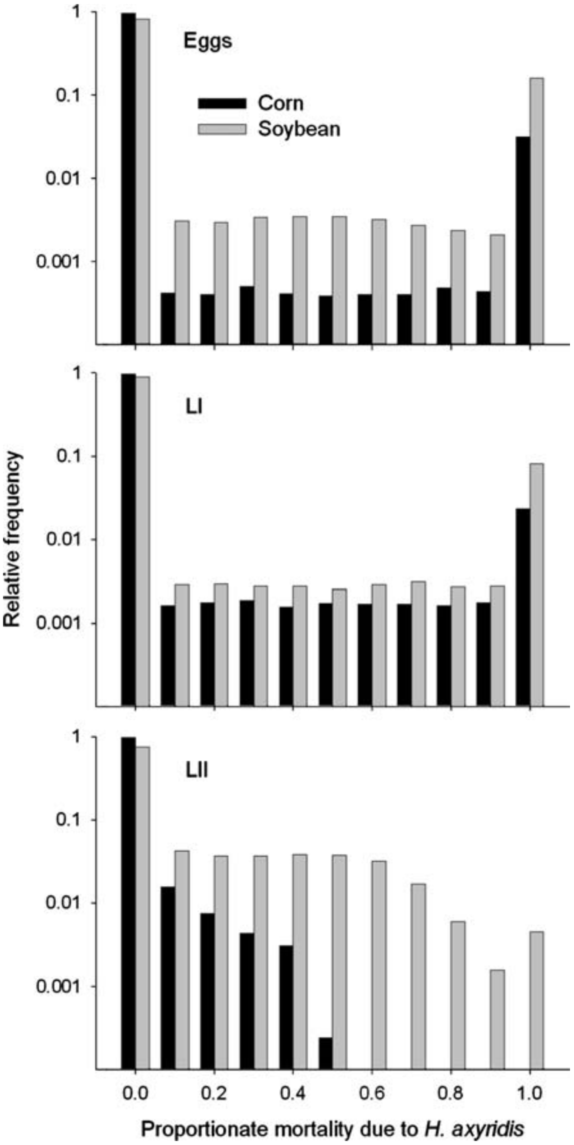

Figure 6. Predicted stage-specific *Danaus plexippus* mortality rates (aq_{ix}) induced by *Harmonia axyridis* for *Danaus plexippus* eggs, and first (LI) and second (LII) instars in Minnesota corn and soybean fields.

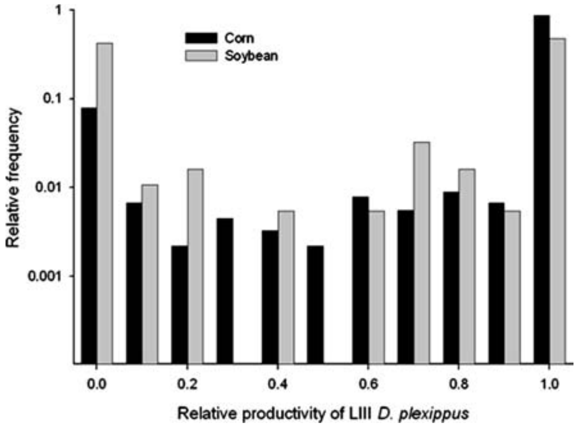

Figure 7. Relative productivity of third instar *Danaus plexippus* in Minnesota corn and soybean fields calculated as the ratio of the number of *D. plexippus* produced with and without *Harmonia axyridis*.

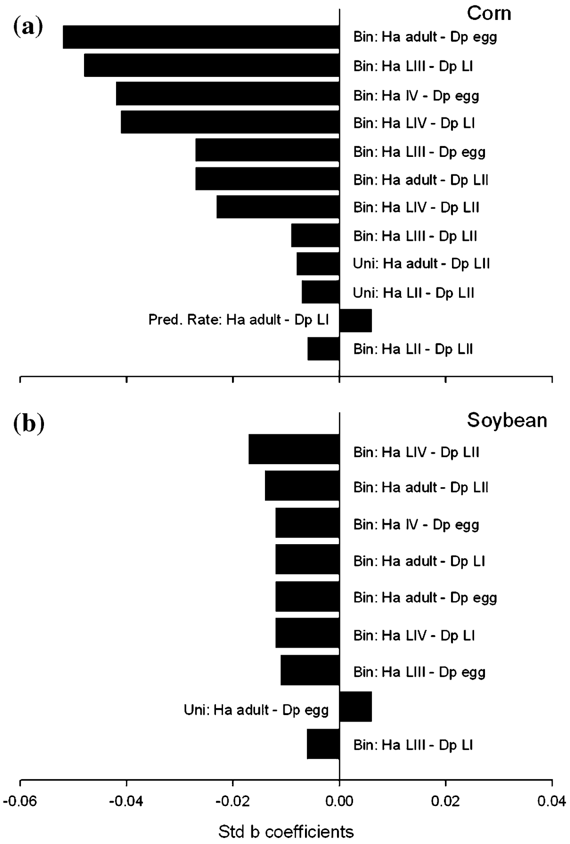

Figure 8. Tornado graphs, based on multivariate stepwise regression, for sensitivity analysis of model calculating the relative productivity third instar *Danaus plexippus* in Minnesota corn and soybean fields with and without *Harmonia axyridis*. The magnitude of each bar on the graphs indicates the number of standard deviations the relative productivity changes after a perturbation of one standard deviation for each input term. In the graphs, 'bin' (i.e., binomial) and 'uni' (i.e., uniform) indicate the component distributions characterizing the exposure, measured in *H. axyridis*-days per *D. plexippus*, of *D. plexippus* to *H. axyridis*. 'Pred. rate' represents the normal distribution used to characterize the rate of *H. axyridis* predation on *D. plexippus*. Ha represents *H. axyridis*, Dp represents *D. plexippus*, and LI, LII LIII, LIV represent the first, second, third, and fourth instars, respectively.

a 42.78% chance of *H. axyridis* killing all *D. plexippus* (Figure 7). The high probability of *D. plexippus* mortality in soybean fields may be attributed to high levels of exposure to *H. axyridis*, likely resulting from the numerical response of *H. axyridis* to *A. glycines*. These results suggest that the risk of *D. plexippus* being impacted by *H. axyridis* in a given system may be dependent upon aphid densities in that habitat.

To determine the relative importance of each term in the model and the sensitivity of the model to perturbations of these terms, multivariate stepwise regression was conducted (@RISK). Results of these analyses are displayed in tornado graphs (Figure 8). The magnitude of each bar on the graphs indicates the number of standard deviations the relative productivity changes after a perturbation of one standard deviation for each input term. For the relative production of third instar *D. plexippus* in corn and soybean fields, the binomial distributions characterizing the frequency of zero vs non-zero values of *H. axyridis*-days per *D. plexippus* (i.e., exposure) had the greatest influence on model results. To determine the sensitivity of the model to changes in these input distributions, the percent change of the mean relative production of third instars was calculated when exponential distributions were used in place of two-stage distributions for *H. axyridis*-days per *D. plexippus*. Using an exponential distribution to characterize exposure greatly increased the impact of *H. axyridis* by overestimating the frequency of low non-zero values of exposure. For instance, in corn fields, stage-specific mortality rates due to *H. axyridis* increased by 690.9%, 340.9%, and 85.7% for eggs, and first and second instars, respectively, when exponential distributions were used in place of the two-stage distributions. In soybean fields, mortality rates increased by 342.5%, 236.2%, and 0% for eggs, and first and second instars, respectively, when the exponential distributions were used. The mean relative production of third instars decreased by 70.5% and 97.7% for corn and soybean fields, respectively. As in the original model, a multivariate stepwise regression sensitivity analysis indicated that the distributions characterizing *H. axyridis*-days per *D. plexippus* were most influential on the relative production of third instars.

Harmonia axyridis is one of many hazards that immature *D. plexippus* must contend with in agricultural systems. Much attention recently focused on the impact of transgenic corn, modified to express toxins from a soil bacterium, *Bacillus thuringiensis* Berliner, (*Bt* corn) (Sears et al. 2001; Koch et al. 2003b; Wolt et al. 2003; Dively et al. 2004). In general, it appears that *Bt* corn poses a negligible threat to *D. plexippus*, due to low levels of exposure. Conversely, pesticides seem to pose a greater threat to immature *D. plexippus* in agricultural systems. Pyrethroid insecticides, commonly used to manage European corn borer, *Ostrinia nubilalis* Hübner, in corn fields and *A. glycines* in soybean, have shown high toxicity to *D. plexippus* larvae (Stanley-Horn et al. 2001); however, the magnitude of *D. plexippus* exposure to insecticides has not been determined. In addition, herbicide use and herbicide-tolerant crops may be suppressing populations of *A. syriaca* within agricultural systems. For instance, in Minnesota, herbicide-tolerant soybean and corn comprised 82% and 17%, respectively, of the total area planted to each crop (NASS 2004).

This risk assessment was conducted as an examination of a potential worst-case scenario. In quantifying the likelihood of predation, the predators were starved for 24 h prior to experimentation; whereas, in the field, it seems unlikely if the surrounding habitat is infested with aphids (e.g., *A. glycines* on soybean plants). In addition, the estimates of exposure in soybean fields presented in this study may be high-end estimates. The two years from which data were collected in soybean fields (2001 and 2003) were high years for *A. glycines* in Minnesota (Hodgson 2005), which likely resulted in greater densities of *H. axyridis* in soybean fields in these years compared to years with lower aphid densities (2002 and 2004). This assessment also neglected factors that might mitigate the impact of *H. axyridis* on *D. plexippus*, such as the presence of alternate prey on *A. syriaca* (Koch et al. 2005) and interactions among predators (Rosenheim et al. 1993). Currently, the likelihood of occurrence for this worst-case scenario remains uncertain. Regardless, this assessment demonstrates that *H. axyridis* has the potential to have a strong adverse impact on *D. plexippus* populations. Most importantly, this research highlights the importance of the exposure analysis in estimating overall risk. In particular, for the *D. plexippus*–*H. axyridis* system, we found that exposure and subsequent risk varied by cropping system. To evaluate the landscape-level effect of *H. axyridis* on *D. plexippus* populations, further information would be required on the recruitment of *D. plexippus* in other habitats, both agricultural and non-agricultural, as well as exposure estimates specific to additional habitats. We hope that this risk assessment can be used as a model for future assessments that may incorporate additional habitats and factors influencing population dynamics.

Classical biological control has been and continues to be an important tactic in pest management. However, for this tactic to remain socially acceptable, its safety must improve. Great advances are being made in the stringency of host range testing and risk analysis to ensure that the release of candidate natural enemies are justified by the likelihood of successful biological control outweighing the likelihood and severity of adverse impacts. With safety ensured through utilization of highly specific natural enemies, classical biological control should remain a viable and acceptable pest management tactic.

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