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Research Article

Treating Prey With Glyphosate Does Not Alter the Demographic Parameters and Predation of the *Harmonia axyridis* (Coleoptera: Coccinellidae)

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Abstract

Glyphosate is an herbicide that is used worldwide with potential environmental risks to nontarget organisms. We applied an age–stage, two-sex life table approach to assess the sublethal effects of short-term oral exposure to a glyphosate-based herbicide on the life table parameters and biocontrol potential of *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae). Aphids (*Metopolophium dirhodum* (Walker) (Sternorrhyncha: Aphididae)) treated with herbicide (an isopropylamine-salt of glyphosate) at low recommended, maximum recommended, and double the maximum recommended concentration for agricultural situations, and untreated controls were offered to the fourth instar of *H. axyridis* for 24 h. Development, consumption, and fecundity were measured daily until death. We detected minor differences in the hatching rate and mean generation time, whereas the longevity, fecundity, net reproductive rate, intrinsic rate of increase, finite rate of increase, and consumption were unaffected across treatments. We conclude that biocontrol potential of *H. axyridis* was not affected by acute oral intoxication by a glyphosate-based herbicide during the larval stage for 24 h under the study design.

Key words: Harlequin lady beetle, Roundup, aphidophagy, life table, biocontrol potential

Since its approval in the 1970s, glyphosate has become the most widely used herbicide ingredient in the world. Glyphosate is a nonselective herbicide when applied in doses of ~1 kg of active ingredient per hectare (Steinmann et al. 2012). The herbicide is taken up by leaves and systemically translocated throughout the entire plant via the vascular system (Grossbard and Atkinson 1985). The effective substance of glyphosate *N*-(phosphonomethyl) glycine inhibits 5-enolpyruvylshikimate 3-phosphate synthase and blocks amino acid synthesis and proteosynthesis, and as a result, treated plants become dry and die several days after application (Grossbard and Atkinson 1985). The use of glyphosate-based herbicides doubled from 1999 to 2010 in Germany, and glyphosate was used on 39% of the total arable land (Steinmann et al. 2012). The manifold increase in glyphosate use in United States farming during the past 20 yr has been primarily driven by the increasing adoption of herbicide-tolerant crops, including cereals (Coupe and Capel 2016). Glyphosate is also used in nonagricultural situations (Woodburn 2000).

Although glyphosate-based herbicides are extremely toxic in aquatic environments (Annett et al. 2014), in terrestrial ecosystems, glyphosate is traditionally thought to have relatively few ecological and toxicological side effects (Giesy et al. 2000). However, according to the recent literature, threats to the terrestrial environment may also occur, e.g., sublethal effects on the behaviors of arthropod predators (Michalková and Pekár 2009, Benamu et al. 2010, Evans et al. 2010, Griesinger et al. 2011), reduced activity and reproduction of earthworms (Santadino et al. 2014, Gaupp-Berghausen et al. 2015), and negative effects on the population parameters of lacewings (Schneider et al. 2009) and aphids (Saska et al. 2016). Commercial products containing glyphosate are apparently more toxic to nontarget organisms than the active ingredient alone, possibly due to the surfactants used in commercial products (Grossbard and Atkinson 1985). Druart et al. (2011) tracked glyphosate in the body of the land snail *Cornu aspersum* (Müller) (Gastropoda: Helicidae) and found that glyphosate can enter the food chain. Although some studies show sublethal effects on selected aspects of predator performance, such as movement, feeding behavior, or development (Michalková, and Pekár 2009, Benamu et al. 2010, Evans et al. 2010, Mirande et al. 2010, Griesinger et al. 2011, Wrinn et al. 2012), whether the biocontrol efficacy of natural enemies is affected by glyphosate treatment has not been investigated. This type of

investigation can be achieved with a detailed study of the demography of predators or parasitoids and their prey (Stark and Banks 2003, Stark et al. 2007) and with the measurement of consumption (Yu et al. 2013) at the same time. From a practical perspective, both population growth and feeding are important to measure simultaneously when the sublethal effects of pesticides on natural enemies are in concern (Desneux et al. 2007, Guedes et al. 2016).

Life tables are strong tools for this type of investigation (Croft 1990) because both acute short-term and long-term effects can be investigated simultaneously, with the results easily extrapolated to the population level. We are aware of only a single study that estimates the effect of glyphosate-treated prey on the population parameters of a predator. Schneider et al. (2009) examined the effects of ingestion of glyphosate-treated lepidopteran eggs on the development, fecundity, and demography of the lacewing *Chrysoperla externa* (Hagen) (Neuroptera: Chrysopidae) and found that feeding on contaminated eggs for 2 d during the third instar negatively influenced survival in the pupal and adult stages, number of eggs laid, and hatching success. Life table analysis revealed that feeding on herbicide-treated prey also shortened the reproductive period by ~60 d and that the intrinsic rate of increase and net reproductive rate of the population decreased by 41% and 94%, respectively, compared with controls (Schneider et al. 2009). Thus, despite no short-term effect on the mortality of *C. externa* larvae after feeding on treated prey, the pronounced negative effects were manifested on the population development of this species in the long-term (Schneider et al. 2009). As Schneider et al. (2009) did not examine how feeding on glyphosate-treated prey affected further consumption, i.e., the biocontrol potential of the surviving predators, and only stated that most of the predator larvae refused to feed on treated prey, the effects on the biocontrol potential after feeding on glyphosate-treated prey remain unexplored.

In this study, we examined the effects of prey contaminated with a glyphosate-based herbicide on the population development and biocontrol potential of an unspecialized aphid predator, *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae). We chose this predator and the aphid *Metopolophium dirhodum* (Walker) (Sternorrhyncha: Aphididae) as the study system because both species are cosmopolitan (Centre for Agriculture and Biosciences International [CABI] 2003, Koch 2003) and occur in cereal fields (Honěk et al. 1998, Nedvěd 2015), including fields of herbicide-tolerant cereals (P. Saska, personal observation) in which glyphosate is abundantly sprayed to control weeds. To evaluate the data, we used the advantages of the age–stage, two-sex life table theory (Chi and Liu 1985, Chi 1988), which integrates the life table and consumption data for a comprehensive assessment of the population development and biocontrol potential of the population. Unlike the traditional female-based life table, this life table approach uses both sexes, the variable developmental rates among individuals, and preadult mortality to describe the development, survival, stage differentiation, reproduction, and predation potential of the population more accurately; therefore, precise predictions of the sublethal effects of a pesticide on biocontrol of a species is provided. Consistent with the general perception, we hypothesized that the population parameters of *H. axyridis* and biocontrol potential would be negatively affected by consuming contaminated prey.

Materials and Methods

Aphid Prey

The aphid *Metopolophium dirhodum* is an oligophagous species (Holman 2009) for which parthenogenetic phases occur on leaves of all cereal crops worldwide ((CABI)(CABI)CABI 2003). In this experiment, we used a laboratory strain that is currently available in the collection of insect pests of the Crop Research Institute, Prague, Czech Republic. The aphids were maintained on young (stages 12–13, according to the Biologische Bundesanstalt, Bundessortenamt und Chemische Industrie [BBCH] scale) plants of winter wheat (*Triticum aestivum* L.) in a greenhouse with a controlled temperature (ca. 20 ± 2°C) and a natural photoperiod. Viviparous wingless or winged females are 2–3 mm long and develop through four instars. This strain of *M. dirhodum* was susceptible to the application of glyphosate-based herbicide (LC_{50} = 174.9 mmol dm^{-3} of the active ingredient [a.i.; CI_{95}: 153.0, 199.0]), with the demography negatively affected by increasing concentrations of the herbicide in both treated and filial generations (Saska et al. 2016).

Coccinellid Predator

Harmonia axyridis is currently the dominant lady beetle in the Czech Republic and occurs in various habitats, including agricultural settings (Nedvěd 2015). Reproduction occurs throughout the season (Koch 2003), making this species an ideal model for laboratory assays. *Harmonia axyridis* is polyphagous, but aphids constitute a sizeable portion of the larval and adult diets (Koch 2003). Adult beetles were collected on hedgerow vegetation untreated with any pesticides in Prague-Ruzyně, Czech Republic (50° 09′ N, 14° 30′ E), during April–May 2014. In the laboratory (climate room at 21 ± 1°C with a photoperiod of 16:8 [L:D] h), groups of 10 individual beetles were placed into plastic containers (15 by 20 by 8 cm) equipped with folded filter paper for oviposition. The stock individuals were fed a mixture of the aphids currently available in the same environment in which *H. axyridis* was collected. Egg batches laid within 24 h were removed from the stock and placed in controlled chambers (21°C and a photoperiod of 16:8 [L:D] h) until hatched. Because newly hatched larvae lingered on eggshells for ~24 h before they started searching for food (usual behavior for Coccinellidae; Hodek et al. 2012) and usually died quickly when removed from the clusters manually (J. Skuhrovec, P. Saska, and J. Lukáš, personal observation), only the newly dispersing larvae were used for life table experiments.

Herbicide Application

Roundup Aktiv (Monsanto, Antwerpen, Belgium) was used as the glyphosate-based herbicide. Roundup Aktiv is approved for use in all types of agricultural and horticultural conditions and for nonagricultural environments in the Czech Republic. This product contains 229 g of the isopropylamine (IPA) salt of glyphosate (molecular weight = 228.18 $g.mol^{-1}$) as the a.i. per liter of solution (1.004 $mol.dm^{-3}$). The experimental protocol followed that described in Saska et al. (2016). Three different molar concentrations of the herbicide (diluted in distilled water) were used in this experiment: 33.5 (low), 66.9 (intermediate), and 133.8 $mmol.dm^{-3}$ of a.i. (high). Pure distilled water was the control. These concentrations reflected the rates recommended for agricultural systems by the manufacturer (product leaflet; Monsanto 2015) after correction for the properties of the spraying device (sprayed area 0.011 m^2, partial adhesion of the aerosol to sides of the chamber). These rates are equivalent to 80, 160, and 320 ml of the commercial product diluted in 2 liters of water applied per 100 m^2 respectively, or 1.832, 3.664, and 7.328 kg of IPA-salt.ha^{-1}, respectively. The highest concentration used in this study was double the maximum recommended concentration, a situation that may occur in the field because of overlaps. The herbicide was applied on aphids using the auto-load Potter-Precision Laboratory Spray Tower (Burkard Scientific,

Uxbridge, United Kingdom) under a pressure of 3 Bars. Fifty N4 *M. dirhodum* were placed on filter paper in a petri dish, and the dish with aphids was positioned on the auto-load arm at the central bottom of the application chamber of the Potter tower. Each time, 2 ml of the solution (either distilled water or a solution of herbicide) was sprayed into the application chamber. The sprayed aphids were immediately presented to the L4 of *H. axyridis* without additional processing. Based on toxicity tests of the herbicide on the prey, most of the aphids in all treatments were expected to survive the application for 24 h because the chosen concentrations were below the LC_{50}; nevertheless, aphid survival declined with the increasing concentration of the herbicide (24-h mortality: 10.4% [low]; 22.2% [medium]; and 33.8% [high concentration]; Saska et al. 2016).

Experimental Setup

Larvae of *H. axyridis* hatched from an egg batch were distributed across the four treatments (control plus three herbicide concentrations), with a maximum of one or two individuals from the same egg batch used in each treatment. The larvae were kept individually in glass petri dishes (5 cm in diameter and 1 cm height) with filter paper on the bottom. Water was supplied by a piece of moist cotton wool. The number and stages (i.e., nymphal stages N1 to N4) of aphids supplied to each individual *H. axyridis* daily were 5–7 N2/N3 to L1, 10 N2/N3 to L2, 15 N3/N4 to L3, 25 N3/N4 to L4, and 40 N4 aphids to adults. Development and survival were monitored and the number of aphids remaining was counted for each individual daily. The aphids were also replenished to the initial number according to the lady beetle instar. Untreated aphids were used during the experiment, except for the one 24-h period when treated aphids were supplied to the L4 of *H. axyridis*, usually the second day after moulting. The L4 stage was selected for acute oral intoxication test because these larvae preferred larger aphids (N3, N4, or adults), which were more easily handled after herbicide application and were more tolerant of the physical aspect of the treatment (i.e., size and density of the droplets; J. Skuhrovec, J. Lukáš, and P. Saska, personal observation). These larvae were also the most voracious stage of larval development as measured by the daily consumption and duration of the feeding phase and therefore potentially ingested the most herbicide. When the larvae pupated, all remaining aphids were removed from the dish. The sex of the emerged adults was determined using labrum and prosternum coloration, recognizing that some individuals would be fully colored as late as 7 d after moulting to the adult stage (McCornack et al. 2007). Mating occurred when adults were 8–9 d in age and then again at 22–23 d, each time for 24 h, using randomly chosen mates from the same treatment. For each mating, different mates were selected. Following the time of the first mating, the fecundity (the number of laid eggs) was quantified daily. The egg batches were collected, transferred to the controlled chamber (conditions as described above), maintained separately, and monitored until hatched to determine the number of hatched eggs per batch and the hatch rate (the number of hatched/the number of laid eggs). In total, the experiment began with 56 L1 of *H. axyridis* per treatment, but the number of replicates used for analyses differed slightly among treatments because some individuals were lost or damaged during manipulations. The experiment was terminated after 95 d.

Data Analyses

Raw data for the survivorship, duration at each developmental stage, and daily female fecundity of individual insects were analyzed according to the age–stage, two-sex life table (Chi and Liu 1985, Chi 1988) using the computer program TWOSEX-MSChart (Chi 2015b). The number of hatched individuals was used as a measure of fecundity in this paper for life table analysis following Mou et al. (2015) because fertility virtually varied among treatments. Because we could not measure the duration of the development of the egg stage in experimental individuals because eggs were laid in clusters and removing eggs from clusters would result in the death of eggs, we assumed that the egg stage generally lasted 3 d, which was justified by measuring the duration of the development of ~100 egg batches of *H. axyridis* under identical light and temperature conditions (J. Skuhrovec and P. Saska unpublished data) and by using published data (Schanderl et al. 1985, Lamana and Miller 1998). Data on the daily predation rates were analyzed with the computer program CONSUME-MSChart (Chi 2015a) following Chi and Yang (2003) and Yu et al. (2013). The following life table and consumption parameters were calculated (x is age and j is the stage): age–stage-specific survival rate (s_{xj}), age-specific survival rate (l_x), age-specific fecundity (m_x), net reproductive rate (R_0), intrinsic rate of increase (r), finite rate of increase (λ), mean generation time (T), age-specific predation rate (k_x), age-specific net predation rate (q_x), net predation (C_0), and transformation ratio from prey population to predator offspring (Q_p; for methods of computation, see Supp. Data analysis [online only]). Bootstrap techniques (100,000 replications to generate less variable results) were used to estimate the variances and standard errors of the population parameters (Akca et al. 2015). Paired bootstrap tests were used to compare the differences in estimated reproduction and life table parameters among treatments (Mou et al. 2015) using TWOSEX-MSChart (Chi 2015b). The sex ratio was compared among treatments using a χ^2 test in the R statistical software package version 3.2.2 (R Development Team 2015).

Results

The average duration of the development of *H. axyridis* per stage and sex was similar across treatments (Suppl. Table 1 [online only]). Because the treatments did not differ until reaching the L4 stage, we statistically compared the duration of development for L4, pupa, and adults only, and no significant differences were detected in any of these stages (Table 1). Although the difference in longevity was up to 10 d among treatments, this variation was not significant based on a paired bootstrap test (Table 1). The age–stage-specific survival rates (s_{xj}; Fig. 1a) were also similar among treatments (Supp. Fig. 1 [online only]), and accordingly, no difference in the survival of L4 and pupa or during the entire preadult period was detected by paired bootstrap tests (Table 1). The only difference detected was for the adult stage, with more females than males at high and low concentrations, but vice versa in the medium treatment (Supp. Fig. 1 [online only]); however, based on the paired bootstrap test, a significant difference in the proportion of the female stage was detected only between the high and medium concentrations (Table 1). The adult sex ratios were not significantly different from the expected even distribution based on a χ^2 test ($\chi^2 = 4.461$, df = 4, $P = 0.347$).

We found no differences in fecundity among the treatments expressed either as laid or hatched eggs, although the hatch rate was significantly higher in the control than that in herbicide treatments (Table 1). Based on age-specific fecundity (m_x) and net maternity ($l_x m_x$), two egg-laying periods were identified for *H. axyridis* (Fig. 1b), and the temporal pattern in m_x and $l_x m_x$ varied among herbicide concentration (Supp. Fig. 2 [online only]). No obvious

Table 1. Reproduction and life table parameters of *H. axyridis* fed glyphosate-treated aphids (*M. dirhodum*)

Parameter	Glyphosate treatment[a]			
	Control	Low conc.	Intermediate conc.	High conc.
L4 (d)	6.49 ± 0.10	6.50 ± 0.10a	6.66 ± 0.10a	6.48 ± 0.12a
Pupa (d)	6.48 ± 0.09a	6.49 ± 0.09a	6.52 ± 0.11a	6.35 ± 0.08a
Adult (d)	56.97 ± 3.64a	61.63 ± 2.54a	64.18 ± 2.62a	61.48 ± 2.99a
Longevity (d)	57.88 ± 5.02a	61.58 ± 5.05a	62.22 ± 5.07a	67.72 ± 4.68a
L4 mortality (rate)	0.120 ± 0.002a	0.058 ± 0.001a	0.137 ± 0.002a	0.038 ± 0.001a
Pupal mortality (rate)	0.004 ± 0.003a	0.006 ± 0.003a	0.010 ± 0.004a	0.008 ± 0.004a
Total preadult mortality (rate)	0.340 ± 0.007a	0.327 ± 0.007a	0.353 ± 0.007a	0.245 ± 0.059a
Survival to female stage (rate)	0.340 ± 0.007ab	0.385 ± 0.007ab	0.255 ± 0.006a	0.472 ± 0.007b
Survival to male stage (rate)	0.320 ± 0.007a	0.288 ± 0.006a	0.392 ± 0.007a	0.283 ± 0.006a
Fecundity (laid eggs.female^{-1})	168.29 ± 25.85a	162.90 ± 18.40a	171.92 ± 29.67a	170.76 ± 22.94a
Fecundity (hatched eggs.female^{-1})	144.06 ± 23.87a	123.40 ± 15.63a	126.08 ± 23.31a	122.76 ± 18.14a
Hatch rate (%)	85.87 ± 0.02b	75.82 ± 0.02a	73.59 ± 0.04a	72.10 ± 0.03a
λ (d^{-1})	1.0814 ± 0.0096a	1.0812 ± 0.0085a	1.0802 ± 0.0099a	1.0790 ± 0.0074a
r (d^{-1})	0.0782 ± 0.0089a	0.0781 ± 0.0079a	0.0772 ± 0.0092a	0.0761 ± 0.0069a
R_0 (offspring.individual^{-1})	48.98 ± 12.50a	47.46 ± 10.22a	32.14 ± 9.63a	57.91 ± 11.95a
T (d)	49.74 ± 3.87ab	49.45 ± 3.56ab	44.97 ± 2.38a	53.36 ± 2.96b

Means ± SE of the parameters were estimated with bootstrapping (100,000 resamplings). The same letters within a row indicate treatments that were not significantly different based on a paired bootstrap test.

[a]Glyphosate was applied at three concentrations: low, 33.5; intermediate, 66.9; and high, 133.8 mmol.dm^{-3} of active ingredient. The control was distilled water.

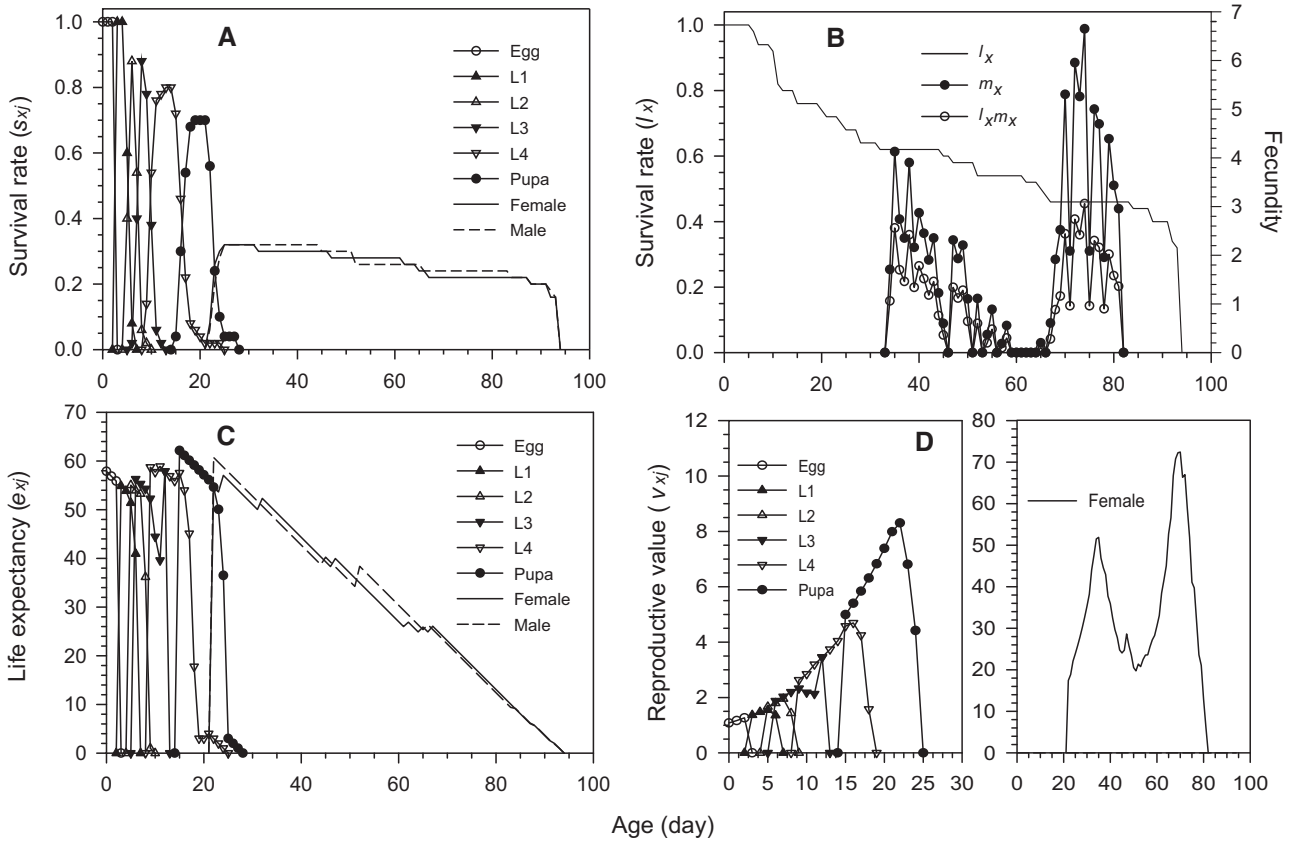

Fig. 1. Life table parameters of *H. axyridis* fed aphids (*M. dirhodum*) in the control treatment. (**a**) Age–stage survival rate (s_{xj}); (**b**) Age-specific survival rate (l_x), fecundity (m_x), and net maternity ($l_x m_x$); (**c**) Age–stage life expectancy (e_{xj}); (**d**) Age–stage reproductive value (v_{xj}).

differences in age–stage-specific life expectancy (e_{xj}; Fig. 1c) of *H. axyridis* were observed (Supp. Fig. 3 [online only]). The age–stage reproductive value (v_{xj}) was the highest at ~70 d of age in the control (Fig. 1d) and at low and high concentrations of herbicide (Supp. Fig. 4 [online only]); at the intermediate herbicide concentration, the age–stage reproductive value (v_{xj}) was only 30 d of age (Supp. Fig. 4 [online only]). Two-sex, age–stage life table analysis further indicated that oral uptake of the glyphosate-based herbicide

Table 2. Consumption parameters of *H. axyridis* fed glyphosate-treated aphids (*M. dirhodum*)

Parameter	Glyphosate treatment[a]			
	Control	Low conc.	Intermediate conc.	High conc.
Consumption L4 (prey)	116.2 ± 2.8a	116.6 ± 2.4a	122.6 ± 2.7a	120.0 ± 2.9a
Consumption adult (prey)	1783.1 ± 108.9a	1954.1 ± 70.7a	2007.8 ± 75.7a	1935.0 ± 94.7a
C_0 (prey)	1327.9 ± 144.2a	1466.9 ± 142.5a	1465.2 ± 146.7a	1624.3 ± 138.5a
ω (prey. viable egg^{-1})	10.63 ± 0.43a	10.92 ± 0.42a	11.31 ± 0.37a	11.57 ± 0.37a
ψ (prey)	9.83 ± 0.37a	10.10 ± 0.36a	10.47 ± 0.31a	10.72 ± 0.32a
Q_p (prey.d^{-1})	27.11 ± 7.52a	30.91 ± 6.69a	45.59 ± 18.30a	28.05 ± 5.74a

Means ± SEs of the parameters were estimated with bootstrapping (100,000 resamplings).

The same letters within a row indicate treatments that were not significantly different based on a paired bootstrap test.

[a]Glyphosate applied at three concentrations: low, 33.5; intermediate, 66.9; and high, 133.8 mmol.dm^{-3} of active ingredient. The control was distilled water.

at any of the three concentrations used in this study did not significantly affect the estimated life table parameters of *H. axyridis* (r, R_0, λ), with the exception of the mean generation time (T), which decreased significantly at the intermediate concentration compared with the high concentration treatment (Table 1) based on a bootstrap test. Therefore, pulse feeding on glyphosate-treated prey by the L4 stage of *H. axyridis* did not cause clear and consistent long-term sublethal effects in this study.

All L4 larvae of *H. axyridis* accepted glyphosate-treated aphids as food in similar numbers to those of untreated ones on days before and after exposure to treated aphids (Supp. Table 2 [online only]). The consumption of aphids by L4 and adults of *H. axyridis* did not differ significantly among the treatments; the mean total consumption was also not significantly different (Table 2). This can be observed in the plots of the age–stage predation rate (c_{xj}; Fig. 2a), predation rate (k_x), and net predation rate (q_x; Fig. 2b; Supp. Figs. 5 and 6 [online only]). Although the stage-specific predation rate of *H. axyridis* is the highest for adult stage, the age–stage-specific predation (Fig. 2c) indicates that a L4 larva consumes more aphids per day than adults (Supp. Fig. 7 [online only]). The predation potential of the *H. axyridis* population remained unaffected by the pulse consumption of prey treated with glyphosate-based herbicides under the conditions of this experiment because for the estimated consumption parameters (ω, ψ, and Q_p), no indication of significant difference was detected among the treatments.

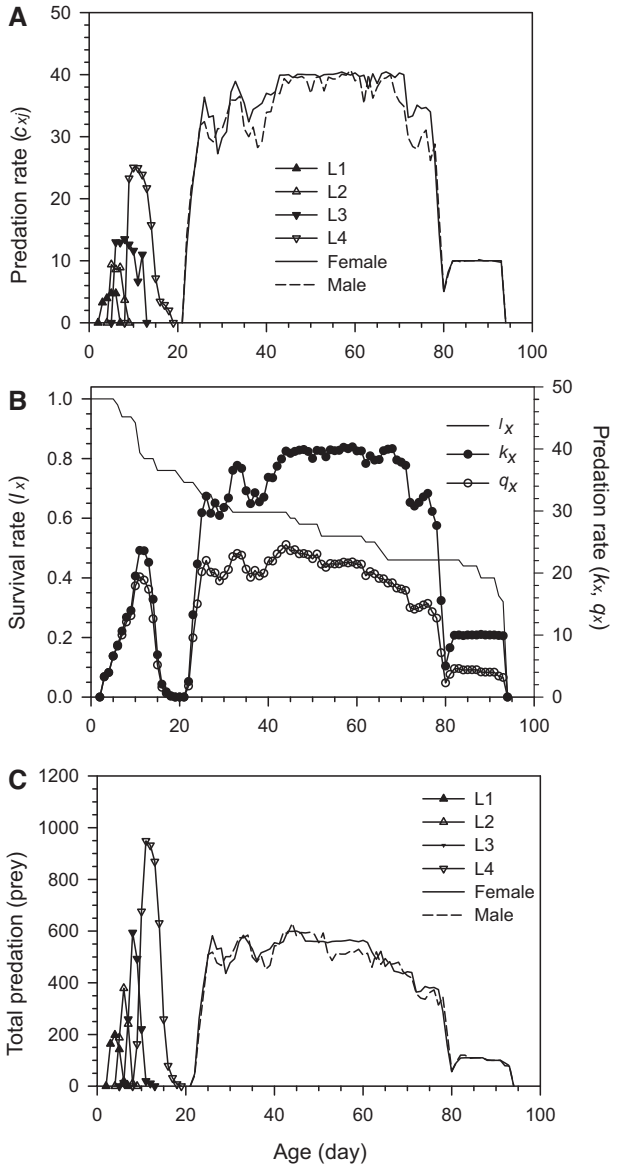

Fig. 2. Consumption parameters of *H. axyridis* fed aphids (*M. dirhodum*) in the control treatment. (**a**) Age–stage predation rate (c_{xj}); (**b**) Age-specific survival rate (l_x), predation rate (k_x), and net predation rate (q_x); (**c**) Age-specific total predation.

Discussion

Contrary to expectations, we found that consuming glyphosate-treated aphids in the fourth instar for 24 h had only minor effects on the generation time and fertility of this predator, and no significant effects of the herbicide on development, population, and consumption parameters were observed. Thus, the feeding of *H. axyridis* larvae on aphids treated with three different concentrations of a glyphosate-based herbicide for a short period altered neither the population biology nor reduced the biocontrol potential of *H. axyridis* under this study design.

The results of our study are incompatible with those of the only other study that applied a two-sex life table approach to assess the effects of a glyphosate-based herbicide on the population biology of a predatory insect (Schneider et al. 2009). Although both Chrysopidae and Coccinellidae are generally relatively tolerant to many pesticides (Bartlett 1964, Grafton-Cardwell and Hoy 1985, Michaud 2002, Benelli et al. 2015), we cannot dismiss the option that the different responses to glyphosate treatment between lacewings and lady beetles were due to the phylogenetic distance between the two taxa or biological differences between the two species. However, this possibility could only be tested if representatives of the two taxa were subjected to the same experimental design; this

was not, however, the aim of this study. Because the two studies differed in herbicide formulation, the method of herbicide application, and exposure time, we consider plausible to attribute disparity in results to methodological differences. These are discussed below.

(i) Herbicide formulation. Surfactants added to commercial solutions can be more harmful than glyphosate itself (Grossbard and Atkinson 1985), and the two studies used two different commercial products (Glifoglex 48 [Glaba, La Plata, Argentina] and Roundup Aktiv) to create the experimental solutions. Different but unknown (to the users) compositions of additives and adjuvants in the two products might be at least partly responsible for the contrasting results. Additionally, any difference might have been significantly increased when the extra surfactant was added to solutions in the study of Schneider et al. (2009), to increase the attachment of the herbicide to the egg chorion.

(ii) Method of application. Schneider et al. (2009) found detrimental effects of the glyphosate-based herbicide when the prey (lepidopteran eggs) was dipped into herbicide, so the eggs were entirely covered by the herbicide solution, a scenario that is unlikely to occur in the field and that might have overestimated the treatment effect. In the present study, we used a Potter tower to spray the solution of herbicide (no supplemental surfactant was added) with droplets of herbicide falling on top of the aphids, which is more likely to be similar to the general application of pesticides in the field. That this method efficiently transferred the herbicide to/into the aphids was proven by Saska et al. (2016) because a single exposure to herbicide in the tower caused a significant decrease in most life table and population parameters in both treated and filial generations of *M. dirhodum* (Saska et al. 2016). Apparently, aphids offered to *H. axyridis* contained the herbicide in physiologically significant amounts, which caused harmful effects to aphids at the population level (Saska et al. 2016), but not to fourth-instar coccinellid larvae when fed on the treated aphid.

(iii) Exposure time. The exposure time of the larvae of the ultimate instar to contaminated prey was different between the two studies. Schneider et al. (2009) presented treated prey for 48 h, whereas in this study, aphids were available for 24 h. Although this difference is minor relative to the entire life span of the predatory phase of these species, it is possible we would have observed a more pronounced effect if *H. axyridis* had been continuously exposed to treated prey. We are aware that a single exposure to treated prey for 24 h as in this study or for 48 h as in Schneider et al. (2009) was not completely consistent with the situation in the field. However, providing a continuous supply of treated aphids to the lady beetles (i.e., testing chronic intoxication) would have been difficult to organize under laboratory conditions. In this experiment, ~250,000 aphids were supplied to *H. axyridis*. Given that a single application is the recommended practice for glyphosate, most of the aphids would have to be sprayed and maintained separately for many days to mimic the field conditions more closely. Moreover, short-term mortality occurs and the population development of treated aphids is retarded after glyphosate treatment (Saska et al. 2016), which would require even more aphids to be treated to assure ad libitum aphid supply for *H. axyridis*. Therefore, such a design would not be achievable, and the single exposure to treated aphids provided a way to circumvent this problem and address the research question.

The impact of pesticides on biocontrol potential of a natural enemy is an important but largely overlooked aspect in insect ecotoxicological studies (Desneux et al. 2007, Guedes et al. 2016). In this study, the biocontrol potential of the *H. axyridis* population was not affected by pulse feeding on contaminated prey in the larval stage. First, the contaminated aphids did not deter *H. axyridis* larvae and practically all of the treated aphids were devoured within one day. This response was in contrast to that of *C. externa* larvae, which largely rejected herbicide-treated prey (Schneider et al. 2009). Second, the single exposure of the L4 to treated aphids did not alter the consumption later in the same stage or during adulthood. Thus, for the first time, our experiment documented that acute oral intoxication by the glyphosate-based herbicide, even at double the recommended concentration, did not affect the biocontrol potential of *H. axyridis*. The values for stage-specific consumption also demonstrated that the population of L4 consumed more aphids per day than adult populations, which highlighted the importance of immature predatory stages for biological control and in food webs in general.

We admit that our laboratory study did not cover the entire complexity of the field situation. Negative effects on demography and consumption might have been observed if earlier instars had been fed treated prey because earlier instars of *H. axyridis* are more susceptible to some pesticides than older ones (Ahn et al. 2001, Youn et al. 2003). However, conducting an experiment using early instars was impracticable, because younger instars are more vulnerable and also require smaller aphids to feed, which are also more sensitive to the physical aspects of sprayed herbicide application. Additionally, the general perception is that residual contact (i.e., tarsal absorption of pesticide that remains on surfaces) is more detrimental to natural enemies than oral uptake (Croft 1990, Pekár 2012), including for *H. axyridis* (Galvan et al. 2006), which was not measured in this study. Therefore, we cannot fully exclude an option that the effect of the combined action of residual contact and oral uptake, which is the situation most likely encountered in the field, would occur in case of *H. axyridis*. However, we are not aware of any study that has investigated such combined action on life table parameters and biocontrol potential.

The life history data collected in our study are comparable with those available in the literature. The duration of development for all stages was within the ranges previously published (Schanderl et al. 1985, Lamana and Miller 1998, Lanzoni et al. 2004), which indicated that the rearing conditions in our experiment were suitable for this species. By contrast, the fecundity (~120–140 hatched or 170 laid eggs per female) observed in this study was notably lower than those values reported previously in the literature. The most commonly reported values are 500–2,000 eggs per female, or even more (Stathas et al. 2001, Lanzoni et al. 2004, Zeki et al. 2015). This difference might be due to interpopulation differences or because females mated only twice in our experiment (because we also measured individual aphid consumption), whereas in other studies, females could mate ad libitum. The two peaks of reproduction observed in this study might be an artefact of that frequency of mating. Life table parameters of *H. axyridis* were estimated previously (Lanzoni et al. 2004, Zeki et al. 2015); however, because these parameters were calculated using female-based life tables, the parameters estimated by those studies are not comparable with those presented here (Huang and Chi 2012). Thus, our study provides the first age–stage, two-sex life table analysis of *H. axyridis*.

Compared with the age–stage, two-sex life table used in this paper, conventional approaches and statistical analyses may produce different results and conclusions on the effects of a glyphosate-based herbicide, even when using subsets of the same data set. For example, we point to the different results that were obtained from the χ^2 test on the sex ratio (not significantly different from the expected even distribution in all treatments) and the paired bootstrap test on the proportion of adult males and females originating from the initial cohort (significant differences in the proportion of females

between intermediate and high concentrations of herbicide). Thus, when sublethal effects of pesticides are the focus of study, an age–stage, two-sex life table provides a more complete evaluation of the research questions related to the population biology of a species.

Although the results of our study did not support the initial hypothesis that treatment of aphids, the prey of *H. axyridis*, with a glyphosate-based herbicide would have negative effects on the demography and biocontrol potential of this coccinellid aphid predator, we recognize that additional studies on the sublethal effects of herbicides on nontarget organisms are required. Biological and ecological interactions are complex, even in simplified systems, such as agricultural crops, and it is in the best interest of the public to understand how pesticides applied during agricultural production modify the roles of individual components of natural food webs and, importantly, those of natural enemies of pests.

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Supplementary Materials

Supplementary data are available at *Journal of Economic Entomology* online.

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