



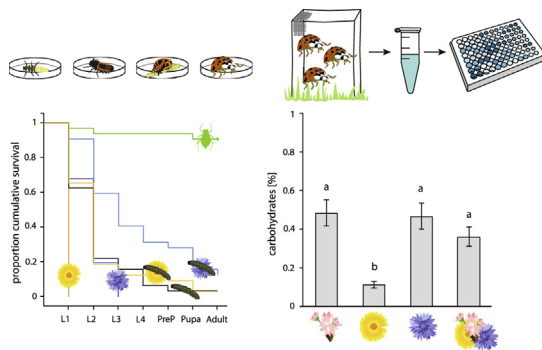
Utilization of plant-derived food sources from annual flower strips by the invasive harlequin ladybird *Harmonia axyridis*

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GRAPHICAL ABSTRACT



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ABSTRACT

The ability to utilize plant-derived food sources and suboptimal prey when the main prey is scarce may enhance competitiveness and invasiveness of entomophagous species such as *Harmonia axyridis*. Alternative food sources are particularly abundant in flower strips and other agri-environment schemes to promote biodiversity and may thus also benefit the invasive species. We investigated the effects of alternative food sources on the development and reproduction of *H. axyridis*. Laboratory experiments demonstrated that larvae of *H. axyridis* developed into adults and produced offspring when reared solely on aphids, lepidopteran eggs or maize pollen but not when they were fed only lepidopteran caterpillars or buckwheat flowers. When fed a combination of the latter two suboptimal food sources, however, some *H. axyridis* larvae developed into fertile adults. Flowering plant species differed in their food quality to sustain ladybird survival and development when fed alone or in combination with suboptimal prey. Differences in food quality of flower species were confirmed in field-cage studies where newly emerged adults were exposed for six days to different plant species and their energetic compartments were analyzed subsequently. Overall *Fagopyrum esculentum* and *Centaurea cyanus* provided a higher food quality than *Calendula arvensis* in those experiments and mixing flower species did not provide an additional benefit. The results show that the harlequin ladybird can sustain itself not only on optimal prey, but also utilize alternative, animal- and plant-derived diets. This could provide *H. axyridis* a competitive advantage over those native ladybird species that depend on aphids for their reproduction.

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1. Introduction

The predacious harlequin ladybird *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae) is native to central and eastern Asia (Dobzhansky, 1933). It has been imported and released for aphid biological control in the United States as early as in 1916 (Gordon, 1985) and in Western Europe first in 1990 (Coutanceau, 2006). After a time lag of several years the species established and spread rapidly with its current distribution including North and South America, Africa, and Europe (Brown et al., 2008; Roy et al., 2016). *H. axyridis* is a strong competitor and populations of several native coccinellid species have markedly declined since its arrival (Brown et al., 2011; Roy et al., 2016) although in certain regions declines began before the arrival of *H. axyridis* (Honek et al., 2016).

One factor for the success of the harlequin ladybird seems to be its particularly wide dietary range, compared to many other ladybird species (Roy et al., 2006). While many primarily aphidophagous ladybird species also accept alternative prey such as lepidopteran and coleopteran larvae, the suitability of the alternative food sources varies greatly among prey and ladybird species (Evans, 2009). Furthermore, plant derived food sources such as pollen, floral and extra floral nectar, fruits and even foliage can be utilized to different extents as additional or alternative food sources (Berkvens et al., 2010; Lundgren 2009a,b). These plant-derived food sources can be used to build up energy reserves before hibernation (Ricci et al., 2005) or to survive when prey is scarce (Lundgren, 2009a). Those situations occur frequently as e.g. aphid populations fluctuate strongly due to weather conditions or due to the fact that they have been exploited by other natural enemies (Hodek & Michaud, 2008). However, only a few species of aphidophagous ladybirds such as *Coleomegilla maculata* De Geer and *H. axyridis* are able to complete development solely on plant food sources (Berkvens et al., 2008; Lundgren & Wiedenmann, 2004). For those ladybirds, and in particular their less mobile larvae, the ability to utilize plant-derived food sources results in a strong competitive advantage.

While a large proportion of the Central European land cover is characterized by managed agricultural ecosystems, many European countries have implemented agri-environmental schemes that foster the establishment of semi-natural habitats for the provision of additional resources to enhance biodiversity. Within these habitats different forms of sown flower strips are increasingly being established (Jacot et al., 2007; Marshall & Moonen, 2002). A number of studies found that insect abundance in such flower strips is higher than in the crop habitat (reviewed in Haaland et al., 2011) and studies from the UK (Ramsden et al., 2015) and from Switzerland (Tschumi et al., 2014) reported high numbers of coccinellids in flower strips. While this is desired for native species, flower strips may at the same time also provide resources for the invasive *H. axyridis*. In fact, *H. axyridis* has been observed as the second most abundant species (after *Propylea quatuordecimpunctata* L.) in several flower strips in Switzerland (Tschumi and Albrecht, personal communication) and in Belgium (Hatt et al., 2017).

Thus, the present study aims to investigate whether and to what extent *H. axyridis* can profit from these additional food sources by unraveling the fitness consequences that result from the utilization of several floral resources commonly found in flower strips. In particular we wanted to i) assess optimal and suboptimal food sources for development of *H. axyridis* and determine their influence on larval fitness, ii) test whether suboptimal food sources would increase in value for *H. axyridis* larvae by dietary mixing, iii) evaluate the nutritional quality of different floral food sources for development of *H. axyridis* and iv) determine if utilization of different floral food sources differs between larvae and adult *H. axyridis*. We conducted laboratory experiments to investigate the utilization of plant-derived food sources by *H. axyridis* larvae and a semi-field experiment with adults to assess the influence of the floral resources on the adult beetles' energetic budget. The results would help to assess whether flower strips could provide *H. axyridis* with an additional competitive advantage when compared to native

species.

2. Material and methods

2.1. Insect material

Adult *H. axyridis* were collected around Zurich, Switzerland in 2013 and reared in 1.8 L plastic containers to establish a breeding colony. They were fed with eggs of *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) (Biotop, Valbonne, France). Egg batches of *H. axyridis* were regularly removed and the hatching larvae were used for the experiments.

Spodoptera littoralis (Boisduval) (Lepidoptera: Noctuidae) (provided as eggs by Syngenta Crop Protection Munchwilten AG, Stein, Switzerland) were reared on *Fagopyrum esculentum* (buckwheat) leaves until they reached a suitable size to be fed to *H. axyridis* (larval stage 1–4 according to the size of *H. axyridis* larvae). *Acyrtosiphon pisum* Harris (Hemiptera: Aphididae) were used from a long term laboratory culture kept at Agroscope on common bean plants (*Vicia faba*) and were fed to *H. axyridis* as mixed stages.

2.2. Plant material

Plants were chosen according to observed positive effects on natural enemies and common presence in flower strips (Tschumi et al., 2016). Seeds (Fenaco Genossenschaft Bern, Switzerland) were either sown into 6 L pots without fertilizer in a greenhouse or directly into the field (semi-field experiment). For laboratory experiments flowers from *F. esculentum*, *Papaver rhoeas* (poppy), *Calendula arvensis* (field marigold), *Sinapis arvensis* (mustard), and *Centaurea cyanus* (cornflower) were grown. Newly opened flowers were cut daily, placed with the stalk into an Eppendorf vial with water and fixed with cotton wool to be used in the experiments. *F. esculentum* and *S. arvensis* flowers both possess a corolla with broad aperture and low to intermediate depth and therefore their nectar and pollen should be well accessible for the ladybirds (Vattala et al., 2006). *Centaurea cyanus* provides easy accessible food due to the presence of extrafloral nectaries, as does *P. rhoeas*, which produces abundant pollen (Bosch et al., 1997). In comparison, *C. arvensis* is a composite with small tubular florets that could be less accessible to the ladybird larvae even though their strong biting mandibles might allow them to reach floral resources by destruction of the flower structure.

Maize plants (*Zea mays* var. Gavott, KWS Saat GmbH, Einbeck, Germany) were grown individually in 12 L plastic pots with 40 g of slow release fertilizer (Osmocote Exact, 16% N, 11% P₂O₅, 11% K₂O, Scotts UK Professional, Bramford, UK). When plants had reached the three leaf stage liquid fertilizer (0.4 L of 0.2% Vegesan standard; Hauert HBG Dünger AG, Grossaffoltern, Switzerland) was added once per week. To collect pollen, air-permeable cellophane bags (19.5 × 37.5 cm, Celloclair AG, Liestal, Switzerland) were clipped over the inflorescences and pollen was collected daily by cutting a small hole into the bottom of each bag. The pollen was passed through a fine mesh (0.2 mm) and dried at room temperature for 1 d before storage in a freezer (−23 °C). Prior to feeding-experiments the pollen was kept for 24 h in a plastic box with saturated humidity.

2.3. Laboratory experiments

The suitability of different food sources for the development and survival of *H. axyridis* larvae was determined in three separate laboratory experiments. The experiments were run in a climate chamber at 24 °C, 75% RH and 16:8h light:dark photoperiodic conditions. The larvae were kept separately in small containers (6 cm dia., 8.5 cm height) and fed *ad libitum* with the respective food source. In addition, a small piece (1.2 × 1.2 cm) of *F. esculentum* leaf (except experiment 3) and a drop of water were added. Larvae were checked daily and their

developmental stage was noted. Adult beetles were weighed within 24 h after emergence and their sex was determined.

2.3.1. Experiment 1. Single food sources

To identify optimal and suboptimal food sources for development of *H. axyridis* and to determine their influence on larval fitness, larvae of *H. axyridis* were fed different single food sources: Aphids (*A. pisum*) were chosen as a natural prey of *H. axyridis* (Specty et al., 2003) and lepidopteran eggs (*E. kuehniella*) as a similarly suitable substitute commonly used in the rearing of predatory arthropods (Berkvens et al., 2008). Other potential food sources from flower strips are *F. esculentum* flowers, caterpillars and pollen. From the latter two *S. littoralis* and maize pollen were readily available and found to be passably suitable during preliminary assays and thus these were included in the assay. The provision of water only was used as a control treatment.

The experiment was conducted in three independent runs. In each run, 25 larvae were tested, resulting in a total sample size of 75 larvae per treatment. For each run, 25 different egg batches were taken out of the *H. axyridis* rearing colony, and one larvae (6–24 h after emergence) of each batch was assigned randomly to one of the food treatments.

2.3.2. Experiment 2. Combined food sources

To test whether suboptimal food sources would increase in value for *H. axyridis* larvae by dietary mixing, the following food sources were tested: *A. pisum*, maize pollen, or *S. littoralis* alone, and combinations of *S. littoralis* either with *F. esculentum* flowers or with maize pollen. The experiment was run as described above, but for each of the three runs 28 larvae (derived from different egg batches) were used per treatment resulting in a total sample size of 84 larvae per treatment. In addition to the standard life-table parameters, freshly emerged (< 24 h) pairs from each treatment (n = 11–29) were kept in 250 mL plastic containers equipped with a piece of cotton tissue as oviposition substrate and fed *ad libitum* with *E. kuehniella* eggs. Each pair was checked daily to avoid cannibalism by emerging larvae, eggs were counted and removed and egg batches kept until hatching. The experiment was terminated for each pair 21 days after laying of the first egg batch.

2.3.3. Experiment 3. Different floral food sources

To evaluate the nutritional quality of different flowers as food sources for development of *H. axyridis* the following flowers were tested either alone or in combination with *S. littoralis* caterpillars: *P. rhoeas*, *C. arvensis*, *S. arvensis* and *C. cyanus*. Two control treatments were run with *A. pisum* or *S. littoralis* as sole food source. This experiment was done in four runs, each using eight larvae (derived from different egg batches) per food treatment resulting in a total sample size of 32 larvae per treatment.

2.4. Semi-field experiment

To determine the suitability of flowers as a food source for adult *H. axyridis*, a semi-field experiment was conducted with five different treatments: one each with flowers from *F. esculentum*, *C. arvensis*, or *C. cyanus*, one with all three flowers together (mixture), and one with no flowering plants (control). The field was divided into plots of 1.5 × 1.5 m that were randomly assigned to one of the treatments and sown respectively, control plots were not managed and from the spontaneously growing grass and weed vegetation all flowering plants were removed manually. Nine plots were prepared for each treatment and divided into three consecutive sowing occasions 14 days apart. That enabled three runs (starting on 26.6., 16.7. and 29.7.2014, respectively), each containing three plots per treatment. Flight cages (1.5 × 1.5 × 2 m, 0.74 × 1.17 mm mesh size) were placed over the plots. At least four days before introduction of the ladybirds the plants inside the flight cages were treated with Pirimor (0.5 g in 1 L water) to remove aphids as unwanted arthropod food source. 45–52 freshly emerged *H. axyridis* adults were introduced into each flight cage when

the plants were flowering. The ladybirds had been reared on *E. kuehniella* eggs in the laboratory and resulted from at least 50 egg batches per run. The ladybirds to be introduced into a flight cage were weighed together and the average weight per beetle was calculated. All beetles were left in the flight cages for six days. Then they were re-collected during 15 min per flight cage once in the morning and once in the afternoon. Collected beetles were cooled and brought immediately into the laboratory where all beetles of one cage were weighed together and the average weight per beetle was calculated.

After the six days of field exposition, five males and females per flight cage were paired and fed with *E. kuehniella* eggs *ad libitum*. Pairs were checked every other day and egg batches were removed until 14 days after the first oviposition. A few instances of egg cannibalism from emerged larvae were visible under these conditions. In these cases the number of eggs was estimated by the remainders of eggs on the cotton tissue. The days until first oviposition as well as the number of eggs and hatched larvae per pair were recorded. The remaining ladybirds were frozen at –23 °C for subsequent analysis of the energy budget. As an additional control, 21 freshly emerged adults of the same egg batches as above were fed *E. kuehniella* eggs for six days in the laboratory and were then frozen for analysis of the energy budget.

2.5. Energy budget analyses

To determine differences in the utilization of flowering plants as food source for adult *H. axyridis*, the energy budget of individual females recollected from the field cages was analyzed. Thereby the contents of the different energy compartments were taken as a proxy for the fitness of adult ladybirds, i.e., their ability to survive and overwinter (lipids), to walk and fly (carbohydrates) and to reproduce (protein). The amount of carbohydrates, proteins, lipids and glycogen for each individual was assessed following a modified protocol developed for small hymenopterans by Foray et al. (2012).

Insect samples were weighed, homogenized in 360 µL of the aqueous lysis buffer solution and centrifuged. For protein determination 5 µL of the resulting supernatant was diluted 1:10, then 5 µL of the dilutions were mixed with 250 µL of Bradford micro-assay reagent (B6916; Sigma, France). 40 µL of sodium sulphate solution and 5 µL of extraction buffer solution were added to the homogenate to dissolve the carbohydrates. 3000 µL of chloroform–methanol mixture was added to solubilize the total lipids and water-soluble carbohydrates. 5 mL Eppendorf tubes were used to comprise the larger volumes of samples. Samples were centrifuged and the pellet kept for glycogen analysis. A hot anthrone reaction with 240 µL of anthrone reagent and 150 µL of supernatant was used to prepare the total water-soluble carbohydrates. Lipids were assayed using 100 µL of the supernatant, 10 µL sulphuric acid and 190 µL of vanillin reagent. Finally glycogen was analysed after washing the pellet twice, using 2 × 800 µL of methanol, centrifuging the samples and incubating the pellet with 2000 µL of anthrone reagent. Determination of energetic compartments was done spectrophotometrically by reading the absorbances (protein: 595 nm; carbohydrates and glycogen: 620 nm; lipids: 492 nm) of the final solutions in a plate reader (SpectraFluor Plus, Tecan).

All colorimetric assays were done in 96-well plates that contained 2 samples from each 5 females (from the same cage) per treatment, and 5 independent repeats of dilution series for the standard curves. In addition each two samples of 0–5 females that had been fed with *E. kuehniella* eggs in the laboratory for six days were added to each plate and four wells were kept as negative controls, i.e. running through the extraction procedure without containing insect tissue. The standard curves (protein: bovine serum albumin (A7030, Sigma-Aldrich); carbohydrates and glycogen: D-Glucose (Fluka 49140); lipids: Triolein (92860, Sigma-Aldrich)) contained 5–7 dilution steps. The mean of the repeats was used to calculate the sample concentrations. It was checked that the mean standard curves fitted with a linear regression (range of R²: protein curves = 0.98–0.99; carbohydrate curves = 0.93–0.99;

lipid curves = 0.93–0.99). The mean value of the two samples per beetle was used for the analysis. Absorbance values of samples that laid outside the range of the standard curve were capped to the highest value from the standard curve. Means of the different energy compartments are given as percent fresh body weight.

2.6. Statistical analyses

Differences in survival over developmental stages were tested with Mantel Cox log rank tests aggregated over levels (i.e. overall effect of treatment) and in pairwise comparisons using the software SPSS (IBM, Version 23; www.ibm.com). All other analyses were performed in R 3.3.3 (R Core Team, 2017).

All dependent variables were analysed for the influence factors “run”, “food” and the interaction of these two factors. To test for the effects of those factors on development time in laboratory experiments a generalized linear model (GLM) with Poisson error distribution was applied. The weight of female and male *H. axyridis* was analysed applying a GLM with gamma error distribution to account for non-normality of data. Due to overdispersion in egg counts when assuming Poisson error distribution (derived from the ratio of residual deviance per degrees of freedom), the number of eggs laid by *H. axyridis* that had been raised on different food sources was modelled with negative binomial error distribution using the `glm.nb` function of the MASS package (Venables & Ripley, 2002).

The energetic compartments protein, lipids, carbohydrates and glycogen were modelled in linear mixed-effects models using the `nlme` package (Pinheiro et al., 2017) and applying cage as a random factor. All final models were achieved by stepwise exclusion of non-significant influence factors from the models by analysis of deviance. Multiple comparisons of the influence factor food were done on the final models by Tukey post hoc tests using the `multcomp` package (Hothorn et al., 2008). Model assumptions were checked according to graphical validation procedures and amount of residual deviance per degree of freedom. Preoviposition period, weight change and number of eggs laid by *H. axyridis* recovered from the field were analysed with analysis of variance after pooling values obtained from each field-cage by using the mean of all beetles (min. 3 max. 5) per cage. Multiple comparisons of the influence factor food were done on the final models by Tukey post hoc tests. Model assumptions were checked by QQ-plots using the package `car` (Fox & Weisberg, 2011) and Levene’s test for homogeneity of variance.

3. Results

3.1. Experiment 1. Single food sources

Food had a significant influence on the survival of *H. axyridis* larvae ($X^2 = 433.64$; $p < 0.001$). When *H. axyridis* larvae were fed with *A. pisum* or *E. kuehniella* eggs, most of them (> 92%) survived, i.e., pupated, emerged as adults and survived for 24 h (Fig. 1A). About 50% of the larvae fed with maize pollen also reached adulthood, while only three larvae fed with *S. littoralis* lived that long and none that was fed with *F. esculentum* flowers or water. Development time was significantly influenced by food ($X^2 = 134.90$; $p < 0.001$) with longer development times in maize pollen-fed larvae compared to *A. pisum*- or *S. littoralis* fed larvae (Table 1). The factor run ($X^2 = 3.42$; $p = 0.18$) and the interaction of run and food ($X^2 = 4.13$; $p = 0.39$) were not significant. Food also had a significant influence on fresh weight at emergence of female ($X^2 = 452.34$; $p < 0.001$) and male ($X^2 = 320.68$; $p < 0.001$) *H. axyridis* (Table 1). Furthermore female ($X^2 = 9.58$; $p = 0.008$), but not male weight ($X^2 = 1.76$; $p = 0.42$), was influenced by the factor run. No interaction between the factors food and run was found for either sex (female: $X^2 = 0.88$; $p = 0.93$; male: $X^2 = 5.30$; $p = 0.26$). Individuals that were fed exclusively with maize pollen were significantly lighter than those fed with *A. pisum* or *E. kuehniella* eggs (Table 1).

3.2. Experiment 2. Combined food sources

If food sources were combined, food significantly influenced survival ($X^2 = 272.63$; $p < 0.001$) (Fig. 1B). From the combined food sources (*S. littoralis* + maize pollen or *S. littoralis* + *F. esculentum* flowers) 55 and 67% of *H. axyridis* larvae, respectively, reached adulthood. Food significantly affected development time ($X^2 = 49.25$; $p < 0.001$) with larvae feeding on maize pollen taking significantly longer to emerge than those feeding on aphids or the combined food sources. Significant differences were also obtained for adult weight (Table 1; females: $X^2 = 174.93$; $p < 0.001$; males: $X^2 = 225.94$; $p < 0.001$), with adults resulting from the maize pollen treatment being significantly lighter than all other beetles and beetles from *A. pisum* being significantly heavier than all other beetles. Run had a significant effect on male weight ($X^2 = 6.03$; $p = 0.049$), but not on female weight ($X^2 = 5.35$, $p = 0.07$), and developmental time ($X^2 = 1.43$; $p = 0.49$). No interaction between run and food was found for either developmental time ($X^2 = 1.60$; $p = 0.95$) or adult weight (female: $X^2 = 7.88$; $p = 0.25$, male: $X^2 = 6.50$; $p = 0.37$).

Pairs of *H. axyridis* derived from all food sources that had supported larval development were able to lay fertile eggs but the number of eggs laid did not differ significantly between food sources ($X^2 = 2.61$; $p = 0.46$). There was no significant effect of run ($X^2 = 3.85$; $p = 0.15$) or the interaction of run and food ($X^2 = 3.40$; $p = 0.76$) on the number of eggs laid.

3.3. Experiment 3. Different floral food sources

None of the *H. axyridis* larvae fed with flowers only reached adulthood (Fig. 2A). However, food significantly influenced survival ($X^2 = 51.69$; $p < 0.001$), with *S. arvensis* and *C. cyanus* flowers supporting development of the larvae to a similar extent as *S. littoralis* caterpillars, while larvae on *P. rhoeas* or *C. arvensis* flowers died earlier. When flowers were combined with *S. littoralis*, some larvae of all combinations reached the pupal or adult stage (Fig. 2B). Flower species significantly influenced survival ($X^2 = 510.0$; $p = 0.019$), whereby the combination of *C. cyanus* with *S. littoralis* supported larval development significantly better than combinations with any of the other flowers.

3.4. Semi-field experiments

Between 50 and 98% of the *H. axyridis* adults were re-collected from each field cage. In all treatments beetles had lost weight during field exposition (Table 2). A significant influence on the amount of weight loss was detected for food source ($F_{4,38} = 9.55$; $p < 0.001$) and run ($F_{2,38} = 15.80$; $p < 0.001$), but not for their interaction ($F_{8,30} = 0.86$; $p = 0.56$).

Beetles from control cages without flowering plants had lost significantly more weight than beetles collected from cages containing *F. esculentum*, *C. cyanus*, or a mixture of flowering plants. The pre-oviposition period of beetles that were fed *E. kuehniella* eggs after field exposure was significantly influenced by food source ($F_{4,38} = 4.59$; $p = 0.004$) and by run ($F_{2,38} = 3.52$; $p = 0.039$) (Table 2). The interaction of the factors run and food was not significant ($F_{8,38} = 0.60$; $p = 0.77$). Beetles from the control treatment took significantly longer to initiate oviposition than beetles from all other treatments except *C. arvensis*. The number of eggs laid was not significantly influenced by food ($F_{4,30} = 1.62$; $p = 0.20$) but by run ($F_{2,30} = 106.69$; $p < 0.001$) and by the interaction of run and food ($F_{8,30} = 2.47$; $p = 0.035$).

Protein, lipid, carbohydrate, and glycogen contents in the female ladybirds were all significantly influenced by food and run but not by the interaction of these two factors (with the exception of protein) (Table 3). The influence of food followed about the same pattern for all energetic compartments with beetles from the *C. arvensis* and control cages containing lower amounts than beetles from the cages with *F. esculentum*, *C. cyanus*, or the flower mixture (Fig. 3). Beetles that had

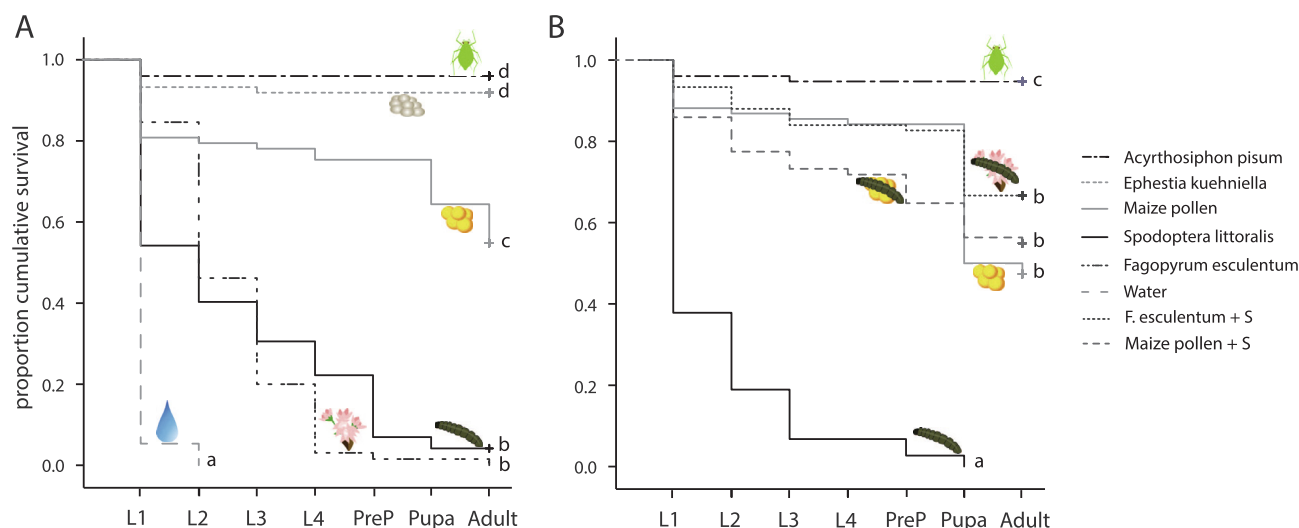


Fig. 1. Stage specific cumulative proportion of surviving *Harmonia axyridis* larvae. A: on food sources *Acyrtosiphon pisum*, *Ephestia kuehniella* eggs, maize pollen, *Spodoptera littoralis* caterpillars, *Fagopyrum esculentum* flowers; B: on food sources *A. pisum*, maize pollen, *S. littoralis* caterpillars and *F. esculentum* flowers and maize pollen each combined with *S. littoralis* caterpillars (+S). Different letters indicate significant differences between curves (Mantel Cox Log rank tests, $p < 0.001$).

remained in the laboratory and were provided *E. kuehniella* eggs for 6 days contained much higher levels of protein (13.14 ± 1.00 , mean \pm SE) and lipids (9.85 ± 0.32) than all beetles that had been exposed for 6 days in the field cages, but lower levels of carbohydrates (0.17 ± 0.02) and glycogen (0.26 ± 0.02) when compared to the beetles that had access to *F. esculentum* and *C. cyanus* in the field cages.

4. Discussion

Laboratory feeding assays with larvae as well as semi-field experiments with adults demonstrated that *H. axyridis* is able to utilize plant-derived food to optimize its nutritional status.

Maize pollen on its own was sufficient to allow development of about 50% of larvae into fertile adult beetles, a value slightly higher than the levels (35–48%) that had previously been reported by Berkvens et al. (2008). Adults that resulted from maize pollen as only food source, were lighter than those reared on an optimal food source (i.e., *A. pisum* or eggs of *E. kuehniella*). Few other species of Coccinellidae are able to develop solely on pollen. For *C. maculata*, 30–40% development on pollen diets has been observed (Smith, 1961) with resulting adults being lighter and less fecund than those fed on aphids (Lundgren & Wiedenmann, 2004). Only 10% of larvae of *Adalia bipunctata* (Linnaeus) were able to develop into adults when fed with bee-collected pollen (De Clerq et al., 2005). Other species such as *Coccinella septempunctata* Linnaeus consume pollen but cannot develop without aphids (Hodek & Michaud, 2008).

Table 1

Development time and adult fresh weight of *Harmonia axyridis* reared on different food sources. Different letters indicate significant differences between means within the “single” or “combined” food groups (generalized linear models followed by Tukey post hoc tests, $p < 0.05$). Treatments that did not allow for adult development (water, *Spodoptera littoralis* and *Fagopyrum esculentum*) were not included in the table.

Food source	Development time \pm SE [d]	Adult weight \pm SE [mg]		Eggs \pm SE	
		female	male		
Single food	<i>Ephestia kuehniella</i> eggs	15.68 \pm 0.11 a	36.04 \pm 0.51 a	30.41 \pm 0.55 a	nr
	<i>Acyrtosiphon pisum</i>	16.50 \pm 0.10 a	37.60 \pm 0.50 a	31.34 \pm 0.36 a	nr
	Maize pollen	24.66 \pm 0.46 b	16.89 \pm 0.91 b	14.16 \pm 0.91 b	nr
Combined food	<i>Acyrtosiphon pisum</i>	16.51 \pm 0.12 a	36.28 \pm 0.57 a	31.04 \pm 0.45 a	719.24 \pm 65.14
	Maize pollen	22.73 \pm 0.39 c	21.59 \pm 1.04 c	18.60 \pm 0.75 c	630.82 \pm 40.28
	<i>Spodoptera littoralis</i> + <i>Fagopyrum esculentum</i>	19.10 \pm 0.18 b	26.44 \pm 0.90 b	22.45 \pm 0.56 b	695.61 \pm 52.27
	<i>Spodoptera littoralis</i> + maize pollen	18.68 \pm 0.17 b	27.06 \pm 0.80 b	23.09 \pm 0.81 b	811.46 \pm 46.26

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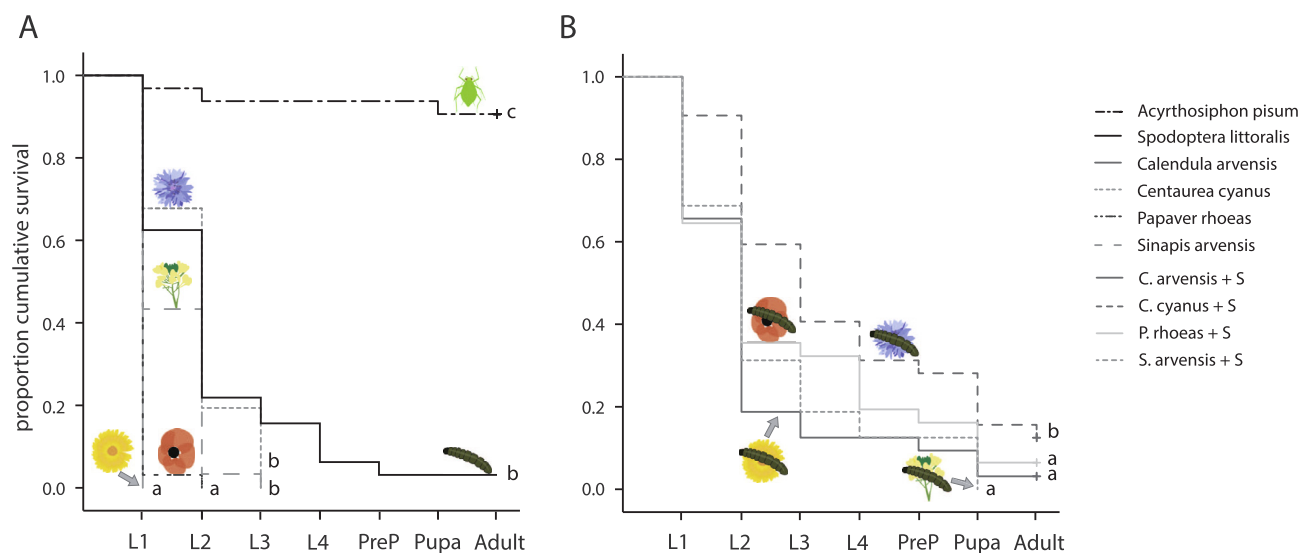


Fig. 2. Stage specific cumulative proportion of surviving *Harmonia axyridis* larvae. A: on food sources *Acyrthosiphon pisum*, *Spodoptera littoralis* caterpillars, *Calendula arvensis*, *Centaurea cyanus*, *Papaver rhoeas*, *Sinapis arvensis*; B: on floral food sources from A combined with *S. littoralis* caterpillars (+S). Different letters indicate significant differences between curves (Mantel Cox Log rank tests, $p < 0.05$).

Hypera postica Gyllenhal (Evans & Gunther, 2005). Mathews et al. (2016) even observed that oviposition took only place when *H. axyridis* were fed sugars from extrafloral nectaries in addition to their aphid prey (*Aphis spiraeicola* Patch), but not when the prey was offered alone. A similar utilization of floral resources has been reported for other predator species. In the green lacewing, addition of pollen and nectar to a suboptimal prey increased survival and adult weight strongly, even though only low assimilation rates of carbon and nitrogen from pollen were detected (Patt et al., 2003). It was assumed that the benefits either resulted from the readily digestible sucrose in the nectar or from micro-nutrients contained in the pollen.

Results from the laboratory feeding studies were largely confirmed in the semi-field experiments, where the two floral food sources *F. esculentum* and *C. cyanus* positively influenced weight and pre-oviposition period in adult *H. axyridis* compared to ladybirds that had no access to flowers. Both plant species are also highly attractive to ladybirds in the field. *Fagopyrum esculentum* is commonly visited by predatory ladybirds including *H. axyridis* (Spellman et al., 2006; Woltz et al., 2012) and field plots of *C. cyanus* attracted the highest numbers of coccinellids among five common flower strip plants in the UK (Fitzgerald & Solomon, 2004).

While in laboratory assays *F. esculentum* was markedly more suitable than *C. cyanus* as a food source for *H. axyridis* larvae, assessment of various parameters for the field exposed adults could not demonstrate such a difference. This difference might be due to the fact that adults and larvae differ in their food requirements. Furthermore, a meta-analysis revealed a smaller effect size for adult coccinellids compared to larvae, when their prey was substituted by plant-derived food sources, i.e. pollen and sugar (Lundgren, 2009a). It is also possible that that small differences became visible under controlled laboratory conditions but could not be detected by our method of field exposition and

subsequent analysis.

In the present study, *H. axyridis* that had no access to sugar sources in the field cages for six days commenced oviposition after ten days of feeding on *E. kuehniella* eggs. Obviously the beetles were able to compensate for the absence of such food sources during the first days as adult by an increased pre-oviposition period once optimal food was available. Moreover, after the pre-oviposition period, no influence of the food-source in the field cages on the number of eggs produced was visible. In many entomophagous predator species the pre-oviposition period is shortened and fecundity is improved when plant food sources are available (Eubanks & Styrsky, 2005; Lundgren, 2009b), whereas only few species such as *C. maculata* (Lundgren & Wiedenmann, 2004) and *Orius insidiosus* (Say) (Kimman & Yeorgan, 1985) are able to mature eggs solely on plant-derived food sources.

The four compartments of the energetic budget in adult *H. axyridis* were positively influenced by the floral food sources *F. esculentum* and *C. cyanus* compared to ladybirds that had no access to flowers. However, none of the floral food sources available during the field exposition resulted in the protein or fat content that was measured in beetles that were kept with *E. kuehniella* eggs in the laboratory. *Ephestia kuehniella* eggs are particularly high in amino acid and fatty acid content, even higher than *A. pisum* (Specty et al., 2003). It seems that *H. axyridis* is able to extract nitrogenous compounds from *F. esculentum* and *C. cyanus* to enhance its protein content in the body. Likely the pollen from these plants is utilized since it contains relatively high amounts of protein compared to nectar (Wäckers et al., 2005). Also the lipid content of adult *H. axyridis* was higher when given access to *C. cyanus* and the flower mixture than when access was given only to *C. arvensis* or control plots. It is known for several ladybird species of the genus *Hippodamia* that they are able to store plant nutrients as fat, even if those are not suitable for egg-production (Hagen, 1962).

Table 2

Mean weight change, pre-oviposition period and number of eggs laid during the first 14 d of oviposition by *Harmonia axyridis* that had been kept in field cages containing individual or mixed floral food sources or no flowers during the first six days after emergence. Different letters indicate significant differences between means in one line (ANOVAs, followed by Tukey post hoc tests, $p < 0.05$).

	<i>Fagopyrum esculentum</i>	<i>Calendula arvensis</i>	<i>Centaurea cyanus</i>	Mixed flowers	Control (no flowers)
Weight loss \pm SE [%]	1.68 \pm 2.26 a	10.87 \pm 2.35 bc	4.61 \pm 1.24 a	5.27 \pm 1.73 ab	11.74 \pm 1.34 c
Pre-oviposition period \pm SE [d]	12.95 \pm 0.54 a	14.81 \pm 0.36 ab	14.16 \pm 0.41 a	14.23 \pm 0.43a	16.67 \pm 0.67 b
Number of eggs laid \pm SE	551.18 \pm 28.81	538.59 \pm 41.03	491.58 \pm 35.95	457.85 \pm 35.57	493.65 \pm 40.98

Table 3

Effect of food, run and their interaction from the full models on energetic compartments of *Harmonia axyridis* that had been exposed to floral food sources in field cages during the first six days after emergence.

Energy compartment	food		run		run × food	
Protein	$F_{4,30} = 9.61$	$p \leq 0.001$	$F_{2,30} = 6.024$	$p = 0.006$	$F_{8,30} = 3.40$	$p = 0.007$
Lipids	$F_{4,30} = 7.13$	$p \leq 0.001$	$F_{2,30} = 62.56$	$p \leq 0.001$	$F_{8,30} = 1.08$	$p = 0.403$
Carbohydrates	$F_{4,30} = 15.46$	$p \leq 0.001$	$F_{2,30} = 3.65$	$p = 0.038$	$F_{8,30} = 2.11$	$p = 0.067$
Glycogen	$F_{4,30} = 7.38$	$p \leq 0.001$	$F_{2,30} = 23.90$	$p \leq 0.001$	$F_{8,30} = 0.64$	$p = 0.735$

Apparently, also *H. axyridis* was able to store fat reserves from the floral food source *C. cyanus*. Those fat reserves may be mobilized for reproduction but also play an important role for the survival of starvation and diapause periods (Arrese & Soulages, 2010).

Harmonia axyridis that had access to *F. esculentum*, *C. cyanus* or a mixture of the flowers had a higher carbohydrate content compared to all other beetles, even those that were fed with *E. keuhniella* eggs in the laboratory. Sugar is only present in low amounts in pollen (Roulston & Buchmann, 2000) but one of the main contents of nectar (Lundgren, 2009a). Contrary to our expectations, sugar content of individuals that had access to *C. cyanus*, which possess extrafloral nectaries, was not higher than sugar content of beetles that were given *F. esculentum*. Extrafloral nectar is suggested to be used more commonly by ladybirds than floral nectar due to its lower content in chemical defense substances and because it is available over longer time-spans (Lundgren, 2009b). However, it is possible that a higher sugar content of individuals that had access to *C. cyanus* was compensated by increased activity, as it has been observed in our laboratory assays with *H. axyridis* larvae.

Laboratory and semi-field experiments demonstrated marked

differences in utilization of the different floral food sources. In both cases, *C. arvensis* was a poor food source providing no benefit over the non-fed control. The low suitability may either arise from non-accessibility, from low dietary quality, or from a low quantity of the floral resources. We assume that the floral resources were accessible to *H. axyridis* due to their strong biting mandibles. In contrast to *F. esculentum* with exposed nectaries (Cawoy et al., 2008) and *C. cyanus* with extrafloral nectaries, however, accessibility of nectar in *C. arvensis* is lower. Studies with the closely related *C. officinalis* have revealed a low suitability of this food source for the syrphid fly *Episyrphus balteatus* (De Geer) when compared to several other flowering plants (Laubertie et al., 2012). Finally, Thom et al. (2016) demonstrated a particular low and variable sugar production in the closely related *C. officinalis*. Thus, low suitability of *C. arvensis* for *H. axyridis* may result from a combination of these three factors. These studies indicate that it is important to carefully select the plants to be provided in flower so that they target beneficial species without fostering unwanted ones (Winkler et al., 2010). However, this challenge is much easier to overcome when beneficials and pest species belong to different families with different morphology and different lifestyle than when a single ladybird species

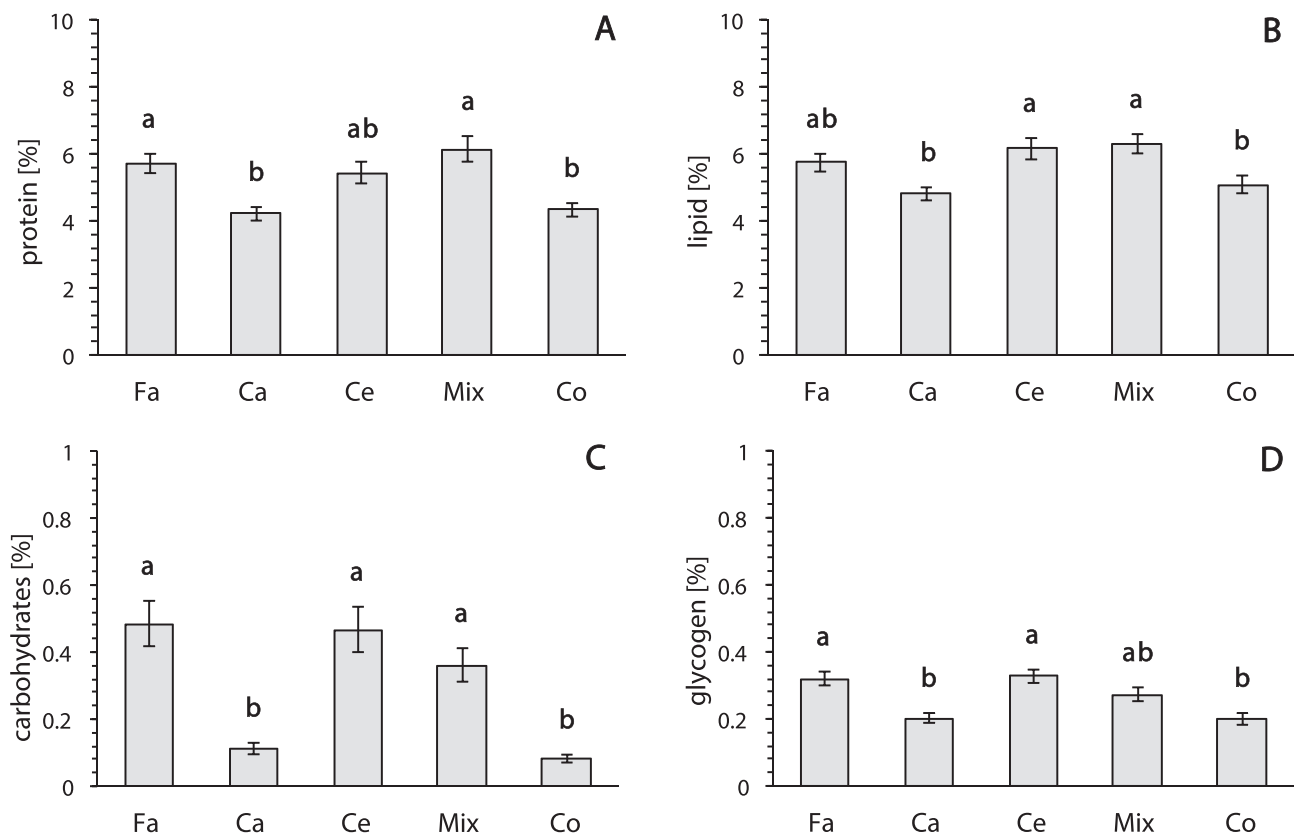


Fig. 3. Energy budget (mean ± SE % body weight) of female *Harmonia axyridis* that had been exposed to floral food sources in field cages during the first six days after emergence. A: carbohydrates, B: lipids; C: protein; D: glycogen. Different letters indicate significant differences between means (linear mixed effects models followed by Tukey post hoc tests, $p < 0.05$). Fa: *Fagopyrum esculentum*; Ca: *Calendula arvensis*; Ce: *Centaurea cyanus*; Mix: flower mixture of all three species; Co: control with no flowering plants.

should be winnowed from numerous closely related species.

The measured parameters from the semi-field experiment did not demonstrate an additional benefit of the plant mixture compared to *F. esculentum* or *C. cyanus* alone. Regarding weight loss and glycogen content the plant mixture was not significantly different from the least suitable flower, *C. arvensis*. Thus, *H. axyridis* does not seem to benefit from mixing two suitable floral food sources but rather experiences a dilution of the best suitable food source. For herbivorous species it is well known that they are able to compensate for a lack of nutritional components in their food plants by dietary selection (Simpson & Simpson, 1990). Likewise the ground beetle *Anchomenus dorsalis* (Pontoppidan) (as *Agonum dorsale*) has been shown to select its prey according to current nutrient needs (Mayntz et al., 2005). In contrast, Nielsen et al. (2002) did not find any benefit for *H. axyridis* in mixing aphid prey species. While many studies have focused on dietary mixing between different prey species or prey and plant food sources in ladybirds, information on the effects of mixed plant food sources on entomophagous insects is scarce. Therefore we cannot judge whether the observed effect in our semi-field experiment is a common phenomenon or specific for *H. axyridis* and the provided plant species.

5. Conclusion

Our results clearly demonstrate that *H. axyridis* can profit from plant food sources available in common flower strips. This could provide *H. axyridis* with a competitive advantage over native ladybird species. For example, it has been suggested that *C. septempunctata* in North America competitively displaces native species due to its ability to tolerate lower aphid densities (Evans, 2004). Similarly, *H. axyridis* can tolerate periods of prey absence by feeding mainly on plant food sources and thus outcompete species that are more dependent on aphids as food source.

Such an ability to utilize alternative food sources and the resulting competitive advantages are currently rarely considered in the environmental risk assessment of predator species that are considered for biological control. These factors might need to be addressed given their potential implications and the fact that this ability may also be of importance in other predatory taxa in which food requirements range from strict predators to omnivorous species.

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Authors' contributions

JC and JR conceived the ideas and designed methodology; SW collected the data; JC and SW analyzed the data; JC led the writing of the manuscript.

All authors contributed critically to the drafts and gave final approval for publication.

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