

## Effects of floral and extrafloral resource diversity on the fitness of an omnivorous bug, *Orius insidiosus*

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### Abstract

Habitat manipulation and increasing biodiversity are important approaches that enhance biological control of pests, but it is important to evaluate the relative benefits of specific plant species when designing conservation programs. *Orius insidiosus* Say (Hemiptera: Anthocoridae) is an important predator of thrips and aphids that also feeds on plants. It is the target of conservation biological control programs. Despite *O. insidiosus*' relevance, little is known about the effects of plant subsidies on predator performance or nutritional status. Here, we examined the influence of restricting the pollen and nectar resources of five plant species (alyssum, buckwheat, phacelia, fava bean, and chamomile), and how increasing plant diversity affects *O. insidiosus* fecundity, survival, and nutritional status. Plant species varied in their suitability for *O. insidiosus*, which was driven in part by the availability of the pollen or nectar resources. Offering plants as a mixture did not improve fecundity; however, the plant least preferred for oviposition under no-choice tests (fava bean) became the preferred egg-laying site when the plants were offered in combination. We conclude that the benefits obtained by *O. insidiosus* vary among plant species, and that increasing plant diversity can have unpredicted, positive effects on insect fitness.

### Introduction

Increasing biodiversity in natural and managed ecosystems can increase pressure on herbivores and improve pest management through biological control (Andow, 1991; Landis et al., 2000; Gurr et al., 2003; Lundgren et al., 2009; Letourneau et al., 2011). Natural enemies could be augmented through either an improvement in their fitness, attraction from neighboring habitats, or an increase in their oviposition rate (Alomar & Wiedenmann, 1996; Lundgren et al., 2009). Fiedler & Landis (2007a,b) suggested that a viable strategy to conserve natural enemies within a habitat would be to mix plant species such that floral resources would be available over a long duration of time. The most beneficial plant species should be identified and provided, either alone or in combination, to selectively enhance the abundance of each natural enemy under study. It is important to determine the extent to which an increase in biodiversity affects beneficial arthropods.

Plant resources (pollen, floral, and extrafloral nectar, or plant sap) are used by natural enemies as food sources (Wäckers et al., 2005; Lundgren, 2009). As a result, the provision of flowering plants can enhance the effectiveness of natural enemies by increasing their longevity, fecundity, and predation or parasitism rates (Berndt & Wratten, 2005; Begum et al., 2006; Lee & Heimpel, 2008). Specifically, sugar feeding improves the fitness and performance, and affects the nutrient status in parasitoids (Olson et al., 2000; Lee et al., 2004; Chen & Fadamiro, 2006; Nafziger & Fadamiro, 2011) and predators (Lundgren & Seagraves, 2011; Seagraves et al., 2011). Research has long supported the importance of nectar feeding for parasitoid life histories, but nectar resources for predators have received less attention until recently. The impact of different pollen and nectar sources on predator survival, longevity, or development should be quantified as insect conservation programs are developed for specific natural enemies.

Several factors affect the relative preferences of natural enemies for specific plant species, of which accessibility and quality of the nectar and pollen are particularly important (Colley & Luna, 2000; Ambrosino et al., 2006; Hogg et al., 2011). Floral architecture restricts which insects can

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access the floral rewards of the plant (Idris & Grafius, 1995; Patt et al., 1997; Vattala et al., 2006). For instance, Stang et al. (2006) found that fewer insects visited flowers with longer and narrower corollas. Studies on the composition of nectar have shown that it is mainly composed of sucrose, glucose, and fructose (Wäckers, 2001; Petanidou, 2005) and it usually contains low levels of amino acids (Baker & Baker, 1973). However, nectar can also contain compounds that are either toxic or repellent to flower visitors (Adler, 2000; Wäckers, 2001). A major difference between floral and extrafloral nectaries comes in their ecological roles: floral nectar is intended to attract pollinators and extrafloral nectar is used to attract natural enemies of herbivores (Lundgren, 2009). As such, extrafloral nectaries are often more exposed to potential visitors and their number and their quantity of nectar may increase in response to herbivory (Heil, 2011). Although plants are known to be important food sources for predators, the physiological benefits and metabolic uses of the different plant subsidies are still poorly understood. Some authors have observed that restricting the access of predators to nectar can reduce their survival and fitness considerably (Lundgren & Seagraves, 2011; Portillo et al., 2012).

*Orius insidiosus* Say (Hemiptera: Anthracoridae) is an important native predator of pests in North America. It consumes various pests such as thrips, aphids, and eggs of Lepidoptera (Phoofolo et al., 2001; Baez et al., 2004; Rutledge & O'Neil, 2005; Harwood et al., 2009; Seagraves & Yeorgan, 2009). Although *O. insidiosus* and congeners mainly feed on prey, these are omnivorous predators that also feed on different plant resources such as sap (Kiman & Yeorgan, 1985; Coll, 1996; Richards & Schmidt, 1996; Lundgren et al., 2008), pollen (Kiman & Yeorgan, 1985; Richards & Schmidt, 1996; Corey et al., 1998), and nectar (Yokoyama, 1978; Bugg, 1987) from several plant species; these foods support variable levels of performance and fitness in *Orius* spp. As far as we know, how nectar and pollen affect the nutrient status (e.g., glycogen and lipid contents) remains to be examined for *O. insidiosus*.

The main objectives of this work are to determine (1) how plants that vary in their accessibility of pollen and nectar affect *O. insidiosus* performance, and (2) whether increasing plant diversity results in additive or synergistic effects on *O. insidiosus* performance.

## Materials and methods

### Sources of insects and plants

*Orius insidiosus* adults were purchased from Koppert Canada (Scarborough, ON, Canada). Upon arrival they were maintained in vermiculite, fed *Ephesia kuehniella* Zeller (Lepidoptera: Pyralidae) eggs (Beneficial Insectary,

Redding, CA, USA), and green bean [*Phaseolus vulgaris* L. (Fabaceae)] pods from a grocery store were used as a water source. Adults were kept in climatic chambers at  $25 \pm 1$  °C,  $70 \pm 10\%$  r.h., and L16:D8 photoperiod until the experiments.

The plant species used were alyssum [*Lobularia maritima* L. cv. Carpet of Snow (Brassicaceae)], buckwheat [*Fagopyrum esculentum* Moench cv. Mancan (Polygonaceae)], phacelia [*Phacelia tanacetifolia* Benth (Boraginaceae)], fava bean [*Vicia faba* L. cv. Negreta (Fabaceae)], and chamomile [*Matricaria chamomilla* L. (Asteraceae)]. Alyssum plants were obtained from a local nursery and maintained in the greenhouse until they were used in the experiments. The rest of the plants were grown in the greenhouse from seed in a compost/peat-moss/perlite mixture (4:2:1), watered daily. The plants were allowed to grow until flowering (or a height in fava bean that was equivalent to flowering chamomile, the shortest of the plants).

These plant species were selected based on their use in conservation biological control programs, and their difference in floral architecture. According to Fiedler et al. (2008), three of the four most-studied plant species in habitat management include alyssum, buckwheat, and phacelia, and thus they were chosen for this study. Another reason these plants were chosen was that alyssum and buckwheat present a short corolla (Vattala et al., 2006), whereas phacelia presents a deep corolla (Baggen et al., 1999). In addition, a plant with extrafloral nectaries, fava bean, and a plant whose nectaries are hidden in a capitulum (Patt et al., 1997), chamomile, were selected for the study. The number of replicates per treatment are provided in the tables and figures.

### Influence of plant sugars on *Orius insidiosus* performance

Potted alyssum, buckwheat, phacelia, chamomile, and fava bean plants were used in the experiment. Stems from those potted plants and a water-saturated cotton wick were enclosed within a plastic cup (710 ml; Solo, Urbana, IL, USA) which was sealed with adhesive clay (Loctite®; Henkel Corporation, Avon, OH, USA). The top of the arena was replaced with cloth mesh to allow ventilation. There were two subtreatments for each plant species, either (1) the flowers (including pollen and floral nectar) or extrafloral nectaries were covered with Parafilm® (Pechiney Plastic Packaging, Menasha, WI, USA) (hereafter, the 'covered treatment') and (2) the flowers or extrafloral nectaries were exposed to the insects (hereafter, the 'uncovered treatment'). In the uncovered treatment, a strip of Parafilm® was wrapped around the stem to standardize the experimental conditions. Care was taken to provide similar surface areas of all the plant species in the individual treatments. Two *O. insidiosus* females (2–6 days old)

were randomly assigned to each treatment for 48 h. After this exposure, the number of *O. insidiosus* females alive, dead, or missing was recorded and the number of eggs laid was counted microscopically. If only one *O. insidiosus* survived the exposure, it was frozen in a microcentrifuge tube at  $-20^{\circ}\text{C}$  for post-mortem nutrient (glycogen and lipids) analyses. If two *O. insidiosus* females were found alive, the first one was kept for the nutrient analyses (described below) and the fecundity and survival of the second one was examined. To determine if prior treatment had any influence on the fecundity and longevity of *O. insidiosus*, this second female was placed in a Petri dish with a green bean pod and a saturated cotton wick; these arenas were checked daily for survival and for the final number of eggs laid. Two other treatments received only water (in the form of a water-saturated cotton wick) or a green bean pod with ad libitum *E. kuehniella* eggs affixed to the sticky portion of a Post-it<sup>®</sup> ( $\approx 1\text{ cm}^2$ ; 3M Office Supplies, St. Paul, MN, USA) also during 2 days. Additional females from the colony, representing a nutritionally satiated cohort, were directly taken out and subjected to the nutrient analyses. Environmental conditions of the experiment were the same as in the rearing operation.

#### Glycogen and lipids assays

Glycogen and lipids were quantified using colorimetric assays modified from methods described by Van Handel (1985a,b). Before the analysis, the bugs were rinsed in water to remove any sugar contamination. Then each insect was individually placed into a sterile microcentrifuge tube and ground in 300  $\mu\text{l}$  of methanol–chloroform (a 2:1 solution) using a sterile plastic pestle. The samples were centrifuged at 16 100 g for 4 min, and the supernatant was placed in a glass tube for the lipid assays; the remaining precipitate was set aside for the glycogen assay. For the analysis of the lipids, the solvent was evaporated at  $90^{\circ}\text{C}$ . Sulfuric acid (40  $\mu\text{l}$ ) was added and the solution was heated for 2 min at  $90^{\circ}\text{C}$ . After cooling the samples, they were incubated in 960  $\mu\text{l}$  of vanillin-phosphoric acid reagent (600 mg vanillin in 100 ml water, diluted in 400 ml of 85% phosphoric acid; vanillin product #V10-100; Fisher Scientific, Pittsburgh, PA, USA) for 25 min at  $23^{\circ}\text{C}$ . An aliquot (200  $\mu\text{l}$ ) of each sample was placed on a 96-well plate and optical density was read at 525 nm using a spectrophotometer ( $\mu\text{Quant}$ ; BioTek Instruments, Winooski, VT, USA). Three positive controls [25  $\mu\text{l}$  of olive oil solution (109  $\mu\text{l}$  extra virgin olive oil in 100  $\mu\text{l}$  of chloroform)] and three negative controls (25  $\mu\text{l}$  of distilled water) on each plate were subjected to the same assay procedure described above.

The glycogen content of the precipitate was quantified using an anthrone assay. One ml of anthrone reagent

(750 mg anthrone in 150 ml water, diluted in 380 ml 98% concentrated sulfuric acid; anthrone product #319899; Sigma-Aldrich, St. Louis, MO, USA) was added to each of the tubes containing the precipitate and heated at  $90^{\circ}\text{C}$  for 15 min. An aliquot (200  $\mu\text{l}$ ) of each sample was placed on a 96-well plate and optical density was read at 630 nm using the spectrophotometer. Three positive controls [25  $\mu\text{l}$  of glycogen solution (25 mg glycogen from oyster, Type II Sigma-Aldrich, in 25 ml water)] and three negative controls (25  $\mu\text{l}$  of distilled water) were subjected to the same procedure described above.

The lipid and glycogen contents per insect were calculated from the absorbance values using a standard curve, as calculated by Olson et al. (2000). To generate the lipid and glycogen curves, extra virgin olive oil and glycogen from oyster were used, respectively. Three replicates for each concentration level (1, 5, 10, 25, 50, 75, and 100  $\mu\text{g}$  brought to a total volume of 1 ml with anthrone and vanillin reagents for glycogen and lipid standards, respectively) were prepared and nutrient amounts were calculated from the resulting linear regression equations. The mean absorbance read from the 48-h-starved insects was subtracted from the experimental samples. The absorbance was then transferred to the standard curve and the glycogen and lipid contents per insect were obtained.

#### Influence of increasing plant diversity on *Orius insidiosus* performance

A second experiment was designed to evaluate how mixtures of three plant species (vs. the individual plant species) affected the fitness of *O. insidiosus*. Fava beans, chamomile, and buckwheat were produced as previously described. Severed plant stems of each species were inserted into a 50-ml centrifuge tube filled with water, and the open top of the tube was sealed around the stem using Parafilm<sup>®</sup>. A final treatment received only water as a saturated cotton wick. Similar surface areas of the three plant species in the individual treatments were used, and this was approximately the same foliage density as in the mixed plant treatment. The severed stems in water were housed within two plastic cups held mouth-to-mouth with Parafilm<sup>®</sup>. The plant stems were inserted through a hole in the base of the bottom cup which was sealed with adhesive clay. A water-saturated cotton wick was also added to each cup. The top of the arena was replaced with cloth mesh to allow ventilation. Two *O. insidiosus* females, received 3 days earlier from Koppert, were placed in each arena for the duration of the experiment. Environmental conditions of the experiment were  $25 \pm 1^{\circ}\text{C}$ ,  $40 \pm 10\%$  r.h., and L16:D8 photoperiod. There were a total of 16 cups assigned to each treatment. Survival and reproduction by the females were monitored. Survival was checked approximately every

48 h until all the bugs have died. As plants deteriorated, they were replaced with fresh ones, and the number of eggs laid on each plant species was recorded at least once per week. Because two females per cup may have oviposited, we standardized the number of eggs laid per 'Orius day', that is, the number of eggs laid per female per day. Any females that were lost or killed accidentally were included in the survival analysis as censored data points.

### Statistical analysis

In the first experiment, the number of eggs laid per female and per plant structure, and the number of females that survived or were missing over the 48-h period of the experiment were compared among treatments using the GLM procedure (with plant species and nectar availability as main effects). The number of eggs laid and the longevity in the Petri dish with green bean pods (subsequent to the plant-exposure period) were compared among treatments using the same procedure. The GLM procedure was also used to determine the effects of plant species and nectar availability on glycogen and lipid contents. The colony females, water only, and green bean pods + *E. kuehniella* eggs treatments were considered as experimental controls and were omitted from these statistical analyses examining glycogen/lipids dynamics. GLMs were used to analyze the plant species and nectar availability factors in the presence and absence of the control treatments. Significantly different means were separated with the Duncan test ( $P < 0.05$ ). Prior to the analysis, the number of eggs laid on green bean and the glycogen contents were square root transformed.

In the second experiment, survival was compared among treatments using a Kaplan–Meier non-parametric survival analysis. For this analysis, each female was regarded as an experimental unit. Subsequently, after we omitted the control, an ANOVA was used to determine differences among treatments. The total number of eggs

laid per treatment and the number of eggs laid per 'Orius day' were compared among treatments with separate ANOVAs. Means were separated with the Duncan test ( $P < 0.05$ ). Prior to the analysis, the total number of eggs in the overall experiment was  $\log x+1$  transformed and the total number of eggs laid per plant species and the number of eggs laid per female within the mixed treatment were square root transformed. All the analyses were conducted using SAS Enterprise Guide 4.2 (Cary, NC, USA).

## Results

### Influence of plant sugars on *Orius insidiosus* performance

**Survival and longevity.** A higher number of females survived during the first 48 h of the experiment on phacelia than on buckwheat or chamomile plants ( $F_{4,266} = 2.75$ ,  $P = 0.03$ ) (Table 1). Females that were on plants with the nectar/pollen uncovered had higher survival during these 48 h ( $F_{1,266} = 6.92$ ,  $P = 0.01$ ). There was no interaction between plant species and nectar availability on survival ( $F_{4,266} = 1.29$ ,  $P = 0.27$ ). The percentage of females that were missing was lower on plants with the nectar/pollen uncovered ( $F_{1,266} = 17.07$ ,  $P < 0.0001$ ). However, there were no effects of the plant species used ( $F_{4,266} = 1.24$ ,  $P = 0.30$ ) or interactions between both factors ( $F_{4,266} = 1.20$ ,  $P = 0.31$ ) (Table 1). The longevity of females after the 48-h plant-exposure period was similar among *O. insidiosus* raised on the different plant species, although the availability of nectar/pollen increased female longevity over treatments without these resources (plant species:  $F_{4,84} = 1.03$ ,  $P = 0.40$ ; nectar/pollen covered or exposed:  $F_{1,84} = 3.90$ ,  $P = 0.05$ ; interaction:  $F_{4,84} = 1.79$ ,  $P = 0.14$ ).

**Fecundity.** During the 48-h exposure period, the availability of floral and extrafloral resources affected the

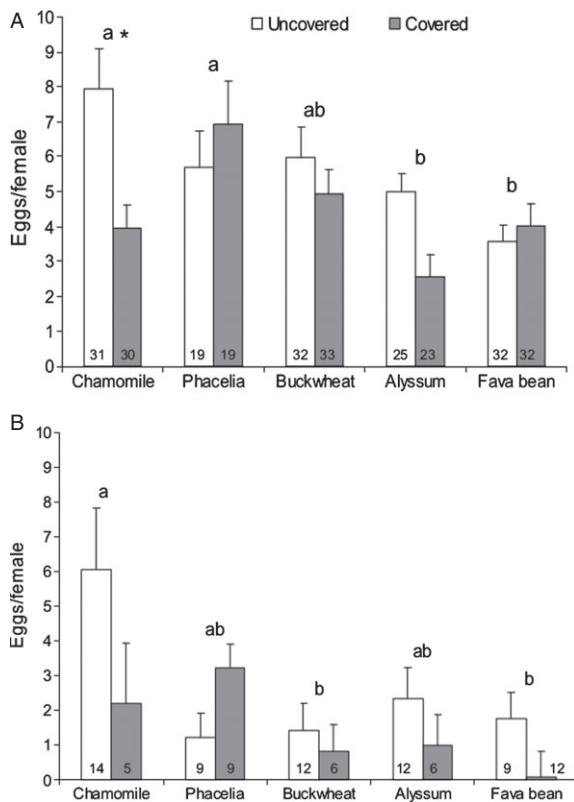
**Table 1** The effects of plant species and nectar/pollen resource availability on the survival, escape, and mean ( $\pm$  SEM) longevity of *Orius insidiosus* females. Sample sizes are indicated in parentheses. Covered treatments had their flowers or extrafloral nectaries covered with Parafilm, and the uncovered treatments had their resources exposed (a piece of Parafilm was placed randomly on the plants). After the assay, *O. insidiosus* was moved onto a green bean section and longevity was recorded

	% survival females		% missing females		Longevity on bean (days)	
	Covered	Uncovered	Covered	Uncovered	Covered	Uncovered
Chamomile	31.67 (30)	50.00b (31)	35.00	14.52	2.00 $\pm$ 0.35 (4)	2.35 $\pm$ 0.30 (14)
Phacelia	63.16 (19)	68.42a (19)	21.05	5.26*	1.78 $\pm$ 0.28 (9)	2.11 $\pm$ 0.54 (9)
Buckwheat	30.30 (33)	53.13b* (32)	25.76	15.63	2.33 $\pm$ 0.61 (6)	1.83 $\pm$ 0.30 (12)
Alyssum	39.13 (23)	62.00ab* (25)	39.13	12.00*	1.33 $\pm$ 0.33 (6)	3.00 $\pm$ 0.44 (12)
Fava bean	53.13 (32)	48.44ab (32)	20.31	15.63	1.50 $\pm$ 0.19 (12)	2.11 $\pm$ 0.39 (9)

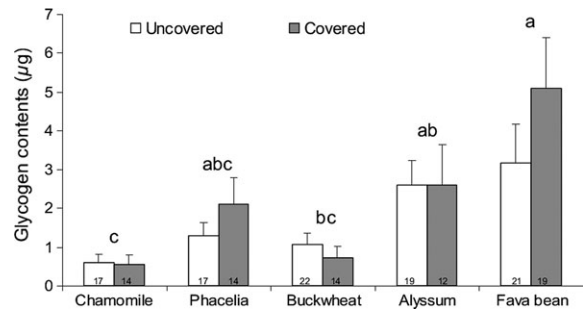
Differences among plants (within a column) are indicated by different lower case letters; asterisks indicate significant differences between columns within a plant species ( $\alpha = 0.05$ ).

number of eggs laid ( $F_{1,266} = 6.32$ ,  $P = 0.01$ ), but in pairwise contrasts, the difference was only significant for chamomile plants: more eggs were laid on uncovered than on covered chamomile plants. Some aphids were found to accidentally occur on the chamomile flowers and this likely increased the number of eggs laid on this treatment. The highest number of eggs was laid on chamomile and phacelia, and those numbers were statistically higher than those laid on fava bean and alyssum ( $F_{4,266} = 4.22$ ,  $P = 0.003$ ) (Figure 1A). There was an interaction between plant species and nectar availability on fecundity during this period ( $F_{4,266} = 3.35$ ,  $P = 0.01$ ).

After the 48-h exposure period, the highest number of eggs laid on green bean was obtained from the females



**Figure 1** The effects of plant species and the availability of nectar/pollen resources on oviposition by *Orius insidiosus*. (A) Mean (+ SEM) number of eggs laid per *O. insidiosus* female during the 48-h exposure period. Plants in the ‘covered’ subtreatments had their floral or extrafloral resources covered with Parafilm. (B) Mean (+ SEM) number of eggs laid per female after a 48-h exposure to each plant species. During this phase of the experiment, females were allowed to lay eggs into green bean pods. Numbers of replicates are shown on the x-axis. Different letters indicate significant differences among plant species, and the asterisk indicates intra-species difference between covered and uncovered subtreatments (Duncan test:  $P < 0.05$ ).



**Figure 2** The effects of plant species and the availability of nectar/pollen resources on the glycogen status of *Orius insidiosus* females. Mean ( $\pm$  SEM) glycogen levels were measured using the colorimetric anthrone assay. Insects in the covered treatment were reared on plants with their floral or extrafloral nectaries covered in Parafilm. Numbers of replicates are shown on the x-axis. Different letters indicate significant differences among plant species (Duncan test:  $P < 0.05$ ).

whose former plant was chamomile, but there was no effect of the previous availability of floral or extrafloral resources on egg production during this stage of the experiment (plant species:  $F_{4,84} = 2.89$ ,  $P = 0.03$ ; covered or uncovered:  $F_{1,84} = 2.32$ ;  $P = 0.13$ ; interaction:  $F_{4,84} = 2.05$ ,  $P = 0.10$ ) (Figure 1B).

**Nutrient analyses.** The GLM procedure showed that the quantity of glycogen per female was higher in *O. insidiosus* raised on fava bean and alyssum than on chamomile plants ( $F_{4,159} = 7.82$ ,  $P < 0.0001$ ) (Figure 2). Covering the nectar sources did not affect glycogen content of the females ( $F_{1,159} = 0.35$ ,  $P = 0.56$ ), nor was there a significant interaction between plant species and nectar availability on glycogen content ( $F_{4,159} = 0.93$ ,  $P = 0.45$ ) (Figure 2). The mean ( $\pm$  SEM) values of glycogen for the controls were:  $3.47 \pm 0.93$ ,  $0.70 \pm 0.23$ , and  $1.21 \pm 0.23 \mu\text{g}$  for the colony, water, and green bean pods + *E. kuehniella* females. The GLM procedure showed that the quantity of lipids per female was not different among plants ( $F_{4,163} = 2.19$ ,  $P = 0.07$ ). The fact of having covered the sugar sources did not show differences among plant species ( $F_{1,163} = 0.06$ ,  $P = 0.80$ ) nor was there a significant interaction between these factors ( $F_{4,163} = 0.79$ ,  $P = 0.53$ ) (Table 2). The mean ( $\pm$  SEM) values of glycogen for the controls were:  $2.99 \pm 0.48$ ,  $2.00 \pm 0.54$ , and  $2.07 \pm 0.47 \mu\text{g}$  for the colony, water, and green bean pods + *E. kuehniella* females.

#### Influence of increasing plant diversity on *Orius insidiosus* performance

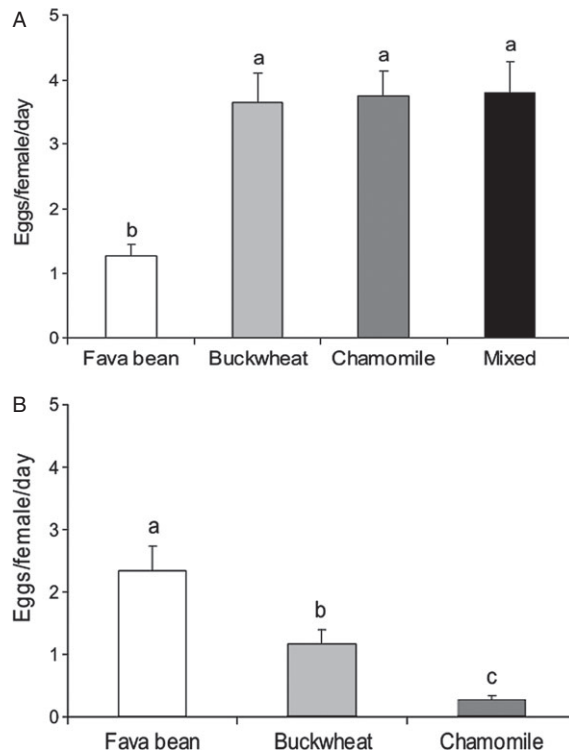
**Survival and longevity.** The females from the four treatments had higher survival than those from the unfed

**Table 2** Effects of plant species and nectar/pollen resources on the lipid status of *Orius insidiosus* females. Mean ( $\pm$  SEM) lipid contents of individual *O. insidiosus* were measured with the vanillin (lipid) reagent. Lipid contents measured in the colony, water, and green bean + *E. kuehniella* controls were excluded from the analyses and these values can be found in the text

Plant species	n	Lipids contents ( $\mu$ g)
Chamomile		
Uncovered	17	1.39 $\pm$ 0.43
Covered	14	1.22 $\pm$ 0.53
Phacelia		
Uncovered	17	1.65 $\pm$ 0.57
Covered	14	2.96 $\pm$ 0.59
Buckwheat		
Uncovered	22	2.62 $\pm$ 0.48
Covered	14	2.67 $\pm$ 0.66
Alyssum		
Uncovered	19	2.88 $\pm$ 0.54
Covered	12	2.89 $\pm$ 0.77
Fava bean		
Uncovered	21	2.31 $\pm$ 0.52
Covered	20	1.67 $\pm$ 0.52

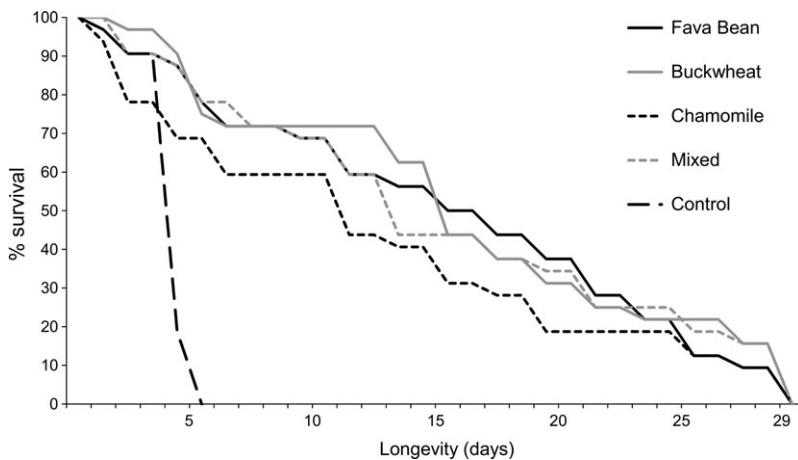
control ( $\chi^2 = 78.36$ , d.f. = 4,  $P < 0.0001$ ). The ANOVA revealed no differences among treatments when the control was not included in the analyses ( $F_{3,124} = 1.77$ ,  $P = 0.16$ ). Mean survival times were  $15.34 \pm 1.59$ ,  $16.09 \pm 1.62$ ,  $11.41 \pm 1.59$ ,  $15.50 \pm 1.64$ , and  $3.97 \pm 0.15$  days for those exposed to fava bean, buckwheat, chamomile, the mixed treatment, and the control, respectively (Figure 3).

**Fecundity.** Under no-choice conditions a higher number of total eggs ( $F_{3,60} = 6.65$ ,  $P < 0.001$ ) as well as of eggs per day ( $F_{3,60} = 9.31$ ,  $P < 0.001$ ) was laid on buckwheat, chamomile, and the mixed treatment compared with fava bean (Figure 4A). However, fava bean plants were the



**Figure 4** Mean (+ SEM) relative oviposition rates of *Orius insidiosus* raised on plants when offered alone or as a mixture. (A) Number of eggs laid per *Orius insidiosus* female in the no-choice experiment. (B) Number of eggs laid per female in the mixed treatment.

preferred oviposition substrate in the mixed treatment for the total number of eggs laid ( $F_{2,45} = 16.02$ ,  $P < 0.0001$ ) as well as the number of eggs laid per day ( $F_{2,45} = 21.40$ ,  $P < 0.0001$ ) (Figure 4B). Under no-choice conditions, the total number of eggs laid was  $32.94 \pm 2.94$ ,  $108.44 \pm 14.01$ ,  $86.44 \pm 11.66$ , and  $114.70 \pm 18.30$  for the



**Figure 3** Survival probability of *Orius insidiosus* raised on three different plant species, or on all of the plant species together as a mixture.

fava bean, buckwheat, chamomile, and mixed treatment, respectively. When given a choice among plants, the total number of eggs laid was  $68.81 \pm 12.52$ ,  $36.62 \pm 8.21$ , and  $9.25 \pm 2.63$  for the fava bean, buckwheat, and chamomile, respectively.

## Discussion

Our study demonstrates that nectar and pollen from different plant species affect the fecundity, survival, and nutrient status of *O. insidiosus*. In addition, increasing plant diversity allows the omnivore to optimize its fitness by exploiting various plant-based resources such as nutrition and oviposition sites. This finding underlines the importance of choosing appropriate plants and providing plant mixtures for obtaining the best performance of predators for conservation biological control programs.

Plant species affected the survival of *O. insidiosus* females. Survival was higher on phacelia than on chamomile or buckwheat during the 48-h exposure rate. However, plant host did not influence longevity or survival once the females were moved over to green bean pods, suggesting that plant species effects were immediate, possibly stemming from nutritional or defensive variations among the plants. Irvin et al. (2007) studied the sugar composition of phacelia, fava bean, alyssum, and buckwheat whole flowers and they determined that phacelia had more total sugars ( $\mu\text{g}$ ) than the other flower species. It could be that the higher survival obtained on phacelia in our study is due to the fact that this plant species provides an extra source of sugar, compared with the other plants.

Plant species also affected the oviposition rate of *O. insidiosus* females; the observed patterns could have been produced by either the nutritional quality of the plant or their morphological characteristics (e.g., epidermal thickness or trichome density) that might restrict their attractiveness to *O. insidiosus*. This predatory bug laid most eggs on chamomile and phacelia, and these numbers were statistically higher than on alyssum and fava bean. These plant effects persisted even after the females were moved onto green bean pods, but only for chamomile: the females that were reared previously on chamomile laid the highest number of eggs and the lowest number of eggs was laid when fava bean and buckwheat were the host plants. There was a low number of aphids found on chamomile flowers, and we believe that this in part produced the strong ovipositional response that we saw on this plant species. Phacelia has been found to be very attractive for some natural enemies (Ambrosino et al., 2006) and our results from the no-choice tests suggests that this species could be a highly

acceptable oviposition substrate for *O. insidiosus* females. Previous work has shown that the morphological characteristics of a plant are correlated with their relative acceptance to ovipositing *O. insidiosus* (Lundgren et al., 2008). However, nutrition also has strong effects on *Orius* oviposition rates (Ferkovich & Shapiro, 2007). Green beans have been widely used as an acceptable oviposition substrate (Richards & Schmidt, 1996; Shapiro & Ferkovich, 2006), and so providing this substrate to all treatments was intended to separate the nutritional suitability of the different plants for oviposition from the morphological/defensive plant characteristics known to influence oviposition. The fact that we saw very similar patterns in oviposition, as indicated in Figure 1A and B, suggest that plant nutrition was a primary driver for the relative ovipositional acceptance of these plant species.

Plant species also affected levels of major storage nutrients in the females. Females on fava bean had the highest levels of glycogen, and these were higher than on buckwheat and chamomile. Glycogen and lipid contents are inversely related to egg production, and we hypothesize that this is a reason for the high storage nutrient levels found in females raised on the plant species that supported the lowest oviposition rate. However, these results support our hypothesis that the extrafloral nectaries in fava bean are a very accessible source of sugar for this bug in that they were able to maintain high levels of glycogen in the absence of other foods. However, nectar alone is seldom sufficient for producing eggs, and the pollen resources found in the flowers, but not extrafloral nectaries, may have helped to support higher oviposition rates in the other plants, which depleted storage nutrient reserves. It is interesting to mention that whereas relative glycogen contents of *O. insidiosus* raised on fava bean were higher than the rest of the plants, the relative lipid contents were not different from the other plant species. Although the differences in lipid contents of *O. insidiosus* reared on different plants were only marginally significant, it is remarkable that the levels obtained from the females that had been on chamomile were much lower than those obtained from the females that had been on alyssum or buckwheat. This is not surprising, as egg production in animals requires protein or lipids and chamomile was the plant where insects laid most of the eggs, which could have caused the depletion of their lipid storages. These marginal differences found in lipid levels are in concordance with other works that have shown that lipids do not vary with sugar-based diets in the case of two different female parasitoid species (Olson et al., 2000; Lee et al., 2004).

Access to floral resources or extrafloral nectar improved the survival of *O. insidiosus* females during the time they were exposed to the plants. This was particularly so for

females raised on buckwheat and alyssum. Therefore, it seems that facultative phytophagy by *O. insidiosus* consists not only of sucking phloem from the plant but also includes the consumption of flower subsidies. Nectar consumption had some lasting effects on *O. insidiosus* survival after they had been removed from the plants and, although the treatment differences were only marginally significant on the longevity recorded on green bean, they suggest that females that had been previously exposed to the floral/nectar resources lived longer than those without floral resources or extrafloral nectar. The importance of nectar for *O. insidiosus* may also be supported by the fact that many of them were lost from the plants with the covered nectaries. The percentages of missing females during the first part of the experiment were higher on the covered plants (more missing on covered phacelia and alyssum than on uncovered). A plausible explanation of these results could be that females on the uncovered treatments had a suitable food source available and they did not invest time in trying to escape. On the contrary, females on the covered treatments, lacking these nutritional resources may have had more incentive to find or create escape routes from the arena (no corpses were found of the missing individuals, reducing the likelihood of cannibalism in the covered treatment). In many other studies, access to sugar sources has improved the survival of predators (Lundgren & Seagraves, 2011; Portillo et al., 2012) and parasitoids (Olson et al., 2000; Lee et al., 2004). We found that *O. insidiosus* females laid more eggs when offered nectar and pollen than when these resources were restricted on three of the four flowering plants, although the greatest benefit of floral resources was observed in chamomile, which had low levels of aphids near the flowers. In contrast, covering the pollen and nectar sources did not produce different levels of glycogen or lipids in the bugs. Other recent work, however, has shown that while access to nectar greatly improved the levels of glycogen in a predatory beetle, the lipid contents were similar when they were compared with no access to nectar (Lundgren & Seagraves, 2011). Access to sugar sources also increased the glycogen levels, but not the lipids on a female parasitoid species when it was compared with starved insects (Olson et al., 2000).

The survival curves demonstrated that longer longevities are obtained on plant diets compared with water alone. This follows the pattern of many other works that have been carried out with other predators or parasitoids (Vattala et al., 2006) which demonstrate the importance of plant-based diets for the survival and thus conservation of omnivorous predators.

Offering the plant species as a mixture gave a much different interpretation of the data, especially with regard to the benefits of fava beans for *O. insidiosus* females. *Orius*

*insidiosus* survived equally well when fava bean, buckwheat, and chamomile were offered alone or in combination. These survivals contrast with those obtained in the first experiment, where the *O. insidiosus* females did not survive longer than 3 days on the green bean pods, after feeding on those plants. This emphasizes the importance of flowering plants compared with only bean pods, which provide similar nutrition to water alone (Pumarino & Alomar, 2012). The results obtained on the number of eggs laid by day per female and the total numbers of eggs laid by female were unexpected. *Orius insidiosus* laid fewer eggs on fava bean in no-choice conditions relative to all of the other treatments. However, when the three plants were offered together, *O. insidiosus* displayed the opposite pattern and fava bean was the preferred plant for oviposition. This predatory bug shows clear preferences for some plants as oviposition substrates (Coll, 1996; Lundgren & Fergen, 2006) and plant species is even more important for oviposition site selection than prey availability (Seagraves & Lundgren, 2010). Our results show that maybe *O. insidiosus* considers fava bean a better oviposition substrate for the development of its offspring, but only when there is high quality nutrition (e.g., pollen) available for the female to mature eggs. Other work has shown that this bug changes its preference for oviposition sites on a plant species depending on whether it was offered singly or with other plants (Lundgren & Fergen, 2006), whereas other work shows that different combinations of plant species did not influence the proportion of eggs laid by *O. insidiosus* on each plant species (Coll, 1996) (although this study just examined small cuttings of trifoliates). Atakan (2010) found that *O. insidiosus* may prefer fava bean plants as a site for egg laying when it was compared with many other plants. In the first experiment, *O. insidiosus* females raised on fava beans with covered nectaries survived well relative to the other plant species when their flowers were covered, which is consistent with the hypothesis that vegetative structures of fava beans may have good nutrition for maintaining *O. insidiosus* survival. Although additional experiments will help elucidate these relationships, our current interpretation of the data is that fava bean could be the most suitable oviposition plant for *O. insidiosus*, but this plant can only be fully exploited when an additional protein or lipid source (either prey or pollen) accompanies it which allows females to mature eggs.

These experiments illustrate that the true conservation benefits of a plant species for a beneficial insect depend on the context in which the plants are examined. Plant species varied substantially in their ability to support survival and reproduction of *O. insidiosus* under no-choice conditions. Although fava bean did not support substantial oviposition in a no-choice situation, when it was offered with other plants it was the preferred oviposition site for



*O. insidiosus* females. Our findings demonstrate that not only is it necessary to provide natural enemies with pollen or nectar sources, but that each predator will respond differently to various plant resources. As such, predicting which plants are most beneficial for conservation biological control programs under various circumstances is inherently very difficult, and it makes the case for conserving plant biodiversity as a whole in and near agroecosystems.

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