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# Importance of wildflowers in *Orius insidiosus* (Heteroptera: Anthocoridae) diet

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**Abstract:** The addition of non-crop floral resources is known to improve the fitness of many beneficial arthropods. *Orius insidiosus* (Say; Heteroptera: Anthocoridae) is a predator of several economically important pests and is known to feed on pollen. Spanish needles, *Bidens alba* L., and Leavenworth's tickseed, *Coreopsis leavenworthii* Torrey & Gray (both Asteraceae), are two Florida native wildflowers that may serve as sources of pollen beneficial to *O. insidiosus*. The current study investigated the effects of adding flowers from *C. leavenworthii* and *B. alba* to the diet of captive *O. insidiosus* on longevity, nymphal development time, and cannibalism. A mixed diet of flowers and thrips prey increased *O. insidiosus* adult female longevity. Nymphal development time was reduced when *B. alba* was added to thrips when compared to a diet of thrips alone. Interestingly, cannibalism was low in all instances, suggesting a negligible effect of cannibalism on *O. insidiosus* except perhaps in extreme situations. These results indicate that *B. alba* and *C. leavenworthii* flowers can increase longevity and fitness of *O. insidiosus*, thus providing support for the use of these plants to enhance natural enemy services.

**Keywords:** Anthocoridae; thrips; plant-feeding predators; omnivory; cannibalism

**Resumen:** Se sabe que la adición de recursos florales no agrícolas mejora la aptitud de muchos artrópodos beneficiosos. *Orius insidiosus* (Say; Heteroptera: Anthocoridae) es un depredador de varias plagas económicamente importantes y se sabe que se alimenta de polen. El mazote blanco, *Bidens alba* L., y la semilla de garrapata de Leavenworth, *Coreopsis leavenworthii* Torrey & Gray (ambas Asteraceae), son dos flores silvestres nativas de la Florida que pueden servir como fuentes de polen beneficioso para *O. insidiosus*.

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El estudio actual investigó los efectos de agregar flores de *C. leavenworthii* y *B. alba* a la dieta de *O. insidiosus* en cautiverio sobre su longevidad, tiempo de desarrollo ninfal y canibalismo. Una dieta mixta de flores y trips aumentó la longevidad de las hembras adultas de *O. insidiosus*. El tiempo de desarrollo ninfal se redujo cuando se añadió *B. alba* a los trips en comparación con una dieta de trips solos. Curiosamente, el canibalismo fue bajo en todos los casos, lo que sugiere un efecto insignificante de canibalismo en *O. insidiosus*, excepto quizás en situaciones extremas. Estos resultados indican que las flores de *B. alba* y *C. leavenworthii* pueden aumentar la longevidad y la aptitud de *O. insidiosus*, brindando así apoyo para el uso de estas plantas en la mejora de los servicios contra los enemigos naturales.

**Palabras Clave:** Anthocoridae; trips; depredadores que se alimentan de plantas; omnívoro; canibalismo

## 1 Introduction

Non-crop floral resources are used to increase abundance and fitness of natural enemies by providing hosts for parasitoids, prey for predators, and additional food resources such as pollen or nectar (Symondson et al. 2002; Wong and Frank 2013). This is particularly critical for omnivorous predators that often benefit from a diet of both prey and plant material (Coll and Guershon 2002; Leon-Beck and Coll 2007; Wong and Frank 2013). Flowering plants can therefore be considered in conservation biological control strategies in the form of banker plants, flower strips or hedgerow plantings to increase recruitment and survival of predators, particularly in monoculture systems with or without a time-limited resource of suitable pollen (Coll and Guershon 2002; Lundgren et al. 2009; Wong and Frank 2013; Waite et al. 2014).

*Orius* species (Hemiptera: Anthocoridae), also called minute pirate bugs, are generalist predators that feed primarily on thrips but also are known to feed and successfully reproduce on other pests affecting vegetable crops such as whiteflies, aphids, and mites (Lundgren et al. 2009; Wong and Frank 2013; Bernardo et al. 2017). Several species are known to feed on pollen at the nymphal and adult stage in addition to prey, which is proposed to affect survival and

development time as well as reduce cannibalism (Kiman and Yeargan 1985; Coll and Guershon 2002; Leon-Beck and Coll 2007; Calixto et al. 2013; Wong and Frank 2013; Bueno et al. 2014). In a previous study, a diet of pollen from pepper *Capsicum annuum* L. cv ‘Black pearl’ (Solanaceae) combined with thrips increased *Orius insidiosus* (Say) longevity and size and decreased nymphal development time (Wong and Frank 2013). There is also evidence that the addition of pollen may reduce cannibalism, which has been described as a potential limiting factor in mass rearing of *O. insidiosus* for commercial use (Leon-Beck and Coll 2007; Calixto et al. 2013; Bueno et al. 2014).

*Bidens alba* L. and *Coreopsis leavenworthii* Torrey & Gray are two Asteraceae host plants of *Orius* spp. native to Florida that have been shown to increase natural enemy and pollinator services (Funderburk 2009; Tyler-Julian et al. 2018; Abdelmaksoud et al. 2020). In addition, these plant species serve as hosts for less damaging native thrips species (Thysanoptera: Thripidae) including *Frankliniella bispinosa* Morgan and *Frankliniella tritici* (Fitch), which have been shown to outcompete the worldwide pest *F. occidentalis* (Pergande) (Baez et al. 2004; Reitz et al. 2006; Paini et al. 2008; Northfield et al. 2011). For this reason, *B. alba* and *C. leavenworthii* are potential candidates to be used as companion plants to enhance *O. insidiosus* fitness (Norcini et al. 2009; Tyler-Julian et al. 2018). Nevertheless, there is limited research documenting the direct effects of *B. alba* and *C. leavenworthii* on the overall fitness of *Orius* spp., perhaps due to their perceived status as weeds. There remains a need to investigate their potential to improve *O. insidiosus* fitness and abundance, and to provide justification for their use in improving conservation biological control of thrips and other pest species.

## 2 Materials and methods

### 2.1 Insect colonies

Thrips (*Frankliniella tritici*) colonies were established with wild populations collected in Gadsden county, Florida and identified to species according to Sprague et al. (2018). Adult thrips were reared in plastic containers (16.2 × 17.7 × 9.3 cm; Rubbermaid, Atlanta, Georgia) vented with thrips-proof mesh (BioQuip, Rancho Dominguez, California) and were provided with green beans washed in a 5 % bleach solution and air dried for food and oviposition. Colonies were kept in an incubator at 25 °C, relative humidity (RH) 60–70 %, and 12:12 h (L:D) photoperiod.

*O. insidiosus* colonies were established with wild populations collected in Gadsden county, Florida. Adult *O. insidiosus* were identified based on the melanization of the femora, basal antennal segments, and wing cuneus (Shapiro et al. 2009). Adult *O. insidiosus* were housed in

plastic containers (18.7 × 18.7 × 14.6 cm; Rubbermaid, Atlanta, Georgia) vented with thrips-proof mesh and were provided with *F. tritici* as prey and flowers from *B. alba* and *L. indica*. Bean pods were provided as supplementary food and an oviposition substrate. Colonies were kept in an incubator at 21.1 °C, RH 60–70 %, and 12:12 h (L:D) photoperiod.

### 2.2 Plants

*B. alba* (identified according to Khamare et al. 2019) seeds were collected from wild plants in Gadsden county, Florida, and *C. leavenworthii* seeds were purchased (Florida Wildflower Cooperative, Crescent City, Florida). Seeds were planted in greenhouse trays (53.3 × 27.9 × 6.3 cm; Growers Solution, Cookeville, Tennessee) and seedlings were transplanted into 3.8 L nursery pots. The plants were irrigated by hand and fertilized every 2 weeks with an all-purpose plant food (Scotts Miracle-Gro Company, Marysville, Ohio).

### 2.3 Diet assays

All assays were carried out in a completely randomized design and were performed in growth chambers at 21.1 °C, RH 60–70 %, and 12:12 h (L:D) photoperiod. Nymphs were removed from the colony and put into a separate container and checked daily. As adult females emerged, they were removed and placed into another container. Each treatment was applied to an individual *O. insidiosus* contained in a Petri dish (100 mm diameter; Fisher Scientific, Hampton, New Hampshire) lined with filter paper (9.0 cm diameter; Fisher Scientific, Hampton, New Hampshire) and with a moistened cotton wick for water. The top of the Petri dish was vented with a 2.54 × 2.54 cm hole covered with thrips-proof mesh. The Petri dishes were sealed with Parafilm® (Bemis Company, Neenah, Wisconsin) wrapped around the lid to prevent insects from escaping.

**2.3.1 Female survival:** One adult female *O. insidiosus* with unknown mating status was placed in a Petri dish in the 24 h after adult emergence and given one of five diets: (1) *C. leavenworthii* and *F. tritici*, (2) *C. leavenworthii* only, (3) *B. alba* and *F. tritici*, (4) *B. alba* only, or (5) *F. tritici* only. For both *C. leavenworthii* and *B. alba* the disc flowers from pseudanthia collected the same day were extracted and placed in the Petri dish. There were nine replicates for each treatment. In the diets with thrips, approximately 20–30 adult and larval *F. tritici* were supplied every day. In the diets with flowers, three flowers per Petri dish were provided every other day. Insects were aspirated out of the flowers to ensure none were present in the treatments with flowers only. The Petri dishes were checked every 24 h for 20 days, and the *O. insidiosus* females were recorded as dead or alive.

**2.3.2 Nymphal development:** A single unsexed first instar nymph (<24 h after hatching) was placed into a Petri dish with one of the five different diets described previously. There were 20 replicates for each treatment. The Petri dishes were checked every 24 h until *O. insidiosus* adults emerged. The days to adult were calculated as the date of the start of the assay subtracted from the date of adult emergence.

**2.3.3 Cannibalism:** One female *O. insidiosus* was placed into a Petri dish and starved for 24 h with no flowers or prey. After 24 h, each was given one of six diets: (1) *C. leavenworthii* + *F. tritici* + 5 *O. insidiosus* nymphs,

(2) *C. leavenworthii* + 5 *O. insidiosus* nymphs, (3) *B. alba* + *F. tritici* + 5 *O. insidiosus* nymphs, (4) *B. alba* + 5 *O. insidiosus* nymphs, (5) *F. tritici* + 5 *O. insidiosus* nymphs, and (6) 5 *O. insidiosus* nymphs only. There were 10 replicates for each treatment. Various nymphal stages were used as supply was limited due to availability in the rearing. The number of *O. insidiosus* nymphs dead or alive was recorded after 2, 4, 8, 16, 24, and 48 h.

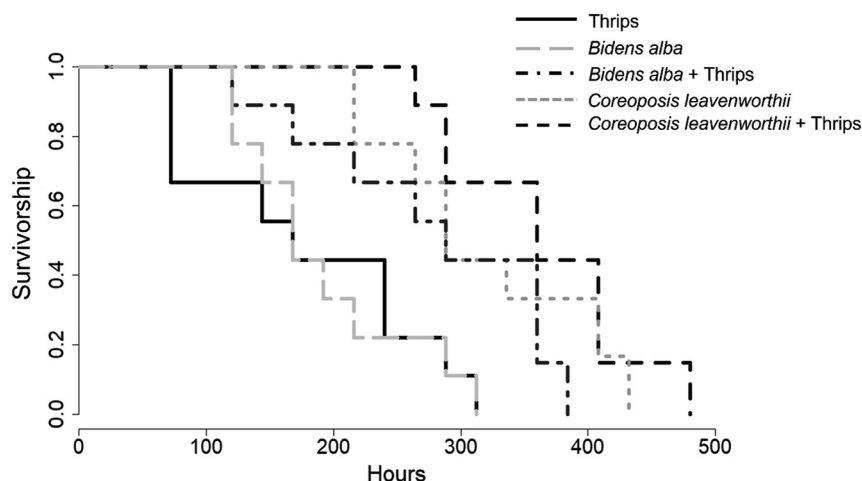
## 2.4 Statistical analysis

All data met the assumptions of normality and homogeneity of variance. Statistical analysis of female survival data was conducted with the statistical software R (R 3.5.1, RStudio, Boston, Massachusetts). Female survival data were analyzed using Cox's proportional hazards model to investigate the association between the survival time of *O. insidiosus* females and diet treatment (Cox 1972). Data from nymphal development and cannibalism assays were analyzed using ANOVA to determine the effects of diet treatment on days to adult and the proportion of cannibalism, respectively (PROC ANOVA, SAS 9.4, SAS Institute Inc., Cary, North Carolina). Pearson's chi-square test was conducted on data from the cannibalism assays pooled for all diet treatments.

# 3 Results

## 3.1 Female survival

There was a significant effect of adding *C. leavenworthii* and *B. alba* in a diet for adult female *O. insidiosus* survivorship (LR = 21, d.f. = 4,  $P < 0.001$ ) (Figure 1). Adults given diets containing *C. leavenworthii* flowers + *F. tritici*, *B. alba* + *F. tritici*, and *C. leavenworthii* flowers alone survived significantly longer ( $11.56 \pm 12.9$ ,  $14.89 \pm 0.96$ , and  $12.89 \pm 1.06$  days, respectively) than those with a diet of *B. alba* flowers only or *F. tritici* thrips only ( $7.44 \pm 1.32$ ,  $8.00 \pm 0.95$  days, respectively). A diet of *B. alba* flowers only did not differ from a diet of thrips alone.



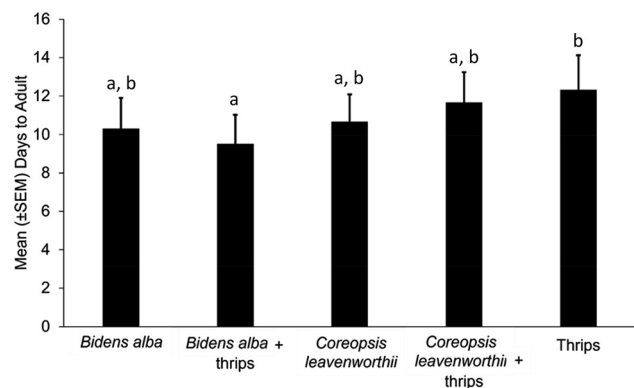
**Figure 1:** Survival distribution of adult female *Orius insidiosus* when fed a diet of thrips *Frankliniella tritici*, flowers of *Bidens alba* only, flowers of *B. alba* + *F. tritici*, flowers of *Coreoposis leavenworthii* only, or flowers of *C. leavenworthii* + *F. tritici* as a function of hours until event up to 480 h (20 days).

## 3.2 Nymphal development

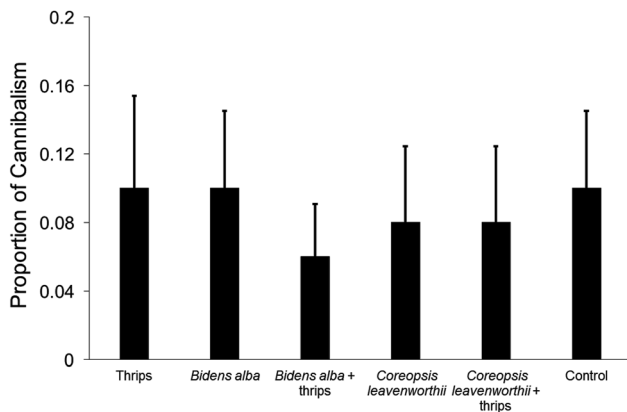
All 100 nymphs survived to adulthood. There was a significant difference among treatments for the nymphal development time ( $F_{4,95} = 3.62$ ;  $P = 0.0086$ ). *O. insidiosus* fed with a diet of *B. alba* and *F. tritici* had a reduced development time as compared to *O. insidiosus* nymphs provided with a diet of *F. tritici* alone ( $P < 0.05$ ). *O. insidiosus* fed on diets with *C. leavenworthii*, *C. leavenworthii* + *F. tritici*, or *B. alba* alone did not differ in development time as compared to the *O. insidiosus* fed on thrips only ( $P > 0.05$ ) (Figure 2).

## 3.3 Cannibalism

Cannibalism events were quite rare throughout all the experiment, with only 10 % for the *O. insidiosus* females provided with *O. insidiosus* nymphs only. After 48 h, there



**Figure 2:** Adult emergence time (mean  $\pm$  SEM days) for *Orius insidiosus* nymphs when fed a diet of thrips *Frankliniella tritici*, *Bidens alba* only, *B. alba* + *F. tritici*, *Coreoposis leavenworthii* only, or *C. leavenworthii* + *F. tritici*. Different lowercase letters indicate a significant difference among treatments ( $P < 0.05$ ).



**Figure 3:** Proportion of *Orius insidiosus* cannibalism after 48 h when fed a diet of thrips *Frankliniella tritici*, *Bidens alba* only, *B. alba* + *F. tritici*, *Coreopsis leavenworthii* only, *C. leavenworthii* + *F. tritici*, or a control with no food. The proportion was determined by the number of nymphs cannibalized divided by the total number of nymphs in each treatment (5). There was no significant differences among treatments ( $P > 0.05$ ).

were no significant differences in instances of cannibalism of *O. insidiosus* nymphs or adults for any of the diet treatments (chi-square = 6.6554; d.f. = 5;  $P = 0.2476$ ) (Figure 3).

## 4 Discussion

The current study suggests that adding flowers from *B. alba* and *C. leavenworthii* has a positive impact on *O. insidiosus* fitness. However, the effects varied by treatment: while *B. alba* reduced nymphal development time and increased survival when combined with thrips prey, *C. leavenworthii* increased *O. insidiosus* female survival by itself as compared to a diet with thrips only. However, no effect on development time was found for *C. leavenworthii*. Survival of *O. insidiosus* females fed with thrips and flowers (15 days for *B. alba* and thrips and 12 days for *C. leavenworthii* and thrips) was slightly higher than from other studies (14 days on average in Calixto et al. (2013) with a commercial mix of pollen from various flowers, 8 days in Wong and Frank (2013) with pepper flowers). Similarly, development times of *O. insidiosus* nymphs in this study (from 9.5 days for *B. alba* + thrips to 12 for thrips only) were longer than in Wong and Frank (2013) (7–8 days) but on a similar range compared with other studies (10–13 days in Mendes et al. (2002), 11–15 days in Bernardo et al. (2017)).

Previous studies have demonstrated the benefits of adding pollen to the diet of *Orius* spp. Wong and Frank (2013) and Waite et al. (2014) found that the addition of pollen from ornamental pepper cultivars (cv. ‘Black Pearl’ and ‘Purple Flash’) increased *O. insidiosus* survival and decreased

nymphal development time, and a pollen blend of several plants including *Eucalyptus* sp. (Myrtaceae), *Bidens pilosa* L. (Asteraceae), and *Citrus* sp. (Rutaceae) shortened nymphal development compared to a diet of prey only (Bernardo et al. 2017). In this study, because both plants were Asteraceae, we did not separate the pollen that are intricated within the tiny disc flowers. Therefore, we cannot rule out that *Orius* may also have fed on other parts of the flower in addition to the pollen. The results from this study regarding *O. insidiosus* female survival differ from that of previous studies where survival was not affected by the addition of pollen to a diet when compared to a diet of thrips prey or pollen only (Wong and Frank 2013). However, in all cases, including in the current study, *Orius* spp. fed a diet containing pollen have similar or increased longevity. The extent to which these effects occur may be dependent on the nutritional value of pollen, which stresses the importance of choosing appropriate pollen sources (Waite et al. 2014; Bernardo et al. 2017).

*O. insidiosus* is a common and popular commercial biological control agent used against *F. occidentalis* and other pests. As an omnivorous predator, they are known to utilize plant materials in addition to prey. When prey availability is low, however, many predators exhibit cannibalistic behaviors, including *O. insidiosus* (Leon-Beck and Coll 2007; Bueno et al. 2014). This study hypothesized that cannibalism would be reduced with the addition of *B. alba* and *C. leavenworthii* flowers than on prey alone as supplementing with plant material is expected to benefit nutrition (Leon-Beck and Coll 2007). Results, however, showed no difference in cannibalism amongst *O. insidiosus* because of diet and are supported by a previous study by Leon-Beck and Coll (2007) who found no differences in cannibalism occurrence in another *Orius* species, *Orius laevigatus* (Fieber), when provided with prey, pollen, or both. Interestingly, there was very low cannibalism ( $\leq 10\%$ ) in all treatments, including no prey or pollen, which suggests cannibalism among *O. insidiosus* may be minimal except in cases of extreme stress that may occur in industrial rearing (i.e., low food availability combined with lack of shelter or hiding spots (Bueno et al. 2014)).

The use of *B. alba* and *C. leavenworthii* flowers to supplement a prey diet can increase *O. insidiosus* survival and decrease nymphal development time, with some variations, depending on the pollen source. A shorter development time is considered a desirable life history trait for a biological control agent as it reduces the non-reproductive period and the stage where the individual is the most susceptible to intra-guild predation or cannibalism, it also allows for the rapid deployment of the agent to control the pest population (Facon 2011; Wong and Frank 2013; Sentis et al. 2022). In addition, shorter development time and higher adult survivorship



is usually correlated to higher fecundity in *Orius* sp. (Tommasini et al. 2004; Calixto et al. 2013). The results from this study suggest that these two plants may be valuable assets for increasing and sustaining *O. insidiosus* populations through conservation biological control and augmentative biological control in the case of mass rearing.

**Research ethics:** Not applicable.

**Author contributions:** Conceptualization IS, XM; Methodology: IS; Formal analysis IS; Investigation IS; Resources XM; Writing IS; Review and Editing IS, XM. The authors have accepted responsibility for the entire content of this manuscript and approved its submission.

**Competing interests:** The authors state no conflict of interest.

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**Data availability:** The raw data can be obtained on request from the corresponding author xmartini@ufl.edu.

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