Entomologia Generalis, Vol. 39 (2019), Issue 3–4, 205–219
Published online 27 September 2019

Article

Why can a predator increase its consumption of prey when it is released along with a parasitoid?

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With 2 figures and 3 tables

Abstract: The mixed release of predators and parasitoids to control a target pest can produce different results. In some cases, this mixed introduction can induce an increase in the predation rates of the pest and even of the parasitoid. To explain this phenomenon, it has been hypothesized that the presence of parasitoids (revealed by their mobility or related products) and interspecific competition (parasitoid vs predator) could influence such rates of predation. Therefore, in the present study we tested the effect of parasitoid mobility, host hemolymph (produced by parasitoid host-feeding) and parasitoid species (competing vs. non-competing species of the predator) on the number of prey consumed by the predator. Additionally, to add weight to the results of the bioassays, we determined whether the gender of the predators and parasitoids induced an effect on prey consumption by the predator by performing three bioassays under randomized block designs. Our results showed that neither parasitoid mobility nor host hemolymph presence modified the number of whitefly nymphs preyed upon by the predator. However, the number of whitefly nymphs consumed was significantly higher when the predator was introduced together with the competing parasitoid species relative to treatments with the non-competing parasitoid. In addition, we found that the predator preyed upon more mobile than immobile parasitoids and more competing than non-competing parasitoids. As for predator gender, we found that female predators consumed more whitefly nymphs relative to male predators and wasp gender did not affect predation. Overall, our results suggest that interspecific competition may be a more important factor regulating predator consumption than parasitoid mobility or the presence of the host hemolymph.

Keywords: *Trialeurodes*, *Geocoris*, *Eretmocerus*, interactions, mixed release

1 Introduction

Biological control is a strategy to control pests that employs natural enemies to reduce population densities of a target pest (Hajek & Eilenberg 2018). One of the essential requirements to make this control strategy work (or improve it) is to understand the ecology of the organisms involved and the way in which they interact (Polis & Strong 1996, Venzon et al. 2001). These interactions (e.g. competition, intraguild predation or parasitism) can modify the outcome of biological control by affecting trophic relationships and population density (Rosenheim et al. 1993, Venzon et al. 2001).

Natural enemies such as parasitoids and predators have been used in mixed release strategies in order to enhance arthropod pest control (Sher et al. 2000, Janssen et al. 2006, Chailleux et al. 2013, 2014, Bao-Fundora et al. 2016). In some cases, mixing natural enemies has resulted in a better control of the target pest relative to the employment of individual natural enemy species (Chailleux et al. 2013, 2014, Bao-Fundora et al. 2016). However, the mixed release of natural enemies has also resulted in a decrease (Rosenheim et al. 1993, Sher et al. 2000) or no change in the control of the target pest (Janssen et al. 2006). Under these scenarios, it becomes difficult to predict the outcome of mixed releases of natural enemies to control a target pest. Therefore, it is important to understand the complex interactions among prey, parasitoids and predators, as well as the factors influencing the performance of natural enemies released in combination.

In a previous study under semi-field conditions, the predator *Geocoris punctipes* Say (Hemiptera: Lygaeidae) increased its consumption of the whitefly *Trialeurodes vaporariorum* Westwood (Hemiptera: Aleyrodidae) during the mixed release of the predator and the parasitoid *Eretmocerus eremicus* Rose & Zolnerowich (Hymenoptera: Aphelinidae) relative to the individual release of the predator (Bao-Fundora et al. 2016). Other studies analyzing the effect of mixed use of predators and parasitoids to control a target pest have

DOI: 10.1127/entomologia/2019/0810
www.schweizerbart.de
0171-8177/2019/0810 $ 6.75

reported similar results (e.g. Rosenheim et al. 1993, Colfer & Rosenheim 2001). However, the mechanisms and factors underlying this predator's response are still unclear. Previous hypotheses explaining the increase of predation attacks, postulate that the presence of parasitoids (revealed by their movement or some related signals [see below]) and/or interspecific competition (predator-parasitoid) may be influencing that predator's response. The biological model consisting of the whitefly *T. vaporariorum*, the predator *G. punctipes* and the parasitoid *E. eremicus* offers a good opportunity to test those hypotheses. Indeed, it is known that *G. punctipes* has acute vision (Readio & Sweet 1982) and responds positively to prey movement (Eubanks & Denno 2000) and to pheromones (Jervis & Kidd 1986, Marques et al. 2000). This predator may therefore be able to detect the parasitoid presence by its movement or related signals (Tapia et al. 2010). For example, the odors or semiochemicals emitted when the parasitoids feed on their prey (i.e. 'host feeding') or during the oviposition process (Vet & Dicke 1992, Dicke & Grostal 2001, Wajnberg et al. 2008). It is known that *E. eremicus* is a parasitoid that performs host feeding whilst foraging for prey (Jervis & Kidd 1986, Headrick et al. 1995, Heimpel & Collier 1996). During host feeding the parasitoid makes a small wound on the dorsal zone of the whitefly nymph and feeds on its exposed hemolymph (Headrick et al. 1995). The exposed hemolymph and its compounds may emit aromatic signals (Vet & Dicke 1992, Dicke & Grostal 2001, Wajnberg et al. 2008) that can be used by the predator to locate potential prey (Epsky et al. 1993, Heath et al. 1995, Piñero et al. 2009). The aim of this study was to test whether the mobility of the parasitoids and the exposed hemolymph of the prey had an effect on the number of prey consumed by the predator. Additionally, to provide weight to these bioassays and new information on our biological model, we aimed to determine the effect of predator sex on the number of whitefly nymphs preyed upon. Previously, it has been reported that females of *G. punctipes* consumed more prey than conspecific males (Crocker et al. 1975, Chivarathanapong & Pitre 1980, Cohen 1984, Bueno et al. 2016). Thus, we predicted that female predators would prey upon more whitefly nymphs than male predators.

On the other hand, some predators respond to the presence of competing species by increasing or decreasing their rates of predation on shared prey (Van Buskirk 1988, Soluk & Collins 1988, Wilbur & Fauth 1990, Fauth 1990, Sih et al. 1998, Losey & Denno 1998). For example, Losey & Denno (1998) found that the combined predation rate of two predators was significantly higher than their individual impact on a shared prey. These authors postulated predator-specific prey defense as the underlying mechanism; prey avoid a predator increasing its exposure to the other predator. In the biological model studied here, the predator *G. punctipes* competes with the parasitoid *E. eremicus* for the use of whitefly nymphs. Therefore, we sought to determine to which extent predator and parasitoid competition influences the predation of the shared prey (whitefly nymphs). In particular, we assessed whether the species of parasitoid (*E. eremicus* [competing species] vs. *Tamarixia triozae* Burks (Hymenoptera: Eulophidae) [non-competing species] could affect the consumption of whitefly nymphs by the predator. Additionally, female parasitoids may represent a greater competitor (relative to the male parasitoids) for the predator because they use the same resource (i.e. whitefly nymphs). The parasitoid females oviposit their eggs in whitefly nymphs while the predator *G. punctipes* feeds them. In contrast, male parasitoids do not lay eggs. To better understand the potential effect of competition on predation of the shared prey, we tested the effect of the parasitoid gender on the predation of *G. punctipes* on whitefly nymphs. We expected that in the presence of female parasitoids (competing individuals) the predator consumed more whitefly nymphs than in the presence of male parasitoids (non-competing individuals).

The whitefly *T. vaporariorum* is one of the most important pests worldwide affecting crops such as tomatoes, cotton and beans (Jong-Kwan et al. 2001, Kennedy 2003, Angeles-López et al. 2012). The whitefly feeds on the sap of these crops, causing damage, either by direct consumption or by infecting the plant with viruses (Jones 2003, Wintermantel 2004). Several aspects of its basic biology and natural history have been previously described (Byrne and Bellows 1991, Martin 1999, Cardona et al. 2005). The predator *G. punctipes* and the parasitoid *E. eremicus* are both natural enemies of *T. vaporariorum*. The predator *G. punctipes* is known as a generalist predator of several pests affecting cotton, sugarcane, and other crops (Champlain and Sholdt 1967), including whitefly nymphs. The parasitoid *E. eremicus* is an endo-ecto parasitoid that can parasitize all nymphal stages of *T. vaporariorum* whitefly nymphs, but prefers the second and third stages (Headrick et al. 1995, Cardona et al. 2005). Both natural enemies are commercially available to control whiteflies (Van Lenteren 2003). On the other hand, *T. triozae* is an ecto-parasitoid of the potato psyllid *Bactericera cockerelli* Sulc (Hemiptera: Triozidae) (Weber 2013).

In this study we aimed to determine whether parasitoid mobility, the prey hemolymph and the parasitoid species had an effect on the number of prey consumed by the predator. We hypothesized that the predators would increase the number of whitefly nymphs consumed when: 1) moving parasitoids were present, 2) the prey's hemolymph was exposed and 3) a competing species of parasitoid was present.

2 Materials and methods

Plants and insects were grown and reared at 24 ± 3 °C, 50 ± 10% relative humidity (RH) and 14:10 h (light:dark) photoperiod as established previously by Velasco-Hernández et al. (2013) and Bao-Fundora et al. (2016).

2.1 Plants

Commercial tomato seeds (*Solanum lycopersicum* L. cv. 'Saladet') were acquired at La Casa del Hortelano (Guadalajara, Jalisco, México) and planted on plastic germinating trays (33.5 × 34.5 × 7 cm) containing Peat-Moss (Floragard, INIMEX, Zapopan, México). After reaching four leaves of development, plants were transferred to individual plastic pots (9 cm high, 11 cm diameter) containing Nutrigarden (Sulfatos y Derivados, S. A. de C. V.). Plants were irrigated with tap water containing "triple 18" fertilizer every third day (0.8 g per 1 L water). This fertilizer contained mainly N, K and P (SQM commercial de México S.A de C.V.). To avoid exposure to herbivores, plants were kept inside containers with their sides covered with anti-aphid mesh. Plants were used in experiments and for rearing insects once they reached seven leaves of development.

2.2 Insects

2.2.1 Whitefly *Trialeurodes vaporariorum*

Whitefly colonies maintained in our laboratory and used in experiments were founded with virus-free whiteflies, provided by Dr. Carla V. Sánchez-Hernández (Universidad de Guadalajara) and reared on tomato plants, placed inside acrylic cages (38.5 × 30.0 × 45.0 cm) with the sides covered with anti-aphid mesh. Taxonomic identification of whiteflies was performed by the Aleyrodidae specialist Dr. Vicente Carapia (Universidad Autónoma del Estado de Morelos). Experiments were performed using second and third instar nymphs, as it is known that *E. eremicus* prefers to parasitize these instars (Headrick et al. 1995, Cardona et al. 2005).

2.2.2 Parasitoids *E. eremicus* and *T. triozae*

Parasitoids were provided by Koppert Mexico S. A. de C. V. (Querétaro, México). *Eretmocerus eremicus* were provided as parasitized nymphs in cardboard shipping containers. These nymphs were placed inside acrylic cages (38.5 × 30.0 × 45.0 cm) to determine adult emergence date. When these adult wasps emerged, they were fed a honey solution (7:3 mL honey:water) and tap water (20 mL) offered each day in paper towels (7 cm × 7 cm) inside a Petri dish. *Eretmocerus eremicus* adults were 2 to 4 days old when used in experiments, taking into account its reproductive biology and lifespan. This parasitoid species can mate and oviposit on their first day as adults (Headrick et al. 1995) and can live up to 11 days (Asplen et al. 2001). *Tamarixia triozae* on the other hand has a mean life span of 20 days. Females are synovogenic and can oviposit an average of 165 eggs throughout their lives (Rojas et al. 2015). This parasitoid was provided as recently emerged adults (1 to 6 days old) and used in experiments when they were 7 to 11 days old, taking into account its reproductive biology and lifespan (Rojas et al. 2015). *Tamarixia triozae* individuals were maintained and fed following the same procedures and conditions as those described previously for *E. eremicus*.

2.2.3 Predator *G. punctipes*

This predator is a hemimetabolous species with the egg, five ninfal instars and the adult stage as part of its life cycle (Champlain and Sholdt 1967). Predator nymphs provided by Organismos Benéficos para la Agricultura SA de CV (Autlán de Navarro, Jalisco, México) were maintained inside polystyrene cages (40 × 30 × 31 cm) and fed *ad libitum* with 5 g of artificial diet (Cohen 1985), tap water (20 mL) offered in a paper towel (7 × 7 cm), commercial pollen (1.8 g, Apiarios Rancaño, Mexico City, Mexico) and sorghum seeds (3.4 g var. UDG-101, Zapopan, Jalisco Mexico) to improve development (Tillman and Mullinix 2003, Dunbar and Bacon 1972). The artificial diet and water were replaced every day, whereas pollen and sorghum seeds were replaced once a week (Velasco-Hernández et al. 2013). Predators were starved for 24h inside a plastic "clip cage" (23 mm high × 63 mm in diameter) that contained only tap water before use in experiments. Predators used in experiments were 8 to 20 days old, taking into account their pre-mating and pre-oviposition period and their lifespan (up to 108 days) (Dunbar 1972, Champlain and Sholdt 1967).

2.3 Bioassays

Bioassays were conducted inside an acclimatized room at 24 ± 3°C, 50 ± 10% RH and 1155 lux of light intensity at the Biological Control Laboratory (BCL) of the University of Guadalajara following Velasco-Hernández et al. (2013, 2017).

Bioassays were performed using 'arenas' that consisted of Petri dishes (15 mm high × 90 mm in diameter) containing a 5 mm layer of 1% (m/V) agar to provide humidity. The agar layer was covered with filter paper (ISOLAB medium porosity, 8.5 cm diameter) to aid predator movement. Each arena was covered with a lid containing two holes (3 cm in diameter) covered with anti- aphid mesh for ventilation, and a third hole (1 cm in diameter) to introduce natural enemies, which was subsequently covered with cotton to avoid insects escaping. All Petri dishes were sealed with Parafilm® (Bemis Company Inc., Neenah, Winsconsin, E. U. A).

The whitefly nymphs were individualized using a 1/8 metal cork-borer (Enestra 3mm diameter) to cut off the leaf area surrounding each nymph. Circles containing only one whitefly nymph were placed randomly and equidistantly over the filter paper inside the arena. All bioassays were performed following a randomized block design, with day as the blocking factor. Each treatment was replicated 20 times and for each replicate new insects and plants were used to avoid pseudoreplication.

2.3.1 Effect of parasitoid mobility on the number of *T. vaporariorum* nymphs preyed upon

The aim of this bioassay was to assess the effect of parasitoid mobility, and parasitoid and predator gender on the number of prey (*T. vaporariorum* nymphs [hereafter referred as 'Tv'] or *E. eremicus* [hereafter referred as 'Ee']) consumed by

G. punctipes (hereafter referred as 'Gp'). We used a randomized block design with the main factors 'parasitoid mobility' (levels: mobile vs. immobile), 'parasitoid gender' (levels: ♀Ee vs. ♂Ee) and 'predator gender' (levels: ♀Gp vs ♂Gp) and the experimental day as the blocking factor. The factor 'parasitoid gender' was included to establish if the predator exhibited different consumption rates depending upon the gender of the parasitoid. This may be possible if it is considered that female parasitoids are the ones who oviposit and use the Tv nymphs, thereby representing a direct competitor for the predator, perhaps more so than male parasitoids.

As a result, we tested 11 treatments (details in Table 1): **i)** Tv alone, **ii)** Tv + ♀Gp, **iii)** Tv + ♂Gp, **iv)** Tv+♀Ee mobile (hereafter referred as 'Mob')+♀Gp, **v)** Tv+♀Ee immobile (hereafter referred as 'Im')+♀Gp, **vi)** Tv+♂Ee Mob+♀Gp, **vii)** Tv+♂Ee Im+♀Gp, **viii)** Tv+♀Ee Mob+♂Gp, **ix)** Tv+♀Ee Im+♂Gp, **x)** Tv+♂Ee Mob+♂Gp and, **xi)** Tv+♂Ee Im+♂Gp. All treatments contained 36 Tv nymphs at 2nd-3rd nymphal stage not previously exposed to wasps or predators. This number was chosen based on previous results investigating the rate of consumption of this predator under similar experimental conditions (see Velasco-Hernández et al. 2013). In treatments where parasitoids were used, we introduced 7 parasitoids to maximize presence (and detection probability) and avoidance of competition among Ee female wasps, accounting for their rate of parasitism (Powell and Bellows 1992). Following Provost et al. (2006) we used a freezing procedure to immobilize individuals. Preliminary tests were then performed to assess a freezing procedure that immobilized parasitoids without

Table 1. Experimental design for the bioassay 1: 'effect of parasitoid mobility on the number of *T. vaporariorum* nymphs consumed by *G. punctipes*'.

	Treatment	Details			Setup description
		parasitoid movement	# individuals, parasitoid gender	# individuals, predator gender	
i	Tv alone [emergence control]	–	–	–	Whitefly nymphs followed the same procedure as those used in the other treatments but without exposure to predators or parasitoids. They were supervised until whitefly adult emergence. The number of whitefly adults was recorded.
ii	Tv+♀Gp [control of female predator]	–	–	1,♀	Once the arena containing Tv nymphs was ready, the predator was released.
iii	Tv+♂Gp [control of male predator]	–	–	1,♂	Once the arena containing Tv nymphs was ready, the predator was released.
iv	Tv+♀Ee Mob+♀Gp	Mob	7,♀	1,♀	Once the arena containing the Tv nymphs was ready, parasitoids were introduced in the arena and immediately after this, the predator was introduced.
v	Tv+♀Ee Im+♀Gp	Im	7,♀	1,♀	Parasitoids were first immobilized following the freezing procedure described in detail in the text. After this, the arena containing the Tv was setup. Ten minutes before the introduction of the predator, parasitoids were ready and placed inside the arena. Just after introduction of parasitoids, the predator was introduced into the arena.
vi	Tv+♂Ee Mob+♀Gp	Mob	7,♂	1,♀	This treatment was similar to treatment iv but using male parasitoids.
vii	Tv+♂Ee Im+♀Gp	Im	7,♂	1,♀	This treatment was similar to treatment v but using male parasitoids.
viii	Tv+♀Ee Mob+♂Gp	Mob	7,♀	1,♂	This treatment was similar to treatment iv but using a male predator.
ix	Tv+♀Ee Im+♂Gp	Im	7,♀	1,♂	This treatment was similar to treatment v but using a male predator.
x	Tv+♂Ee Mob+♂Gp	Mob	7,♂	1,♂	This treatment was similar to treatment iv but using male parasitoids and a male predator.
xi	Tv+♂Ee Im+♂Gp	Im	7,♂	1,♂	This treatment was similar to treatment v but using male parasitoids and a male predator.

Tv = *Trialeurodes vaporariorum* nymphs; Gp = *Geocoris punctipes*; Ee = *Eretmocerus eremicus*; Mob = mobile; Im = immobile

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killing wasps or affecting predator consumption. As a result, Ee parasitoids were immobilized by freezing them at –14°C during 90 min.

For all treatments, each arena was first set up without predators and subsequently predators were released and allowed to forage for 6 hours. After 6 hours, predators were extracted and the number of Tv and Ee preyed upon were recorded by checking each arena and the individuals within it under a Carl Zeiss stereoscope (Carl Zeiss de México S. A. de C. V. Mexico City, Mexico). In addition, we recorded the time at which predators made the first attack on Tv by observing the arenas after the predators were released.

At all bioassays and for each factor the selection of the best approximating model was made using the Akaike's information criterion (AIC) (Symonds and Mousalli 2011). In addition, when the control group (Tv + Gp) was included in an analysis (e.g. for the analysis of 'Tv preyed upon' and 'time to first attack on Tv' in bioassays 1 and 3), a 1-way analysis of variance per factor was performed because the control group included levels not shared with the others factors. When the control group (Tv + Gp) was not included in an analysis (e.g. for the analysis of 'Ee preyed upon' in bioassay 1 and 3 or response variables in bioassay 2), full factorial (2 or 3-way) analyzes of variance were performed. All analyses were performed using R, V.3.5.1 (R Core Team 2014) and comparisons among treatments were performed using the contrast derived from the models (Crawley 2007). When specified, data transformations were performed to fit the requirements of normality and homoscedasticity (Zar 1998).

For the first bioassay, the effect of the factors 'parasitoid mobility' and 'parasitoid gender' on the response variable 'mean number of Tv preyed upon', were analyzed using linear mixed models (LMMs) with those factors as fixed effects and the block (days) as the random factor. The effect of the factor 'predator gender' on the Tv nymphs consumed was analyzed using a generalized linear model (GLM) with a Gaussian error and an identity link function (Crawley 2007). The 'mean number of Ee preyed upon' was analyzed via a linear three-way ANOVA after data were subjected to a square root (x + 0.5) transformation (Zar 1998). In this 3-way ANOVA, the three factors were integrated into the model as fixed effects and the block (days) as the random factor. The effect of factors 'parasitoid mobility' and 'predator gender' on the response variable 'time to first attack on Tv' was analyzed using a one-way ANOVA per factor, after response variable was log (x + 1) transformed (Zar 1998). The effect of factor 'parasitoid gender' on the time to first attack was analyzed using a LMM with the host species as the fixed factor and the block (days) as the random factor (Crawley 2007).

2.3.2 Effect of recently exposed Tv hemolymph on the number of Tv nymphs preyed upon

The aim of this bioassay was to assess the effect of recently exposed Tv hemolymph on the number of prey consumed by *G. punctipes*. We performed a randomized block design with the factors 'Tv hemolymph' (levels: presence vs. absence) and 'predator gender' (levels: ♀Gp vs. ♂Gp) as main factors while the day of experimentation was the blocking factor. To obtain enough Tv nymphs with presence of hemolymph, Tv nymphs were prepared with a puncture simulating the one made by the parasitoid during host-feeding (according to preliminary observations). The required Tv nymphs were therefore punctured once in the dorsal part of their body, using a very fine pin (13 mm length and 0.25 mm diameter) (Terumo, Terumo Medical de Mexico SA de CV, Mexico City, Mexico). All the wounds were made just before starting the observations to ensure the presence of recently exposed hemolymph before the introduction of the predators. It should be noted that Tv were punctured only once and this was done using a Carl Zeiss DV4 stereoscope in order to achieve high precision (Carl Zeiss de México S. A. de C. V. Mexico City, Mexico). The factor ´predator gender´ was included in this second bioassay by taking the results of the bioassay 1 into account (see Fig. 1A) and aiming to increase robustness to this second bioassay. As a result, we tested five treatments (details in Table 2): **i)** Tv alone, **ii)** Tv hemolymph absent + ♀Gp, **iii)** Tv hemolymph present + ♀Gp, **iv)** Tv hemolymph absent + ♂Gp, **v)** Tv hemolymph present + ♂Gp.

After all arenas were set up we released the predators and allowed them to forage for 6 hours. After 6 hours, the number of Tv preyed upon by *G. punctipes* was recorded using a Carl Zeiss DV4 stereoscope (Carl Zeiss de México S. A. de C. V. Ciudad de México, México). In addition, we recorded the time to first attack on Tv.

The effect of the factors 'Tv hemolymph' and 'predator gender' on the 'mean number of Tv nymphs preyed upon' by *G. punctipes* was analyzed using a two-way ANOVA, after data were square root (x + 0.5) transformed (Zar 1998). The effect of the factors 'Tv hemolymph' and 'predator gender' on the response variable 'time to first attack on Tv' was analyzed using a two-way ANOVA, after the response variable was log (x + 1) transformed (Zar 1998).

2.3.3 Effect of parasitoid species on the number of Tv nymphs preyed upon

This bioassay was performed mainly to assess the effect of two parasitoid species (Ee [competing species] and *Tamarixia triozae*, hereafter Tt [non-competing species]) on the number of Tv preyed upon by Gp. We then performed a randomized block design with the factors 'parasitoid species' (levels: Ee vs. Tt), 'parasitoid gender' (level: ♀ vs. ♂) and 'parasitoid mobility' (levels: mobile vs. immobile) as main factors and the experimentation day as the blocking factor. The factors 'parasitoid gender' and 'parasitoid mobility' were also included in this bioassay taking into account results of bioassay 1 (see Figure 1B) and aiming to increase robustness to this third bioassay. Only female predators were used in this bioassay because they had higher rates of predation (Figure 1A) and to focus on the factors related to the

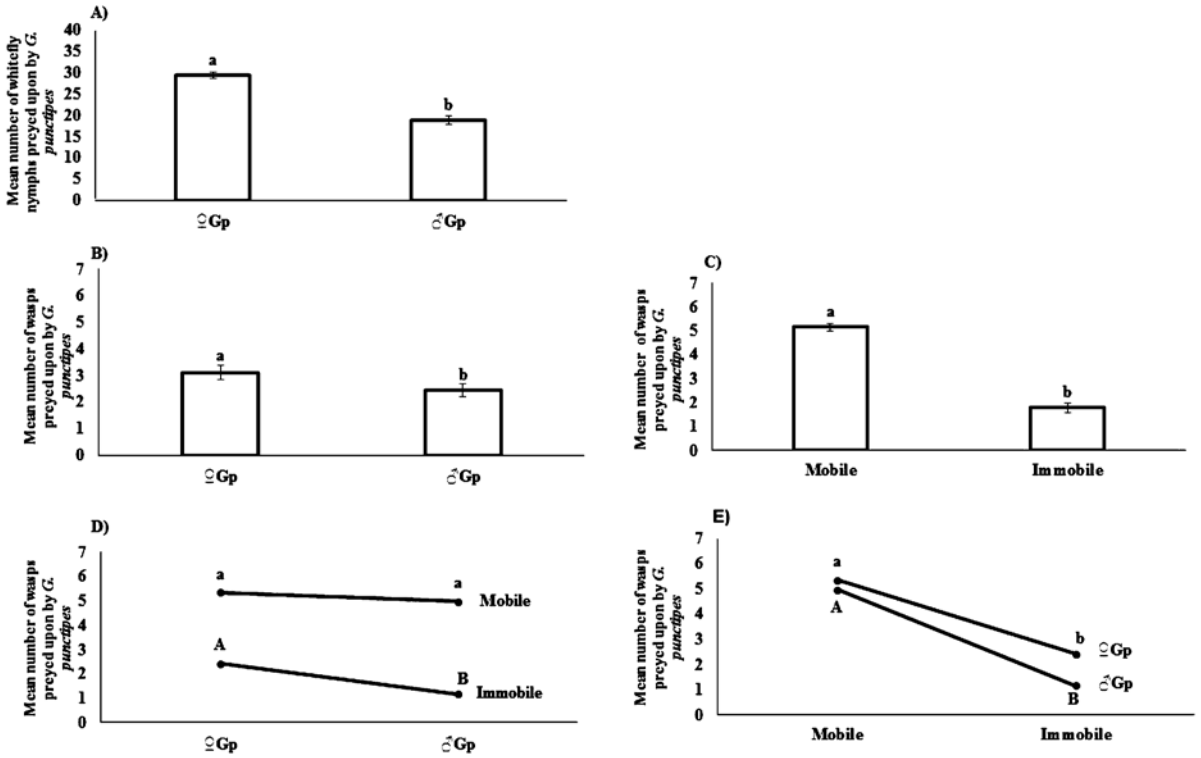

Fig. 1. Mean (± SE) number of whitefly nymphs (A) and parasitoids (B–E) preyed upon by *Geocoris punctipes*. ♀Gp = female *Geocoris punctipes*, ♂Gp = male *Geocoris punctipes*, Mobile = mobile parasitoids present, Immobile = immobile parasitoids present. Different letters indicate significant differences ($P < 0.05$).

Table 2. Experimental design for the bioassay 2: 'effect of T. vaporariorum heolymph on the numer of T. vaporariorum nymphs consumed by *G. punctipes*'.

	Treatment	Details		Setup description
		prey hemolymph	# individuals, predator gender	
i	Tv alone [emergence control]	–	–	Whitefly nymphs followed the same procedure as those used in the other treatments but without exposure to predators or parasitoids. They were supervised until whitefly adult emergence. The number of whitefly adults was recorded.
ii	Tv hemolymph absent +♀Gp	Absent	1,♀	Once the arena containing Tv nymphs was ready, the predator was released and allowed to forage for 6 h.
iii	Tv hemolymph present +♀Gp	Present	1,♀	All Tv nymphs were first punctured to simulate a host-feeding wound. Immediately after Tv puncture, nymphs were placed in the arena and the predator was released and allowed to forage for 6 h.
iv	Tv hemolymph absent +♂Gp	Absent	1,♂	This treatment was similar to treatment ii but introducing a male predator.
v	Tv hemolymph present+♂Gp	Present	1,♂	This treatment was similar to treatment iii but introducing a male predator.

Tv = *Trialeurodes vaporarioum* nymphs; Gp = *Geocoris punctipes*; Ee = *Eretmocerus eremicus*; Mob = mobile; Im = immobile

20191215-103834
C7285/16634/B083B3A6

parasitoids. As a result, we tested 10 treatments (details in Table 3): **i)** Tv alone, **ii)** Tv + Gp, **iii)** Tv + ♀Ee Mob + Gp, **iv)** Tv + ♀Ee Im + Gp, **v)** Tv + ♂Ee Mob + Gp, **vi)** Tv + ♂Ee Im + Gp, **vii)** Tv + ♀Tt Mob + Gp, **viii)** Tv + ♀Tt Im + Gp, **ix)** Tv + ♂Tt Mob + Gp, **x)** Tv + ♂Tt Im + Gp. The number and stages of Tv nymphs employed were the same as those in bioassay 1. To immobilize parasitoids, we followed the freezing procedure described in bioassay 1. However, Tt was immobilized by freezing individuals without killing them or affecting predation rate at –14°C during 22 hours. For all treatments, the time that the predators were allowed to forage was 6 hours. After this period of time, predators were extracted and the number of Tv, Ee and Tt preyed upon were recorded. In addition, the time to the first attack on Tv was also recorded.

The effect of factors 'parasitoid species', 'parasitoid gender' and 'parasitoid mobility' on the 'mean number of Tv nymphs preyed upon' was analyzed using a LMM per factor modeling those factors as fixed effects and the block (days) as the random factor. The effect of the factors 'parasitoid species', 'parasitoid gender' and 'parasitoid mobility' on the 'mean number of Ee preyed upon' was analyzed using a LMM with the three factors integrated into the model as fixed effects and the block (days) as the random factor. The effect of the factors 'parasitoid species', 'parasitoid gender' and 'parasitoid mobility' on the 'time to first attack on Tv' was analyzed using a one-way ANOVA per factor, after a square root (x + 0.5) transformation of the response variable (Zar 1998).

Table 3. Experimental design for the bioassay 3: 'effect of parasitoid species on the number of *T. vaporariorum* nymphs consumed by female *G. punctipes*'.

	Treatment	Details			Setup description
		# individuals, parasitoid species	Parasitoid gender	Parasitoid movement	
i	Tv alone [emergence control]	–	–	–	Whitefly nymphs followed the same procedure as those used in the other treatments but without exposure to predators or parasitoids. They were supervised until whitefly adult emergence. The number of whitefly adults was recorded.
ii	Tv+Gp [control of female predator consumption]	–	–	–	Once the arena containing Tv nymphs was ready, a female predator was released.
iii	Tv+♀Ee Mob+Gp	7, Ee	♀	Mob	Once the arena containing the Tv nymphs was ready, parasitoids were introduced in the arena and immediately after this, a female predator was introduced.
iv	Tv+♀Ee Im+Gp	7, Ee	♀	Im	Parasitoids were first immobilized following the freezing procedure described in detail in the text (bioassay 1). After this, the arena containing the Tv was setup. Ten minutes before the introduction of the predator, parasitoids were ready and placed inside the arena. Just after introduction of parasitoids, a female predator was introduced into the arena.
v	Tv+♂Ee Mob+Gp	7, Ee	♂	Mob	This treatment was similar to treatment iii but using male parasitoids.
vi	Tv+♂Ee Im+Gp	7, Ee	♂	Im	This treatment was similar to treatment iv but using male parasitoids.
vii	Tv+♀Tt Mob+Gp	7, Tt	♀	Mob	This treatment was similar to treatment iii but using Tt as the parasitoid species.
viii	Tv+♀Tt Im+Gp	7, Tt	♀	Im	This treatment was similar to treatment iv but using Tt as the parasitoid species.
ix	Tv+♂Tt Mob+Gp	7, Tt	♂	Mob	This treatment was similar to treatment iii but using Tt male parasitoids.
x	Tv+♂Tt Im+Gp	7, Tt	♂	Im	This treatment was similar to treatment iv but using Tt male parasitoids.

Tv = *Trialeurodes vaporarioum* nymphs; Gp = female *Geocoris punctipes*; Ee = *Eretmocerus eremicus*; Mob = mobile; Im = immobile

3 Results

3.1 Effect of parasitoid mobility on the number of *T. vaporariorum* nymphs preyed upon

The factors 'parasitoid mobility' and 'parasitoid gender' did not significantly affect the mean number of Tv nymphs preyed upon by *G. punctipes* ($F_{2,190}$ = 1.628; P = 0.198 and $F_{2,190}$ = 1.593; P = 0.206, respectively). The predator preyed upon (mean ± SE) 23.5 (± 1.1) Tv nymphs in presence of mobile parasitoids and 23.4 (± 1.2) when immobile wasps were present. When female parasitoids were present *G. punctipes* consumed 22.9 (± 1.2) Tv nymphs and 24.0 (± 1.1) in presence of male parasitoids. Contrastingly, the factor 'predator gender' significantly influenced the number of Tv nymphs preyed upon ($F_{1,191}$ = 121.77; P < 0.001). Female predators consumed significantly more Tv nymphs than male predators (Figure 1A).

The factors 'parasitoid mobility', 'predator gender' and their interaction showed a significant effect on the number of Ee preyed upon ($F_{1,145}$ = 197.003, P < 0.001; $F_{1,145}$ = 10.258, P = 0.001 and $F_{1,145}$ = 4.587, P = 0.033, respectively). Female predators preyed upon significantly more parasitoids than male predators (Figure 1B). The predator consumed significantly more mobile parasitoids than immobile counterparts (Figure 1C). Additionally, when parasitoids were mobile, female and male predators preyed upon similar number of parasitoids (Figure 1D); however, when parasitoids were immobile, female predators significantly preyed upon more parasitoids than male predators (Figure 1D). Our findings also indicate that both, female and male predators consumed significantly more mobile parasitoids than immobile counterparts (Figure 1E). On the other hand, the factor 'parasitoid gender' did not significantly affect the number of parasitoids preyed upon by *G. punctipes* ($F_{1,145}$ = 2.222, P = 0.138). The predators consumed 3.3 (± 0.2) female parasitoids and 3.6 (± 0.2) male parasitoids.

Regarding the time to first attack on Tv nymphs, results indicate that none of the tested factors modified this response variable significantly (parasitoid mobility: $F_{2,190}$ = 1.055, P = 0.349; parasitoid gender: $F_{2,190}$ = 1.013, P = 0.364; predator gender: $F_{1,191}$ = 2.259, P = 0.134). The predators performed their first attack on Tv nymphs after 4332.4 s (± 666.5 s) when mobile parasitoids were present and after 5065.9 s (± 734.4 s) when immobile parasitoids were introduced. The time to first attack of predators on Tv nymphs was 4941.5 s (± 757.5 s) when female parasitoids were present and of 4456.8 s (± 641.5 s) when male counterparts were introduced. Female predators took 3513.2 s (± 531.6 s) to perform their first attack on Tv nymphs while male predators took 5208.3 s (± 663.7 s).

3.2 Effect of recently exposed Tv hemolymph on the number of Tv nymphs preyed upon

Our results showed that the number of Tv nymphs preyed upon by *G. punctipes* was not significantly affected by the presence of Tv hemolymph ($F_{1,70}$ = 0.091; P = 0.763). Predators consumed 23.1 (± 1.6) nymphs in the presence of Tv hemolymph and 22.9 (± 1.6) Tv nymphs in the absence of Tv hemolymph. In contrast, the gender of the predator significantly influenced the number of Tv nymphs preyed upon ($F_{1,70}$ = 39.937; P <0.001). Female predators consumed 28.5 (± 1.0) Tv nymphs while male predators consumed 17.4 (±1.6) Tv nymphs. The interaction of both factors was not significant ($F_{1,70}$ = 1.462; P = 0.230).

Similar results were found when the response variable 'time to first attack on Tv nymphs' was analyzed. The factor 'Tv hemolymph' did not influenced the time to first attack of the predator on Tv nymphs ($F_{1,70}$ = 1.156; P =0.285). The mean time that predators took to perform their first attack on Tv nymphs in the presence of Tv hemolymph was 4511.4 s (± 1103.8 s) while in the absence of hemolymph it took 5946.5 s (± 1197.5 s). Contrastingly, the gender of the predator significantly influenced the time at which they performed their first attack on Tv nymphs ($F_{1,70}$ = 8.727; P = 0.004). Female predators took 2680.8 s (± 697.0 s) to perform their first attack on Tv nymphs while male predators took 7777.1 s (± 1363.9 s). The interaction of both factors was not significant ($F_{1,70}$ = 0.087; P = 0.768).

3.3 Effect of parasitoid species on the number of Tv nymphs consumed

Our results showed that parasitoid species significantly influenced the number of Tv nymphs preyed upon by *G. punctipes* ($F_{2,170}$ = 5.626; P = 0.004). The predators consumed significantly more Tv nymphs in the presence of Ee (the competing species) than Tt (the non-competing species) (Fig. 2A). When the factors 'parasitoid gender' and 'parasitoid mobility' were analyzed, we found a significant difference between the control group and the two treatments ($F_{2,170}$ = 7.268, P < 0.001 and $F_{2,170}$= 7.582; P < 0.001, respectively). However, when subsequent paired comparisons between 'female' (♀) *vs.* 'male' (♂) and 'mobile' *vs.* 'immobile' treatments were performed, we found no significant differences ($t_{1,170}$ = 1.098, P = 0.273 and $t_{1,170}$ = 0.100, P = 0.919, respectively). Predators preyed upon (mean ± SE) 27.6 (± 1.2) Tv nymphs in the presence of female (♀) parasitoids and 29.3 (± 1.0) Tv nymphs in the presence of male (♂) parasitoids. When mobile parasitoids were present, the predators consumed 28.4 (± 1.1) Tv nymphs compared to 28.5 (± 1.1) nymphs when immobile parasitoids were present.

The factors 'parasitoid species' and 'parasitoid mobility' significantly affected the number of parasitoids preyed upon by *G. punctipes* ($F_{1,145}$ = 8.590, P = 0.003; $F_{1,145}$ = 11.428, P < 0.001, respectively). Predators consumed significantly

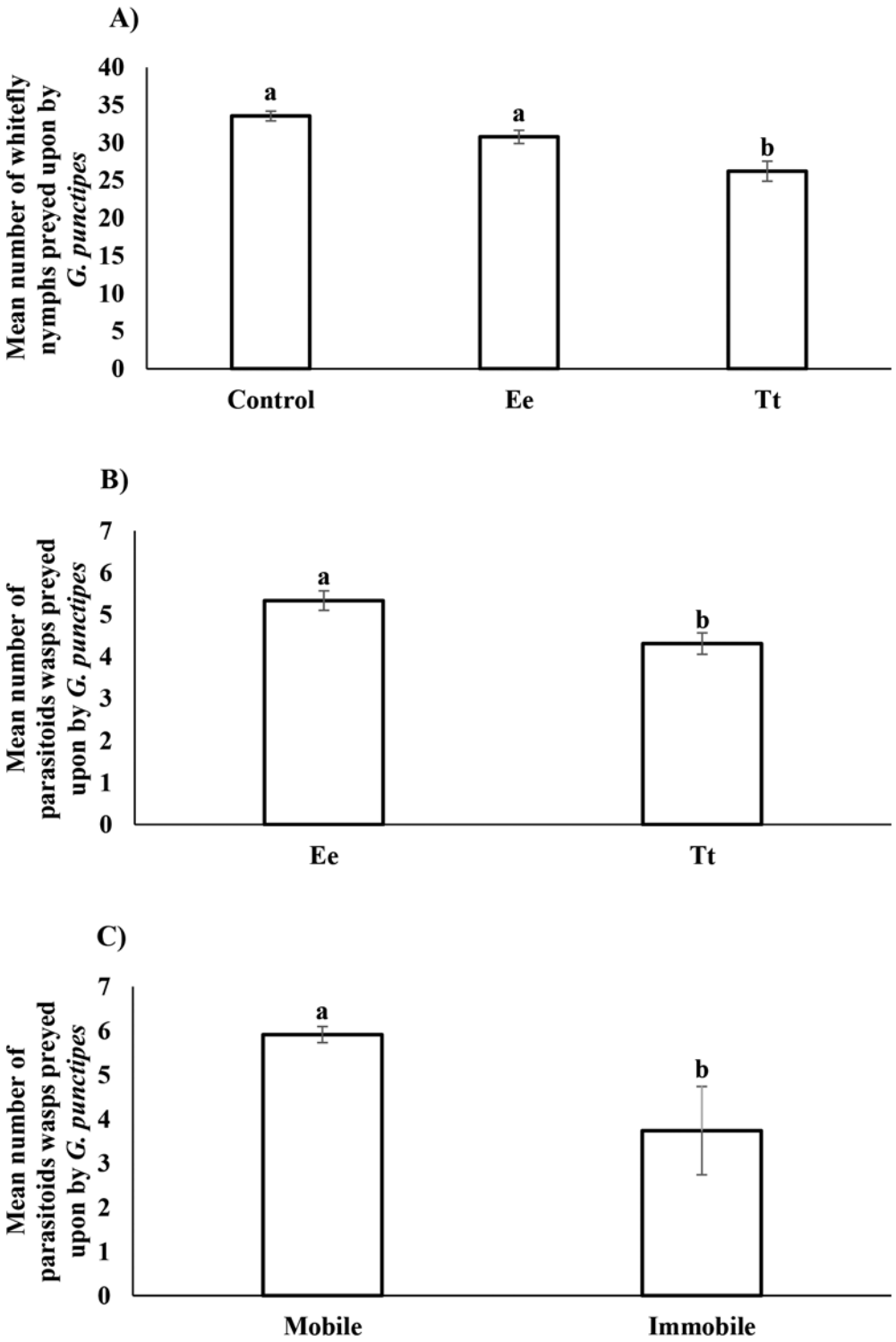

Fig. 2. Mean (±SE) number of whitefly nymphs (A) and parasitoids (B and C) preyed upon by *Geocoris punctipes*. Ee= *Eretmocerus eremicus* parasitoids, Tt= *Tamarixia triozae* parasitoids, Mobile=mobile parasitoids present, Immobile=immobile parasitoids present. Different letters indicate significant differences ($P < 0.05$).

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more Ee (the competing species) than Tt (the non-competing species) parasitoids (Fig. 2B). In addition, predators consumed significantly more mobile parasitoids than immobile counterparts (Fig. 2C). In contrast, the factor 'parasitoid gender' did not significantly affect the number of parasitoids preyed upon by *G. punctipes* ($F_{1,145}$ = 0.223, P = 0.636). When predators were introduced together with female parasitoids, the mean number of consumed parasitoids was 4.9 (± 0.2), while 4.7 (± 0.2) parasitoids were consumed in the presence of male parasitoids.

The time that predators took to perform the first attack on Tv nymphs was not significantly affected by parasitoid species ($F_{2,170}$ = 0.211, P = 0.809), parasitoid gender ($F_{2,170}$ = 0.539, P = 0.584) or parasitoid mobility ($F_{2,170}$ = 1.207, P = 0.301). Predators took (mean ± SE) 1711.5 seconds (± 228.4 s) to attack Tv nymphs in presence of Ee parasitoids and, 1464.9 s (±185.8 s) when Tt counterparts were present. When female parasitoids were present, predators took 1688.9 s (± 216.4 s) to perform the first attack on Tv nymphs, while they took 1487.6 s (± 166.3 s) in presence of male parasitoids. As for parasitoid mobility, predators took 1596.2 s (± 204.8 s) to perform the first attack on Tv nymphs in presence of mobile parasitoids, and 1580.2 s (± 212.5 s) in presence of immobile parasitoids.

4 Discussion

Previous studies have reported that the mixed release of natural enemies (predators and parasitoids) can induce an increase in pest predation rates (Rosenheim et al. 1993, Finke and Denno 2003, Bao-Fundora et al. 2016, Tan et al. 2016). Several factors have been put forth to explain this phenomenon, including the proximal detection of parasitoids by the predator (revealed by their mobility or related semiochemicals) and the role of inter-specific competition (predator-parasitoid). However, to our knowledge, the effects of parasitoid mobility and the presence of the hemolymph on the prey (produced during host-feeding) on pest predation levels have not been tested empirically. In the present study, we aimed to determine whether parasitoid mobility, the presence of nymphs' hemolymph and the species of parasitoid affected the number of nymphs preyed upon by *G. punctipes*. Our results showed that neither parasitoid mobility nor the presence of hemolymph of the nymphs induced a significant change in the number of nymphs preyed upon by *G. punctipes*. In contrast, the species of parasitoid affected the number of Tv nymphs preyed upon by the predator. The predator consumed more Tv nymphs when it was in the presence of *E. eremicus* parasitoids (the competing species) than when it was in the presence of *T. triozae* (the non-competing species) (Figure 2A). Other results of this study are discussed in detail below.

4.1 Effect of parasitoid mobility on the number of *T. vaporariorum* nymphs preyed upon

We initially tested the hypothesis that parasitoid mobility would induce an increase in the number of nymphs preyed upon by the predator. However, we found no evidence to support this hypothesis. The number of nymphs preyed upon was similar in the presence of mobile and immobile parasitoids. As *G. punctipes* consumes more mobile than immobile prey (Eubanks and Denno 2000), we postulated that the presence of mobile wasps would cause a higher overall consumption of all organisms present in the arenas, including the Tv nymphs. However, our results show that this is not the case. Parasitoid mobility induced a higher attack on mobile parasitoids (Figure 1C, 1E), but not on the Tv nymphs present in the arenas. Our results therefore confirm that the predator *G. punctipes* consumes more mobile prey (Figure 1C, 1E) but showed that the presence of mobile prey does not affect the rate of predation of other prey present in the system. In terms of trophic interactions, when both whitefly natural enemies are present (predator and parasitoid), the whitefly may benefit if the predator attacks the parasitoid. However, that benefit does not seem to occur because parasitoid presence resulted in greater whitefly predation (Bao-Fundora et al. 2016). As discussed below, our results indicate that this negative inter-guild interaction for the whitefly is perhaps more closely related to inter-guild competition. In addition, the higher predation of mobile parasitoids helps to better understand why the rates of parasitism are significantly reduced in mixed releases of predator-parasitoid compared to the isolated release of parasitoids (Bao-Fundora et al. 2016). Parasitoid mobility leads to a higher rate of mortality and consequently, a potential reduction in parasitism.

We expected that the number of nymphs consumed by the predator would increase when it was found in the presence of female parasitoids as females can compete with the predator either by parasitizing the Tv nymphs or by consuming them (Headrick et al. 1995, Giron et al. 2002, Cardona et al. 2005, Hirose et al. 2009, Zang and Liu 2010, Yang et al. 2012). However, our results showed that parasitoid gender did not significantly influence the consumption of nymphs by the predator. This suggests that the predator cannot discriminate parasitoid gender, and is therefore not able to identify which individuals are competitors at this level. *Geocoris punctipes* has an acute vision (Readio and Sweet 1982) enabling it to distinguish mobile from immobile prey (Eubanks and Denno 2000). However, its apparent inability to recognize *E. eremicus* females from males might be explained by the similar size and appearance of both genders (Rose and Zolnerowich 1997). In applied terms, our results therefore indicate that similar rates of pest control may be obtained using arrhenotokous parasitoids (individuals of both genders) or thelytokous wasps (only female individuals) (Ramirez-Romero et al. 2012) as parasitoid gender will not affect predator consumption.

We found that female *G. punctipes* consumed significantly more Tv nymphs compared to male predators (Figure 1A). This result is in line with previous studies reporting that *G. punctipes* females consumed significantly more eggs of *Pseudoplusia includens* (Walker), than males (Crocker et al. 1975). This result is potentially related to the fact that females need to consume more prey due to the metabolic demand of egg production (Crocker et al. 1975). In terms of biological pest control, our results indicate that females should preferably be released because they would consume more individuals of the pest than males. However, additional observations are needed to verify this hypothesis.

We found no significant influence of wasp gender on the number of 'Ee preyed upon'. However, mobile wasps were consumed more by *G. punctipes* than immobile parasitoid counterparts (Figure 1C) and female predators consumed more wasps than male predators (Figure 1B). Additionally, we found a significant interaction between the factors 'parasitoid mobility' and 'predator gender', indicating that when wasps were mobile, both female and male predators consumed a similar number of wasps (Figure 1D). Contrastingly, when parasitoids were immobile, female predators consumed significantly more parasitoids than males (Figure 1D). This indicates that female predators may have better abilities to detect immobile prey than male predators. Studies at the level of signal perception are necessary to determine if differences between female and male predators exist at that level. Alternatively, female predators may be more willing to attack immobile prey, as they require less investment of time and energy compared to mobile prey. Such energy saved could be invested in the high metabolic demand of egg production and reproduction (Calow 1979).

4.2 Effect of recently exposed Tv hemolymph on the number of Tv nymphs preyed upon

We tested the hypothesis that the presence of hemolymph of Tv nymphs would increase their rates of predation by *G. punctipes*. When *E. eremicus* practice 'host-feeding' on Tv nymphs, they may generate a signal revealing the presence of wasps or nymphs and thereby cause an increase in predation rates. In fact, as a result of its organic and inorganic components (Jervis and Kidd 1986, Godfray 1994, Handke et al. 2013, Gill et al. 2017) hemolymph can emit aromatic signals that can be detected by a predator (Rittschof et al. 1992, Epsky et al. 1993, Heath et al. 1995, Small and Thacker 1994, Piñero et al. 2009). Previous studies have shown that predators can modify their predation rates due to the influence of preys' hemolymph (Müller and Brakefield 2003, Lundgren et al. 2009). Additionally, *G. punctipes* has been reported to respond positively to pheromones (Marques et al. 2000). However, contrary to our predictions, for our biological model and under our experimental conditions, we found that the number of Tv nymphs preyed upon by the predator was similar in the treatments with and without hemolymphs. This suggests that the hemolymph produced through host-feeding of *E. eremicus* on Tv nymphs, does not influence the rate of predator consumption of prey. This result also indicates that other mechanisms of finding prey are more important for this predator species (e.g. vision [Readio and Sweet 1982]) or require further investigation (e.g. mouthpart structures [Shimoda et al. 1997]). Finally, the results of 'predator gender' were similar to those of bioassay 1, confirming that females of *G. punctipes* consume more Tv nymphs than male predators.

4.3 Effect of parasitoid species on the number of Tv nymphs consumed

Some predators respond to the presence of competing species by increasing or reducing their rates of predation of the shared prey (Wilbur and Fauth 1990, Wissinger and McGrady 1993, Leppanen et al. 2012). For example, Leppanen et al. (2012) found that predators can reduce or increase their rates of predation depending on the competitor species that they are confronting. Similarly, competition between parasitoids can also reduce or increase pest control. For instance, Tang et al. (2016) reported that the combination of two parasitoid species produced different pest control outcomes. These authors highlighted the importance of analyzing these interactions in mixed release programs. In the biological model studied here, the predator *G. punctipes* competes with the parasitoid *E. eremicus* for the use of whitefly nymphs. As a result, we predicted that in the presence of the competing species, the predator would increase its consumption of whitefly nymphs (the shared prey) to secure resources. In line with our predictions, the number of Tv nymphs preyed upon by *G. punctipes* in the presence of *E. eremicus* (Ee) was higher relative to treatments where *T. triozae* (Tt) was present (Figure 2A). In addition, *G. punctipes* preyed upon more Ee parasitoids than Tt counterparts (Figure 2B). These results suggest that *G. punctipes* may be able to detect a competing species and modulate its consumption behavior accordingly. Vision may be important in discriminating prey. However, the process of selection and discrimination of prey seem to be more complex than simply choosing a more conspicuous prey (in terms of color and size) (Velasco-Hernández et al. 2013) as the less conspicuous parasitoid (*E. eremicus*) was the most preyed upon by predators. Mechanisms other than vision such as contact discrimination using cuticular composition (Singer 1998) or parasitoid escape behaviors (Card 2012) might therefore also be operating to aid in competitor identification and predator selection. While *G. punctipes* seem to discriminate competitor species, this predator does not discriminate at the level of parasitoid gender. This suggests that the discrimination of competitors could occur at an inter-specific level but not at an intra-specific one. A possible explanation for this may be that inter-specific differences are greater than those at intra-specific level. Subsequent analyses of mechanisms underlying *G. punctipes* prey selection

and recognition of competitors is warranted. As for the effect of parasitoid mobility on the number of parasitoids preyed upon by *G. punctipes,* we found similar results to those discussed in the first bioassay; predators consumed significantly more mobile parasitoids than immobile counterparts.

5 Conclusions

Integrated pest management (IPM) programs are strongly reliant on the understanding of the role of natural enemies in ecosystems and the study of their ecology is fundamental to make better decisions (Kogan 1998). Results of the present study are an important contribution showing that neither wasp mobility nor the presence of host's hemolymph modify the number of Tv nymphs preyed upon by *G. punctipes*. However, the number of whitefly nymphs consumed was significantly higher when the predator was introduced with a competing parasitoid species. In addition, we found that the predator preyed upon more mobile than immobile parasitoids and more competing than non-competing parasitoids. These results suggest that interspecific competition may be a stronger factor regulating predator consumption than parasitoid mobility or the presence of a host's hemolymph. Finally, our results showed that female predators consumed more whitefly nymphs than male conspecifics and preyed upon more immobile prey than male predators. This last finding suggests that female predators have a better ability to detect prey relative to male counterparts.

Acknowledgments: We thank Luis Enrique Chavarín-Gómez and Pedro Torres Enciso (BCL- UdG) for their technical assistance. Dr. Denise Spaan is acknowledged for assistance with language editing. We acknowledge CONACyT (Grant Number 157259) and the UC-Mexus-Conacyt Program (Grant Number CN12608) which provided grant support to RRR. This work formed part of the requirements for the undergraduate degree of PCC, supervised by RRR. We thank Koppert-México SA de CV and Organismos Benéficos para la Agricultura SA de CV for the provision of the natural enemies.

References

Ángeles-López, Y. I., Martínez-Gallardo, N. A., Ramírez-Romero, R., López, M. G., Sánchez-Hernández, C., & Délano-Frier, J. P. (2012). Cross-kingdom effects of plant-plant signaling via volatile organic compounds emitted by tomato (Solanum lycopersicum) plants infested by the greenhouse whitefly (Trialeurodes vaporariorum). *Journal of Chemical Ecology, 38*(11), 1376–1386. https://doi.org/10.1007/s10886-012-0201-z

Asplen, M. K., Bellamy, D. E., & Byrne, D. N. (2001). *Eggs of Eretmocerus eremicus, a whitefly parasitoid. Vegetable Report* (pp. 1–3). Tuczon, AZ: Department of Entomology, The University of Arizona.

Bao-Fundora, L., Ramirez-Romero, R., Sánchez-Hernández, C. V., Sánchez-Martínez, J., & Desneux, N. (2016). Intraguild predation of *Geocoris punctipes* on *Eretmocerus eremicus* and its influence on the control of the whitefly *Trialeurodes vaporariorum. Pest Management Science, 72*(6), 1110–1116. https://doi.org/10.1002/ps.4163

Bueno, V. H. P., Silva, D. S., Calixto, A. M., Montes, F. C., & Lenteren, J. (2016). *Geocoris punctipes* nymphs and adults easily prey on leaf-mining larvae of *Tuta absoluta* on tomato. *Bulletin of Insectology, 69*, 271–276.

Byrne, D. N., & Bellows, T. S., Jr. (1999). Whitefly biology. *Annual Review of Entomology, 36*(1), 431–457. https://doi.org/10.1146/annurev.en.36.010191.002243

Calow, P. (1979). The cost of reproduction-a physiological approach. *Biological Reviews of the Cambridge Philosophical Society, 54*(1),23–40.https://doi.org/10.1111/j.1469-185X.1979.tb00866.x

Card, G. M. (2012). Escape behaviors in insects. *Current Opinion in Neurobiology, 22*(2), 180–186. https://doi.org/10.1016/j.conb.2011.12.009

Cardona, C., Rodríguez, I., Bueno, J., & Tapia, X. (2005). Biología y manejo de la mosca blanca *Trialeurodes vaporariorum* en habichuela y frijol. Cali, Colombia. *CIAT, 345*, 50.

Chailleux, A., Bearez, P., Pizzol, J., & Desneux, N. (2013). Potential for combined use of parasitoids and generalist predators for biological control of the key invasive tomato pest *Tuta absoluta. Journal of Pest Science, 86*(3), 533–541. https://doi.org/10.1007/s10340-013-0498-6

Chailleux, A., Mohl, E., Teixeira-Alves, M., Messelink, G., & Desneux, N. (2014). Indirect interaction in agro-ecosystems: Potential for optimizing integrated pest management. *Pest Management Science, 70*, 1769–1779. https://doi.org/10.1002/ps.3916

Champlain, R. A., & Sholdt, L. L. (1967). Life history of *Geocoris punctipes* (Hemiptera: Lygaeidae) in the Laboratory. *Annals of the Entomological Society of America, 60*(5), 881–883. https://doi.org/10.1093/aesa/60.5.881

Chivarathanapong, S., & Pitre, H. N. (1980). Effects of *Heliothis virescens* larval size on predation by *Geocoris punctipes. The Florida Entomologist, 63*(1), 146–151. https://doi.org/10.2307/3494667

Cohen, A. C. (1984). Food consumption, food utilization, and metabolic rates of *Geocoris punctipes* [Het.: Lygaeidae] fed *Heliothis virescens* [Lep.: Noctuidae] eggs. *Entomophaga, 29*(4), 361–367. https://doi.org/10.1007/BF02372157

Cohen, A. C. (1985). Simple method for rearing the insect predator *Geocoris punctipes* (Heteroptera: Lygaeidae) on a meat diet. *Journal of Economic Entomology, 78*(5), 1173–1175. https://doi.org/10.1093/jee/78.5.1173

Colfer, R. G., & Rosenheim, J. A. (2001). Predation on immature parasitoids and its impact on aphid suppression. *Oecologia, 126*(2), 292–304. https://doi.org/10.1007/s004420000510

Crawley, M. J. (2007). *The R Book*. Silwood Park, Berks, UK: Wiley. https://doi.org/10.1002/9780470515075

Crocker, R. L., Whitcomb, W. H., & Ray, R. M. (1975). Effects of sex, developmental stage, and temperature on predation by *Geocoris punctipes. Environmental Entomology, 4*(4), 531–534. https://doi.org/10.1093/ee/4.4.531

Dicke, M., & Grostal, P. (2001). Chemical detection of natural enemies by arthropods: An ecological prespective. *Annual Review*

of Ecology and Systematics, 32(1), 1–23. https://doi.org/10.1146/annurev.ecolsys.32.081501.113951

Dunbar, D. M. (1972). Notes on the Mating Behavior of *Geocoris punctipes* (Hemíptera: Lygaeidae). *Annals of the Entomological Society of America, 65*(3), 764–765. https://doi.org/10.1093/aesa/65.3.764

Dunbar, D. M., & Bacon, O. G. (1972). Feeding, Development and Reproduction of *Geocoris punctipes* (Heteroptera: Lygaeydae) on Eight Diets. *Annals of the Entomological Society of America, 65*(4), 892–895. https://doi.org/10.1093/aesa/65.4.892

Epsky, N. D., Heath, R. R., Sivinski, J. M., Calkins, C. O., Baranowski, R. M., & Fritz, A. H. (1993). Evaluation of protein bait formulations for the Caribbean fruit fly (Diptera: Tephritidae). *The Florida Entomologist, 76*(4), 626–635. https://doi.org/10.2307/3495797

Eubanks, M. D., & Denno, R. F. (2000). Health food versus fast food: The effects of prey quality and mobility on prey selection by a generalist predator and indirect interactions among prey species. *Ecological Entomology, 25*(2), 140–146. https://doi.org/10.1046/j.1365-2311.2000.00243.x

Fauth, J. E. (1990). Interactive effects of predators and early larval dynamics of the treefrog *Hyla chrysoscelis* . *Ecology, 71*(4), 1609–1616. https://doi.org/10.2307/1938296

Finke, D. L., & Denno, R. F. (2003). Intra-guild predation relaxes natural enemy impacts on herbivore populations. *Ecological Entomology, 28*(1), 67–73. https://doi.org/10.1046/j.1365-2311.2003.00475.x

Gill, T. A., Chu, C., & Pelz-Stelinski, K. S. (2017). Comparative proteomic analysis of hemolymph from uninfected and *Candidatus Liberibacter asiaticus*-infected *Diaphorina citri. Amino Acids, 49*(2), 389–406. https://doi.org/10.1007/s00726-016-2373-2

Giron, D., Rivero, A., Mandon, N., Darrouzet, E., & Casas, J. (2002). The physiology of host feeding in parasitic wasps: Implications for survival. *Functional Ecology, 16*(6), 750–757. https://doi.org/10.1046/j.1365-2435.2002.00679.x

Godfray, H. C. J. (1994). Parasitoids: behavioral and evolutionary ecology. USA: Princeton University Press.

Hajek, A., & Eilenberg, J. (2018). Natural Enemies: An Introduction to Biological Control. Cambridge. UK: Cambridge University Press. https://doi.org/10.1017/9781107280267

Handke, B., Poernbacher, I., Goetze, S., Ahrens, C. H., Omasits, U., Marty, F., … Lehner, C. F. (2013). The hemolymph proteome of fed and starved Drosophila larvae. *PLoS One, 8*(6), e67208. https://doi.org/10.1371/journal.pone.0067208

Headrick, D. H., Bellows, T. S., Jr., & Perring, T. M. (1995). Behaviors of female *Eretmocerus sp* . nr. *californicus* (Hym. Aphelinidae) attacking *Bemisia argentifolii* (Homoptera: Aleyrodidae) on sweetpotato. *Environmental Entomology, 24*(2), 412–422. https://doi.org/10.1093/ee/24.2.412

Heath, R. R., Epsky, N. D., Guzman, A., Dueben, B. D., Manukian, A., & Meyer, W. L. (1995). Development of a dry plastic insect trap with food-based synthetic attractant for the Mediterranean and Mexican fruit flies (Diptera: Tephritidae). *Journal of Economic Entomology, 88*(5), 1307–1315. https://doi.org/10.1093/jee/88.5.1307

Heimpel, G. E., & Collier, T. R. (1996). The evolution of host-feeding behavior in insect parasitoids. *Biological Reviews of the Cambridge Philosophical Society, 71*(3), 373–400. https://doi.org/10.1111/j.1469-185X.1996.tb01279.x

Hirose, Y., Mitsunaga, T., Yano, E., & Goto, C. (2009). Effects of sugars on the longevity of adult females of *Eretmocerus eremicus* and *Encarsia formosa* (Hymenoptera: Aphelinidae), parasitoids of *Bemisia tabaci* and *Trialeurodes vaporariorum* (Hemiptera: Aleyrodidae), as related to their honeydew feeding and host feeding. *Applied Entomology and Zoology, 44*(1), 175–181. https://doi.org/10.1303/aez.2009.175

Huang, C., & Sih, A. (1991). Experimental studies on direct and indirect interactions in a three trophic-level stream system. *Oecologia, 85*(4), 530–536. https://doi.org/10.1007/BF00323765

Janssen A, Montserrat M, HilleRisLambers R, Roos AM, Pallini A, Sabelis MW (2006): Intraguild Predation usually does not disrupt biological control. In: Brodeur J., Boivin G. (eds) Trophic and Guild in Biological Interactions Control. Progress in Biological Control. Springer, Dordrecht, Netherlands.

Jervis, M. A., & Kidd, N. A. C. (1986). Host-feeding strategies in hymemopteran parasitoids. *Biological Reviews of the Cambridge Philosophical Society, 61*(4), 395–434. https://doi.org/10.1111/j.1469-185X.1986.tb00660.x

Jones, D. R. (2003). Plant viruses transmitted by whiteflies. *European Journal of Plant Pathology, 109*(3), 195–219. https://doi.org/10.1023/A:1022846630513

Jong-Kwan, K., Jung-Joon, P., Heungsun, P., & Kijong, C. (2001). Ubiased estimation of greenhouse whitefly, *Trialeurodes vaporariorum,* mean density using yellow sticky trap in cherry tomato greenhouses. *Entomologia Experimentalis et Applicata, 100*(2), 235–243. https://doi.org/10.1046/j.1570-7458.2001.00868.x

Kennedy, G. G. (2003). Tomato, pests, parasitoids, and predators: Tritrophic interactions involving the genus *Lycopersicon. Annual Review of Entomology, 48*(1), 51–72. https://doi.org/10.1146/annurev.ento.48.091801.112733

Kogan, M. (1998). Integrated pest management: Historical perspectives and contemporary developments. *Annual Review of Entomology, 43*(1), 243–270. https://doi.org/10.1146/annurev.ento.43.1.243

Leppanen, C., Alyokhin, A., & Gross, S. (2012). Competition for aphid prey between different lady beetle species in a laboratory arena. *Psyche,* 890327.

Losey, J. E., & Denno, R. F. (1998). Positive predator-predator interactions: Enhanced predation rates and synergistic suppression of aphid populations. *Ecology, 79*, 2143–2152.

Lundgren, J. G., Haye, T., Toepfer, S., & Kuhlmann, U. (2009). A multifaceted hemolymph defense against predation in *Diabrotica virgifera* virgifera larvae. *Biocontrol Science and Technology, 19*(8), 871–880. https://doi.org/10.1080/09583150903168549

Marques, F. D. M., Elfresh, M. C., & Millar, J. G. (2000). Female produced sex pheromone of the predatory bug *Geocoris punctipes. Journal of Chemical Ecology, 26*(12), 2843–2855. https://doi.org/10.1023/A:1026498028531

Martin NA (1999): Whitefly biology, identification and life cycle. New Zealand Institute for Crop & Food Research 91: 71-85.

Müller, C., & Brakefield, P. M. (2003). Analysis of a chemical defense in sawfly larvae: Easy bleeding targets predatory wasps in late summer. *Journal of Chemical Ecology, 29*(12), 2683–2694. https://doi.org/10.1023/B:JOEC.0000008012.73092.01

Piñero, J. C., Mau, R. F., & Vargas, R. I. (2009). Managing oriental fruit fly (Diptera: Tephritidae), with spinosad-based protein bait sprays and sanitation in papaya orchards in Hawaii. *Journal of Economic Entomology, 102*(3), 1123–1132. https://doi.org/10.1603/029.102.0334

Polis, G. A., & Strong, D. R. (1996). Food web complexity and community dynamics. *American Naturalist, 147*(5), 813–846. https://doi.org/10.1086/285880

Powell, D. A., & Bellows, T. S., Jr. (1992). Development and reproduction of two populations of *Eretmocerus* species (Hymenoptera: Aphelinidae) on *Bemisia tabaci* (Hymenoptera: Aphelinidae). *Environmental Entomology, 21*(3), 651–658. https://doi.org/10.1093/ee/21.3.651

Provost, C., Lucas, E., Coderre, D., & Chouinard, G. (2006). Prey selection by the lady beetle *Harmonia axyridis*: The influence of prey mobility and prey species. *Journal of Insect Behavior, 19*(2), 265–277. https://doi.org/10.1007/s10905-006-9023-6

Ramirez-Romero, R., Sivinski, J., Copeland, C. S., & Aluja, M. (2012). Are individuals from thelytokous and arrhenotokous populations equally adept as biocontrol agents? Orientation and host searching behavior of a fruit fly parasitoid. *BioControl, 57*(3), 427–440. https://doi.org/10.1007/s10526-011-9413-3

Readio J, Sweet M (1982): A review of the Geocorinae of the United States East of the 100 Meridian (Hemiptera: Lygaeidae). Thesis by J. Readio for a Master of Science Degree-Miscellaneous Publications.

Rittschof, D., Tsai, D. W., Massey, P. G., Blanco, L., Kueber, G. L., Jr., & Haas, R. J., Jr. (1992). Chemical mediation of behavior in hermit crabs: Alarm and aggregation cues. *Journal of Chemical Ecology, 18*(7), 959–984. https://doi.org/10.1007/BF00980056

R Core Team. (2014). *R: A Language and Environment for Statistical Computing*. Vienna, Austria: R Foundation for Statistical Computing.

Rojas, P., Rodriguez-Leyva, E., Lomeli-Flores, J. R., & Liu, T. X. (2015). Biology and life history of *Tamarixia triozae* , a parasitoid of the potato psyllid *Bactericera cockerelli. BioControl, 60*(1), 27–35. https://doi.org/10.1007/s10526-014-9625-4

Rose, M., & Zolnerowich, G. (1997). *Eretmocerus haldeman* (Hymenoptera: Aphelinidae) in the United States with descriptions of new species attacking *Bemisia* (tabaci complex) (Homoptera: Aleyrodidae). *Proceedings of the Entomological Society of Washington, 99*, 1–27.

Rosenheim, J. A., Wilhoit, L. R., & Armer, C. A. (1993). Influence of intraguild predation among generalist insect predators on the suppression of an herbivore population. *Oecologia, 96*(3), 439–449. https://doi.org/10.1007/BF00317517

Sher, R. B., Parella, M. P., & Kaya, H. K. (2000). Biological control of the leafminer *Liriomyza trifolii* (Burgess): Implication for intraguild predation between *Diglyphus begini* Ashmead and *Steinernema carpocapsae* (Weiser). *Biological Control, 17*(2), 155–163. https://doi.org/10.1006/bcon.1999.0794

Shimoda, T., Shinkaji, N., & Amano, H. (1997). Prey stage preference and feeding behavior of *Oligota kashimirica benefica* (Coleoptera: Staphylinidae), an insect predator of the spider mite *Tetranychus urticae* (Acari: Tetranichidae). *Experimental & Applied Acarology, 21*(10-11), 665–675. https://doi.org/10.1007/BF02803509

Sih, A., Englund, G., & Wooster, D. (1998). Emergent impacts of multiple predators on prey. *Trends in Ecology & Evolution, 13*(9), 350–355. https://doi.org/10.1016/S0169-5347(98)01437-2

Singer, T. L. (1998). Roles of hydrocarbons in the recognition systems of insects. *American Zoologist, 38*(2), 394–405. https://doi.org/10.1093/icb/38.2.394

Small, M. P., & Thacker, R. W. (1994). Land hermit crabs use odors of dead conspecifics to locate shells. *Journal of Experimental Marine Biology and Ecology, 182*(2), 169–182. https://doi.org/10.1016/0022-0981(94)90049-3

Soluk, D. A., & Collins, N. C. (1988). Synergistic interactions between fish and stoneflies: Facilitation and interference among stream predators. *Oikos, 52*(1), 94–100. https://doi.org/10.2307/3565987

Symonds, M. R. E., & Moussalli, A. (2011). A brief guide to model selection, multimodel inference and model averaging in behavioural ecology using Akaike's information criterion. *Behavioral Ecology and Sociobiology, 65*(1), 13–21. https://doi.org/10.1007/s00265-010-1037-6

Tan, X., Hu, N., Zhang, F., Ramirez-Romero, R., Desneux, N., Wang, S., & Ge, F. (2016). Mixed release of two parasitoids and a polyphagous ladybird as a potential strategy to control the tobacco whitefly *Bemisia tabaci. Scientific Reports, 6*(1), 28245. https://doi.org/10.1038/srep28245

Tapia, D. H., Morales, F., & Grez, A. A. (2010). Olfactory cues mediating prey-searching behavior in interacting aphidophagous predators: Are semiocjemicals key factors in predator-facilitation? *Entomologia Experimentalis et Applicata, 137*(1), 28–35. https://doi.org/10.1111/j.1570-7458.2010.01034.x

Tillman, P. G., & Mullinix, B. G. (2003). Effect of Prey species on Plant Feeding Behavior by the Big-Eyed Bug, *Geocoris punctipes* (Say) (Heteroptera: Geocoridae), on Cotton. *Environmental Entomology, 32*(6), 1399–1403. https://doi.org/10.1603/0046-225X-32.6.1399

Van Buskirk, J. (1988). Interactive effects of dragonfly predation in experimental pound communities. *Ecology, 69*(3), 857–867. https://doi.org/10.2307/1941035

Van Lenteren, J. C. (2003). Comercial availability of biological control agents. In J. C. Van Lenteren (Ed.), *Quality control and production of biological control agents, theory and testing procedures*. Wallingford, UK: CABI. https://doi.org/10.1079/9780851996882.0167

Velasco-Hernández, M. C., Ramirez-Romero, R., Cicero, L., Michel-Rios, C., & Desneux, N. (2013). Intraguild predation on the whitefly parasitoid *Eretmocerus eremicus* by the generalist predator *Geocoris punctipes*: A behavioral approach. *PLoS One, 8*(11), e80679. https://doi.org/10.1371/journal.pone.0080679

Velasco-Hernández, M. C., Desneux, N., Ramírez-Martínez, M. M., Cicero, L., & Ramirez-Romero, R. (2017). Host species suitability and instar preference of *Aphidius ervi* and *Aphelinus abdominalis. Entomologia Generalis, 36*(4), 347–367. https://doi.org/10.1127/entomologia/2017/0500

Venzon, M., Pallini, A., & Janssen, A. (2001). Interaction mediated by predators in arthropod food webs. *Neotropical Entomology, 30*(1), 1–9. https://doi.org/10.1590/S1519-566X2001000100002

Vet, L., & Dicke, M. (1992). Ecology of infochemical use by natural enemies in a tritrophic context. *Annual Review of Entomology, 37*(1), 141–172. https://doi.org/10.1146/annurev.en.37.010192.001041

Wajnberg, E., Bernstein, C., & van Alphen, J. (2008). *Behavioral ecology of insect parasitoids, from theoretical approaches to field applications*. USA: Blackwell Publishing. https://doi.org/10.1002/9780470696200

Weber, D. C. (2013). Biological control of potato insect pests. In P. Giordanengo, C. H. Vincent, & A. Alyokhin (Eds.), *Insect Pests of Potato. Global Perspectives on Biology and Management* (pp. 399–437). Oxford, U. K.: Academic Press. https://doi.org/10.1016/B978-0-12-386895-4.00014-4

Wilbur, H. M., & Fauth, J. E. (1990). Experimental aquatic food webs : Interations between two predators and two prey. *American Naturalist, 135*(2), 176–204. https://doi.org/10.1086/285038

Wintermantel AM (2004): Emergence of greenhouse whitefly (*Trialeurodes vaporariorum*) transmitted criniviruses as threats to vegetable and fruit production in North America. APSnet Features. Doi:10.1094/APSnetFeature-2004-0604.

Wissinger, S., & McGrady, J. (1993). Intraguild predation and competition between larval dragonflies : Direct and indirect effects on shared prey. *Ecology, 74*(1), 207–218. https://doi.org/10.2307/1939515

Yang, N. W., Ji, L. L., Lövei, G. L., & Wan, F. H. (2012). Shifting preference between oviposition vs. host-feeding under changing host densities in two aphelinid parasitoids. *PLoS One, 7*(7), e41189. https://doi.org/10.1371/journal.pone.0041189

Zang, L. S., & Liu, T. X. (2010). Effects of food deprivation on host feeding and parasitism of whitefly parasitoids. *Environmental Entomology, 39*(3), 912–918. https://doi.org/10.1603/EN09266

Zar, J. H. (1998). *Biostatistical Analysis*. Upper Saddle River, NJ, USA: Prentice Hall.

Manuscript received: 30 December 2018
Revisions requested: 27 February 2019
Accepted: 8 April 2019