Research Article

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Intraguild predation of *Geocoris punctipes* on *Eretmocerus eremicus* and its influence on the control of the whitefly *Trialeurodes vaporariorum*

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Abstract

BACKGROUND: *Geocoris punctipes* (Hemiptera: Lygaeidae) and *Eretmocerus eremicus* (Hymenoptera: Aphelinidae) are whitefly natural enemies. Previously, under laboratory conditions, we showed that *G. punctipes* engages in intraguild predation (IGP), the attack of one natural enemy by another, on *E. eremicus*. However, it is unknown whether this IGP interaction takes place under more complex scenarios, such as semi-field conditions. Even more importantly, the effect of this interaction on the density of the prey population requires investigation. Therefore, the present study aimed to establish whether this IGP takes place under semi-field conditions and to determine whether the predation rate of *G. punctipes* on the whitefly decreases when IGP takes place.

RESULTS: Molecular analysis showed that, under semi-field conditions, *G. punctipes* performed IGP on *E. eremicus*. However, although IGP did take place, the predation rate by *G. punctipes* on the whitefly was nevertheless higher when both natural enemies were present together than when the predator was present alone.

CONCLUSION: While IGP of *G. punctipes* on *E. eremicus* does occur under semi-field conditions, it does not adversely affect whitefly control. The concomitant use of these two natural enemies seems a valid option for inundative biological control programmes of *T. vaporariorum* in tomato.

Keywords: Lygaeidae; Aphelinidae; Aleyrodidae; intraguild predation

1 INTRODUCTION

The whitefly *Trialeurodes vaporariorum* (Hemiptera: Aleyrodidae) is a sucking pest, native to Central and South America.[1,2] This whitefly is a cosmopolitan species considered to be an important pest, as it can lead to substantial economic losses in different crops, such as tomato, cotton and beans.[2–4] Its hemimetabolous life cycle includes cylindrical eggs, four nymphal instars and adult development.[2] The whole life cycle can last up to 30–80 days (depending upon temperature), with multiple generations per year.[2] The rates of oviposition and infestation are influenced by factors such as environmental conditions, host plant, inter- and intraspecific competition and presence of natural enemies.[2,5,6]

One of the whitefly natural enemies used successfully as a biocontrol agent is the parasitoid *Eretmocerus eremicus* Rose and Zolnerowich (Hymenoptera: Aphelinidae).[7–9] This solitary endoparasitoid prefers to oviposit on second- and third-instar whitefly nymphs.[10–12] Its pre-imaginal developmental period can last from 16 to 32 days, and females can live for up to 11 days and oviposit on average about five eggs per day on *Bemisia argentifolii* Bellows & Perring nymphs as hosts.[13]

In turn, the predator *Geocoris punctipes* (Hemiptera: Lygaeidae) is another common natural enemy of this pest in the Americas.[14–17] This polyphagous predator has a nymphal development of 23–36 days and a subsequent adult stage than can last up to 108 days.[18] Adults of this species can mate 3 days after emergence and can start oviposition after 5 days following emergence.[18,19] In a companion study, we found that, under laboratory conditions, *G. punctipes* prey upon nymphs and adults of the wasp *E. eremicus*.[20] This kind of trophic interaction, in which one natural enemy of a target pest preys on another natural enemy of the same pest, is known as intraguild predation (IGP) and can take place with various natural enemies.[21,22] Until now, it was unknown whether IGP between these two species took place under more complex scenarios, such as field or semi-field conditions. Also unknown was the effect of IGP occurrence on *T. vaporariorum* control.

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Complementary studies under more natural conditions are important because factors related to the complexity of agroecosystems, such as environmental variations, plant size or structure and leaf properties (e.g. epicuticular waxes, trichomes or domatia), can influence interactions among insects.[23–26] More specifically, these properties of environmental complexity can affect IGP.[27,28] For example, the IGP rate was reduced when plants were used in bioassays, compared with bioassays using arenas without plants.[28] Moreover, Seelman *et al.*[27] found that the rate and direction of IGP was influenced by the pubescence of the leaves. Clearly, it is important to determine whether a more complex scenario modifies the IGP observed under laboratory conditions and, more importantly, the potential effect of this interaction on pest control.

Indeed, it has been shown that IGP can influence pest control by reducing,[29–31] increasing[32] or maintaining the pest control unchanged.[33] For example, it has been proposed that the preference of IG predators can play a role in the net effect of the predation rate of the IG predator on pests.[32] The IG predator *G. punctipes* is known to prefer moving prey to immobile prey.[34] Therefore, if this IG predator were confronted with mobile prey (adult wasps) versus immobile prey (whitefly nymphs), *G. punctipes* might preferably prey upon adult wasps (i.e. exhibit IGP). If so, a reduction in the predation rate of *G. punctipes* on the whitefly could be expected (relative to conditions in which *G. punctipes* is confronted with whitefly nymphs only). Therefore, as a second objective of this study, we aimed to determine whether the whitefly predation rate by *G. punctipes* decreases when IGP is taking place.

2 MATERIALS AND METHODS

2.1 Plants

Tomato seeds (*Solanum lycopersicum* L. cv. 'Saladette') were bought at La Casa del Hortelano (Guadalajara, Jalisco, Mexico). Seeds were planted on plastic germinating trays under environmental conditions of 24 ± 3 °C, 50 ± 10% relative humidity and a 12:12 h (light:dark) photoperiod. When plants reached the third leaf of development, they were transferred to individual plastic pots (16 cm high, 18 cm in diameter). Each pot contained a mixture consisting of vermicompost (Lombrifert®; CUCBA, Universidad de Guadalajara, Zapopan, Jalisco, Mexico), pumice and coir (Germinza®; Germinaza SA de CV, Guadalajara, Jalisco, Mexico). Plants were grown under natural conditions in experimental cages (see below) and were used in experiments once they had reached the fourth leaf of development. Plants were protected from exposure to herbivores using plastic cylinders containing anti-aphid mesh-covered windows (see below). Any type of pesticide application was strictly avoided.

2.2 Insects

All insects were reared in acclimatised chambers at a temperature of 24 ± 3 °C, a relative humidity of 50 ± 10% and a 14:10 h (light:dark) photoperiod.

2.2.1 *Whitefly Trialeurodes vaporariorum*

Whiteflies used in the experiments came from our colonies founded by individuals provided by Dr Carla Sanchez-Hernández (Universidad de Guadalajara) and taxonomically verified by the Aleyrodidae specialist Dr Vicente Carapia (Universidad Autónoma del Estado de Morelos, Cuernavaca, Morelos, Mexico). These were virus-free whiteflies. These colonies were reared on tomato plants placed in acrylic cages (38.5 × 30.0 × 45.0 cm) with sides covered with anti-aphid mesh.

2.2.2 *Parasitoid Eretmocerus eremicus*

Parasitoids were provided by Koppert México SA de CV (El Marqués, Querétaro, Mexico) as parasitised nymphs in cardboard shipping containers. The nymphs were placed inside acrylic cages (38.5 × 30.0 × 45.0 cm) and inspected daily to determine adult emergence date. Upon emergence, adults were fed a honey solution (7:3 mL, honey:water), which was offered on a paper towel (5 cm^2), and tap water; food was replaced every day.[22] Parasitoids were used when they were 2–4 days old because it is known that they can mate and oviposit when they are 1 day old[35] and can live up to 11 days.[13]

2.2.3 *Predator Geocoris punctipes*

Predators were provided by Organismos Benéficos para la Agricultura (Autlán de Navarro, Jalisco, Mexico) as nymphs. Upon their arrival in our laboratory, nymphs were placed inside polystyrene cages (40 × 63 × 63 cm) and fed *ad libitum* with ≈5 g of artificial diet,[20] pollen (5 g; Apiarios Rancaño, Mexico City, Mexico) and sorghum seeds (10 g, var. UDG-110; Universidad de Guadalajara). Artificial diet was replaced every day, and pollen and sorghum seeds once a week.[20] Female predators were used in experiments when they were 8–20 days old because after 5 days females can produce fertile eggs and they can live up to 108 days.[18]

2.3 Experimental set-up

Bioassays were carried out under field conditions within wood field cages (3 × 3 × 1.8 m) with sides containing anti-aphid mesh, placed in campus experimental grounds (20° 44′ 47″ N, 103° 30′ 43″ W) (CUCBA, Universidad de Guadalajara). During the experimental period, a mean temperature (± SD) of 25.31 ± 10.8 °C and a mean relative humidity (± SD) of 55.48 ± 28.16% were recorded (Datalogger RHT10; Extech® Instruments, Waltham, MA, USA).

Within the field cages we set up microcosms containing the tested treatments. Each microcosm consisted of a tomato plant enclosed in a plastic cylinder (28 cm high and 18 cm in diameter) with six holes (each 9.5 cm in diameter) covered with anti-aphid mesh for ventilation. Before use in experiments, each of these microcosms was infested with 150 adult whiteflies, which were allowed to oviposit for 48 h. After this period, whitefly adults were removed from the microcosms, and laid eggs were allowed to develop for 14 days. After this period of time, we recorded the number of nymphs at the second and third stage using a 40× magnifying glass (Victorinox®, Mexico City, Mexico). Any first-stage nymphs that were present at this time point were removed from the plants. Thus, the experimental set-up contained 200–230 nymphs at the second or third nymphal stage.[20]

We established five treatments: (1) control (only whitefly nymphs); (2) whitefly nymphs and predators; (3) whitefly nymphs, predators and parasitoids, introduced concomitantly; (4) whitefly nymphs, parasitoids and predators, introduced consecutively; (5) whitefly nymphs and parasitoids.

Treatment 1 (hereafter referred to as the 'control') consisted of microcosms containing whitefly nymphs and submitted to the same environmental and experimental conditions as the other treatments, but without natural enemy exposure. This control allowed us to establish a benchmark predation rate for *G. punctipes*.

In treatment 2 (hereafter referred to as '*Tv* + *Gp*'), a microcosm containing whitefly nymphs was set up, and six female predators were released within the microcosm. Taking into account preliminary observations, we used six females in each microcosm

to determine nymph consumption rate per female per day, with an initial number of available nymphs of 200–230. After release, the female predators were allowed to forage within the microcosm for 48 h.[36] After this period of time, predators were removed from microcosms, preserved in 70% ethanol (v:v) and stored at 4 °C for subsequent analysis for whitefly DNA detection.[37]

In treatment 3 (hereafter referred to as '*Tv* + *Gp* + *Ee*'), we simultaneously released six female predators and 19 parasitoid pairs (19 females + 19 males) into a microcosm containing whitefly nymphs. This wasp number was chosen in accordance with the daily rate of oviposition of *E. eremicus* and host density.[13] Both natural enemies were allowed to forage during 48 h concomitantly. After this period of time, predators were captured and preserved in 70% ethanol (v:v) at 4 °C for subsequent analysis for whitefly and parasitoid DNA detection.[37] At the same time, wasps were also removed from microcosms and preserved in ethanol.

In treatment 4 (hereafter referred to as '*Tv* + *Ee* ⇒ *Gp*'), we released 19 parasitoids pairs (19 females + 19 males) into a microcosm containing whitefly nymphs. After introducing the wasps, we let them forage for 48 h, a similar time to that for predators in the previous treatment. After this period of time, the adult wasps were removed from the microcosm, and after 18–20 days (when the developing wasps had reached the last larval stage[38]) six female predators were introduced into the microcosm and allowed to forage for 48 h. The female predators were then recovered and preserved in 70% ethanol (v:v) at 4 °C until further analysis (prey DNA detection; see below).

Treatment 5 (hereafter referred to as '*Tv* + *Ee*') consisted of a microcosm with whitefly nymphs and 19 pairs of parasitoids. These parasitoids were allowed to forage for 48 h and were then removed from the microcosm. After removing the natural enemies, the microcosms were promptly put back into field cages.

For treatments 2, 3 and 4, we recorded the number of whitefly nymphs preyed upon by *G. punctipes*. For these treatments, the counting of nymphs preyed upon was performed the same day that predators were extracted. For this count, all leaves of each plant were scrutinised under a stereomicroscope (DV4; Carl Zeiss de Mexico SA de CV, Mexico City, Mexico). For all treatments, the number of emerged adult whiteflies was recorded 30 days following the whitefly oviposition period, in consideration of whitefly development time.[2,39] The number of parasitised nymphs was recorded 18 days following the parasitism period. Finally, the number of emerged wasps was recorded 25 days following the parasitism period. These time points for counting of parasitised nymphs and adult wasps were determined in accordance with earlier observations and previous literature on the development of *E. eremicus*.[20,38,40] We followed a randomised block design, with time as the blocking factor. Each treatment was replicated 11 times.

2.4 IGP assessment by molecular analysis

To determine whether IGP occurs under natural conditions, we looked for traces of prey DNA in predator homogenates from treatments 2 (*Tv* + *Gp*), 3 (*Tv* + *Gp* + *Ee*) and 4 (*Tv* + *Ee* ⇒ *Gp*). In these analyses, DNA (including possible whitefly and parasitoid DNA) was extracted from predators using whole predator bodies following the protocol described by Cenis *et al.*[41] Briefly, individual insects were crushed in 2.0 mL microcentrifuge tubes and homogenised with a Kontes pellet pestle in 100 µL of extraction buffer (200 mM of Tris-HCl, pH 8.5, 250 mM of NaCl, 25 mM of EDTA, 0.5% SDS). A quantity of 100 µL of 3 M sodium acetate (pH 5.2) was added, and tubes were placed in a −20 °C freezer for 10 min. After centrifugation, the supernatant was transferred to another tube, and the DNA was precipitated with isopropanol. The pellet was washed with 70% ethanol and resuspended in 35 µL of TE buffer (10 mM of Tris-HCl, 1 mM of EDTA, pH 8.0). DNA integrity was checked by agarose gel electrophoresis and quantified by a Q5000 Quawell spectrophotometer (Quawell Technology, San Jose, CA, USA). DNA was stored at −20 °C until required. For PCR analysis, the mitochondrial cytochrome oxidase subunit I (COI) gene was targeted for *T. vaporariorum* detection using previously reported Tv1F/Tv1R primers.[42] *E. eremicus*-specific primers (5′-TTGGTGTCTCAATTTTATATC-3′/5′-ATACTCAAAATCCTTCCCGC-3′) were designed from the internal transcribed spacer (ITS-1) region (Genbank AB662973) to generate a ∼143 bp amplicon. Primers were tested for cross-reactivity and performance in PCR. PCR amplifications were performed in a Swift maxi thermo cycler (ESCO, Philadelphia, PA, USA). Reactions were prepared in a total reaction volume of 25 µL containing 500 ng of DNA, 1× PCR buffer (50 mM of KCl, 20 mM of Tris-HCl, pH 8.4), 1.5 mM of $MgCl_2$, 0.5 mM of each dNTP, 0.5 µM of each primer and 2 U of Invitrogen Taq DNA polymerase (Life Technologies, Sao Paulo, Brazil). The cycling conditions were set as follows: initial denaturation step at 95 °C for 3 min, followed by 45 cycles of denaturation at 95 °C for 3 min, annealing at 63 °C for 30 s, extension at 72 °C for 40 s and a final extension step at 72 °C for 10 min. Prey DNA (*T. vaporariorum* or *E. eremicus*) and *G. punctipies* DNA or autoclaved distilled water were included as positive and negative controls respectively. PCR products were separated by electrophoresis in 1.5% agarose gels stained with ethidium bromide and visualised under UV light. A predator was considered to be negative for prey DNA if prey DNA was not detected after two separate analyses. The results are reported as the proportion of predators in which prey DNA was detected (i.e. the number of predators with prey DNA divided by the total number of analysed predators).

2.5 Whitefly predation rate by *G. punctipes* also engaging in IGP

To determine whether the predation rate of *G. punctipes* on whiteflies decreased under IGP scenarios, we focused on the percentage of whitefly nymphs preyed upon by *G. punctipes* in treatments *Tv* + *Gp* + *Ee* (simultaneous release) and *Tv* + *Ee* ⇒ *Gp* (sequential release) and compared these with the percentage of whitefly nymphs preyed upon in treatment *Tv* + *Gp* (individual release).

2.6 Data analysis

To compare the mean percentage of nymphs preyed upon by *G. punctipes*, i.e. [number of whitefly nymphs preyed upon by predator/initial number of whitefly nymphs] × 100, among treatments, we used a one-way ANOVA, as data met normality and homoscedasticity requirements. For whitefly density, we compared the mean percentage of emerged whitefly adults, i.e. [number of emerged whitefly adults/initial number of whitefly nymphs] × 100, among all treatments using a generalised linear model (GLM) with a gamma error and a log link function.[43] The mean percentage of parasitised nymphs, i.e. [number of parasitised whitefly nymphs/initial number of whitefly nymphs] × 100, and the mean percentage of emerged wasps, i.e. [number of emerged wasps/initial number of whitefly nymphs] × 100, were also subjected to a one-way ANOVA, but after log (x + 1) transformations[44] to fit normality and homoscedasticity requirements. For all models, the block was included as an explanatory variable in each model. *Post hoc* tests were performed, with contrasts derived from the linear model.[43] All analyses were performed using R, v.3.1.1.[45]

Table 1. Molecular detection percentages of *T. vaporariorum* and *E. eremicus* in *G. punctipes*[a]

Treatment	Number of analysed predators	Individuals in which DNA was detected (%)	
		T. vaporariorum	*E. eremicus*
Tv + Gp	63	38.1	
Tv + Gp + Ee	58	55.2	89.7
Tv + Ee ⇒ Gp	54	*	90.7

[a] *Tv + Gp* = *T. vaporariorum* nymphs and *G. punctipes*; *Tv + Gp + Ee* = *T. vaporariorum* nymphs, *G. punctipes* and *E. eremicus* adults released simultaneously; *Tv + Ee ⇒ Gp* = *T. vaporariorum* nymphs, *E. eremicus* and *G. punctipes* released consecutively.
*At this time point, predators were confronted only to parasitised whitefly nymphs.

3 RESULTS

3.1 IGP of *G. punctipes* on *E. eremicus*, molecular assessment

Our results show that, under semi-field conditions, the predator *G. punctipes* engages in IGP on the wasp *E. eremicus* (Table 1). High percentages of predators containing wasp DNA were found both in the concomitant release treatment (*Tv + Gp + Ee*: 89.7%) and in the sequential release treatment (*Tv + Ee ⇒ Gp*: 90.7%).

3.2 Whitefly predation rate by *G. punctipes* also engaging in IGP

When the mean percentage of nymphs preyed upon by *G. punctipes* was compared among treatments, we found significant differences ($F_{2,28} = 97.273$; $P < 0.001$) (Fig. 1a). Unexpectedly, the highest number of preyed upon nymphs was recorded in the treatment where both natural enemies were concomitantly present (*Tv + Gp + Ee*), followed in descending order by the predator-only treatment (*Tv + Gp*) and the sequential introduction of both natural enemies (*Tv + Ee ⇒ Gp*) (Fig. 1a).

3.3 Emerged adult whiteflies, parasitised nymphs and emerged wasps

As for the mean percentage of emerged whiteflies, we found significant differences when treatments were compared ($F_{4,48} = 18.574$; $P < 0.001$) (Fig. 1b). The treatments with the highest rate of emerged flies were the control, *Tv + Gp* and *Tv + Ee*. Those with the lowest rate of emerged flies were *Tv + Gp + Ee* and *Tv + Ee ⇒ Gp* (Fig. 1b).

The mean percentage of parasitised nymphs was significantly different among treatments ($F_{2,28} = 39.585$; $P < 0.001$) (Fig. 2a). The highest number of parasitised nymphs was recorded in the treatments *Tv + Ee* and *Tv + Ee ⇒ Gp*, followed by the treatment *Tv + Gp + Ee* (Fig. 2a). A similar trend was found when the mean percentage of emerged wasps was compared ($F_{2,28} = 27.328$; $P < 0.001$). The treatment with the highest percentage of emerged wasps was *Tv + Ee*, followed by *Tv + Ee ⇒ Gp* and *Tv + Gp + Ee* (Fig. 2b).

4 DISCUSSION

Our results show that the predator *G. punctipes* engages in IGP on the wasp *E. eremicus* under natural conditions (Table 1). However, although IGP occurred, the predation rate of *G. punctipes* on whitefly nymphs was higher when both natural enemies were concomitantly released (i.e. under IGP conditions) than when *G. punctipes* was released alone (Fig. 1a).

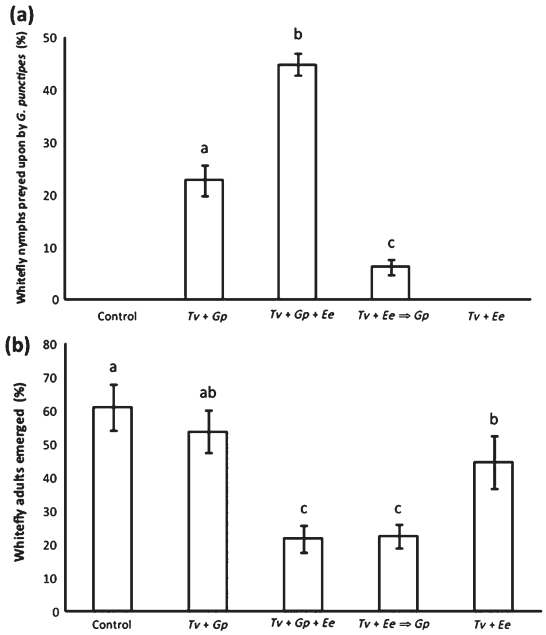

Figure 1. (a) Mean percentage (± SEM) of *T. vaporariorum* nymphs preyed upon by *G. punctipes* under semi-field conditions. (b) Mean percentage (± SEM) of *T. vaporariorum* adults emerged. Analysed treatments: control (*T. vaporariorum* nymphs only); *Tv + Gp* (*T. vaporariorum* nymphs and *G. punctipes*); *Tv + Gp + Ee* (*T. vaporariorum* nymphs, *G. punctipes* and *E. eremicus* released simultaneously); *Tv + Ee ⇒ Gp* (*T. vaporariorum* nymphs, *E. eremicus* and *G. punctipes* released consecutively); *Tv + Ee* (*T. vaporariorum* nymphs and *E. eremicus*). Columns bearing different letters represent significant differences (at $P < 0.05$).

4.1 Intraguild predation assessment

Previously, under laboratory conditions, we found that *G. punctipes* exhibited IGP on *E. eremicus* nymphs and adults.[20] However, it was unknown whether IGP occurred under more complex conditions. This is important because effects observed under laboratory conditions will not necessarily take place in field or semi-field conditions.[46–48] Some of the factors that could be related to these differential results include experimental conditions or environment complexity (e.g. climate, plant structure, leaf properties, light or season).[23–26,46,47] For IGP specifically, it is known that the use of plants, as opposed to bare artificial arenas, and the pubescence of leaves can modify the rate at which IGP occurs.[27,28] As a result, the complexity of analysed systems can influence the outcome of interactions and particularly that of IGP. Therefore, we were first interested in determining whether the predator G. *punctipes* exhibited IGP on the parasitoid *E. eremicus* under semi-field conditions. Our results showed that the predator did in fact engage in IGP on the parasitoid. High percentages of analysed predators contained wasp DNA when the two natural enemies were released into a microcosm together (89.7%) or sequentially (90.7%). In the treatment with concomitant release (*Tv + Gp + Ee*), the predators remained with the adult wasps for 48 h. At this point, the wasp DNA detected in predators was more likely obtained from adult wasps than from immature wasps. This is because the parasitoid egg is oviposited externally under the whitefly nymph and the invasion of the host nymph can take two or more days after parasitoid larvae hatching.[49,50] In contrast, in the treatment with sequential release of natural enemies (*Tv + Ee ⇒ Gp*), the predators were confronted with parasitised nymphs only. In this case, the wasp DNA detected in the predators was from these immature parasitoids developing in the host. These results are in line

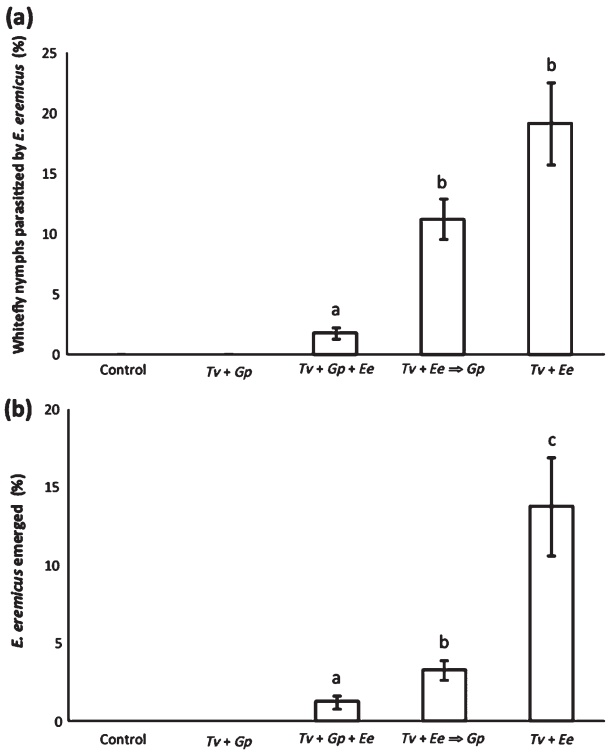

Figure 2. (a) Mean percentage (± SEM) of *T. vaporariorum* nymphs parasitised by *E. eremicus* under semi-field conditions. (b) Mean percentage (± SEM) of *E. eremicus* adults emerged. Analysed treatments: control (*T. vaporariorum* nymphs only); *Tv* + *Gp* (*T. vaporariorum* nymphs and *G. punctipes*); *Tv* + *Gp* + *Ee* (*T. vaporariorum* nymphs, *G. punctipes* and *E. eremicus* released simultaneously); *Tv* + *Ee* ⇒ *Gp* (*T. vaporariorum* nymphs, *E. eremicus* and *G. punctipes* released consecutively); *Tv* + *Ee* (*T. vaporariorum* nymphs and *E. eremicus*). Columns bearing different letters represent significant differences (at $P < 0.05$).

with previous observations under laboratory conditions indicating that *G. punctipes* can prey upon adult and immature stages of *E. eremicus*.[20] This could be relevant in terms of planned biological control programmes. For example, if a joint release were intended (concomitant or sequential), our results indicate that parasitoids (immature or adults) would be preyed upon and, as a result, a low rate of parasitism (Fig. 2a) and wasp emergence (Fig. 2b) would arise. Therefore, the joint release of both natural enemies (concurrent or sequential) seems not to favour (at least on the side of the wasp) an inoculative biological control programme where the reproduction of natural enemies is sought.[51] If what is sought is the establishment of *E. eremicus*, our results suggest that an individual release (without predators) would be more favourable (Fig. 2b). In contrast, if what is planned is inundative releases (where the main goal is the control of the pest, without the need for reproduction of natural enemies), the concomitant or sequential release of *G. punctipes* and *E. eremicus* could be much more successful than individual releases (Fig. 1b). These results point to the importance of analysing different combinations of natural enemies before combined releases are implemented, to optimise the effectiveness of a biological control programme.[31]

4.2 Whitefly predation rate by *G. punctipes* also engaging in IGP

The other important point we analysed in the present study was whether IGP occurrence reduced the predation rate of *G. punctipes* on the pest and consequently the efficacy of biological pest control. Previous literature reported that, in fact, occurrence of IGP could result in a reduction in pest control.[29–31] For example, IGP of *Pardosa littoralis* (Araneae: Lycosidae) on *Tyttus vagus* (Hemiptera: Miridae) resulted in an increase in their shared prey, the *Pronkelisia* planthoppers.[30] However, in other cases it has been documented that, in spite of the presence of IGP, control of pests increases[32] or remains unchanged.[33] These heterogeneous outcomes indicate the variability of the influence of interactions such as IGP on pest control. In the specific case of our short-term biological model, we expected that IGP of *G. punctipes* on adult *E. eremicus* would reduce nymph whitefly predation and consequently would reduce pest control efficacy. This hypothesis was postulated taking into account the fact that *G. punctipes* exhibits a preference for mobile prey over non-mobile targets.[34] That is to say, we expected that when *G. punctipes* was confronted with both options (adult wasps versus whitefly nymphs), it would preferably attack adult wasps, reducing its rate of predation on whitefly nymphs. Consequently, the rates of consumed whitefly nymphs would be lower in the treatment with concomitant release (*Tv* + *Gp* + *Ee*) relative to that with the predator alone (*Tv* + *Gp*). Nevertheless, our results showed that, contrary to our expectations, the rate of consumed whitefly nymphs was significantly higher in the *Tv* + *Gp* + *Ee* treatment relative to *Tv* + *Gp* (Fig. 1a). This indicates that, in spite of the consumption of the parasitoids (i.e. IGP) (Table 1), for some reason the predator increases the rate of consumed nymphs relative to conditions in which it is the lone natural enemy. That reason is likely related to the perception of adult wasps and a proximal response of the predator to the detection of that prey. Wasp presence can be revealed through semiochemical signals or movements. Semiochemical signals could be those traces left by wasps while walking, parasitising or feeding hosts,[52–54] and these signals can be detected by predators.[19,55–58] Movement could be the other signal revealing the presence of wasps, because the predator *G. punctipes* has been reported to be positively attracted to mobile prey.[34] The perception of wasps could then trigger an increase in predation rate by *G. punctipes*, because some predators (including *G. punctipes*) increase kills when prey density increases[34,36,59,60] or as a response to competition represented by wasps.[61,62] Further experiments are required for a better assessment of the reason for this predator's response and the potential outcomes on the prey–predator–parasitoid long-term population dynamics.

One final point, from the perspective of applied entomology, is related to the rate of emergence of adult whiteflies. We found that the lowest rates of whitefly emergence were found in treatments with joint release of natural enemies (Fig. 1b). This indicates that pest control with the joint release of natural enemies (in spite of IGP occurrence) is more effective than that with the release of a single natural enemy. This suggests that both natural enemies are inducing pest kills additively. Indeed, when we analysed concomitant release (*Tv* + *Gp* + *Ee*), we observed that the predator exhibited a higher rate of nymph consumption (Fig. 1a). Parasitoids, meanwhile, parasitised fewer nymphs (Fig. 2a) in that treatment, but, regardless, joint action (*Tv* + *Gp* + *Ee*) resulted in the lowest rate of whitefly emergence (Fig. 1b). A similar rate of pest emergence was obtained in the *Tv* + *Ee* ⇒ *Gp* treatment (Fig. 1b), in which the predator consumed fewer nymphs (Fig. 1a) but the wasps parasitised more nymphs (Fig. 2a). These results are in line with other studies that have reported that natural enemies that participate in IGP act additively in pest control.[63]

5 CONCLUSION

Our results showed that *G. punctipes* engages in IGP on immature and adult stages of *E. eremicus* under semi-field conditions. However, contrary to our expectations that, in the presence of IGP, pest control might be reduced, the whitefly consumption rate was instead increased with a simultaneous release of both natural enemies. Consequently, whitefly infestation was lowest when both natural enemies were released. These results indicate that the joint release of these natural enemies should be effective in controlling whitefly, within a framework of inundative release, in spite of the presence of IGP.

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