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The potential of *Dicyphus hesperus* as a biological control agent of potato psyllid and sweetpotato whitefly in tomato

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Abstract

The potential of the mirid predator *Dicyphus hesperus* Knight (Heteroptera: Miridae) as a biological control agent of the sweetpotato whitefly, *Bemisia tabaci* Gennadius (Hemiptera: Aleyrodidae) and the potato psyllid, *Bactericera cockerelli* Sulcer (Hemiptera: Psyllidae) in tomato was investigated in two experiments. The first experiment focused on the study of the life history traits of *D. hesperus* when fed on nymphs of the potato psyllid compared with the factitious prey *Ephestia kuehniella* Zeller (Lepidoptera: Pyrallidae) eggs. Although reproductive and development rates were higher on *E. kuehniella* eggs, the predator exhibited a good intrinsic rate of natural increase (r_m) when feeding on *B. cockerelli* nymphs (r_m: *B. cockerelli* 0.069 ± 0.0001; *E. kuehniella* 0.078 ± 0.0001), thus reflecting good potential as a biocontrol agent of this pest. The second experiment focused on the efficacy of *D. hesperus* as a biocontrol agent of the potato psyllid and the sweetpotato whitefly in a tomato greenhouse. Prey species were offered individually or together in a series of five treatments in greenhouse cages. Results showed that the predator was able to establish and suppress populations of both pests inhabiting tomato plants when pests occurred alone or together. Thus, *D. hesperus* was demonstrated to be a suitable biocontrol agent of these two important pests that could be used in tomato greenhouses.

Keywords: greenhouse crops, Miridae, *Bemisia tabaci*, *Bactericera cockerelli*, augmentation

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Introduction

The potato psyllid, *Bactericera cockerelli* Sulcer (Hemiptera: Psyllidae) and the sweetpotato whitefly, *Bemisia tabaci* Gennadius (Hemiptera: Aleyrodidae), are major pests in greenhouse-grown tomatoes in North American countries (Garzón-Tiznado *et al*., 2009; Butler & Trumble, 2012*a*). Both species directly damage plants when feeding and produce honeydew that serves as a substrate for sooty mould. The sweetpotato whitefly feeding may also induce irregular tomato ripening (Schuster, 2001). However, they are even more important due to their role in the transmission of plant viruses and bacteria (Jones, 2003; Butler & Trumble, 2012*a*). The sweetpotato whitefly is an effective vector of a long list of plant viruses (Jones, 2003), and the potato psyllid transmits the bacterial pathogen '*Candidatus* Liberibacter solanacearum' (syn. '*Ca. L. psyllaurous*'), which causes a disease referred to as 'yellows' (Munyaneza *et al*., 2007; Secor *et al*., 2009).

The list of natural enemies of *B. cockerelli* in North America includes several parasitoids and predators (Butler & Trumble, 2012*a*, *b*). However, so far none of the evaluated parasitoids or predators have been demonstrated to be effective on a large commercial scale under greenhouse conditions (Workman & Whiteman, 2009; Banks, 2012; Butler & Trumble, 2012*a*, *b*;

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Rojas *et al.*, 2015). Biocontrol of whitefly in tomato in North America has been typically achieved by releasing the parasitic wasps *Eretmocerus eremicus* Rose & Zolnerowich and *Encarsia formosa* Gahan (Hymenoptera: Aphelinidae) (Hoddle & van Driesche, 1999; van Driesche *et al.*, 2001*a*; Greenberg *et al.*, 2002) but this approach requires weekly releases of the parasitoids and often has to be supplemented with pesticide applications (van Driesche *et al.*, 2001*b*). As a consequence, current biocontrol-based integrated pest management (IPM) programmes present limited effectiveness against these two major pests, so growers often rely on pesticides for their pest control needs. In Europe, biological control-based IPM programmes for tomato crops are based on the use of mirid bugs such as *Macrolophus pygmaeus* (Rambur) and *Nesidiocoris tenuis* (Reuter) (Heteroptera: Miridae). These mirid-based IPM programmes provide excellent control of the whiteflies *B. tabaci* and *Trialeurodes vaporariorum* Westwood (Hemiptera: Aleyrodidae), the invasive pest *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae), and contribute to the control of other secondary pests in more than 8000 ha of tomato greenhouses (Calvo *et al.*, 2009, 2012*a*, *b*, *c*; Urbaneja *et al.*, 2009, 2012). Implementation of a similar programme in North America could have the same effect and serve to increase adoption of biocontrol in tomato. Some years ago, McGregor *et al.* (1999) initiated a research project aimed at finding a natural enemy native to North America and suitable for tomato crops with the characteristics of *M. pygmaeus*. Among the tested species, *Dicyphus hesperus* Knight (Heteroptera: Miridae) was the most promising (Gillespie *et al.*, 2007). These authors found, that the predator exhibited good development and reproductive parameters when reared on whitefly on tomato. However, life-history traits when fed on *B. cockerelli* are still unknown. Knowledge of these traits would provide useful information about its potential for *B. cockerelli* control. We, therefore, conducted a first experiment designed to study the suitability of *B. cockerelli* nymphs for *D. hesperus* compared with the factitious prey *Ephestia kuehniella* Zeller (Lepidoptera: Pyrallidae) eggs, which is known to be a suitable food.

Shipp & Wang (2006) and Gillespie *et al.* (2007) reported that *D. hesperus* was able to establish in tomato greenhouses and suppress populations of different pests, including *T. vaporariorum* and the western flower thrips *Frankliniella occidentalis* Pergande (Thysanoptera: Thripiidae). This list was extended by Calvo *et al.* (2016*a*), who observed *D. hesperus* suppressing simultaneous infestations of *B. tabaci* and *B. cockerelli*. Nevertheless, the potato psyllid and the sweetpotato whitefly that may appear simultaneously or individually are suitable prey for *D. hesperus*. Although the presence of one or more suitable insect prey can affect the predator's control capacity, when possible, the option of targeting two pests with the same natural enemy is preferred. This may reduce the number of beneficial species and individuals necessary to provide adequate control, leading to economic benefits as well as a more easily manageable IPM programme. Nevertheless, the use of a predatory mirid for biological control is sometimes controversial due to their potential for plant damage (Sánchez 2008; Calvo *et al.*, 2009; Castañé *et al.*, 2011). Phytophagy can, however, provide benefits for omnivorous insects, as it may allow them to survive by feeding on plants in periods of insect prey scarcity (Alomar & Wiedenmann, 1996; Naranjo & Gibson, 1996), and may enhance their fitness when they also feed on insect prey (Naranjo & Gibson, 1996; Coll & Guershon, 2002). In the case of *D. hesperus*, McGregor *et al.* (2000) suggested that its use on tomato crops should not be constrained by fruit damage. Plant-feeding of *D. hesperus* seems to increase with prey feeding, but has also been related with prey quality and abundance (Gillespie & McGregor, 2000; Shipp & Wang, 2006; Vankosky & van Laerhoven, 2015). Presence of psyllids and/or whiteflies, could, therefore, have some unknown effects on control capacity and plant feeding of *D. hesperus*. Thus, knowledge about the establishment of *D. hesperus* on tomato, i.e. capacity to grow and reproduce on the crop, its control capacity and risk for plant damage when whiteflies and psyllid are present either alone or together, is also useful information in determining its potential as a biocontrol agent for tomato crops. We thus designed a second experiment to evaluate the effectiveness and risk for plant damage of augmentative biocontrol of *D. hesperus* under different scenarios of pest entry into a tomato crop, i.e. sweetpotato whitefly and potato psyllid entering either alone or together.

Materials and methods

Laboratory experiment: biology of B. cockerelli

Insects

Dicyphus hesperus used in the assay was obtained from a rearing colony maintained on tomato and fed with *E. kuehniella* eggs at 25°C, 75% relative humidity (RH), and 16 : 8 h (L : D) photoperiod at the Koppert Mexico facilities located in Queretaro (Queretaro, Mexico). *Bactericera cockerelli* adults to infest the tomato plants were collected from a mass rearing colony maintained on tomato plants and originally obtained from field samples from several locations within Mexico. Eggs of *E. kuehniella* were obtained from the commercial product Entofood™ (Koppert Biological Systems, The Netherlands) in bottles containing 10 g of frozen eggs.

Developmental time

Initially, five potted tomato cv. Merlice (De Riuter, St. Louis, Missouri, USA) seedlings, approximately 30 cm high, were placed into mesh-walled wooden-frame cages (rearing cages) ($50 \times 50 \times 50$ cm^3), and then 50 *D. hesperus* adults were released into each rearing cage in order to establish a colony. Two of these cages were used for each prey species, with all cages being simultaneously initiated using adults belonging to the same cohort collected from the colony. All cages were maintained after the predator release at 25°C, 75% RH, and 16 : 8 h (L : D) photoperiod inside a climatic walk-in room ($3 \times 5 \times 3$ m^3). In cages designated for *E. kuehniella*, adults were fed *ad libitum* with frozen *E. kuehniella* eggs, which were provided, glued to 5×1 cm^2 paper strips. The strips were renewed twice a week. In cages for *B. cockerelli*, highly psyllid nymph-infested plants (presenting an *ad libitum* quantity of nymphs per leaf), were placed before the release of the adults. One of the plants was replaced weekly with a new highly psyllid nymph-infested plant. Nymphs on replaced plants were collected and released again into the cage.

Plants were inspected daily and newly emerged nymphs were transferred individually to a mesh-lid 60 ml translucent cup (Solo™, USA, IL) with a fresh leaf-disk containing 50–60 second-third *B. cockerelli* instar nymphs or *E. kuehniella* eggs (*ad libitum*) on a fine (ca. 2 mm) layer of agar (2% w/v). Excess of psyllid nymphs was removed with a fine paintbrush. Leaf-disks infested with second-third psyllid nymphs were

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obtained by infesting ca. 75 cm high tomato plants 2 weeks before being used in the experiment with 100 psyllid adults each. Adults were removed 72 h after the infestation and plants were always maintained during this 2-week period at 25°C, 75% RH, and 16 : 8 h (L : D) photoperiod. All cups were maintained in a climatic cabinet (ICP20, Lumistel, Celaya, Mexico) at 25.0 ± 2.5°C with a 16 : 8 h (L : D) photoperiod and 75 ± 5% RH. Mirid nymphs were inspected daily using a 40× stereoscopic microscope and their developmental stage noted. Nymphs were transferred to a new set-up every 2–3 days, or when the nymphs moulted. Nymphal developmental time, survivorship, and the sex ratio of emerged adults were calculated. Fifty nymphs were bred on each prey species.

Fecundity and egg hatching time

Nymphs reaching adulthood on each prey species were used to estimate reproductive and demographic parameters on the same prey. Newly emerged adults were transferred in pairs (male × female) to a 350 ml plastic container containing a two-leaved tomato seedling and *E. kuehniella* eggs (*ad libitum*), or two-leaved tomato seedling and a leaf disk infested with ca. 60 *B. cockerelli* second-third instar nymphs. Psyllid nymph-infested leaf-disks were obtained as above. This container was placed inside another 350 ml cup containing water. The tomato seedling was pushed through a hole in the inner container to reach the water inside the outer one. Twenty containers were prepared for each prey species and they were maintained at 25°C, 75% RH, and 16 : 8 h (L : D) photoperiod. During the experiment, pairs were checked daily and dead males replaced, which served to estimate the lifetime of adult females. Additionally, pairs were transferred to a new set-up every 2–3 days during the first 7 days, after which they were transferred daily, for 3 days, to a new set-up. After this 3-day period, they were transferred again to a new set-up every 2–3 days until the female had died. All replaced seedlings were extracted from the plastic containers and then inspected using a 40× stereoscopic microscope to count the number of laid eggs. This served to estimate lifetime and daily fecundity of adult females.

The 3-day period served to provide the eggs used to estimate egg hatching time and fertility. For each prey species, 40 eggs were selected from seedlings being replaced during the 3-day period. For that, seedlings of each day were consecutively inspected until having 40 eggs, with the remaining seedlings being discarded. Selected seedlings containing the 40 eggs were returned to the containers, which were labelled (day in which pair was replaced), and then maintained with the rest of the containers inside the same climatic cabinet. Eggs were inspected daily using a 40× stereoscopic microscope until all eggs had hatched or died (dehydrated).

Data analysis

Developmental and hatching time, as well as female fecundity, were log $(x + 1)$ transformed, whereas fertility of eggs were arcsin$\sqrt{(x)}$ transformed prior to analysis. Untransformed values (expressed as percentages in the case of proportions) are given in all tables and figures. Data from all nymphs that reached the adult stage were used to evaluate the effects of prey species on the development time of *D. hesperus* males and females, for which they were subjected to a two-way analysis of variance (ANOVA) ($\alpha = 0.05$) with sex and prey species as fixed factors. The analysis revealed that the sex–diet interaction and sex were non-significant (sex-diet: $F_{1,90} = 0.217$, P$P = 0.642$; sex: $F_{1,90} = 0.070$, $P = 0.792$), and thus were removed from the analysis. The effects of diet on the development time of *D. hesperus* males and females were then tested with a one-way ANOVA ($\alpha = 0.05$).

Effects of prey species on reproductive and demographic parameters were tested using a one-way ANOVA ($\alpha = 0.05$), and effects on longevity of ovipositing females of *D. hesperus* were tested using Cox Regression models ($\alpha = 0.05$). Values for age-specific survivorship beginning with 1-day-old eggs and age-specific fecundity for females was used to estimate life history parameters. The intrinsic rate of natural increase (r_m) was computed using the equation $\sum e^{-rm} l_x m_x$, where l_x is survivorship of the original cohort over the age interval from day $x - 1$ to day x, and m_x is the mean number of female offspring produced per surviving female during the age interval x (Birch, 1948). Values of m_x for the population were calculated from the mean number of eggs laid per female per day. Other parameters, including net reproductive rate and mean generation time ($T = \ln R_0/r_m$) were calculated for each prey species (Birch, 1948; Andrewartha & Birch, 1954; Laughlin, 1965; Southwood & Henderson, 1978; Mackauer, 1983). All life table parameters for each prey species were calculated using the jack-knife technique (Maia *et al*., 2000), and then were subjected to a one-way ANOVA ($\alpha = 0.05$). All tests were performed using the software IBM SPSS Statistics 24.0.

Greenhouse experiment

Experimental greenhouse

The experiment was conducted in a multi-tunnel greenhouse located in Amexe (Guanajuato, Mexico). Twenty walk-in cages were constructed inside the greenhouse to accommodate plants and isolate treatments. Each walk-in cage ($1.5 \times 2.5 \times 3$ m^3) was constructed of 'anti-thrips' polyethylene screen with 220×331 µm interstices and supported by heavy wires. Floors were covered with woven 2-mm-thick polyethylene cloth and access to each cage was through a zippered doorway. The greenhouse was equipped with a climate control system for temperature and RH. Temperature and RH were monitored in four randomly selected walk-in cages with a HOBO H8 RH/Temp Loggers (Onset Computer, Bourne, MA, USA).

Plants and cultural practices

Tomato seeds cv. Merlice (De Riuter, St. Louis, Missouri, USA) were sown into 15 cm^3 peat moss root cubes, and the experiment initiated on 17 September 2013 when seedlings were at the five-leaf stage. Plants were transplanted into a composted coconut fibre 6.3 l white polyethylene flower pots, with 12 being placed in each walk-in cage. They were grown according to typical cultivation techniques for tomato. Plants were trained by the main stem to a black polyethylene string tied to a stainless-steel overhead wire. Additionally, secondary shoots were removed as required and each plant was provided with a drip emitter delivering 2 l h^{-1} through which water and fertilizers were supplied as required.

Experimental design and procedure

Five treatments were compared in a complete randomized block design with four replicates: (i) *B. cockerelli*; (ii) *B. tabaci*;

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(iii) *B. cockerelli* + *D. hesperus*; (iv) *B. tabaci* + *D. hesperus; and* (v) *B. cockerelli* + *B. tabaci* + *D. hesperus*. In all cages with *B. cockerelli*, one insect was released per plant for 3 weeks, beginning the week of planting for a total of three psyllid adults per plant. In all cages with *B. tabaci*, plants were infested by releasing ten whitefly adults per plant for 3 weeks, beginning the week of planting for a total of 30 whitefly adults per plant. In all cages receiving *D. hesperus*, the predator was released during the week of planting and at a rate of 1 *D. hesperus* per plant. This release schedule for pests was used to simulate gradual but heavy immigration of both pests into the greenhouse. Timing and rate for the predator release were chosen based on recommended methods for in field releases of other commercially available mirid bugs (Calvo *et al.*, 2009).

Adults of *B. cockerelli* for the experiment were obtained from the same rearing colony used for the first experiment and adult whitefly were obtained from a rearing colony maintained on tomato for several generations before the start of the experiment and originally collected on tomato in several locations within Mexico. Adult pests to be released into cages were collected each week from a single colony cohort to ensure homogeneity of age and sex ratio (whitefly). They were later cooled briefly in a cold room at 8°C for counting and then released into the designated walk-in cages at the above-mentioned rate and in the case of the psyllid at a sex ratio 1 : 1 (male : female). Whitefly adults were not sexed, as we considered that sex ratio of the released populations in each plot was similar to that estimated for our rearing colony due to the relatively high number released in each cage. The sex-ratio of our rearing colony was estimated before the experiment at ca. 1 : 1 (male : females), as 50.4% were females. *D. hesperus* were obtained following the method adopted in the first experiment. Adults of *D. hesperus* were collected from a single colony cohort to ensure homogeneity of age (less than 3 day-old adults) and were later cooled briefly in a cold room at 8 °C for counting. Adults were released in the designated walk-in cages at the above-mentioned rate and at a sex ratio of 1 : 1 (male : female). Eggs of *E. kuehniella* were sprinkled on all plants from cages receiving *D. hesperus* at a rate of 0.01 g per cage, beginning just after the predator release, and for 4 weeks thereafter. Availability of whitefly and/or psyllid nymphs was expected to be low during this period and the supplementary food was added to increase the likelihood of establishment, due to the incapability of *D. hesperus* nymphs to reach maturity in the absence of prey (Sánchez *et al.*, 2004).

Sampling

Plants were monitored weekly for 10 weeks, beginning 1 week after transplanting on 25 September 2013. On each sampling occasion, five plants were randomly selected in each walk-in cage. Nymphs, pupae, and adults of whitefly and psyllid, as well as mirid nymphs and adults, were counted on three leaves from each of the five selected plants. One leaf was selected at random from the upper, one from the middle, and one from the bottom third of the plants. In each case, leaves were turned carefully to count first whitefly, psyllid and *D. hesperus* adults and then the other insect stages using a 15× hand lens.

Plant-feeding of *D. hesperus* on leaves was assessed weekly by recording (on the same above-mentioned leaves) the number of necrotic rings present on the rachis and petioles, characteristic symptoms of plant feeding on petioles and rachis (Arnó *et al.*, 2010). Additionally, the percentage of punctured leaf area, a characteristic symptom of plant feeding on the blade of the leaf, was recorded. The punctured area was rated visually as 1, 2, 3 or 4 where 0 was no damage, 1 = 1–25%, 2 = 26–50%, 3 = 51–75%, and 4 > 76% of the leaf surface damaged, respectively. Upon the start of flowering, the effects of plant feeding on flowering were assessed weekly by counting the number of affected (flower presenting a necrotic ring in the petiole), aborted or healthy flowers in five flowering clusters in each cage. Clusters belonged to different plants and in all plants, the third cluster from the top was selected. Once fruits were present, feeding on fruits was also assessed by counting the number of feeding punctures surrounded by a whitish halo (blemishing, McGregor *et al.*, 2000) in ten fruits per cage. Fruits were collected from different plants but always from the fifth cluster from the top of the plant. The selection of clusters to assess feeding on flowers and fruits was done in order to select the youngest possible fruits and fully-opened flowers.

Ambient conditions

Mean daily temperature during the experimental period varied from 15.2 ± 0.2 to 26.4 ± 0.6°C with absolute maxima and minima during the experiment estimated at 44.4 ± 0.2 and 6.6 ± 0.2°C, respectively. The mean daily RH ranged from 50.4 ± 2.1 to 88.2 ± 0.8 % during the experiment, with the lowest and highest values of RH during the experiment estimated at 37.5 ± 2.6 and 100.0 ± 0.0, respectively.

Data analysis

Treatment effects on *B. tabaci*, *B. cockerelli*, and *D. hesperus* were analyzed using linear mixed effects models (α = 0.05), with time (weeks) as random factor nested in blocks (replicates) to correct for pseudoreplication due to repeated measures (see Messelink *et al.*, 2008; Calvo *et al.*, 2016*b*). Thereafter, treatments were compared, contingent on a significant model, through model simplification by combining treatments (Crawley, 2002). Insect numbers per leaf were $\log(x + 1)$ transformed prior to analysis to stabilize error variance, although untransformed values are given in the text. All tests were performed using the software IBM SPSS Statistics 24.0.

Results

Biology on B. cockerelli

Development and survival

Hatching time was nearly 1 day shorter when *D. hesperus* females fed on *B. cockerelli* nymphs (12.6 ± 0.18 days) than on *E. kuehniella* eggs (13.5 ± 0.17 days) ($P < 0.001$; $F_{1,69} = 15.329$), but prey type had no effect on the fertility ($P = 1.000$; $F_{1,79} = 0.00$), as 87.6 ± 0.15 % of eggs hatched when both prey species were offered. We observed that 88% and 94% of nymphs reached the adult stage, of which the 48% and 54% were females, when fed on potato psyllid nymphs and *E. kuehniella* eggs, respectively. Prey species also affected total developmental time of both sexes (Males: $F_{1,43} = 15.845$, $P < 0.001$; Females: $F_{1,45} = 12.209$; $P = 0.001$), as females and males developed ca. 2 days faster when nymphs fed on *E. kuehniella* (Females: 19.2 ± 0.21 days; Males: 19.1 ± 0.33) compared with psyllid nymphs (Females: 20.9 ± 0.50 days; Males: 21.2 ± 0.38). Sex had no effect on development time,

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Table 1. Reproductive and demographic parameters (Mean ± SE) of ovipositing females of *Dicyphus hesperus* reared on *Ephestia kuehniella* eggs or second-third instar *Bactericera cockerelli* nymphs on tomato at 25°C, 75% RH, and 16 : 8 (L : D) photoperiod.

	Ephestia kuehniella	*Bactericera cockerelli*	Statistics
Longevity (days)	48.2 ± 4.87	40.5 ± 2.23	HR = 0.421; $P = 0.019$
Pre-oviposition (days)	7.5 ± 0.44	6.6 ± 0.23	$F_{1,36} = 2.993$; $P = 0.092$
Oviposition (days)	32.2 ± 4.18	29.1 ± 2.32	$F_{1,36} = 1.500$; $P = 0.229$
Post-oviposition (days)	7.0 ± 2.07	4.5 ± 1.29	$F_{1,36} = 0.116$; $P = 0.735$
Fecundity (eggs/female)	138.9 ± 21.7	97.5 ± 10.7	$F_{1,39} = 3.096$; $P = 0.087$
Daily oviposition rate (eggs/female/day)	4.5 ± 0.28	3.7 ± 0.17	$F_{1,36} = 6.560$; $P = 0.015$
R_0	49.4 ± 0.39	31.1 ± 0.17	$F_{1,39} = 1789.2$; $P < 0.001$
T	49.7 ± 0.02	49.5 ± 0.02	$F_{1,39} = 62.691$; $P < 0.001$
r_m	0.078 ± 0.0001	0.069 ± 0.0001	$F_{1,39} = 5540.3$; $P < 0.001$

R_0, net reproductive rate; T, mean generation time; r_m, intrinsic rate of natural increase.
Means followed by the same letter within the same row were not significantly different (ANOVA, $P > 0.05$).

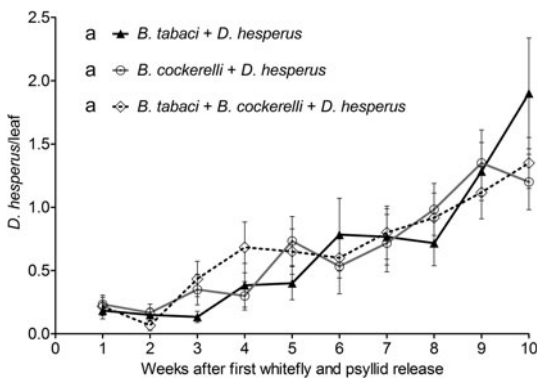

Fig. 1. Mean (±SE) of nymphs plus adults of *Dicyphus hesperus* per leaf in treatments receiving the predator during the Greenhouse Experiment. Treatments with the same letters (shown in the legends) are not significantly different (GLMM, $P > 0.05$).

as males and females developed equally fast when fed on the same prey species.

Reproductive and demographic parameters

Ovipositing females of *D. hesperus* survived longer when fed on *E. kuehniella*, but fecundity was comparable when they were fed with either *E. kuehniella* eggs or *B. cockerelli* nymphs (table 1). Duration of pre, post, and oviposition periods was not affected by the offered prey species, although the daily ovipositing rate was higher when females fed on *E. kuehniella* eggs. Higher oviposition rate and longer lifetime, together with a shorter developmental time, resulted in a higher net reproductive rate, shorter generation time and higher growth rate when *D. hesperus* was reared on *E. kuehniella* eggs compared with psyllid nymphs.

Greenhouse experiment

D. hesperus

The numbers of nymphs and adults of *D. hesperus* per leaf increased in all treatments progressively after the release of the predator until the end of the experiment (fig. 1). Predator population size was similar in all cages with predator release, regardless of the prey available ($F_{2,1.977} = 0.019$; $P = 0.981$).

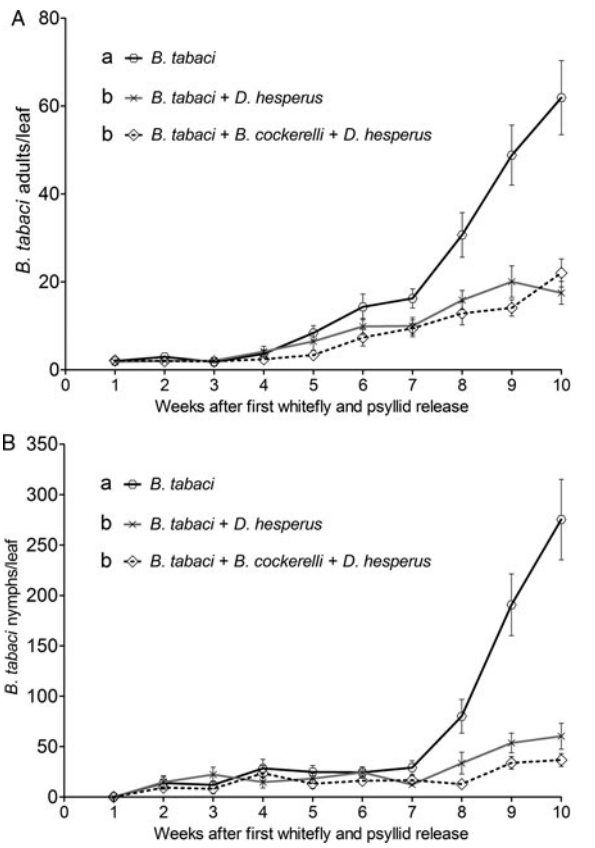

Fig. 2. Dynamics (mean ± SE) of adults (a) and nymphs plus pupae (b) of *Bemisia tabaci* per leaf in each treatment during the Greenhouse Experiment. Treatments with the same letters (shown in the legends) are not significantly different (GLMM, $P > 0.05$).

Whitefly

Density of whitefly adults per leaf was similar among treatments until week 5 (fig. 2a), after which greater numbers of whitefly per leaf were recorded in cages with the pest only, compared with cages with predator release. The abundance of whitefly adults was therefore significantly higher in plots with the pest only, whereas it was similar in treatments with predator release (table 2). Similar results were observed on

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Table 2. Comparison among treatments (GLMM, $\alpha < 0.05$).

	Statistics			
	B. tabaci		*B. cockerelli*	
Compared treatments	Immatures/leaf	Adults/leaf	Immatures/leaf	Adults/leaf
All treatments	$F_{2,1.917} = 69.231$; $P < 0.001$[1]	$F_{2,1.917} = 62.281$; $P < 0.001$[1]	$F_{2,1.917} = 51.969$; $P < 0.001$[2]	$F_{2,1.917} = 34.026$; $P < 0.001$[2]
Wf vs. Wf + Dh	$F_{1.917} = 59.973$; $P < 0.001$	$F_{1.917} = 14.005$; $P < 0.001$		
Wf vs. Wf + Ps + Dh	$F_{1.917} = 57.614$; $P < 0.001$	$F_{1.917} = 13.444$; $P < 0.001$		
Wf + Dh vs. Wf + Ps + Dh	$F_{1.917} = 3.641$; $P = 0.516$	$F_{1.917} = 0.560$; $P = 0.701$		
Ps vs. Ps + Dh			$F_{1.917} = 61.267$; $P < 0.001$	$F_{1.917} = 17.088$; $P < 0.001$
Ps vs. Wf + Ps + Dh			$F_{1.917} = 69.827$; $P < 0.001$	$F_{1.917} = 18.535$; $P < 0.001$
Ps + Dh vs. Wf + Ps + Dh			$F_{1.917} = 8.561$; $P = 0.261$	$F_{1.917} = 1.447$; $P = 0.571$

Wf: *Bemisia tabaci*; Ps: *Bactericera cockerelli*; Dh: *Dicyphus hesperus*.
Treatments were: (1) Ps: *B. cockerelli*; (2) Wf: *B. tabaci*; (3) Ps + Dh: *B. cockerelli* + *D. hesperus*; (4) Wf + Dh: *B. tabaci* + *D. hesperus*; and (5) Wf + Ps + Dh: *B. tabaci* + *B. cockerelli* + *D. hesperus*.
[1]Compared treatments: *B. tabaci*; *B. tabaci* + *D. hesperus*; *B. tabaci* + *B. cockerelli* + *D. Hesperus*.
[2]Compared treatments: *B. cockerelli*; *B. cockerelli* + *D. hesperus*; *B. tabaci* + *B. cockerelli* + *D. Hesperus*.

whitefly nymphs plus pupae. Again, numbers of whitefly nymphs plus pupae per leaf were similar among treatments during the first weeks of the experiment, but later these numbers increased more rapidly in untreated cages (fig. 2b). This resulted in a greater abundance of nymphs plus pupae (table 2) compared with cages receiving the predator where similar numbers were recorded. At the end of the study, the number of whitefly nymphs plus pupae per leaf was ca. 7 times greater in cages with the pest only, compared with cages with *D. hesperus*.

Psyllid

Numbers of *B. cockerelli* adults increased more rapidly at the end of the experiment in cages with the pest only (fig. 3a). In these cages, more than 100 adults of *B. cockerelli* per leaf were recorded at the end of the experiment, which were significantly, and nearly four times, greater than those recorded in cages receiving *D. hesperus* (table 2). In these latter treatments, similar numbers of psyllid adults were recorded throughout the entire experiment, and thus the abundance of adults of *B. cockerelli* was similar. During the first 6 weeks of the experiment, the number of psyllid nymphs and pupae per leaf was similar among treatments (fig. 3b). Nevertheless, abundance of psyllid nymphs plus pupae increased more rapidly afterwards in the treatment with psyllid alone, and exceeded 350 nymphs plus pupae per leaf at the end of the experiment, which was much higher compared with levels observed in cages with either *D. hesperus* and psyllid, or *D. hesperus* plus whitefly and psyllid (table 2).

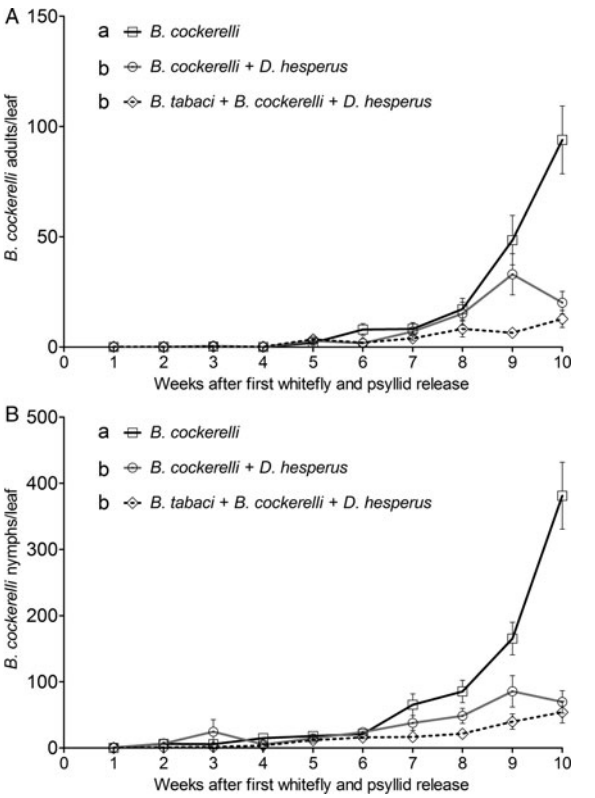

Fig. 3. Dynamics (mean ± SE) of adults (a) and nymphs plus pupae (b) of *Bactericera cockerelli* per leaf in each treatment during the Greenhouse Experiment. Treatments with the same letters (shown in the legends) are not significantly different (GLMM, $P > 0.05$).

Phytophagy

Neither necrotic rings nor effects on flowering were observed in response to the predator release during the experiment. Only 0.1 ± 0.1 feeding punctures per fruit were observed 8 weeks after the predator release in cages with *D. hesperus* plus *B. cockerelli*.

Discussion

The laboratory experiment showed that *D. hesperus* was able to develop and reproduce on *B. cockerelli*, although the predator exhibited better reproductive and development parameters when fed on *E. kuehniella* eggs. This reflects that the factitious prey is more suitable for the predator than psyllid nymphs, though our estimate of r_m for *D. hesperus* on the potato psyllid was above some r_m estimates for *B. cockerelli* in tomato, bell pepper or eggplant (Xiang-Bing *et al.*, 2013; Vargas-Madríz 2010). These rates, together with those estimated for the predator on whitefly (McGregor *et al.*, 1999),

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suggest that *D. hesperus* has potential as a biocontrol agent of the potato psyllid and whiteflies.

The greenhouse experiment confirmed the results from the laboratory experiment, as the predator established well and provided good pest control in cages simulating different scenarios of simultaneous and single infestation in tomato of *B. cockerelli* and *B. tabaci*. This correlates with earlier research in which whiteflies and psyllids were used as prey (Gillespie *et al*., 2007; Calvo *et al*., 2016*a*), but also extends the possibilities to situations of single psyllid infestation, or simultaneous infestation with *B. tabaci* and *B. cockerelli*. McGregor *et al*. (1999), Shipp & Wang (2006) and Gillespie *et al*. (2007) demonstrated the capability of *D. hesperus* to reduce populations of *T. vaporariorum*, *F. occidentalis* or *Tetranychus urticae* Koch (Acari: Tetranychidae), demonstrating overall the potential of the predator as a biological control agent of different important pests attacking tomato crops. The option of targeting more than one pest with a single natural enemy has positive implications for biocontrol. Technically, it may reduce the complexity and costs of the biological control programme by reducing the number of beneficial insect species that have to be released. This is the key of the success of programmes based on augmentation of the mirid *N. tenuis*, which currently provides excellent whitefly and *T. absoluta* control in more than 8000 ha of tomato greenhouses (Calvo *et al*., 2012*c*). Biologically, the option of feeding on several insect pests increases the likelihood of successful establishment and persistence in the crop, as the predator has more options to find a suitable prey. This is particularly interesting as Sánchez *et al*. (2004) reported that nymphs of *D. hesperus* cannot reach maturity in the absence of prey. Additionally, Sánchez *et al*. (2004), and our laboratory experiment demonstrated that *D. hesperus* has better reproductive and developing parameters when it had access to *E. kuehniella* eggs. This revealed that, as it is the case under greenhouse conditions for other mirid predators (Calvo *et al*., 2009, 2012*a*, *b*), *E. kuehniella* eggs could be used as a supplementary food for *D. hesperus*. Artificial provision of *E.kuehniella* eggs in the crop could, therefore, enhance establishment and persistence of the predator in the crop in periods of prey scarcity, by allowing faster development and greater survival of nymphs, and greater longevity and fecundity of adult females. In our greenhouse experiment, the addition of *E. kiehniella* eggs could have therefore contributed to a more rapid increase in predator numbers in the crop, ultimately resulting in better pest control.

During our experiment, we recorded no evidence of plant-feeding by *D. hesperus* on leaves, and fruit damage was insignificant regardless of the presence of single or simultaneous infestations of *B. tabaci* and *B. cockerelli*. This could be explained by the fact that the predator always had access to prey, as the intensity of plant-feeding by *D. hesperus* seems to increase with prey feeding and be affected by prey quality and abundance (Gillespie & McGregor, 2000; McGregor *et al*., 2000; Shipp & Wang, 2006). Nevertheless, Gillespie *et al*. (2007) suggested that the use of the *D. hesperus* on tomato crops should not be constrained by fruit damage, which agrees with our findings.

In conclusion, the present experiment provides evidence for the possible successful control of high initial pest populations of whitefly and potato psyllid in tomato, based exclusively on augmentative biological control with *D. hesperus*. Additionally, it showed that use of *D. hesperus* was safe in terms of plant-feeding, demonstrating overall the usefulness of this predator as a biological control agent against these two important pests in tomato. Further study, to confirm these results under real greenhouse conditions, would determine whether this beneficial insect could be used for biological control purposes.

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