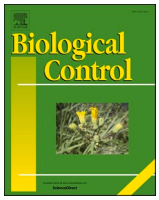
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Role of tomato plant-derived food sources on *Dolichogenidea gelechiidivoris*, parasitic wasp of *Tuta absoluta*

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HIGHLIGHTS

- Honeydew and plant guttation are the main plant-derived food sources in tomato crops.
- Honeydew improves the fitness and performance of the parasitoid *Dolichogenidea gelechiidivoris*.
- Tomato guttation does not affect the biological parameters of *Dolichogenidea gelechiidivoris*.
- Chemical analyses demonstrated that tomato guttation is poor in carbohydrates and proteins.

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ABSTRACT

Tuta absoluta (Meyrick) (Lepidoptera: Gelechiidae) poses a significant threat to tomato crops worldwide. Following its detection in the Mediterranean basin, considerable efforts have been made to develop biological control programs against this pest. The larval parasitoid *Dolichogenidea gelechiidivoris* Marsh (Hymenoptera: Braconidae) is widely distributed in northeastern Spain and has shown promise as a natural enemy in this region. Recent studies have demonstrated that access to floral nectar can improve the biological parameters of this parasitic wasp. However, the effects of other plant-derived food sources available in tomato crops, such as plant guttation and honeydew, have yet to be explored. This study assessed the impact of plant guttation and the honeydew of two phloem-feeding insects, *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) and *Bemisia tabaci* (Gennadius) (Hemiptera: Aleurodidae), on the fitness (longevity and fecundity) and performance (parasitism and sex ratio) of *D. gelechiidivoris* under controlled laboratory conditions. Our results demonstrated that both honeydews enhanced the fitness and performance of this parasitoid, while tomato plant guttation did not have an effect on its fitness and negatively impacted parasitism. These findings highlight honeydew as a potential food source in integrated pest management (IPM) strategies. Further research is recommended to explore the specific chemical composition of tomato plant guttation and its broader effects on beneficial insects in tomato systems.

1. Introduction

The tomato moth *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae), recently reinstated as *Phthorimaea absoluta* Meyrick (Chang and Metz, 2021), was first detected outside its native range in Spain at the end of 2006 (Urbaneja et al., 2007), and from there it spread quickly across the Mediterranean area, causing severe damage to tomato crops (Desneux et al., 2010). Although insecticides continue to be the main control tool for this pest in many areas of the world, significant investments have been made in the development of biological control methods (Biondi et al., 2018). So far, in the areas of the Mediterranean basin where it initially invaded, its biological control is based mainly on the use of the predatory mirid bugs *Macrolophus pygmaeus* (Rambur) and *Nesidiocoris tenuis* (Reuter) (Hemiptera: Miridae) (Desneux et al., 2022), as both species prey mostly on the eggs of *T. absoluta* (Arnó et al., 2009; Urbaneja et al., 2009). To complement the low predatory action of these predators on *T. absoluta* larvae, surveys have been conducted to detect new species of larval parasitoids capable of controlling this pest (Gabarra et al., 2014; Zappalà et al., 2013).

In Spain, numerous species of larval parasitoids of *T. absoluta* have been recorded, mostly belonging to the Braconidae, Eulophidae, and Ichneumonidae families (Gabarra et al., 2014). *Necremnus tutae* Ribes &

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Bernardo (Hymenoptera: Eulophidae), previously referred to as *Necremnus artynes* (Walker), is the species with the highest abundance in the field and wider geographical distribution (Gebiola et al., 2015). In many tomato-growing regions of Europe, conservation biological control through the manipulation of widely distributed *N. tutae* could serve as a viable strategy against *T. absoluta* (Calvo et al., 2016). *Dolichogenidea gelechiidivoris* Marsh (Hymenoptera: Braconidae) is a koinobiont solitary larval endoparasitoid that is native to South America and has been widely distributed in Catalonia (northeast Spain) since 2016, where it was probably unintentionally introduced with the pest (Denis et al., 2022). This braconid parasitoid is one of South America's most important biological control agents for *T. absoluta* (Salas Gervassio et al., 2019). It was imported into Kenya from Peru in 2017 (Aigbedion-Atalor et al., 2020), and it was recently detected in tomato crops in Algeria (Krache et al., 2021). According to van Lenteren et al. (2021), behavior studies of this parasitoid have been conducted outside its native range, particularly in East Africa (Aigbedion-Atalor et al., 2020; Mama Sambo et al., 2022). However, in the Mediterranean basin, the behavior of *D. gelechiidivoris* remains unknown.

Numerous species of beneficial insects require plant-provided food sources as an indispensable part of their diets (Benelli et al., 2017; Urbaneja-Bernat et al., 2020; Wäckers et al., 2008). Thus, understanding the role of plant-derived food sources in the biological parameters of natural enemies is crucial for optimizing integrated pest management (IPM) programs (Benelli et al., 2017; Wäckers et al., 2008). Among plant-derived food sources, extra and floral nectars are rich-nutritive food sources for insects (Heil, 2015; Wäckers et al., 2005). Although these food sources are not present in the tomato system, two plant-derived food sources can be found in this crop: honeydew and plant guttation. Honeydew and plant guttation are nutritive carbohydrate-rich food sources present in many agricultural systems, and they can enhance the fitness of natural enemies (Tena et al., 2016; Urbaneja-Bernat et al., 2020). Guttation is found in many crops, including fruits, cereals, vegetables, and cover crops (Singh, 2020), and its nutritional value depends on the morphological and physiological characteristics of each plant species, such as xylem and phloem composition (Urbaneja-Bernat et al., 2024a).

Recent research has demonstrated the importance of the nectar of the insectary plant *Lobularia maritima* L. (Brassicaceae) as a reliable food source that enhances the biological parameters of *D. gelechiidivoris* (Urbaneja-Bernat et al., 2024b). However, the potential impact on this parasitic wasp of plant-derived food sources present in tomato, such as plant guttation and honeydew of phloem feeders, has yet to be explored. We hypothesized that the presence of these plant-derived food sources in the tomato crop system could enhance the efficacy of *D. gelechiidivoris*. The aim of this study was to investigate the role of plant-derived food sources present in tomato crop systems on parasitic wasp fitness (longevity and fecundity) and performance (parasitism and sex ratio).

2. Materials & methods

2.1. Plants and insect rearing

All experiments and insect rearing were conducted at IRTA facilities (Cabrils, Barcelona, Spain) under controlled conditions (25 ± 1 °C, 70 ± 10 % RH, and 16:8h L:D photoperiod). Pesticide-free tomato (*Solanum lycopersicum* L. (Solanaceae)) plants (cv. Roma; Semillas Fitó) were grown by sowing seeds on a mixture of 70 % peat and 30 % perlite in seed-raising plastic trays of 28 cells (7.5 × 7.5 cm) and maintained in a greenhouse. When the seedlings reached 20–30 cm in height, the plants were transferred to the laboratory to initiate insect colonies and bioassays. Herbivore and parasitic wasp colonies were established using individuals collected from commercial tomato fields infested with *T. absoluta* in northeastern Spain (Maresme County) and refreshed annually. *Tuta absoluta* larvae were reared on caged tomato plants (47.5 cm × 47.5 cm × 47.5 cm; BugDorm-2; MegaView Science, Taiwan), where adult females and males of *D. gelechiidivoris* were then introduced. To obtain individuals for the experiments, leaflets containing parasitized *T. absoluta* larvae were maintained in aerated cages until parasitoids emerged.

2.2. Honeydew and plant guttation collection

We collected honeydew from two phloem feeders, *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) and *Bemisia tabaci* (Gennadius) (Hemiptera: Aleurodidae), typically found in greenhouse-grown tomato crops across the Mediterranean basin (Arnó et al., 2018; Lacasa and Contreras, 1995). Fresh honeydew was collected daily from colonies of *M. persicae* and colonies of *B. tabaci* reared on tomato plants under the same controlled conditions mentioned above. Colonies were kept in separate cages, ensuring that the plants were not co-infested. The density of both phloem feeders was high-enough to collect *ad libitum* honeydew for the experiments. The collection was realized by placing Parafilm® squares of 10 cm × 10 cm below the infested plants for 24 h. Samples of *M. persicae* and *B. tabaci* honeydew were stored in 15 cm diameter plastic Petri dishes, sealed with Parafilm®, at −20 °C until their use (Urbaneja-Bernat et al., 2023). Honeydew from each phloem feeder was provided on 1 cm^2 square pieces of Parafilm®, cut from the collected Parafilm® squares, and offered to the parasitic wasp during the experiments. Guttation droplets were collected daily early in the morning hours from the leaves of greenhouse-grown and pest-free tomato plants in IRTA, Cabrils. Plants were daily irrigated to maintain soil moisture and the occurrence of plant guttation. Droplets were collected using a single channel pipette of 200–1,000 μL volume range with sterile tip refills of 1,000 μL volume. Then, droplets were stored in 0.5 ml Eppendorf® tubes at −20 °C in Cryoboxes labeled by date until used. Both plant-derived food sources were collected from April to September 2023.

2.3. Effect of plant-derived food sources on D. gelechiidivoris fitness

To study the effect of plant-derived food sources on the longevity of female and male *D. gelechiidivoris*, six diets were tested on newly emerged parasitoids (< 24 h): (i) water-only, (ii) sugar-only (1 M sucrose), (iii) sugar (1 M sucrose) + superoxide dismutase (SOD) protein (Superoxide Dismutase bovine, SIGMA-ALDRICH, S9697-15KU) (7 μg/ml), (iv) *M. persicae* honeydew, v) *B. tabaci* honeydew, and vi) tomato plant guttation. The selection of sucrose as a positive control was based on previous studies that have evaluated the effect of honeydew on parasitic wasps (Tena et al., 2013; Urbaneja-Bernat et al., 2023). Plant expressed SODs have been found in the honeydews of phloem feeders. These defense-related proteins have shown before a positive impact on the fitness of a parasitoid used as a biological control agent (Urbaneja-Bernat et al., 2024c). Twenty newly emerged unfed parasitoids of each sex (20 males, and 20 females), per diet were placed individually in glass vials (7 cm height, 1.2 cm diameter), with the corresponding diet offered *ad libitum*. Water was provided in cotton balls that also sealed the vials. Although honeydew was provided *ad libitum* on 1 cm^2 Parafilm® pieces, the volume of this plant-derived food source was measured to analyze the carbohydrate and protein content (section 2.6). Subsequently, similar volumes of all diets were provided using a micropipette. Food sources and water were renewed daily. Survival was checked daily until the individuals died.

Next, to evaluate the effect of the six diets described above on the fecundity of *D. gelechiidivoris,* the egg load (the number of mature eggs in the abdomens of females) was counted. Fifteen unfed newly emerged females (< 24 h old) were collected and then killed immediately at −20 °C. To ensure mating, 75 individuals (2:1 female:male) were placed inside a transparent polypropylene plastic cup (946 ml; 114 mm diameter; 127 mm height; Paper Mart, CA, USA) and were provided *ad libitum* one of the six diets for 24 h. After 24 h, 15 of these *D. gelechiidivoris* females per diet were killed and maintained at −20 °C. Another 30 females per diet were individually placed in glass vials (7 cm height, 1.2

cm diameter) with the corresponding diet. Food and water were offered as in the longevity assay. Three and six days after emergence, 15 females per diet and day were killed and maintained at −20 °C. Therefore, we had females that were 0, 1, 3, and 6 days old. To count the eggs, all frozen females were dissected, and the number of mature eggs was recorded. All frozen females were dissected to count the eggs by placing them on a microscope slide with a drop of water under a coverslip. To expose the ovaries with mature eggs out of the abdomen, light pressure was applied to the thorax with a pair of pins. A total of 15 replicates were conducted for each day and per diet for this experiment.

2.4. Effect of plant-derived food sources on D. gelechiidivoris parasitism

To assess the impact of plant-derived food sources on the parasitism of *D. gelechiidivoris,* the six diets mentioned above were tested. Thirty newly emerged parasitoids (15 females and 15 males) per diet were collected and paired individually in aerated polypropylene 100 ml urine sample containers 2 h before the experiment. Each pair was provided with their respective diets *ad libitum*, and water was supplied via a wet cotton ball. The experimental setup consisted of a rearing cage (30 × 30 × 30 cm) with a single tomato plant (20-30 cm height) infested with 15 L_1larvae of *T. absoluta*. To ensure uniformity in larval stage, cohorts of *T. absoluta* larvae were used, following the insect rearing methodology described above. Tomato plants were infested with *T. absoluta* adults for 24 h, allowing for oviposition and ensuring that all larvae were at the L_1 stage at the time of the experiment. A pair of parasitoids, along with their diet offered *ad libitum* in a piece of Parafilm® (1 cm^2) and distilled water, was introduced into the cage. The parasitoids remained in the setup for 3 days under the specified climatic chamber conditions. Daily renewal of diet, water, and tomato plants was ensured, and parasitoid survival was recorded. A total of 15 replicates per diet were conducted for this assay. Additionally, there were 15 replicates with cages containing only *T. absoluta* larvae (control). Plants were maintained in a climatic chamber, and after 5 days, the number of alive and dead *T. absoluta* larvae found on each plant and treatment per day was counted. Alive *T. absoluta* larvae were introduced in a polypropylene 100 ml urine sample container for 24 h with water and sodium hypochlorite solution (1:49 mL). After 24 h, *T. absoluta* larvae were dissected under a stereomicroscope (ZEISS Stemi 508) to record the parasitized and non-parasitized larvae.

2.5. Effect of plant-derived food sources on D. gelechiidivoris sex ratio

To evaluate the effect of plant-derived food sources on the sex ratio (female proportion), 1–3-day-old female and male *D. gelechiidivoris* were introduced in the same container as in the parasitism bioassay with their corresponding diet *ad libitum* and water for 24 h to ensure mating. Females were then introduced into the same setup as in the parasitism bioassay to allow *T. absoluta* parasitization for 24 h with their corresponding diet and water. Before starting the experiment, alive *T. absoluta* larvae per plant were recorded (8.9 ± 0.11), with no significant differences between treatments. After 24 h, female parasitoids were removed from cages, and plants with *T. absoluta* larvae were kept under control conditions until *T. absoluta* or parasitoids emerged. Then, the *D. gelechiidivoris* sex ratio was recorded. A total of 15 replicates per diet were conducted for this experiment.

2.6. Carbohydrate and protein content in plant-derived food sources

The concentration of total sugars and proteins was estimated in the above-mentioned honeydew and guttation samples that were previously collected and measured (Section 2.2). The concentration of total sugars in the honeydew droplets and guttation was determined using a quantitative anthrone assay (Morse, 1947) with modifications. The volume of each droplet was estimated as (4/3 × π × r3) × 1/2, where r is the radius of the droplet. To analyze the honeydew, we processed six samples (biological replicates) of each honeydew studied. For *B. tabaci,* each sample contained 8977.7 ± 1601.6 (mean ± s.e.) drops, with a diameter of 0.04 ± 0.01 mm, a volume of 0.17 ± 0.31 μl. For *M. persicae*, each sample contained 61.1 ± 25.9 (mean ± s.e.) drops. Each, with a diameter of 0.21 ± 0.4 mm and a volume of 0.10 ± 0.01 μl. For analyzing the nutritional content of honeydew drops, samples were diluted by adding 150 μl of phosphate-buffered saline (8 mM Na_2HPO_4, 2 mM KH_2PO_4, and 150 mM NaCl (pH 7.4). However, further dilutions were made if needed, depending on the sugar concentrations. To determine total sugar and protein concentrations in the guttation, we used liquid collected directly from tomato plants. No dilution was needed. Ten microliters of each diluted sample were placed in a 96-well plate and mixed with 90 μl of anthrone reagent (1.5 mg ml^{-1} in 95 % sulfuric acid). The plate was stirred for 10 s and incubated for 2 h at 65 °C (hot anthrone test). Then, the absorbance at 620 nm was measured in a 96-well plate reader (Tecan Infinite M200Pro; Tecan Austria GmbH, Grödig, Austria). The concentration of total sugars was estimated using known concentrations of glucose as standards. The quantification of total proteins in the same six samples of honeydew and guttation was carried out by fluorimetry using a Qubit 3.0 fluorometer (Thermo Fisher, Hercules, CA, USA). One microliter of each sample was examined using a Qubit Protein Assay Kit (Thermo Fisher, Hercules, CA, USA) according to the manufacturer's recommendations.

2.7. Statistical analysis

Kaplan–Meier survival analysis, followed by a log-rank equality test and a complete pairwise analysis, was used to analyze differences among survival curves for the six different diets for each sex of the parasitoid species. Bonferroni correction was used to find statistical differences for each pair of treatments. Generalized linear models (GLMs) with a Poisson distribution and a log link function were applied to determine differences in the number of mature eggs for each diet. When statistically significant differences appeared in the GLM, the means were separated using estimated marginal means and adjusted with the Bonferroni correction for multiple comparisons. For the egg load on day 6, we performed a *t*-test. We used GLM with a Poisson distribution and a log link function to determine differences in the number of *T. absoluta* larvae parasitized per parasitic wasp. Means were separated using estimated marginal means and adjusted with the Bonferroni correction for multiple comparisons when statistically significant differences were found. GLM was also used to analyze the sex ratio, with an analysis of deviance, assuming binomial errors, and a logit link function. The number of female wasps was used as the response variable, and the total offspring number was used as the binomial denominator. An analysis of deviance was performed to assess the overall differences among the treatments. When statistically significant differences were found in the GLM, post hoc pairwise comparisons were conducted with a Bonferroni correction to control for multiple comparisons. All statistical analyses were conducted using R Studio software (R version 4.3.1) with a nominal significance of 5 % ($P < 0.05$).

3. Results

3.1. Effect of plant-derived food sources on D. gelechiidivoris fitness

The survival of *D. gelechiidivoris* females depended on the diet provided ($\chi^2 = 357.84$; $P < 0.001$) (Fig. 1a). *Dolichogenidea gelechiidivoris* females lived significantly longer when fed sucrose and sucrose + SOD (positive controls) compared to those fed honeydew of both phloem feeders, guttation and water-only diets. Furthermore, survival rates were higher in females fed on *B. tabaci* and *M. persicae* honeydew compared to those that fed on plant guttation, and water-only (negative control). Females fed on water and plant guttation had the lowest survival rates (Supplementary material, Table 1S). Similarly, access to sugary foods, including positive controls and both honeydews,

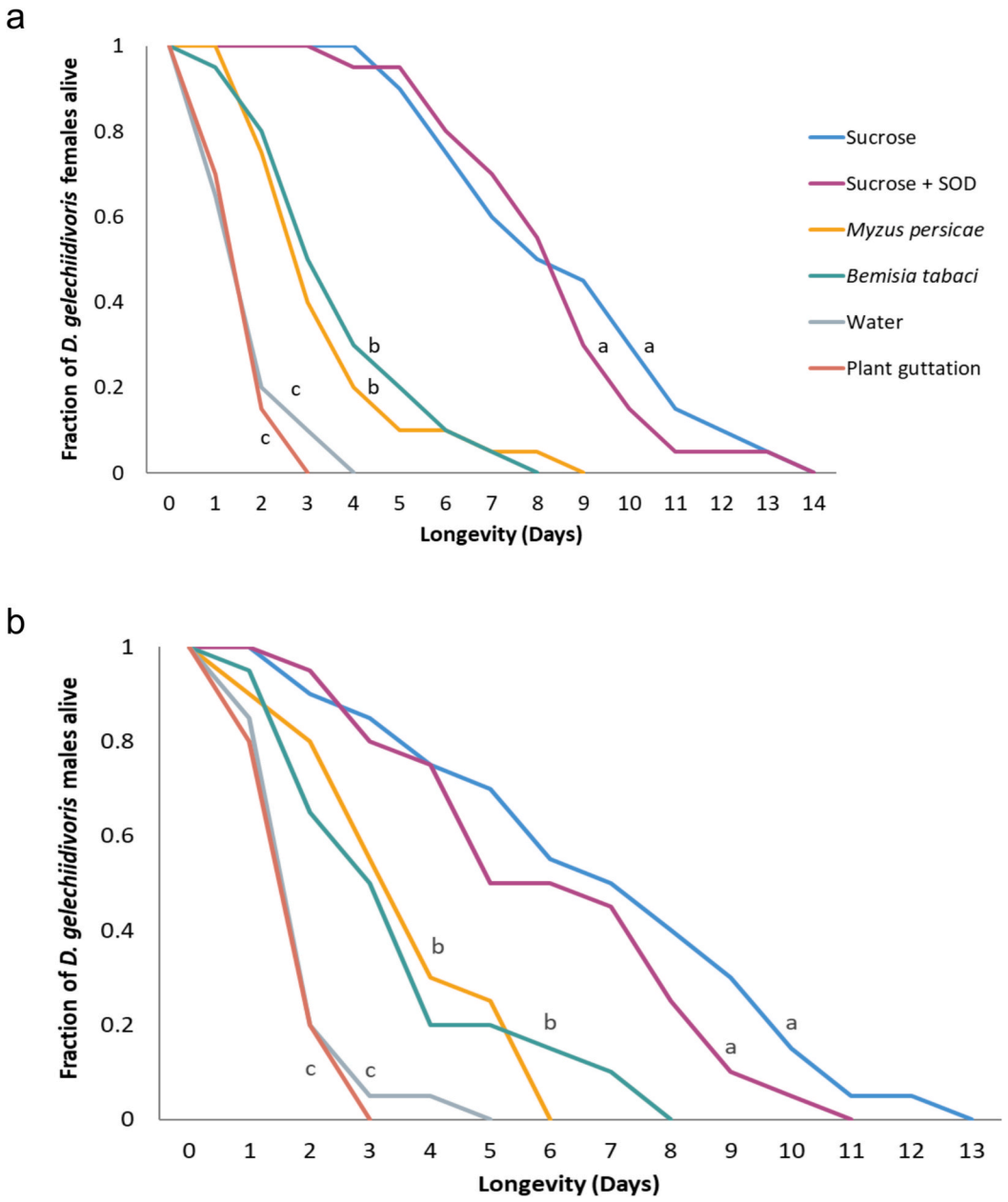


Fig. 1. Survival curves for *Dolichogenidea gelechiidivoris* females (a) and males (b) fed on six different diets: water, sugar-only (1 M sucrose), sugar (1 M sucrose) + superoxide dismutase (SOD) protein (7 μg/ml), *Myzus persicae* honeydew, *Bemisia tabaci* honeydew, and plant guttation. Different letters indicate significant differences among the treatments after pairwise comparison of the survival curves (Bonferroni correction was used to weigh the multiple comparisons done).

significantly increased the longevity of *D. gelechiidivoris* males (χ^2 = 254.22, $P < 0.05$) (Fig. 1b), but was lowest when fed on plant guttation and water-only diets.

Newly emerged females of *D. gelechiidivoris* (< 12 h) had an egg load of 55 ± 3 eggs per female. One day after their emergence, the egg load of *D. gelechiidivoris* females differed significantly depending on the diet (χ^2 = 179.67; *df* = 5, $P < 0.001$). *Dolichogenidea gelechiidivoris* females significantly increased their egg load when fed all diets compared to water-only (Fig. 2). Three days after parasitoid emergence, females fed only on water and guttation had already died, and those fed on sucrose maintained a significantly higher egg load compared to the sucrose + SOD diet (χ^2 = 9.01, $P < 0.05$), while females fed on both honeydew diets showed intermediate values. Finally, 6 days after their emergence, females fed on honeydews were not alive, and there were no significant differences in females' egg load between the two sugary control diets (*t* = −0.14, *df* = 27.09, $P = 0.89$) (Fig. 2).

3.2. Effect of plant-derived food sources on D. gelechiidivoris performance

Diet significantly affected the number of parasitized *T. absoluta* larvae provided (χ^2 = 71.92, *df* = 5, $P < 0.001$) (Fig. 3). *Dolichogenidea gelechiidivoris* females parasitized more *T. absoluta* larvae when fed with the sucrose + SOD diet than when they were fed on guttation (χ^2 = 63.10, $P < 0.001$). Both honeydew diets, along with sucrose and water, also led to a higher number of parasitized larvae compared to guttation. The presence of *D. gelechiidivoris* significantly increased the number of dead larvae compared to the control (χ^2 = 23.59, *df* = 6, $P < 0.001$). The sucrose + SOD diet yielded a higher number of dead larvae compared to water, the *M. persicae* honeydew diet, and the control, while the rest of the diets had intermediate values.

In the sex ratio experiment, the number of female offspring of *D. gelechiidivoris* was influenced by the diet provided. A higher number of female offspring was obtained when the parasitic wasp was fed

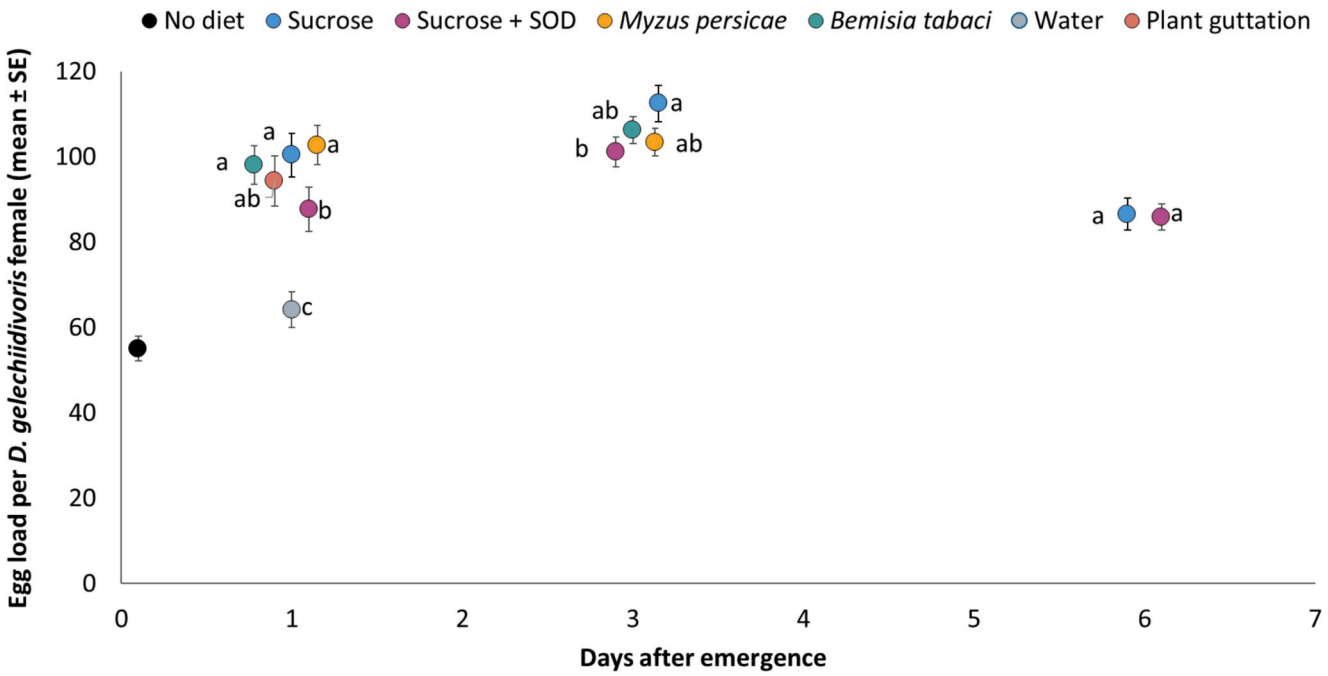


Fig. 2. Mean number (± SE) of mature eggs produced by newly emerged *D. gelechiidivoris* (< 24 h old) and those fed on the six different diets (water, sugar-only (1 M sucrose), sugar (1 M sucrose) + superoxide dismutase (SOD) protein (7 μg/ml), *M. persicae* honeydew, *B. tabaci* honeydew, and plant guttation) for 1, 3, and 6 days after emergence. Different letters indicate significant differences among treatments according to the Bonferroni pairwise tests for days 1 and 3. No significant differences in the mean number of mature eggs were found on day 6 (*t*-test $P < 0.05$).

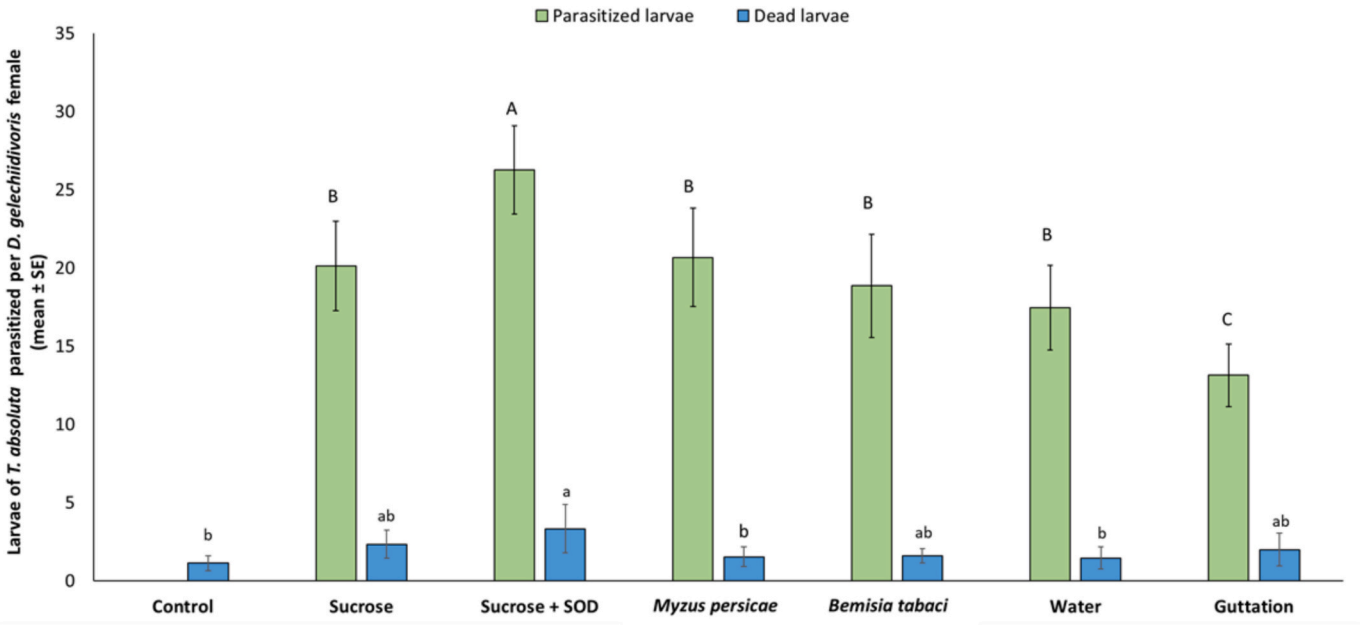


Fig. 3. Mean number of *T. absoluta* dead and parasitized larvae per *D. gelechiidivoris* female when fed on six different diets (water, sugar-only (1 M sucrose), sugar (1 M sucrose) + superoxide dismutase (SOD) protein (7 μg/ml), *M. persicae* honeydew, *B. tabaci* honeydew, and plant guttation). Different letters indicate significant differences among treatments in the mean number of parasitized and dead larvae, according to Bonferroni correction ($P < 0.05$).

sucrose ($\chi^2 = 11.05$, $P < 0.05$) and *B. tabaci* honeydew ($\chi^2 = 9.53$, $P < 0.05$) than water. Sucrose + SOD, *M. persicae* honeydew, and plant guttation had intermediate values that were not significantly different from water or each other (Table 1).

3.3. Carbohydrate and protein content in plant-derived food sources

The total concentrations of sugars and proteins per guttation droplet were estimated at 0.15 ± 0.2 mg ml^{-1} and 2.5 ± 0.5 μg/ml^{-1} (mean ± s.e.), respectively. For *B. tabaci* honeydew, the sugar content was 32.7 ± 7.5 mg/ml^{-1}, while the protein content was 5.1 ± 0.5 μg/ml^{-1}. Similarly, in *M. persicae* honeydew, sugar levels were estimated at 39.6 ± 6.4 mg/ml^{-1}, with a protein concentration of 8.9 ± 0.6 μg/ml^{-1} (Table 2).

Table 1
Sex ratio (mean ± SE) when fed on six different diets (water, sugar-only, sugar + (SOD) protein, *M. persicae* honeydew, *B. tabaci* honeydew, and plant guttation. Values in the same column for the sex ratio followed by the same letter are not significantly different (Bonferroni correction, $P < 0.05$).

Diet	*D. gelechiidivoris* sex ratio
Water	0.47 ± 0.11b
Plant guttation	0.49 ± 0.07 ab
Bemisia tabaci honeydew	0.74 ± 0.07 a
Myzus persicae honeydew	0.66 ± 0.08 ab
Sucrose	0.77 ± 0.05 a
Sucrose + SOD	0.69 ± 0.07 ab
Statistics	$\chi^2 = 21.84$, $df = 5$, $P < 0.001$

Table 2
Carbohydrate (mg/ml) and protein(μg/ml) concentrations of the diets used in the experiments.

Diet	Carbohydrates (mg/ml)	Proteins (μg/ml)
Plant guttation	0.15 ± 0.2	2.5 ± 0.5
Bemisia tabaci honeydew	32.7 ± 7.5	5.1 ± 0.5
Myzus persicae honeydew	39.6 ± 6.4	8.9 ± 0.6
Sucrose	342	–
Sucrose + SOD	342	7

4. Discussion

Our study demonstrates that honeydews produced by *B. tabaci* and *M. persicae* in tomato significantly enhance the fitness, parasitism, and female offspring of *D. gelechiidivoris*. In contrast, when this parasitoid feeds on tomato plant guttation, it does not confer any beneficial effects on biological parameters. Many studies have highlighted the positive impact of carbohydrate sources in honeydews on the biological parameters of parasitoids (Tena et al., 2016; Wäckers et al., 2005). These results are likely due to the significant differences in the sugar content between these food sources, as both honeydews contain more than 200 times the sugar content found in plant guttation. Our findings align with those of Urbaneja-Bernat et al. (2024b), highlighting the importance of rich carbohydrate food sources, such as *L. maritima* nectar, in enhancing the biological parameters of *D. gelechiidivoris*.

Honeydew might be the predominant sugar source in agricultural systems; nevertheless, it is more variable in quality than other plant-derived food sources (Tena et al., 2016; Wäckers et al., 2008). Our results demonstrate that *B. tabaci* and *M. persicae* honeydew enhanced the longevity of both female and male *D. gelechiidivoris* as well as the egg load compared to water. Although our study found no significant differences in protein content between honeydews and the sucrose + SOD diet, high sugar content, particularly sucrose, was key to sustaining the egg load three days after the emergence of parasitoids. Many koinobiont endoparasitoids lay hydropic eggs that are typically yolkless because they absorb host hemolymph proteins to complete embryogenesis (Ode et al., 2021). Therefore, additional dietary proteins might not significantly impact egg production. When we tested these food sources on the parasitism of *D. gelechiidivoris,* our results revealed that the percentage of parasitized *T. absoluta* larvae was similar to that of the water diet. According to a review by Lee (2024), while sugar feeding can enhance the longevity and fecundity of parasitoids, it does not always result in higher parasitism rates or improved biological control in the field. It is commonly known that the sex ratio of parasitoids can shift to produce a higher proportion of female offspring when female parasitoids are well fed (Benelli et al., 2017). In the sex ratio bioassay, *B. tabaci* honeydew and sucrose produced a higher number of female *D. gelechiidivoris* offspring compared to water. These findings are aligned with the study by Fuchsberg et al. (2007), in which the egg parasitoid *Trichogramma ostriniae* Pang & Chen (Hymenoptera: Trichogrammatidae) produced a higher proportion of females when parasitic wasps were fed with aphid honeydew in comparison to unfed individuals. Moreover, when *Pachycrepoideus vindemmiae* (Rondani) (Hymenoptera: Pteromalidae) females were fed honey, they produced more offspring, with a higher proportion of females. In the absence of this diet, parasitic wasps produced fewer offspring, and the proportion of male offspring increased, although it remained female-biased overall (Hu et al., 2012). Moreover, a biological tendency towards a lower proportion of female offspring was observed when parasitoids fed on tomato plant guttation compared to both honeydews (~0.2), suggesting the need for further research.

Then, although the presence of honeydew in the field is not desired because it serves as a substrate for sooty molds that can disrupt the photosynthetic capacity of plants, thus leading to reduced crop quality and yield, it is worth perceiving honeydew in agriculture not only as a challenge but also as an opportunity to improve pest management (Fernández de Bobadilla et al., 2024). Strategies such as promoting *B. tabaci* and *M. persicae* populations when they do not reach the economic threshold can be valuable tools in the integrated pest management of *T. absoluta.*

One of the hypotheses of our study focused on the potential positive effects of plant guttation. However, when evaluating the impact of tomato plant guttation on a natural enemy for the first time, we found that plant guttation serves as a poor-quality food source for *D. gelechiidivoris*. This is evidenced by reduced longevity, lower parasitism rates, and a higher proportion of male offspring when the parasitic wasp feeds on tomato plant guttation. A plausible explanation for this may lie in the lower sugar and protein content of plant guttation compared to honeydew, but also in the potential presence of secondary plant metabolites, such as alkaloids (Singh and Singh, 2013), which could inhibit feeding behavior or exert toxic effects on *D. gelechiidivoris*. Our findings differ from studies on plant guttation from other plants, such as highbush blueberries, where plant guttation was found to be a rich food source for beneficial insects and enhanced the longevity of three different insect taxa: an herbivore, a predator, and a parasitic wasp (Urbaneja-Bernat et al., 2020).

In conclusion, our study demonstrates that honeydew produced by *M. persicae* and *B. tabaci* positively influences the fitness and performance of *D. gelechiidivoris*, while tomato plant guttation does not provide similar benefits. On the contrary, our results indicate that tomato plant guttation does not enhance—and may even negatively affect—the biological parameters of this parasitic wasp. Given these findings, the presence of honeydew from these two phloem feeders may play a beneficial role in enhancing the biological control of *T. absoluta* by improving the fitness and performance of *D. gelechiidivoris*. Future research should further investigate the role of tomato plant guttation and its potential implications for other beneficial insects in tomato crops, such as mirid predatory bugs. Although our results suggest that tomato plant guttation has no positive effect on *D. gelechiidivoris*, reducing its parasitism, it is crucial to explore the specific mechanisms responsible for these effects. Identifying secondary compounds in plant guttation, such as alkaloids, that could detrimentally impact parasitoid biological traits is important. Additionally, evaluating different tomato varieties to assess variations in the nutritional composition of plant guttation will provide deeper insights into its broader influence on beneficial insects in tomato agricultural systems.

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CRediT authorship contribution statement

Angeliki Syropoulou: Writing – review & editing, Writing – original

draft, Visualization, Methodology, Investigation, Formal analysis, Data curation. **Joel González-Cabrera:** Writing – review & editing, Writing – original draft, Methodology, Formal analysis, Data curation. **Judit Arnó:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Funding acquisition, Conceptualization. **Pablo Urbaneja-Bernat:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Methodology, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biocontrol.2025.105719.

References

Aigbedion-Atalor, P.O., Mohamed, S.A., Hill, M.P., Zalucki, M.P., Azrag, A.G.A., Srinivasan, R., Ekesi, S., 2020. Host stage preference and performance of *Dolichogenidea gelechiidivoris* (Hymenoptera: Braconidae), a candidate for classical biological control of *Tuta absoluta* in Africa. Biol. Control 144, 104215. https://doi.org/10.1016/j.biocontrol.2020.104215.

Arnó, J., Sorribas, R., Prat, M., Matas, M., Pozo, C., Rodríguez, D., Garreta, A., Gómez, A., Gabarra, R., 2009. *Tuta absoluta*, a new pest in IPM tomatoes in the northeast of Spain. IOBC/WPRS Bull. 49, 203–208.

Arnó, J., Castañé, C., Alomar, O., Riudavets, J., Agustí, N., Gabarra, R., Albajes, R., 2018. Forty years of biological control in Mediterranean tomato greenhouses: the story of success. Isr. J. Entomol. 48, 20–29. https://doi.org/10.5281/zenodo.1486574.

Benelli, G., Giunti, G., Tena, A., Desneux, N., Caselli, A., Canale, A., 2017. The impact of adult diet on parasitoid reproductive performance. J. Pest. Sci. 90, 807–823. https://doi.org/10.1007/s10340-017-0835-2.

Biondi, A., Narciso, R., Guedes, C., Wan, F.H., Desneux, N., 2018. Ecology, worldwide spread, and management of the invasive South American tomato pinworm, *Tuta absoluta*: Past, present, and future. Annu. Rev. Entomol. 63, 239–258. https://doi.org/10.1146/annurev-ento-031616.

Calvo, F.J., Soriano, J.D., Stansly, P.A., Belda, J.E., 2016. Can the parasitoid *Necremnus tutae* (Hymenoptera: Eulophidae) improve existing biological control of the tomato leafminer *Tuta aboluta* (Lepidoptera: Gelechiidae)? Bull. Entomol. Res. 106, 502–511. https://doi.org/10.1017/S0007485316000183.

Chang, P.E.C., Metz, M.A., 2021. Classification of *Tuta absoluta* (Meyrick, 1917) (Lepidoptera: Gelechiidae: Gelechiinae: Gnorimoschemini) based on cladistic analysis of morphology. Proc. Entomol. Soc. Washingt. 123, 41–54. https://doi.org/10.4289/0013-8797.123.1.41.

Denis, C., Riudavets, J., Alomar, O., Agustí, N., Gonzalez-Valero, H., Cubí, M., Matas, M., Rodríguez, D., van Achterberg, K., Arnó, J., 2022. Naturalized *Dolichogenidea gelechiidivoris* complement the resident parasitoid complex of *Tuta absoluta* in Northeastern Spain. J. Appl. Entomol. 146, 461–464. https://doi.org/10.1111/jen.12994.

Desneux, N., Han, P., Mansour, R., Arnó, J., Brévault, T., Campos, M.R., Chailleux, A., Guedes, R.N.C., Karimi, J., Konan, K.A.J., Lavoir, A. violette, Luna, M.G., Perez-Hedo, M., Urbaneja, A., Verheggen, F.J., Zappalà, L., Abbes, K., Ali, A., Bayram, Y., Cantor, F., Cuthbertson, A.G.S., De Vis, R., Erler, F., Firake, D.M., Haddi, K., Hajjar, M.J., Ismoilov, K., Jaworski, C.C., Kenis, M., Liu, H. tian, Madadi, H., Martin, T., Mazih, A., J. Messelink, G., Mohamed, S.A., Nofemela, R.S., Oke, A., Ramos, C., Ricupero, M., Roditakis, E., Shashank, P.R., Wan, F.H., Wang, M. hui, Wang, S., Zhang, Y.B., Biondi, A., 2022. Integrated pest management of *Tuta absoluta*: practical implementations across different world regions. J Pest Sci (2004). https://doi.org/10.1007/s10340-021-01442-8.

Desneux, N., Wajnberg, E., Wyckhuys, K.A.G., Burgio, G., Arpaia, S., Narváez-Vasquez, C. A., González-Cabrera, J., Ruescas, D.C., Tabone, E., Frandon, J., Pizzol, J., Poncet, C., Cabello, T., Urbaneja, A., 2010. Biological invasion of European tomato crops by *Tuta absoluta*: Ecology, geographic expansion and prospects for biological control. J. Pest. Sci. 83, 197–215. https://doi.org/10.1007/s10340-010-0321-6.

Fernández de Bobadilla, M., Ramírez, N.M., Calvo-Agudo, M., Dicke, M., Tena, A., 2024. Honeydew management to promote biological control. Curr. Opin. Insect Sci. 61, 101151. https://doi.org/10.1016/j.cois.2023.101151.

Fuchsberg, J.R., Yong, T.-H., Losey, J.E., Carter, M.E., Hoffmann, M.P., 2007. Evaluation of corn leaf aphid (*Rhopalosiphum maidis*; Homoptera: Aphididae) honeydew as a food source for the egg parasitoid *Trichogramma ostriniae* (Hymenoptera: Trichogrammatidae). Biol. Control 40, 230–236. https://doi.org/10.1016/j.biocontrol.2006.10.009.

Gabarra, R., Arnó, J., Lara, L., Verdú, M.J., Ribes, A., Beitia, F., Urbaneja, A., Téllez, M. del M., Mollá, O., Riudavets, J., 2014. Native parasitoids associated with *Tuta absoluta* in the tomato production areas of the Spanish Mediterranean Coast. BioControl. 59, 45–54. Doi: 10.1007/s10526-013-9545-8.

Gebiola, M., Bernardo, U., Ribes, A., Gibson, G.A.P., 2015. An integrative study of *Necremnus* Thomson (Hymenoptera: Eulophidae) associated with invasive pests in Europe and North America: taxonomic and ecological implications. Zool. J. Linn. Soc. 173, 352–423. https://doi.org/10.1111/zoj.12210.

Heil, M., 2015. Extrafloral nectar at the plant-insect interface: A spotlight on chemical ecology, phenotypic plasticity, and food webs. Annu. Rev. Entomol. 60, 213–232. https://doi.org/10.1146/annurev-ento-010814-020753.

Hu, H.Y., Chen, Z.Z., Duan, B.S., Zheng, J.T., Zhang, T.X., 2012. Effects of female diet and age on offspring sex ratio of the solitary parasitoid *Pachycrepoideus vindemmiae* (Rondani) (Hymenoptera, Pteromalidae). Rev. Bras. Entomol. 56, 259–262. https://doi.org/10.1590/S0085-56262012005000028.

Krache, F., Boualem, M., Fernandez-Triana, J., Bass, A., Arnó, J., Benourad, F., 2021. First record in Africa of the parasitoid *Dolichogenidea gelechiidivoris* (Hymenoptera: Braconidae) on tomato leafminer *Tuta absoluta* (Lepidoptera: Gelechiidae) from tomato fields in Algeria. J. Hymenopt. Res. 88, 115–131. https://doi.org/10.3897/jhr.88.75279.

Lacasa, A., Contreras, J., 1995. Las plagas, in: Nuez, F. (Ed.), El Cultivo del Tomate. Mundi-Prensa, Madrid, pp. 385–467.

Lee, J.C., 2024. Flourishing with sugars - following the fate of parasitoids in the field. Curr. Opin. Insect Sci. 61, 101158. https://doi.org/10.1016/j.cois.2023.101158.

Mama Sambo, S., Ndlela, S., du Plessis, H., Obala, F., Mohamed, S.A., 2022. Ratio dependence effects of the parasitoid *Dolichogenidea gelechiidivoris* on its associated host *Tuta absoluta*. Biocontrol Sci. Tech. 32, 497–510. https://doi.org/10.1080/09583157.2022.2033702.

Morse, E.E., 1947. Anthrone in estimating low concentrations of sucrose. Anal. Chem. 19, 1012–1013. https://doi.org/10.1021/ac60012a021.

Ode, P.J., Vyas, D.K., Harvey, J.A., 2021. Extrinsic inter-and intraspecific competition in parasitoid wasps. Annu. Rev. Entomol. 67, 305–328. https://doi.org/10.1146/annurev-ento-071421.

Salas Gervassio, N.G., Aquino, D., Vallina, C., Biondi, A., Luna, M.G., 2019. A re-examination of *Tuta absoluta* parasitoids in South America for optimized biological control. J. Pest. Sci. 92, 1343–1357. https://doi.org/10.1007/s10340-018-01078-1.

Singh, S., 2020. Guttation: Fundamentals and Applications. Cambridge University Press.

Singh, S., Singh, T.N., 2013. Guttation 1: Chemistry, crop husbandry and molecular farming. Phytochem. Rev. 12, 147–172. https://doi.org/10.1007/s11101-012-9269-x.

Tena, A., Llácer, E., Urbaneja, A., 2013. Biological control of a non-honeydew producer mediated by a distinct hierarchy of honeydew quality. Biol. Control 67, 117–122. https://doi.org/10.1016/j.biocontrol.2013.07.018.

Tena, A., Wäckers, F.L., Heimpel, G.E., Urbaneja, A., Pekas, A., 2016. Parasitoid nutritional ecology in a community context: the importance of honeydew and implications for biological control. Curr. Opin. Insect Sci. 14, 100–104. https://doi.org/10.1016/j.cois.2016.02.008.

Urbaneja, A., Montón, H., Mollá, O., 2009. Suitability of the tomato borer *Tuta absoluta* as prey for *Macrolophus pygmaeus* and *Nesidiocoris tenuis*. J. Appl. Entomol. 133, 292–296. https://doi.org/10.1111/j.1439-0418.2008.01319.x.

Urbaneja, A., Vercher, R., Navarro, V., García Marí, F., Porcuna, J.L., 2007. La polilla del tomate. *Tuta Absoluta*. Phytoma España. 194, 16–23.

Urbaneja-Bernat, P., González-Cabrera, J., Hernández-Suárez, E., Tena, A., 2023. Honeydew of HLB vector, *Trioza erytreae*, increases longevity, egg load and parasitism of its main parasitoid *Tamarixia dryi*. Biol. Control 179, 105169. https://doi.org/10.1016/j.biocontrol.2023.105169.

Urbaneja-Bernat, P., Tena, A., González-Cabrera, J., Rodriguez-Saona, C., 2024a. An insect's energy bar: The potential role of plant guttation on biological control. Curr. Opin. Insect Sci. 61, 101140. https://doi.org/10.1016/j.cois.2023.101140.

Urbaneja-Bernat, P., Riudavets, J., Denis, C., Ojeda, J., Alomar, O., Arnó, J., 2024b. *Lobularia maritima* as a nutrient-rich floral food source for two parasitoid wasps of *Tuta absoluta*. Entomol. Gen. 44, 339–346. https://doi.org/10.1127/entomologia/2024/2299.

Urbaneja-Bernat, P., Rodriguez-Saona, C., Valero, M.L., González-Cabrera, J., Tena, A., 2024c. Not just candy: a herbivore-induced defence-related plant protein in honeydew enhances natural enemy fitness. Funct. Ecol. 38, 1822–1834. https://doi.org/10.1111/1365-2435.14605.

Urbaneja-Bernat, P., Tena, A., González-Cabrera, J., Rodriguez-Saona, C., 2020. Plant guttation provides nutrient-rich food for insects. Proc. R. Soc. B Biol. Sci. 28. https://doi.org/10.1098/rspb.2020.1080.

van Lenteren, J.C., Lanzoni, A., Hemerik, L., Bueno, V.H.P., Bajonero Cuervo, J.G., Biondi, A., Burgio, G., Calvo, F.J., de Jong, P.W., López, S.N., Luna, M.G., Montes, F. C., Nieves, E.L., Aigbedion-Atalor, P.O., Riquelme Virgala, M.B., Sánchez, N.E., Urbaneja, A., 2021. The pest kill rate of thirteen natural enemies as aggregate evaluation criterion of their biological control potential of *Tuta absoluta*. Sci. Rep. 11, 10756. https://doi.org/10.1038/s41598-021-90034-8.

Wäckers, F., Rinj, J., Bruin, J., 2005. Plant-provided food for carnivorous insects: a protective mutualism and its applications, Cambridge. ed. Cambridge Univ. Press. Cambridge, UK.

Wäckers, F.L., van Rijn, P.C.J., Heimpel, G.E., 2008. Honeydew as a food source for natural enemies: making the best of a bad meal? Biol. Control 45, 176–184. https://doi.org/10.1016/j.biocontrol.2008.01.007.

Zappalà, L., Biondi, A., Alma, A., Al-Jboory, I.J., Arnò, J., Bayram, A., Chailleux, A., El-Arnaouty, A., Gerling, D., Guenaoui, Y., Shaltiel-Harpaz, L., Siscaro, G., Stavrinides, M., Tavella, L., Vercher Aznar, R., Urbaneja, A., Desneux, N., 2013. Natural enemies of the South American moth, *Tuta absoluta*, in Europe, North Africa and Middle East, and their potential use in pest control strategies. J Pest Sci (2004) 86, 635–647. https://doi.org/10.1007/s10340-013-0531-9.