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## **Reduced phytophagy in sugar provisioned mirids**

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## **Abstract**

Zoophytophagous mirids (Hemiptera: Miridae) are one of the most studied and successful group of natural enemies used as biological control agents in horticultural crops. When prey is scarce, some species, such as *Nesidiocoris tenuis* (Reuter) may damage plant tissue by increasing feeding on vegetative and reproductive organs. Despite the importance of mirids, the provision of a sugar source as an alternative or complement to plant feeding has never been addressed to reduce mirid phytophagy. Here we analyzed the nutritional status and phytophagy of *N. tenuis* in the presence of tomato plants with and without sugar dispensers. Our study demonstrated how nymphs and adults obtained carbohydrates when fed on tomato plants. Phytophagy was reduced more than two-fold with the provision of sugar dispensers. Both nymphs and adults contained higher carbohydrate levels when they had access to plants with sugar dispensers than without. Nymphs, which are generally responsible for more serious plant damage, had a higher content of carbohydrates than adults independently of the diet provided. Our findings contribute not only to improve the use of zoophytophagous predators as biological control agents, but also to understand the nutritional ecology of the Miridae, a group with a very diverse diet.

**Keywords:** *Nesidiocoris tenuis*; nutritional ecology; biological control; tomato; artificial food.

## **Key Message**

- *Nesidiocoris tenuis* causes damage when it punctures tomato plants to obtain carbohydrates.
- Provision of artificial sugars reduced its phytophagy and increased the carbohydrate content of *N. tenuis* nymphs and adults.
- Our results provide useful information for using zoophytophagous predators questioned by their phytophagy.

## INTRODUCTION

Conservation biological control is increasingly important as a biological control strategy (Gurr et al. 2017; Landis et al. 2000; Tena et al. 2016). The majority of predators and parasitoids are facultative or obligate omnivores as they commonly feed on plant-derived food sources like pollen and nectar (Wäckers et al. 2005). Hence, facilitating the access to these rich natural carbohydrate sources, as well as to artificial sugar supplements, could improve natural enemy nutrition and its life history traits, such as: longevity, fecundity, and/or foraging behavior (Wade et al. 2008). However, the efficacy of carbohydrate provisioning in biological control programs has been limited and inconsistent (Heimpel and Jervis 2005; Wade et al. 2008). Miridae (Hemiptera: Miridae) is one of the most commonly used group of natural enemies in biological control programs in horticultural crops (Bueno et al. 2013; Pérez-Hedo and Urbaneja 2015). The success of this zoophytophagous group lies in the adaptability to feed not only on prey and nectar but also on plant tissue throughout all of the developmental stages (Naselli et al. 2016; Naranjo and Gibson 1996; Coll 1998). This trait facilitates the establishment of zoophytophagous predators and their conservation in periods of prey deficiency. Moreover, the use of alternative carbohydrate sources for these predators has been largely neglected, until recently (Portillo et al. 2012; Put et al. 2012; Urbaneja-Bernat et al. 2015; Biondi et al. 2016).

The nutritional requirements of mirids as well as the type and amount of nutrients obtained when feeding on plant tissues have not yet been investigated in detail. It has been suggested that mirids feed on plant tissue to obtain only the necessary water for vital functions (Cohen 1990; Gillespie and McGregor 2000). However, *N. tenuis* uses a lacerate-and-flush feeding strategy to reach phloem tissue which is rich in carbohydrates. This feeding behavior, typical of *N. tenuis*, results in the hypertrophy and hyperplasy of cells surrounding the phloem that produce characteristic necrotic rings at feeding sites (Raman and Sanjayan 1984; Raman et al. 1984; Sanchez et al. 2016). Furthermore, the action of the salivary enzymes on the tissue and the response reaction of the plant (toxemia) produce this damage on stems and leaves (Arnó et al. 2010; Castañé et al. 2011).

For this reason, the main problem of zoophytophagous mirids in agriculture is that some species can damage the plant when they feed on vegetative and reproductive organs (Calvo et al. 2009; Pérez-Hedo and Urbaneja 2015, 2016; Sanchez 2008). This damage is economically significant and considered to be the most important constraint which circumvents the selection of mirid species in biological control programs (Bueno et al. 2013). Mirid damage usually occurs at the end of a crop cycle when they are abundant and prey is scarce. Under these conditions mirids feed on plant tissue to obtain nutrients (Sanchez 2008, 2009; Sanchez and Lacasa 2008; Calvo et al. 2009; Arnó et al. 2010). This observation suggests that the presence of additional food in the crop might reduce the damage caused by zoophytophagous mirids. In fact, mirids, as other predators, increase their fitness when feeding on nectar and artificial sugars (Portillo et al. 2012; Urbaneja-Bernat et al. 2013). However, these studies did not elucidate whether this sugar provisioning contributes to cover the carbohydrate requirements of mirids and decrease their phytophagy and, consequently, the damage caused when prey is scarce.

In this research we chose the zoophytophagous mirid *Nesidiocoris tenuis* (Reuter) (Hemiptera: Miridae), one of the most successful natural enemies to control pests in tomato crops in southern Europe, which is also used worldwide (Calvo et al. 2012; Bueno et al. 2013; Pérez-Hedo and Urbaneja 2016; Pérez-Hedo et al. 2017). *Nesidiocoris tenuis* is a polyphagous predator commonly present in natural vegetation and horticultural crops of the Mediterranean basin (Pérez-Hedo and Urbaneja 2016). This mirid lays its eggs inside plant tissue, mainly in tender flushes and leaves. Nymphs and adults feed on eggs and young larvae of the tomato moth *Tuta absoluta* (Meyrick) (Lepidoptera: Gelichiidae) (Urbaneja et al. 2009), on nymphs of the whitefly *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) (Calvo et al. 2009) as well as on several aphid species (Pérez-Hedo and Urbaneja 2015). In tomato greenhouses, *N. tenuis* is released at the beginning of the crop cycle to regulate populations of different pests, thus increasing the resilience of the crop (González-Cabrera et al. 2011; Mollá et al. 2011; Calvo et al. 2012; Urbaneja et al. 2012; Pérez-Hedo et al. 2015; 2017). However, *N. tenuis* nymphs and adults feed on tomato plants producing necrotic rings in vegetative structures as well as flower abortion thereby reducing

tomato production (Sanchez 2008; Sanchez and Lacasa 2008; Calvo et al. 2009; Arnó et al. 2010; Castañé et al. 2011). Apart from feeding on prey and plant tissue *N. tenuis* also feeds on rich sugar sources such as nectar, pollen, and honeydew excreted by hemipterans in the field (personal observation) as well as on 0.5 M sucrose in laboratory and field experiments (Urbaneja-Bernat et al. 2013, 2015). In these experiments the addition of artificial sugars upon releasing improved *N. tenuis* establishment in the crop (Urbaneja-Bernat et al. 2015).

The first aim of this study was to describe and compare the carbohydrate content of nymphs and adult females of *N. tenuis* with and without access to plants. The second aim was to determine if sugar provisioning improves the carbohydrate content of *N. tenuis* and reduces its phytophagy.

## **MATERIAL AND METHODS**

### **Plants and insects**

Pesticide-free tomato plants (30 cm high) variety “Optima” (Seminis Vegetable Seeds, Inc., Almería, Spain) were used in all the trials. The plants were irrigated every two days. *Nesidiocoris tenuis* adults were obtained from a commercial supplier (NESIBUG®; Koppert Biological Systems, S.L., Águilas, Murcia, Spain). Each bottle contained approximately 500 specimens of less than 3-day-old individuals (F.J. Calvo, Koppert BS; Personal Communication). For assays with adults, we used four pairs of *N. tenuis* obtained directly from the bottle. To obtain newly emerged third-instar nymphs, around 100 individuals were isolated in 40 x 25 x 25 cm methyl acrylate cages with green bean pods to lay eggs in and feed on. They were also provided *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) eggs, *ad libitum*, as additional food. The cages were placed in a climate controlled chamber ( $25 \pm 1$  °C,  $60 \pm 5$  % RH, 16:8 h L:D) for one day. After the 24 hour period, *N. tenuis* adults were removed and green bean pods were checked for oviposition scars. Green bean pods with eggs were placed in 30 x 10 x 10 cm plastic cages. Nymphs were fed with green beans and *E. kuehniella* eggs until they reached the third instar (N3); 14 days from egg to N3.

### ***Effect of sugar addition on the carbohydrate contents of *N. tenuis****

In this assay we first evaluated whether *N. tenuis* nymphs and adults obtain carbohydrates when they puncture tomato plants; it has been suggested that *N. tenuis* feeds on plants to primarily obtain water (Gillespie and McGregor 2000), but also carbohydrates as phloem feeders (Sanchez et al. 2016). Next, we tested whether *N. tenuis* nymphs and adults are able to find and feed on artificial sugar dispensers in the presence and absence of tomato plants. Finally, we evaluated whether or not the provision of artificial sugar dispensers increases the carbohydrate levels of both life stages on tomato plants. Dispensers consisted of 1.5 ml centrifuge tubes filled with water or 0.5 M sucrose (catalog number: S0389; Sigma-Aldrich, UK) sealed with cotton. To test these hypotheses, carbohydrate levels in *N. tenuis* adults and nymphs were compared when they had access to: i) only water dispensers vs tomato plants and water dispensers; ii) water dispensers vs sugar dispensers; and iii) tomato plants and water dispensers vs tomato plants and sugar dispensers. Each replicate entailed a 60 x 60 x 60 cm plastic cage (ventilated BugDorm-2; MegaView Science Co., Ltd.; Taichung, Taiwan) with one tomato plant (30 cm high) inside an 8 x 8 x 8 cm plastic pot or one wooden stick (30 cm high). On each plant or stick, we placed three dispensers with either water or 0.5 M sucrose, depending on the treatment, as explained above. Dispensers were provided in the apical part of the plant, the preferred plant feeding part of *N. tenuis* (Castañé et al. 2011; Mollá et al. 2011, 2014; Calvo et al. 2012; Pérez-Hedo et al. 2015), and were replaced on the second day. Twelve, less than 3-day-old, adult *N. tenuis* females per plant were released in each Bugdorm cage. Three days later females were collected with a manual aspirator, introduced individually in microcentrifuge tubes (1.5 ml) and frozen in liquid nitrogen. Individuals were labeled and kept frozen (-80°C) until used. The same procedure was followed to collect and preserve nymphs (N3). Since some individuals died during the assay, mostly in the “without tomato plant and with water” treatment, or were lost during the analytical process, the final number of individuals and replicates used in the analysis is provided in Table 1.

### ***Measurement of carbohydrate levels of *N. tenuis****

The tubes containing the insects were vortexed for 10 sec in 1 mL warm (60°C) autoclaved deionized water to remove carbohydrates from the outside of the insect body. Finally, the mirid samples were transferred to new tubes and kept on ice.

Carbohydrate levels, in particular fructose, total sugars and glycogen were measured using a quantitative anthrone assay (modified from Olson et al. 2000; Lee et al. 2004; Wyckhuys et al. 2008; Foray et al. 2012). Each mirid was homogenized in 5 µl 2 % (w/v) sodium sulfate using autoclaved plastic pestles. Pestles were rinsed with 45 µL of methanol:chloroform (2:1), which was added to the homogenate. The homogenate was centrifuged for 2 min at 13,000 rpm and the supernatant, containing all soluble sugars, was transferred to a 100 ml glass test tube. The white pellet, containing insoluble high molecular weight sugars (e.g. glycogen), was kept on ice until used to determine glycogen level.

A cold anthrone test was used to determine the fructose content. Each glass tube with the homogenate was incubated at 90°C for approximately 1.5 min in a dry bath incubator until all liquid had evaporated. Next, the tubes were cooled on ice for 15 min after which 100µL of anthrone reagent (1.42 mg/ml anthrone in 70 % sulfuric acid) was added. The tubes were vortexed for 10 sec and incubated for 1.5 hours at room temperature. The absorbance at 620 nm was measured in a 96-well plate reader (Biochrom® Anthos 2010, Biochrom, UK). To determine the total sugar content the samples were incubated at 65°C for 2 hours (hot anthrone test) and the absorbance was recorded at 620 nm.

The white pellet containing insoluble high molecular weight sugars (glycogen) was suspended (vortexing) in 200 µL of anthrone reagent. Part of the mixture (100 µL) was transferred to a new 1.5 mL centrifuge tube, avoiding floating insect appendages, and incubated at 90°C for 3 min. Next, the tubes were cooled on ice for 15 min and the absorbance read as above at 620 nm. The absorbance of all three assays were compared to absorbance values of known fructose, sucrose [0.001, 0.0025, 0.005, 0.01, 0.05, 0.1 and 0.25 µg/µL (in 25% ethanol (v/v))] and glucose standards [0.01, 0.025, 0.05, 0.075, 0.1, 0.25 and 0.3 µg/µL (in distilled water)] with two replicates per read.

Here, we refer to the cold anthrone reading as fructose. Similarly, it has been assumed that if the amount of fructose detected by the cold anthrone test multiplied by two is subtracted from the sugars detected by the hot anthrone test it represents the body sugar levels of the insect (Olson et al. 2001). Thus, we prefer to refer to it as total sugars. Finally, we calculated the total amount of carbohydrates by summing the amount of sugars obtained with the hot anthrone test and the glycogen. Therefore, we had four measurements to describe the nutritional state of *N. tenuis*: fructose, total sugar, glycogen, and total carbohydrates. To adjust the carbohydrate levels for mirid size, the carbohydrate concentrations were expressed relative to the hind tibia length of the mirid (Gillespie and Mcgregor 2000; Castañé and Zapata 2005; Urbaneja-Bernat et al. 2013).

### ***Effect of sugar addition on N. tenuis phytophagy and fecundity***

This experiment was conducted in a glasshouse located at IVIA (Insituto Valenciano de Investigaciones Agrarias), Moncada, Spain, under controlled climatic conditions (23-27 °C, 65 ± 10 % RH and the natural May photoperiod). Three treatments: i) control (plant only), ii) three water dispensers per plant and iii) three sugar dispensers per plant, were compared to determine whether the provision of water or sucrose decreases the phytophagy of *N. tenuis* nymphs and adults. Each replicate entailed a plastic cage 60 x 60 x 60 cm (same ventilated BugDorms as above) with four tomato plants (30 cm high) in individual 8 x 8 x 8 cm pots. Each treatment was replicated 10 times (total of 40 plants). We used the same dispensers as above. They were placed at the apical part of the plant and replaced every two days.

Using a destructive protocol we evaluated the phytophagy of *N. tenuis* adults at three and seven days after releasing four pairs into the cages. On the third day two plants per replicate (total of 20 plants) were transported to the lab after ensuring the absence of *N. tenuis*. The number of necrotic rings was counted with a magnifying glass as a measure of phytophagy. The plants were cut at ground level and placed individually in ventilated Petri dishes in a climate controlled chamber (25 ± 1 °C, 60 ± 5 % RH, 16:8 h L:D) to evaluate the fecundity of the females ten days later. This protocol was repeated again four days later (one week after releasing *N. tenuis*) with the remaining

plants. The interval of seven days was selected to avoid the emergence of new nymphs under the conditions of the assay (Sanchez et al. 2009). We evaluated the phytophagy of *N. tenuis* nymphs using the same procedure as above, except in this assay eight N3 *N. tenuis* were released per plant.

### ***Statistical analysis***

The amount of the different carbohydrates was compared using ANOVA with treatment as explanatory factor and plant (replicate) as a random factor using the function LME from the LME4 package for R. The normality assumption was assessed using Shapiro's test, and the homoscedasticity assumption was assessed with Levene's test. We applied generalized linear modeling (GLM) techniques with a logit link function assuming Poisson error variance for the count variables, including damage and offspring, and treatment as the explanatory variable. Initially, we assumed a Poisson error variance. When an over or underdispersion was detected, we re-evaluated the significance of the explanatory variables using an *F*-test after rescaling the statistical model by a Pearson's chi-square divided by the residual degrees of freedom (Crawley 2007). Moreover, Bonferroni's post hoc tests were performed using the function GLHT from the MULTCOMP package for R. All data analyses were performed with the R free-ware statistical package (<http://www.R-project.org/>).

## **RESULTS**

### ***Carbohydrate levels of *N. tenuis* with access to water dispensers with and without tomato plants***

When *N. tenuis* nymphs had access to water dispensers, they contained a significantly higher level of carbohydrates in microcosms with tomato plants than without ( $t = 2.67$ ;  $df = 27$ ;  $P = 0.013$ ) (Table 2; columns of water dispensers). Among the different carbohydrates, nymphs contained higher levels of total sugars ( $t = 3.03$ ;  $df = 27$ ;  $P = 0.005$ ) and glycogen ( $t = 2.35$ ;  $df = 27$ ;  $P = 0.027$ ) in microcosms with tomato plants than without (Table 2; columns of water dispensers). The statistical analysis of the content of fructose in nymphs was not carried out because individuals without plants did not contain fructose (Table 2; columns of water dispensers).

Similarly, *N. tenuis* adult females contained a significantly higher level of total carbohydrates in microcosms with tomato plants than without ( $t = 2.12$ ;  $df = 29$ ;  $P = 0.043$ ) (Table 3; columns of water dispensers). Among carbohydrates, nymphs contained higher levels of total sugars ( $t = 2.32$ ;  $df = 29$ ;  $P = 0.027$ ), fructose ( $t = 2.12$ ;  $df = 29$ ;  $P = 0.042$ ) and glycogen ( $t = 2.19$ ;  $df = 29$ ;  $P = 0.037$ ) in microcosms with tomato plants than without (Table 3; columns of water dispensers).

#### ***Carbohydrate levels of *N. tenuis* with access to water dispensers or artificial sugar dispensers***

When *N. tenuis* nymphs did not have access to tomato plants, they contained a significantly higher level of total carbohydrates in microcosms with artificial sugar dispensers than in those with water dispensers ( $t = 3.6$ ;  $df = 26$ ;  $P = 0.0014$ ) (Table 2, grey columns). Among the different carbohydrates, nymphs contained higher level of total sugars ( $t = 4.57$ ;  $df = 26$ ;  $P < 0.0001$ ) and glycogen ( $t = 2.93$ ;  $df = 26$ ;  $P = 0.007$ ) when they had access to artificial sugar dispensers than to water dispensers (Table 2, grey columns). Statistical analysis was not carried out on fructose concentrations because individuals without access to plants did not contain fructose (Table 2, grey columns).

Similarly, when *N. tenuis* adult females did not have access to tomato plants, they contained a significantly higher level of total carbohydrates in microcosms with artificial sugar dispensers than in those with water dispensers ( $t = 2.87$ ;  $df = 25$ ;  $P = 0.0084$ ) (Table 3, grey columns). Among carbohydrates, adult females also contained a significantly higher level of total sugars ( $t = 2.52$ ;  $df = 25$ ;  $P = 0.019$ ) and glycogen ( $t = 2.39$ ;  $df = 25$ ;  $P = 0.025$ ) when they had access to artificial sugar dispensers (Table 3, grey columns). We did not, however, observe differences in the levels of fructose ( $t = 1.61$ ;  $df = 25$ ;  $P = 0.12$ ) (Table 3, grey columns).

#### ***Carbohydrate levels of *N. tenuis* on tomato plants with and without access to artificial sugar dispensers***

Finally, we tested whether the presence of artificial sugar dispensers improves the carbohydrate content of *N. tenuis* in the presence of tomato plants. Nymphs contained a significantly higher level of total carbohydrates in microcosms with artificial sugar dispensers than in those with water

dispensers ( $t = 3.59$ ;  $df = 49$ ;  $P < 0.001$ ) (Table 2, white columns). Among the different carbohydrates, nymphs contained significantly higher levels of total sugars ( $t = 3.71$ ;  $df = 49$ ;  $P < 0.001$ ) and glycogen ( $t = 3.18$ ;  $df = 49$ ;  $P = 0.0026$ ) in the microcosm with tomato plants and artificial sugar dispensers than in those without sugars. However, we did not observe differences in the fructose level ( $t = 0.99$ ;  $df = 49$ ;  $P = 0.32$ ) (Table 2, white columns).

Similarly, when *N. tenuis* adult females had access to tomato plants, they also contained a significantly higher level of total carbohydrates in microcosms with artificial sugar dispensers than in those with water dispensers ( $t = 2.51$ ;  $df = 39$ ;  $P = 0.017$ ) (Table 3, white columns). Among carbohydrates, *N. tenuis* adult females contained significantly higher levels of total sugars ( $t = 2.48$ ;  $df = 39$ ;  $P = 0.018$ ) and glycogen ( $t = 2.43$ ;  $df = 39$ ;  $P = 0.02$ ) when they had access to artificial sugar dispensers; nevertheless, we did not observe differences in fructose concentrations ( $t = 1.48$ ;  $df = 39$ ;  $P = 0.15$ ) (Table 3, white columns).

#### ***Effect of sugar provisioning on N. tenuis phytophagy***

Sugar dispensers reduced the phytophagy of *N. tenuis* nymphs and adults. Three days after their release, the number of necrotic rings per plant (measure of *N. tenuis* phytophagy) was significantly higher in plants without sugar dispensers than in plants with sugar dispensers (nymphs:  $F_{2, 27} = 11.05$ ,  $P < 0.001$ ; adults:  $F_{2, 29} = 7.41$ ,  $P < 0.001$ ) (Fig. 1a and b, respectively). The number of necrotic rings per plant seven days after the release of the nymphs remained significantly higher in plants without sugar dispensers than in plants with sugar dispensers (nymphs:  $F_{2, 27} = 33.66$ ,  $P < 0.001$ ; adults:  $F_{2, 29} = 37.96$ ,  $P < 0.001$ ) (Fig. 1a and b).

#### ***Effect of sugar provisioning on N. tenuis fecundity***

*N. tenuis* fecundity, measured as the number of nymphs per plant, was significantly lower in plants without sugar dispensers than in plants with sugar dispensers three days after the release of the adults ( $F_{2, 27} = 48.12$ ,  $P < 0.001$ ) (Fig. 3a). *N. tenuis* fecundity remained significantly lower in plants without sugars than in plants with sugar dispensers seven days after the release ( $F_{2, 27} = 44.98$ ,  $P < 0.001$ ) (Fig. 3b).

## DISCUSSION

When nymphs and adult females of the zoophytophagous mirid *N. tenuis* feed on plants they obtain carbohydrates and their phytophagy is reduced by the provision of artificial sugars is herein demonstrated. These results contribute to the understanding of the nutritional ecology of the Miridae, a group with a very diverse diet; they range from monophytophagous to zoophytophagous that feed on numerous plant and prey species (Wheeler 2001; Cassis and Schuh 2012; Wäckers et al. 2007). Moreover, this contribution is important for biological control practitioners being that the use of zoophytophagous predators as biological control agents has sometimes been neglected/questioned due to their phytophagy (Sanchez 2008; Castañé et al. 2011).

To our knowledge, this is the first study that analyses the nutritional state of a Mirid, measured herein as carbohydrate content. Our results have several implications. First, *N. tenuis* obtained a high amount of carbohydrates when nymphs and adult females had access to tomato plants, carbohydrate levels increased twice as much compared to those without access to plants. This result suggests, as expected, that *N. tenuis* ingests mainly phloem sap, which has a higher concentration of carbohydrates compared to that of xylem (Pérez-Alfocea et al. 2000). This feeding behavior results in the hypertrophy and hyperplasy of cells surrounding the phloem that produce characteristic necrotic rings at feeding sites, typical of *N. tenuis* (Raman and Sanjayan 1984; Raman et al. 1984; Sanchez et al. 2016). Secondly, nymphs contained higher levels of sugars than adult females; they also benefit more from sugar dispensers. This greater sugar requirement of mirid nymphs might explain why they tend to produce more damage in tomato plants than adults (Arnó et al. 2006; Calvo et al. 2009; Perdakis et al. 2009; Sanchez 2009). Thirdly, our results challenge the commonly held notion that mirids damage plants when they feed on plant tissues only to obtain water to digest their prey, as demonstrated by Gillespie and McGregor (2000) for *Dicyphus hesperus* (Hemiptera: Miridae). In our behavioral bioassay, where we measured the damages produced by *N. tenuis*, the provision of water in dispensers did not reduce the number of necrotic rings. This implies that mirids feed on plants to obtain nutrients

and not just water. Here, we have analyzed the carbohydrate content of adult females, further research should evaluate whether male feeding also benefits of artificial sugars. Female mirids generally have a higher nutrient demand than males (Wheeler 2001). Recently, Naselli et al. (2016) evaluated the capacity of both, *N. tenuis* females and males, to induce plant defenses in tomato plants, and they also observe that the feeding intensity of females was higher than males. Apart from obtaining carbohydrates from the plant *N. tenuis* exploited the artificial sugar dispensers which reduced their phytophagy. Carbohydrate sources have been widely provided in biological control programs to increase the abundance and impact of natural enemies (Gurr et al. 2005; Wade et al. 2008), but their use to reduce the phytophagy of mirids has not been previously examined. According to the results of the present study, this mirid has the ability to detect and feed on artificial sugar sources with or without the presence of a plant. Our behavioral and physiological assays demonstrated a reduction in *N. tenuis* phytophagy (measured as the number of necrotic rings) and increased carbohydrate levels when alternative sugar dispensers were provided. Altogether, this suggests that either *N. tenuis* prefers to feed on sugar dispensers over feeding on plant tissue or the quality/concentration of the sugar dispensers (0.5 M sucrose) is higher than the quality of the phloem content. Previously published studies featured the effect of sugars, either as nectar or artificial sugars, on the survival, immature development, fecundity and distribution of zoophytophagous predators (Portillo et al. 2012; Put et al. 2012; Igarashi et al. 2013; Urbaneja-Bernat et al. 2013, 2015) but not on their phytophagy and plant damage. The present research represents a novel strategy to decrease phytophagy in zoophytophagous mirids, thus increasing their potential as biological control agents. Finally, further research might investigate whether this technique can be used to reduce the damage produced by zoophytophagous mirid pests as for example *Apolygus lucorum* (Meyer-Dür) (Hemiptera: Miridae). This mirid bug is an important pest in China, feeding on more than 200 plant species, including cotton, tea, cereals, and vegetable and fruit crops (Lu et al. 2008, 2010; Pan et al. 2015). This method, however, should only be considered as a short-term method because the addition of sugars also increases the fecundity of *N. tenuis* as we have demonstrated in this research and in a previous study (Urbaneja-Bernat et al. 2013). The fecundity, measured as mean number of

nymphs per plant, was higher in plants with sugar dispensers than without. This result can be due to a prolonged oviposition period, an increase in the daily fecundity, or both. Since the surviving adults were not counted at the end of the assay we cannot determine the reason behind this increase in fecundity. However, in the previous study (Urbaneja-Bernat et al. 2013) the increase was due to an increase in the daily fecundity.

We propose three methods to increase the presence of carbohydrate sources to reduce zoophytophagy by mirids: i) maintain populations of hemipterans that excrete honeydew of high value for the mirid in hedges ii) grow non-crop plants providing nectar and iii) provide artificial sugars. To our knowledge, the effect of honeydew on *N. tenuis* fitness has not been tested. Honeydew quality for parasitoid fitness is known to depend on the honeydew-producing species (Tena et al. 2016). Therefore, several honeydews should be evaluated to determine the most suitable one for *N. tenuis*. Mirids are known to feed on extrafloral nectar (Portillo et al. 2012); Biondi et al., (2016) have recently shown the potential role of *Sesamum indicum* (L.) (Pedaliaceae) as an alternative host plant for *N. tenuis*. Urbaneja-Bernat et al. (2015) have also demonstrated the potential of artificial sugars to maintain and increase mirid populations. Therefore, these two latter approaches seem to be the most promising. In the case of artificial sugars, at least two alternative sugar dispensers could be tested: hydrocapsules filled with sugars and sprayed sugars. Both *N. tenuis* nymphs and adults feed on hydrocapsules filled with sugars under laboratory conditions (Urbaneja-Bernat et al. 2013) and greenhouse conditions (Urbaneja-Bernat et al. 2015). Sprayed sugars have been widely used for other groups of predators (Wade et al. 2008). For example, sprayed sugars increase the number of coccinellids in soybean crops (Seagraves et al. 2011), their survival and longevity in the absence of prey in laboratory assays has been increased (Matsuka et al. 1982; Dreyer et al. 1997; Lundgren 2009) along with their flight capacity (Nedvěd et al. 2001). In the case of hemipteran predators, sugars sprayed on alfalfa fields increased the number of *Orius tristicolor* (White) (Hemiptera: Anthocoridae) (Evans and Swallow 1993). All these methods could be implemented, yet at the end of the crop cycle when *N. tenuis* causes the most damage.

Our study represents the first step to understanding the nutritional ecology of zoophytophagous mirids and how we can use sugar sources to decrease their phytophagy. Further research should explore the content of proteins and lipids to understand the complete nutritional necessities of *N. tenuis* as well as the nutrients obtained from the plant. In this sense, it would be interesting to understand the nutrients obtained from both crops and alternative host plants used to promote the presence of *N. tenuis* such as *Sesamum indicum* L. (Biondi et al. 2016). In this study, Biondi and his colleagues found, under laboratory conditions, that the presence of *S. indicum* reduces the damage of the mirid on tomato. From the behavioral perspective, further research should analyze the effect of sugar sources on their zoophagy. In this sense, our previous study (Urbaneja-Bernat et al. 2013) shows how the addition of sucrose reduces the number of *E. kuehniella* eggs consumed. Therefore, the addition of sugar dispensers should be avoided when pest density is high because they might reduce the potential of *N. tenuis*.

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## **Author Contribution Statement**

PU-B, AU, JG-C and AT designed the research. PU-B and PB conducted experiments. PU-B and AT analyzed the data. All authors commented on the manuscript.

## **Compliance with Ethical Standards**

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Ethical approval: This article does not contain any studies with human participants performed by any of the authors. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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**Tables:**

**Table 1.** Number of nymphs and adults of *Nesidiocoris tenuis* of each treatment/diet used in the analysis of carbohydrates.

**Table 2.** Mean ( $\pm$ SE) content of carbohydrates (fructose, total sugars, glycogen and total carbohydrates measured as  $\mu\text{g} / \text{mm}$  of tibia length) of *Nesidiocoris tenuis* nymphs in microcosms with access to different food sources.

**Table 3.** Mean ( $\pm$ SE) content of carbohydrates (fructose, total sugars, glycogen and total carbohydrates measured as  $\mu\text{g} / \text{mm}$  of tibia length) of *Nesidiocoris tenuis* adults in microcosms with access to different food sources.

**Table 1.**

<b>Replicate</b>	<b>Water dispensers</b>				<b>Sugar dispensers</b>			
	<b>Nymphs</b>		<b>Adults</b>		<b>Nymphs</b>		<b>Adults</b>	
	No plant	Plant	No plant	Plant	No plant	Plant	No plant	Plant
1	1	8	2	5	9	10	3	4
2	5	6	3	2	6	6	8	8
3	0	4	6	6	6	6	4	5
4	-	4	-	6	-	6	-	4
<b>Total</b>	<b>6</b>	<b>22</b>	<b>11</b>	<b>19</b>	<b>21</b>	<b>28</b>	<b>15</b>	<b>21</b>

**Table 2.**

<b>Carbohydrates</b>	<b>Water dispensers</b>		<b>Sugar dispensers</b>	
	No plant	Plant	No plant	Plant
Total sugars	1.40 ± 0.50	4.75 ± 0.64	6.63 ± 0.65	7.97 ± 0.58
Fructose	0	0.09 ± 0.02	0.17 ± 0.03	0.11 ± 0.02
Glycogen	5.39 ± 2.27	13.07 ± 2.05	13.97 ± 1.83	23.48 ± 2.41
<b>Total*</b>	<b>6.80 ± 2.71</b>	<b>17.82 ± 2.53</b>	<b>20.61 ± 2.32</b>	<b>31.45 ± 2.71</b>

\*Total = Total sugars + glycogen

**Table 3.**

<b>Carbohydrates</b>	<b>Water dispensers</b>		<b>Sugar dispensers</b>	
	No plant	Plant	No plant	Plant
Total sugars	0.53 ± 0.29	2.24 ± 0.43	3.28 ± 0.9	4.53 ± 0.79
Fructose	0.04 ± 0.01	0.093 ± 0.02	0.28 ± 0.13	0.23 ± 0.09
Glycogen	5.90 ± 1.05	10.57 ± 1.49	11.50 ± 1.85	19.44 ± 3.20
<b>Total</b>	<b>6.43 ± 1.08</b>	<b>12.81 ± 1.83</b>	<b>14.78 ± 2.35</b>	<b>23.98 ± 3.90</b>

\*Total = Total sugars + glycogen