Sugars as complementary alternative food for the establishment of *Nesidiocoris tenuis* in greenhouse tomato

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**Keywords**

_Ephestia kuehniella_, hydrocapsules, inoculative releases, Miridae, nutritional ecology, _Tuta absoluta_

**Abstract**

*Nesidiocoris tenuis* (Reuter) (Hemiptera: Miridae) is an omnivorous generalist predator which is augmentatively released and conserved for control of whiteflies (Hemiptera: Aleyrodidae) and *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) in tomato crops. Eggs of _Ephestia kuehniella_ Zeller (Lepidoptera: Pyralidae) are often provided as factitious prey to improve the establishment of _N. tenuis_ after its release. We first tested different amounts of _E. kuehniella_ eggs per plant to optimize _N. tenuis_ establishment and then investigated whether the amount of eggs that optimized _N. tenuis_ establishment might be reduced by adding sugars (hydrocapsules filled with 0.5 M sucrose) under walk-in cage and commercial greenhouse conditions. These experiments demonstrated that the addition of sugar to the diet of _N. tenuis_ could half the amount of _E. kuehniella_ eggs required to establish _N. tenuis_. Under greenhouse conditions, the progeny of _N. tenuis_ per plant did not differ significantly between _E. kuehniella_ alone or the half amount of _E. kuehniella_ plus hydrocapsules. These results demonstrated that the sugar could partially substitute for _E. kuehniella_ eggs improve establishment of _N. tenuis_ and suggest that natural sugars such as nectar and honeydew might also beneficial.

**Introduction**

The sweetpotato whitefly _Bemisia tabaci_ (Gennadius) (Hemiptera: Aleyrodidae) and the south American tomato pinworm _Tuta absoluta_ (Meyrick) (Lepidoptera: Gelechiidae) are considered major pests of tomato (_Lycopersicon esculentum_ Mill) in the Mediterranean basin (Calvo et al. 2009; Desneux et al. 2011; Urbaneja et al. 2012). _Bemisia tabaci_ debilitates the crop through sap removal, secrets large amount of honeydew with the subsequent build-up of sooty mould, causes tomato irregular ripening, and vectors the tomato yellow leaf curl virus (TYLCV) (Stansly and Naranjo 2010). _Tuta absoluta_ larvae, an invasive pest of tomato in Europe (Desneux et al. 2010, 2011), feed on leaves, flowers, stems and fruit causing losses of up to 100% if control measures are not taken (Desneux et al. 2010).

_Nesidiocoris tenuis_ Reuter (Hemiptera: Miridae) is an omnivorous and polyphagous predator commonly present in natural vegetation and horticultural crops of the Mediterranean basin (Arnó et al. 2010). This mirid lays its eggs into plant tissue, mainly on tender flushes and leaves, and the nymphs and adults feed on eggs and larvae of _T. absoluta_ (Urbaneja et al. 2009) as well as nymphs of _B. tabaci_ (Calvo et al. 2009). _Nesidiocoris tenuis_ feeds also on tomato plants, producing necrotic rings in vegetative structures as well as flower abortion (Castañé et al. 2011). The advantage of generalist omnivorous predators such as _N. tenuis_ in biological control is the ability to use various food resources (such as alternative prey or plant material), successfully colonize the crop prior to pest infestation, resulting in crop systems that are more resilient to pests (Thompson 1999). In greenhouse tomato, _N. tenuis_ is able to regulate populations of...
B. tabaci and T. absoluta when it is augmentatively released at the beginning of the crop (Gonzalez-Cabrer et al. 2011; Mollà et al. 2011; Calvo et al. 2012a, b; Urbaneja et al. 2012).

Two strategies are presently underway to control T. absoluta and B. tabaci in tomato greenhouses using N. tenuis. The first strategy is based on the release of 1–2 individuals of N. tenuis/m² between 2 and 4 weeks after transplanting tomato plants in the greenhouse (Calvo et al. 2009). To shorten the establishment period and improve distribution in the crop, especially when weather conditions are not favourable, a second strategy consists of releasing N. tenuis in seedling nurseries (pre-plant release) (Calvo et al. 2012a). This strategy entails transplanting tomato plants on which N. tenuis individuals have already laid eggs. For this, 0.25–0.5 N. tenuis couples per plant are released in the nursery. Because N. tenuis is not able to survive on a strictly phytophagous diet (Urbaneja et al. 2005), Ephesia kuehniella Zeller (Lepidoptera: Pyralidae) eggs are supplemented with both release techniques, as alternative food to facilitate N. tenuis establishment (Calvo et al. 2012a). Provision of this alternative diet increases cost because of the high price of E. kuehniella, approximately $400 per kg of fresh eggs.

The most obvious alternative to lower costs would be to reduce the amount of E. kuehniella eggs deployed. To our knowledge, a study of the optimal amount of E. kuehniella required to guarantee establishment of N. tenuis has not been conducted or at least published. Second, alternative and more economic diets for predaceous mirids have not been evaluated (Grenier et al. 1989; Thompson 1999; Cohen 2003; Castaño and Zapata 2005; Vandekerkhove et al. 2006). Such diets might also be used in the field to improve the establishment of mirids and reduce the cost. For example, different artificial diets based on egg yolk (Thompson 1999; Cohen 2003; Vandekerkhove et al. 2006) and on bovine meat (Grenier et al. 1989; Castaño and Zapata 2005) have been compared with E. kuehniella to rear other mirids with different results. However, developing and evaluating an artificial diet is time-consuming activity (Vandekerkhove et al. 2006). Third, different pollen and nectar alone or in combination with E. kuehniella eggs may improve the establishment and dispersal of mirids. Laboratory studies have reported that Macrolelophus pygmaeus can complete its development when feeding exclusively on pollen of the plant Ecballium elaterium (L.) (Perdikis and Lykouressis 2000) or bee pollen (Perdikis and Lykouressis 2000; Vandekerkhove and De Clercq 2010) even though developmental time, survival and fecundity are inferior compared with a mixed diet of prey and plant material or a diet of E. kuehniella eggs. More recently, Portillo et al. (2012) have demonstrated that M. pygmaeus survival and fecundity are prolonged on broad bean plants that provide extrafloral nectar as compared to broad bean with extrafloral nectaries removed. Following a similar approach, Urbaneja-Bernat et al. (2013) have recently demonstrated that N. tenuis feeds on sugar sources and that the addition of a 0.5-M sucrose solution inside hydrocapsules (HYDROCAPSULE®) to a diet of E. kuehniella eggs increased the fecundity of N. tenuis and reduced the number of eggs consumed under laboratory conditions. Consequently, a possible alternative to E. kuehniella eggs could be the addition of sucrose in the release diet.

The first aim of this study was to evaluate N. tenuis establishment as a function of the quantity of E. kuehniella eggs deployed in the release. Then, based on an established minimum of E. kuehniella eggs, walk-in cage and one greenhouse trial were conducted to assess effects of addition of sugars to a less than optimum amount of E. kuehniella eggs on the establishment of N. tenuis in tomato.

**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 experiments. Tomato seedlings were transplanted into 8 × 8 × 8 cm pots. Nesidiocoris tenuis adults were obtained from a commercial supplier (NESIBUG®, Koppert Biological Systems, S.L., Águilas, Murcia, Spain). Each bottle contained approximately 500 3-day-old specimens (FJ Calvo and BS Koppert, Personal Communication).

**Application rates of E. kuehniella eggs**

This experiment was conducted in a glasshouse located at Instituto Valenciano de Investigaciones Agrarias IVIA (Moncada, Valencia, Spain). The climatic conditions were 23–27°C, 65 ± 10% RH and natural photoperiod (from September 6 to October 4).

To determine the amount of E. kuehniella eggs that maintain the higher number of N. tenuis nymphs, four quantities of eggs were sprinkled once on the plants: 0.1, 0.05, 0.02 g and one control (without E. kuehniella eggs). The trial was conducted in 12 plastic cages 60 × 60 × 60 cm (ventilated BugDorm-2; MegaView Science Co., Ltd.; Taichung, Taiwan). In each cage
(plot), five tomato plants (30 cm high) and one couple of *N. tenuis* per plant were introduced the same day as the eggs. In total, there were three replicates per treatment with five plants in each plot.

During the following 5 weeks, one plant per cage was removed weekly and the number of *N. tenuis* nymphs and adults was counted in the greenhouse. After counting adults and nymphs, each plant was cut into small sections (approximately 4–5 cm), which were introduced into Petri dishes (9 cm in diameter) and held in a climatic chamber (25 ± 1°C, 60 ± 5% HR, 16:8 h L:D photoperiod) for the development of *N. tenuis* eggs (humidity inside Petri dishes was not measured). Two weeks later, the number of emerging nymphs was counted again to estimate the total number of nymphs emerged (the sum of both counts).

Sugar as complement for *N. tenuis* establishment

**Chamber trial**

This experiment was conducted in the same glasshouse and under the same conditions as previously described but in April (from March 3 to May 4). Four diets were tested: (i) 0.1 g of *E. kuehniella* eggs per plant (previously obtained as adequate amount of eggs and significantly different from 0.05 g for *N. tenuis* establishment), (ii) 0.05 g of eggs plus 0.6 g of hydrocapsules filled with 0.5 M sucrose (Urbaneja-Bernat et al. 2013), (iii) 0.6 g of hydrocapsules filled with 0.5 m sucrose alone and (iv) a control (without alternative food).

Six tomato plants (30 cm high) and one couple of *N. tenuis* per plant were introduced into each Bugdorm the same day as diets. Cages were set out in a completely randomized block design with four replicates per treatment with six plants each replicate. The same procedure described above was followed to determine the presence of *N. tenuis* nymphs in plants during 6 weeks.

**Greenhouse trial**

This trial was conducted in a greenhouse of 170 m² in Vila-real (39°56′16″N, 0°06′05″O) (Castellón), covered with plastic. Tomato plants, variety ‘Virgilio’ tolerant to TYLCV (Clause Vegetable Seeds, UK), were grown following typical practices for tomato greenhouse cultivation in Spain: the plant main stem was trained with plastic rings to a wire structure, secondary shoots and senescent leaves were pruned weekly, and a standard nutrient solution for tomato was added directly to the soil (Gonzalez-Cabrera et al. 2011). In addition, bumblebees were provided for pollination (Natupol®, Koppert BS, Águilas, Murcia) inside the greenhouse (5 April 2011).

Tomato seedlings were transplanted with *N. tenuis* (pre-plant releases) following the procedure described in Calvo et al. (2012a). Briefly, adult *N. tenuis* was released 1 March at a sex ratio of 1 : 1 and a rate of one per two plants in a cage containing 40 seedling tomato plants. Four paper strips (3 × 1 cm) with eggs of *E. kuehniella* glued to one side were also placed inside the inoculating cages to serve as a food source for the mirids (Calvo et al. 2012a). Tomato plants were transplanted 6 days later. Three diets described above (i) 0.1 g of *E. kuehniella* eggs per plant, (ii) 0.05 g of eggs plus 0.6 g of hydrocapsules filled with 0.5 m sucrose and (iii) a control without alternative food were tested in a randomized complete block design with five replicates. Additional diet applications were made weekly for 5 weeks. Each replicate consisted of five rows (blocks) with 24 tomato plants each row. Each row contained three groups of eight plants (plot) and randomly assigned to one of the treatments. The four middle plants of each row were evaluated with two plants on each side serving as a border between treatments. The number of *N. tenuis* nymphs found on the upper third of the plant was counted weekly for 7 weeks (until April 28).

Data analysis

The number of adults and nymphs of *N. tenuis* on tomato plants in the different experiments was analyzed using a generalized linear mixed model with repeated measures. Treatment was considered as a fixed factor and time as a random one. Pairwise comparisons of the fixed factor levels were made using the least significant difference (LSD) post hoc test (P < 0.05) upon a significant treatment effect. Each GLMM used a normal distribution and identity link function.

Results

**Application rates of *E. kuehniella* eggs**

The mean number of *N. tenuis* nymphs per plant throughout the experiment depended on the amount of *E. kuehniella* eggs provided (F = 12.97; d.f. = 3, 56; P < 0.001) (fig. 1). The number of nymphs found on plants provided with 0.02 g of eggs was not significantly different from those with none. At application rates of 0.05 and 0.1 g, significantly more nymphs were found compared with plants with no eggs. The mean number of nymphs was significantly greater
when 0.1 g eggs were provided compared with 0.05 g of eggs.

Sugar as complementary alternative food for the establishment of \textit{N. tenuis}

\textit{Chamber trial}

The mean number of \textit{N. tenuis} nymphs and adults per plant throughout the trial depended on the provided alternative food (fig. 2) (nymphs: \( F = 16.47; \) d.f. = 3, 92; \( P < 0.001 \); adults: \( F = 16.28; \) d.f. = 3, 92; \( P < 0.001 \)). The number of nymphs or adults was significantly greater when 0.1 g of \textit{E. kuehniella} eggs half as many eggs plus 0.6 g of hydrocapsules filled with 0.5 M sucrose was provided than the remaining treatments. The provision of only sucrose (hydrocapsules) did not significantly increase the number of nymphs and adults when

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig1.png}
\caption{Number of \textit{Nesidiocoris tenuis} nymphs (mean \( \pm \) SE) per plant with different amounts of \textit{Ephestia kuehniella} eggs: 0.1, 0.05, 0.02, g per plant and control (without eggs). Treatments labelled with different letters are significantly different at \( P < 0.05 \). Data are presented slightly displaced from their originally date in order to better represent number of nymphs.}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig2.png}
\caption{Number of \textit{Nesidiocoris tenuis} nymphs (a) and adults (b) (mean \( \pm \) SE) per plant with different alternative diets: no additions (control); 0.6 g of hydrocapsules filled with 0.5 M sucrose (sucrose); 0.1 g of \textit{Ephestia kuehniella} eggs; 0.05 g of \textit{E. kuehniella} eggs; and 0.6 g of hydrocapsules filled with 0.5 M sucrose (1/2 \textit{E. kuehniella}+sucrose). One \textit{N. tenuis} couple was released 3 weeks before the beginning of the experiment. Treatments labelled with different letters are significantly different at \( P < 0.05 \). Data are presented slightly displaced from their originally date in order to better represent number of mirids.}
\end{figure}
compared with the control treatment with no supplementary nutrition provided.

**Greenhouse trial**

In the commercial greenhouse trial (Vila-real), the number of nymphs again depended on the provided alternative food ($F = 9.78; \text{d.f.} = 2, 41; P < 0.001$) (figs 3 and 4). The number of nymphs was significantly higher when 0.1 g of *E. kuehniella* eggs or when 0.05 g of eggs and 0.6 g of hydrocapsules filled with 0.5 M sucrose were provided than in the control treatment (control vs. 0.1 g of *E. kuehniella* eggs: $t = 2.71; P = 0.007$; control vs. 0.05 g of *E. kuehniella* eggs and 0.6 g of hydrocapsules filled with 0.5 M sucrose: $t = 4.38; P < 0.001$). There were no significant differences between the two alternative foods (0.1 g of *E. kuehniella* eggs vs. 0.05 g of *E. kuehniella* eggs and 0.6 g of hydrocapsules filled with 0.5 M sucrose: $t = 1.62; P = 0.096$).

**Discussion**

The first experiment showed that a weight of 0.1 g of *E. kuehniella* eggs per plant increased the offspring of *N. tenuis* per tomato plant when one couple of the mirid was released per plant. Lower amounts of *E. kuehniella* eggs (0.05 and 0.02 g per plant) resulted in lower number of *N. tenuis* nymphs per plant. *E. kuehniella* eggs are generally used at the beginning of the tomato crop when prey is scarce because *N. tenuis* nymphs cannot complete their development and establish in the crop if they do not have access to prey (Urbanaje et al. 2005). Moreover, this addition increases lifetime fecundity, shortens developmental time and avoids cannibalism between nymphs (Calvo et al. 2009, 2012a,b).

Our semifield and greenhouse trails demonstrated that the provision of sugars halved the amount of *E. kuehniella* eggs needed for the establishment of *N. tenuis* in tomato plants. Recently, it was shown that addition of sucrose to a diet of *E. kuehniella* doubled fecundity of *N. tenuis* during the first 15 days and reduced the number of eggs consumed (Urbanaje-Bernat et al. 2013). These two advantages observed under laboratory conditions might explain why *N. tenuis* populations did not decrease when the *E. kuehniella* amount was halved. Another non-exclusive hypothesis is that the hydrocapsules used in these experiments can maintain the sugars from degrading throughout the week, whereas *E. kuehniella* eggs might degrade faster under field conditions (same authors, per observations). Consequently, *N. tenuis* might use these sugars when *E. kuehniella* eggs have already degraded. Apart from artificial sugars, our results suggest that natural sugar sources such as nectar and honeydew could facilitate the establishment of zoophytophagous insects as shown by Pumariño and Alomar (2012). Although *N. tenuis* can reach the adult stage feeding on 0.5 M sucrose and tomato plants under laboratory conditions (Urbanaje-Bernat et al. 2013), the mirid did not establish in our trials when we used sucrose without *E. kuehniella* eggs in our semifield assay. Similarly, the addition of crystallized fructose (one of the main nectar components beside sucrose) and pollen did not improve the establishment of *M. pygmaeus* in tomato crop when compared to the control (Put et al. 2012).

Finally, this study was focused and designed to measure the establishment of *N. tenuis* in greenhouse tomato prior to pest infestation and, consequently, increase the resilience of the crop. For this, we did not measure the population levels of potential pests because they were either low or null when field trails started. Several studies have shown that the addition of non-prey foods, such as sugar, negatively influence predation rate and might even

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**Fig. 3** Number of *Nesidiocoris tenuis* nymphs (mean ± SE) per plant without (no releases of the mirid) and with *N. tenuis* that received different nutritional supplements: no additions (control); 0.1 g of *Ephestia kuehniella* eggs (*E. kuehniella*); 0.05 g of *E. kuehniella* eggs; and 0.6 g of hydrocapsules filled with 0.5 M sucrose ($\frac{1}{2} E. kuehniella +$ sucrose). One *N. tenuis* couple per plant was released in the seedling nurseries (predator in first) in 1 March 2011. Tomato plants were planted 6 days later in a greenhouse from Vila-real, Spain.
increase pest population (Wade et al. 2008). However, we did not expect it in our system because, the main tomato pests cannot feed on hydrocapsules and increase their populations as occurs when floral nectar is provided and lepidopteran pests feed on them (Wackers et al. 2007). For the mirid, the trade-off between feeding on sugars or pests would only occur during the first weeks. Therefore, a decrease of predation rates would only be expected at the beginning of the crop if it occurred at all.

In conclusion, the addition of hydrocapsules filled with 0.5 m sucrose at the beginning of the tomato crop reduced the amount of E. kuehniella eggs needed to maintain N. tenuis in tomato crop, suggesting that sugars can be used to improve and substitute partially the addition of E. kuehniella eggs.

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