Induction of glandular trichomes to control *Bemisia tabaci* in tomato crops: modulation by the natural enemy *Nesidiocoris tenuis*

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Short title: Trichomes induction by *N. tenuis*

Abstract

Whitefly-transmitted viruses are one of the biggest threats to tomato growing worldwide. Strategies based on the introgression of resistance traits from wild relatives are promoted to control tomato pests and diseases. Recently, a trichome-based resistance characterizing the wild species *Solanum pimpinellifolium* was introgressed into a cultivated tomato. An advanced backcross line (BC_5S_2) exhibiting the presence of acylsugars-associated type IV trichomes, which are lacking in cultivated tomatoes, was effective at controlling whiteflies (Hemiptera: Aleyrodidae) and limiting the spread of whitefly-transmitted viruses. However, at early growth stages, type IV trichomes density and acylsugars production are limited; thus, protection against whiteflies and whitefly-transmitted viruses remains irrelevant. In this work, we demonstrate that young BC_5S_2 tomato plants feeding-punctured by the zoophytophagous predator *Nesidiocoris tenuis* (Reuter) (Hemiptera: Miridae) displayed an increase (above 50%) in type IV trichomes density. Acylsugars production was consistently increased in *N. tenuis*-punctured BC_5S_2 plants, which was more likely associated with upregulated expression of *BCKD-E2* gene related to acylsugars biosynthesis. In addition, the infestation of BC_5S_2 plants with *N. tenuis* effectively induced the expression of defensive genes involved in the jasmonic acid signaling pathway, resulting in strong repellence to *B. tabaci* and attractiveness to *N. tenuis*. Thus, by pre-plant release of *N. tenuis* in tomato nurseries carried out in some integrated pest management programs, type

IV trichome-expressing plants can be prepared to control whiteflies and whitefly-transmitted viruses at early growth stages. This study emphasizes the advantage of reinforcing constitutive resistance using defense inducers to guarantee robust protection against pests and transmitted viruses.

Keywords: Acylsugars, *Bemisia tabaci*, defense induction, tomato, type IV glandular trichomes, whitefly-transmitted viruses.

Introduction

Tomato (*Solanum lycopersicum* L.) is one of the most widely produced and consumed crop species in the Mediterranean and elsewhere (Research and Markets World – Tomato 2022). Many arthropod pests and diseases threaten tomato production and can cause severe yield losses. The tomato plant is susceptible to attack by several phytophagous insects, including the South American tomato pinworm *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) and the tobacco whitefly *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae), which are listed as harmful pests of both protected and field tomatoes (Stansly et al. 2010; Desneux et al. 2022). *Bemisia tabaci* causes direct damage by sucking on the plants' phloem sap, and by producing honeydew providing a favorable medium for sooty mold fungi (Byrne and Miller 1990). However, the most important damage caused by *B. tabaci* is the transmission of phloem-restricted viruses, primarily the persistently transmitted begomoviruses (genus *Begomovirus*, family Geminivirida*e*) (Moriones and Navas-Castillo 2000; Gilbertson et al. 2015; Brown and Czosnek 2002; Stansly et al. 2010).

Isolates of the begomovirus species *Tomato yellow leaf curl virus* (TYLCV) transmitted by the *B. tabaci* whitefly are widely distributed worldwide and cause one of the most devastating viral diseases in cultivated tomatoes (Lefeuvre et al. 2010; Moriones and Navas-Castillo 2000). As agricultural practices are increasingly based on pesticides and chemicals, together with the production of genetically uniform crops, TYLCV has become a major limiting factor for production in tomato-growing areas (Pico et al. 1996; Basak 2016). Host plant resistance is the most effective and environmentally sustainable management approach to control viruses transmitted by whiteflies (Nombela and Muñiz 2010; Firdaus et al. 2012; McDaniel et al. 2016; Rojas et al. 2018). The strategies to reduce the damage caused by TYLCV are shifting toward restricting insect vector performance (Fereres and Moreno 2009). In fact, plant virus transmission relies on host plant selection, feeding behavior, and the dispersion capacity of their insect vectors to spread the virus from plant to plant (Fereres and Moreno 2009).

Tomato plants' resistance to *B. tabaci* has been explored in sources of wild species to control this whitefly and the transmitted viruses. Accordingly, different resistance levels to whiteflies have been reported from wild tomato species such as *S. pennellii, S. habrochaites, S. galapagense, S. pimpinellifolium*, and *S. chilense* (Lapidot et al. 1997; Toscano et al. 2002; Maruthi et al. 2003; Firdaus et al. 2012; Vendemiatti et al. 2022). Plant resistance to whiteflies and other herbivore species in wild tomatoes has been associated with the presence of physical barriers and phytochemicals that negatively affect pest feeding and performance. Physical traits on the aerial plant surface, including hairiness and cuticle features, alter host plant selection and pest preference (Berlinger 1986). Among these resistant traits are trichomes: appendages covering plant leaves and stems that influence herbivore settling and growth on host plants (Wagner et al. 2004). Notably, allelochemicals produced by glandular trichomes, *i.e.*, acylsugars, methylketones, terpenoids, phenylpropanoids, and flavonoids, have been reported to strongly confer resistance against whiteflies in wild tomato species (Simmons and Gurr 2005; Glas et al. 2012). These volatile and non-volatile secondary metabolites act as anti-herbivore substances which can have an antibiotic and/or antixenosis effect on diverse arthropod species (Yao et al. 2019; Paspati et al. 2021; Wagner et al. 2004). In this context, trichome-mediated resistance in the wild tomato species *S. pimpinellifolium* L. accession TO-937 was introgressed into recipient-cultivated tomato *S. lycopersicum* cv. Moneymaker (Rodriguez-Lopez et al. 2011). The resulting inbred lines showed resistance to whitefly and TYLCV transmission (Rodríguez-López et al. 2020, 2012). This resistance was associated with type IV leaf glandular trichomes and the production of antiherbivore acylsugars by glandular exudates. The cultivated tomato is susceptible to many arthropod attacks as it lacks the presence of type IV glandular trichomes, and the level of acylsugars produced by the other glandular trichomes is insufficient to guarantee protection against pests (McDowell et al. 2011; Rodríguez-López et al. 2020; Blanco-Sánchez et al. 2021).

In previous research by Escobar-Bravo et al. (2016), a BC_5S_2 introgressed line derived from the initial cross between *S. pimpinellifolium* accession TO-937 and recurrent tomato cv. Moneymaker was selected for high type IV trichomes density and acylsugars level. These traits made this introgressed line very promising for managing whiteflies and their associated viruses. However, the advanced backcross BC_5S_2 does not fully express trichomes type IV density and acylsugars secretion until the plants reach the 10-leaf growth stage. Therefore, young tomato plants might be unprotected at early growth stages after being transplanted to the field. However, this setback could be overcome when treating seedlings with methyl jasmonate (MeJA) (Escobar-Bravo et al. 2016). Jasmonic acid-mediated defense activated the expression of the genes involved in the biosynthesis of type IV trichomes and the defensive acylsugars leading to higher trichomes type IV density and the acylsugars secretion, assuring expression of trichomes-mediated resistance at early developmental stages. This can enhance plant protection against whiteflies and avoid devastating damage to tomatoes at susceptible young stages (Rodriguez-Lopez et al. 2011).

The omnivorous predatory bug *Nesidiocoris tenuis* (Reuter) (Hemiptera: Miridae) is an effective predator largely used in protected and open field tomato crops to control *B. tabaci* among others (Calvo et al., 2009; Pérez-Hedo, Riahi, et al., 2021). Pre-plant release of *N. tenuis* in tomato nurseries has been adopted in some integrated pest management programs to ensure that the predator becomes established in tomato crops (Calvo et al. 2012). This mirid has a zoophytophagous behavior, which means that in addition to feeding on different prey, it feeds directly on the plant. By inserting its stylet into the plant tissue (Pérez-Hedo and Urbaneja 2016; Chinchilla-Ramírez et al. 2021), the tomato plant responds by activating various defense mechanisms, including the induction of the JA pathway (Pérez-Hedo et al. 2015b; Bouagga et al. 2020; Pérez-Hedo et al. 2022).

We undertook this research to study whether *N. tenuis* might induce type IV trichomes-based resistance traits in acylsugars-producing introgressed line BC_5S_2

at early plant growth stages. For this purpose, we compared the type IV glandular trichomes density and the acylsugars production in BC_5S_2 intact and *N. tenuis*-infested plants. Moreover, on these experimental treatments, we assessed the expression of genes related to jasmonic acid and acylsugars synthesis pathways in tomato cv. Moneymaker and its near-isogenic line BC_5S_2. We also studied the effect of type IV trichomes-based resistance induction by *N. tenuis* on the host plant selection by *B. tabaci* and *N. tenuis*. Knowledge derived from our study might provide further insight into the management of arthropod pests and diseases and help identify potential strategies for integrated pest management combining the classical breeding approaches and the use of natural enemies to induce defenses.

Materials and Methods

Plants and Insects. The cultivated tomato variety Moneymaker and its near-isogenic line BC_5S_2 that, additional to the trichome types present in Moneymaker, shows the acylsugar-producing glandular type IV trichomes were used in the experiments. The BC_5S_2 line was obtained in IHSM “La Mayora”, Malaga, Spain after repeated backcrossing towards Moneymaker, selection and final fixation steps for presence of type IV trichomes introgressed from *S. pimpinellifolium* L. wild species [see breeding program in supplementary Figure S1 of Escobar-Bravo et al. (2016)]. Seeds were sown in a mixture of soil and local peat moss. Two weeks after germination, seedlings were individually transplanted into plastic pots (8 x 8 x 8 cm) and maintained in a climatic chamber at 25 ± 2 ºC, 60 ± 5% RH, and 14:10 h day:night (long day, LD) photoperiod until use.

Adults of *N. tenuis* were provided by Koppert Biological Systems, S.L. (Águilas, Murcia, Spain) and housed in a climatic chamber at 25 ± 2 ºC, 60-80% RH, and 14:10 h (LD) photoperiod. *N. tenuis* was caged in BugDorm-1 Insect Tents; MegaView Science Co., Ltd. (Taichung, Taiwan) (30 x 30 x 30 cm) containing green bean pods (*Phaseolus vulgaris* L. Fabales: Fabaceae) and supplemented with eggs of *Ephestia kuehniella* (Zeller) (Lepidoptera: Pyralidae) until use. Individuals of *B. tabaci* (Mediterranean species) were obtained from a colony originated from individuals collected during field visits in Malaga (southern Spain) and reared on melon plants (*Cucumis melo* L. cv. ANC42, IHSM seedbank collection) within wooden cages covered with insect-proof nets, in an insect-proof glasshouse with temperature control [60-70% ± 10% RH, with loose temperature control (16-20 °C night, 22-28°C day) and natural photoperiod] and light supplementation when needed. Newly emerged adults of *B. tabaci* (less than two days old) were placed on tomato plants caged in 60 x 60 x 60 cm BugDorm-2 insect tents. Five-day-old adults of *B. tabaci* and *N. tenuis* were used in the experiments.

Type IV trichome density determination. Moneymaker tomato plants and their near-isogenic line BC_5S_2 were infested by *N. tenuis* (hereafter referred to as activated plants). Two experiments were conducted in a glasshouse located at the IHSM “La Mayora” at 60-70% ± 10% RH, with loose temperature control (16-20 °C night, 22-28°C day) and natural photoperiod (approx. 14:10, LD). In the first experiment, two pairs of *N. tenuis* per plant were used to activate tomato plants (Moneymaker and

BC_5S_2) at the 1-leaf growth stage. In the second, 4-leaf growth stage Moneymaker and BC_5S_2 tomato plants were activated using two pairs of *N. tenuis* per plant. Each experiment consisted of two treatments: tomato intact plants and activated plants. Three replicates per treatment and eight plants per replicate in each experiment were performed for each tomato genotype. The cages were placed on 2 x 1 m benches, and all *N. tenuis* specimens were left undisturbed on the plants throughout all the time-course experiments. *Ephestia kuehniella* eggs were added weekly to these plants as supplementary food. At 12 and 18 days post-*N. tenuis* infestation (dpi), two diagonal leaflets of the third youngest apical leaf of intact and activated BC_5S_2 plants were collected. The density of trichomes type IV on the abaxial surface was recorded on four positions of leaf lamina (~ 0.2 cm^2) using a stereo microscope (40x) (Leica Microsystems, Wetzlar, Germany). As Moneymaker plants lack type IV glandular trichomes, the leaflets were collected only to quantify acylsugars content.

Acylsugars analysis. Two leaflets of the third apical leaf of both Moneymaker and BC_5S_2 plants arranged diagonally (the two remaining to those previously collected to estimate the trichomes density) were used to analyze the acylsugars production. Trichome counts and extractions were made on leaflets of the same position and developmental stage following Alba et al. (2009) to minimize the interference caused by slight differences in acylsugars accumulation between leaves with different positions in the plant that we detected in previous work (data not presented). The epicuticular leaf acylsugars extraction was carried out according to the modified method described by Mutschler et al. (1996). Leaflets were placed in a scintillation vial (20 ml) with 4 ml of dichloromethane and gently mixed for 30 seconds. The leaflets were removed, and the extracts were rinsed twice with 2 ml of distilled water, fully dried under vacuum, and then dissolved in 2 ml of methanol with gentle shaking for 12 hours. After removal, an area (cm^2) of the rinsed leaflets was measured to estimate the acylsugars accumulation per unit area, using a ΔT Area Meter, MK2 model (Delta-T Devices, Cambridge, UK). Acylsugars content of the samples was concentrated four times and redissolved in methanol. Then, the sugar ester secreted by type IV trichomes was quantified using Rhodamine B (Lin and Wagner 1994). In short, 100 µl of the concentrated sample was placed in 96-well plates, evaporated, and 100 µl of Rhodamine B at 0.5% (p/v) (Sigma-Aldrich, Barcelona, Spain) was added. After 10 min of incubation at room temperature, the sample was gently rinsed five times with 200 µl of water, dried, and dissolved in 250 µl of acetone 50%. Then, the absorbance was recorded at 550 nm in Anthos 2010 Microplate reader (Biochrom Ltd, UK).

Y-tube bioassay. The behavioral responses of *N. tenuis* and *B. tabaci* females to BC_5S_2 and Moneymaker intact plants and BC_5S_2 and Moneymaker activated plants after being exposed for 14 days to two pairs of *N. tenuis* were analyzed in a Y-tube olfactometer (Analytical Research Systems, Gainesville, FL, USA). BC_5S_2 and Moneymaker tomato seedlings at the 1-leaf growth stage were used in this experiment. Two pairs of *N. tenuis* per plant were introduced in 24.5 x 24.5 x 63.0 cm individual plastic cages (BugDorm-4; MegaView Science Co., Ltd.; Taichung, Taiwan) containing 1-leaf growth stage BC_5S_2 or Moneymaker tomato plants. Intact and *N. tenuis*-released treatments were conducted in separate greenhouses under identical conditions, at 60-80% RH, 20-28 ºC, and 16:8 LD photoperiod. After 14 days, *N. tenuis* individuals were removed, and the plants were directly used in the Y-tube experiment. A transparent glass Y-shaped tube (diameter 2.4 cm, base 13.5 cm, two arms 5.75 cm each at a 70º angle) was used where each arm was connected via high-density polyethylene tubes to two identical glass jars (5-l volume), each containing a *N. tenuis* plant exposed for 14 days or an intact control plant. An air pump was used to flow a unidirectional filtered air at 150 ml/min from the arms to the base of the tube. Each jar was connected to a flow meter, a charcoal filter, and a water bubbler. Four 60-cm-long fluorescent tubes (OSRAM, L18 W/765, OSRAM GmbH, Germany) were positioned 40 cm above the arms. The light intensity over the Y-tube was measured with a ceptometer (LP-80 AccuPAR, Decagon Devices, Inc., Pullman, WA) at 2,516 lux. The environmental conditions in the Y-tube experiments were 23 ± 2 °C and 60 ± 10 % RH (Pérez-Hedo et al. 2015b). *B. tabaci* and *N. tenuis* females were starved for at least 6 h before the olfactory test. A single female was introduced into the tube and observed until it had walked at least 3 cm up one of the side arms or until 15 min had elapsed. Adults that did not choose a sidearm within 15 min were considered 'non-responders' and excluded from subsequent data analysis. 35 to 38 responsive individuals were tested, and each individual was used only once. After testing five individuals, the olfactometer arms were flipped around (180°) to minimize the spatial effect on arm choice. After

10 females had been tested, the olfactometer setup was rinsed with neutral detergent Extran® (Merck KGaA, Darmstadt, Germany), water, and acetone and air dried for 5 min. New intact and activated BC_5S_2 plants were then placed inside. The percentage of individuals that have chosen one side arm of the total responder females was calculated (% olfactory response).

Gene expression analysis using RT-qPCR. Plants of Moneymaker and its near-isogenic line BC_5S_2 at the 1-leaf growth stage were enclosed in individual plastic cages (BugDorm-4) with two pairs of *N. tenuis* for 14 days and placed in greenhouses, as explained above. The total RNA of the apical part of *N. tenuis*-punctured plants and intact plants (six biological replicates per treatment, i.e., each obtained from an individual plant) was isolated using the NZYol (NZYTech, Lisboa, Portugal) based extraction. A 5 µg total RNA was treated with TURBO DNA-freeTM Kit (AmbionR, Life Technologies, CA, USA) in a 25 µl reaction, after which 500 ng of cDNA was synthesized using Prime ScriptTM RT Reagent Kit (TAKARA Bio, CA, USA). Real-time PCR amplification was performed in LightCycler R 480 System (Roche Molecular Systems, Inc., Switzerland), using NZYSupreme qPCR Green Master Mix (2x) (NZYTech, Lisbon, Portugal). Reactions were performed in a 10 µl volume containing 0.5 µM of each primer and 1 µg of cDNA template. The cycling program was set to 5 min of the pre-cycling stage (95 °C), 40 cycles of 15 s at 95 °C, 30 s at 58 °C and 30 s at 72 °C followed by a melting curve analysis. The primers sequences of defensive genes *PIN2* (*proteinase inhibitor II*), *LoxD* (*lipoxygenaseD*), *BCKD-E2* (*branched chain keto-acid dehydrogenase subunit 2*), and the housekeeping genes *Actin* (Solyc03g078400.2.1) and *EF1* (*Elongator factor 1*) used as a standard control gene for normalization are shown in (Table 1). The gene BCKD-E2 was selected due to its effectiveness in signaling the induction of the acylsugars pathway over other acylsugars-related genes (Escobar-Bravo et al. 2016).

Statistical analysis. Differences in type IV trichome density of intact and *N. tenuis*-punctured BC_5S_2 plants were tested by two-tailed Student's t-test ($P < 0.05$). Differences in acylsugars content in leaf exudates of infested vs non-infested Moneymaker and BC_5S_2 plants at 12 and 18 days post-infestation were analyzed by two-way analysis of variance (ANOVA, $P < 0.05$) and mean comparison at each time point were performed by Tukey's test. Data obtained from Y-tube olfactometer experiments were statically analyzed by chi-square goodness of fit test based on a null model in which the two odor sources are selected with equal frequency. Data of gene expression quantification were analyzed by one-way ANOVA, and Tukey's test was used for mean comparison ($P < 0.05$). All statistical analyses were performed using GraphPad Prism 9 for Windows (GraphPad Software, San Diego, CA, USA).

Results

***N. tenuis* phytophagy increased type IV trichomes density**. To test whether *N. tenuis* puncturing might induce an early and higher density of type IV glandular trichomes, the number of trichomes covering the abaxial surface of the BC_5S_2

tomato plants was counted. At the 1-leaf growth stage, the number of type IV trichomes was significantly higher on *N. tenuis*-punctured BC_5S_2 plants than on intact BC_5S_2 plants, at both 12 ($t_{1\text{-}4}$ = 8.74, P = 0.0009) and 18 ($t_{1\text{-}4}$ = 12.52; P = 0.0005) days after *N. tenuis* infestation (Figure 1A). Similarly, BC_5S_2 plants punctured by *N. tenuis* at the 4-leaf growth stage exhibited a significantly higher density of type IV trichomes when compared with BC_5S_2 intact plant at both 12 ($t_{1\text{-}4}$ = 4.49, P = 0.0109) and 18 ($t_{1\text{-}4}$ = 5.47; P = 0.0109) days post plant infestation (Figure 1B). At 12 and 18 days post-infestation (dpi), the BC_5S_2 plants reached 4-5 and 6-8 fully developed leaves when infested by *N. tenuis* at the 1-leaf growth stage (Figure 1A). Instead, when BC_5S_2 plants were infested at 4-leaf growth age, the 12 and 18 dpi corresponded to 6-8 and 9-10 leaf growth stages, respectively (Figure 1B).

Acylsugars production increased in *N. tenuis*-punctured BC_5S_2 plants. The analysis of type IV trichomes associated traits revealed an increased production of acylsugars in *N. tenuis*-punctured BC_5S_2 1-leaf growth stage plants when compared with BC_5S_2 intact plants at 12 dpi ($F_{3\text{-}8}$= 4.67; P= 0.021) and 18 dpi ($F_{3\text{-}8}$= 5.36; P = 0.0079) (Figure 2A). Similarly, BC_5S_2 plants exposed to *N. tenuis* secreted significantly higher amounts of acylsugars than Moneymaker intact plants at 12 dpi ($F_{3\text{-}8}$= 8.94; P< 0.0001) and 18 dpi ($F_{3\text{-}8}$= 12.34; P< 0.0001), and than *N. tenuis*-punctured Moneymaker plants ($F_{3\text{-}8}$ = 7.73; P = 0.0003, $F_{3\text{-}8}$ = 10.78; P < 0.0001, 12 and 18 dpi respectively) (Figure 2A). No significant differences for acylsugars production of 4-leaf growth stage plants were observed between *N.*

tenuis-punctured BC_5S_2 plants and BC_5S_2 intact plants at 12 dpi ($F_{3\text{-}8}$ = 0.28; P = 0.99) (Figure 2B). Conversely, *N. tenuis* puncturing of BC_5S_2 of 4-leaf growth stage plants resulted in significantly enhanced acylsugars production at 18 dpi compared with BC_5S_2 intact plants ($F_{3\text{-}8}$ = 8.15; P = 0.0002) (Figure 2B). For 1-leaf growth stage plants, there was no interaction between both factors ($F_{3\text{-}8}$ = 8.15; P = 0.0002). However, for the 4-leaf growth stage, the interaction was significant due to the greater increase in acyl sugar content over time in BC_5S_2 compared to Moneymaker plants ($F_{3\text{-}8}$ = 17.96; P = 0.0007).

***N. tenuis* phytophagy alters insect behavior.** To better understand the effect of the early expression of type IV leaf glandular trichomes and their associated traits, the behavioral response of *B. tabaci* and *N. tenuis* were assessed in a Y-tube olfactometer. *N. tenuis*-punctured BC_5S_2 plants significantly repelled individuals of the *B. tabaci* tomato pest (χ^2 = 8.26; P = 0.0041), as shown in Figure 3A where 74% of the female *B. tabaci* preferred intact BC_5S_2 plants over *N. tenuis*-punctured plants. However, *N. tenuis* individuals significantly preferred (68%) BC_5S_2 *N. tenuis*-punctured versus intact plants (χ^2 = 5.16; P = 0.0231) (Figure 3A). Accordingly, the predatory bug *N. tenuis* was significantly more attracted to BC_5S_2 *N. tenuis*-punctured (69%) than *N. tenuis*-punctured Moneymaker plants (χ^2 = 4.83; P = 0.0279). In contrast, *N. tenuis*-punctured BC_5S_2 plants were significantly repellent (68%) to the phytophagous pest *B. tabaci* than *N. tenuis*-punctured Moneymaker (χ^2 = 4.57; P = 0.0326) (Figure 3B).

***N. tenuis* puncturing induces plant defenses**. To further investigate the role of *N. tenuis* in plant defense induction, quantification of the expression levels of some defensive genes and an acylsugars biosynthesis-related gene was performed. The *BCKD-E2* gene involved in the biosynthesis of acylsugars (Slocombe et al. 2008) was significantly upregulated in *N. tenuis*-punctured BC_5S_2 plants at 14 days post-infestation by *N. tenuis* in comparison with BC_5S_2 intact plants ($F_{3\text{-}20}$ = 11.86; $P <$ 0.0001) (Fig. 4 A,B). Interestingly, genetic expression of *BCKD-E2* in *N. tenuis*-punctured Moneymaker plants and BC_5S_2 intact plants was higher in comparison with Moneymaker intact plants ($F_{3\text{-}20}$ = 13.79; $p <$ 0.0001 and $F_{3\text{-}20}$ = 11.95; $P <$ 0.0001, respectively). The quantification of *PIN2* showed upregulation of this JA-response-related gene in BC_5S_2 plants punctured by *N. tenuis* when compared with Moneymaker ($F_{3\text{-}20}$ = 6.70; P = 0.0007) and BC_5S_2 ($F_{3\text{-}20}$ = 6.71; $P <$ 0.0007) intact plants (Figure 4C,D). Furthermore, the expression of *PIN2* increased significantly in *N. tenuis*-punctured Moneymaker plants compared with Moneymaker ($F_{3\text{-}20}$ = 4.31; P = 0.0297) and BC_5S_2 ($F_{3\text{-}20}$ = 4.32; P = 0.0292) intact plants. *N. tenuis* puncturing upregulated the expression of *LoxD*, a JA-biosynthesis-related gene, in BC_5S_2 plants when compared with Moneymaker ($F_{3\text{-}20}$ = 10.68; $P <$ 0.0001) and BC_5S_2 ($F_{3\text{-}20}$ = 8.50; $P <$ 0.0001) intact plants (Figure 4E,F). This defensive gene was also upregulated in *N. tenuis*-punctured Moneymaker plants in comparison with Moneymaker ($F_{3\text{-}20}$ = 8.95; $P <$ 0.0001) and BC_5S_2 ($F_{3\text{-}20}$ = 6.77; P = 0.0006) intact plants.

Discussion

Induction of defensive responses, mediated by exogenous elicitors, has been shown to promote plant resistance against pests and diseases, enhancing plant protection (Agrawal 1999; Dicke and Hilker 2003; Yao et al. 2019). Indeed, natural and synthetic elicitors have been described extensively to trigger constitutive plant defenses. In particular, the exogenous application of chemical compounds such as methyl jasmonate (MeJA) increased the density of structural trichomes type IV and VI, enhancing resistance to the western flower thrips, *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae), and to the tobacco whitefly *B. tabaci* in tomato plants (Boughton et al. 2005; Escobar-Bravo et al. 2017, 2016). Furthermore, microbial elicitors have been reported to induce trichomes-based defenses establishing direct and indirect responses, deterring herbivore colonization, reproduction, and reducing tomato plant damage (Chen et al. 2018; Javed and Qiu 2020). Some plants, as well, might trigger the increase of trichomes density in newly formed leaves upon herbivory (Gonzáles et al. 2008; Traw and Dawson 2002; Dalin et al. 2008; Agrawal 1999). Our results show for the first time that the phytophagy of *N. tenuis* induces an early expression of type IV trichomes-mediated resistance in tomato plants. Herein, the findings of this work provide insights into the role of the predator mirid *N. tenuis* in the enhancement of structural plant defenses and the increase of type IV trichomes densities and acylsugar secretions in young plants of advanced backcross tomato lines. *N. tenuis* induction can then be proposed as an alternative to prepare young plants of type IV trichome-expressing tomatoes to resist whiteflies and to limit the spread of whitefly-transmitted viruses.

Many studies have revealed the successful use of *N. tenuis* in integrated pest management programs for tomatoes in southern Europe (Van Lenteren 2012; van Lenteren et al. 2018; Calvo et al. 2012; Pérez-Hedo and Urbaneja 2016). Reasons behind the effective performance of this omnivore predatory bug in tomato growing systems are associated with the high potential of preying upon several tomato key pests (Pérez-Hedo et al. 2020, 2022; Desneux et al. 2022). *Nesidiocoris tenuis* is a generalist predator and feeds actively on a wide range of prey, including *B. tabaci*, *T. absoluta*, *F. occidentalis*, and *Tetranychus* urticae Koch (Acari: Tetranychidae), which enables these plant herbivores to be controlled where *N. tenuis* is released (Urbaneja et al. 2009; Biondi et al. 2013). Biological control of plant herbivores by *N. tenuis* can have increased effects if plant herbivores transmit viruses. This is the case of *B. tabaci* as a vector of TYLCV in tomatoes; thus, *N. tenuis* can provide increased virus control (Tsuchida et al. 2017). Additionally, benefits derived from the phytophagous behavior of this biocontrol agent regarding the induction of plant defensive signaling pathways have enhanced the advantageous use of *N. tenuis* in biological control strategies (Tortorici et al. 2022; Pérez-Hedo et al. 2022). As shown here, when feeding on plants, *N. tenuis* activates several metabolic pathways related to plant defenses such as jasmonic transduction signaling pathway, which are consequently responsible for triggering the release of herbivore-induced plant volatiles (HIPVs) (Pérez-Hedo et al. 2015a; Bouagga et al.

2018; Naselli et al. 2016). Highlighting the added value of *N. tenuis* revealed in our study, in terms of inducing constitutive defense, *N. tenuis* might provide robust and effective protection against tomato pests and associated diseases.

Our acylsugars quantification analysis demonstrated a time-increased production of antiherbivore substance in *N. tenuis*-punctured BC_5S_2 plants, presumably associated with the induction of type IV glandular trichomes on leaf abaxial surface by *N. tenuis*. Type IV glandular trichomes, characteristic of the BC_5S_2 plants used in this study and some wild tomato species such as *S. pimpinellifolium*, *S. galapagense*, and *S. pennellii*, exude and store allelochemical compounds (Goffreda et al. 1988; Liedl et al. 1995; Hawthorne et al. 1992; Fan et al. 2019; Firdaus et al. 2013; Simmons and Gurr 2005) that act as insecticidal metabolites against herbivores. The results obtained here are consistent with those from Lucini et al. (2015) and Alba et al. (2009), who reported a positive correlation between the density of glandular trichomes, abundantly type IV, and the content of acylsugars of some tomato genotypes, which consequently reduced the oviposition, survival and host plant preference of the two-spotted spider mite *T. urticae*. In our study, Moneymaker plants – whether *N. tenuis*-punctured or intact plants – slightly secreted acylsugars in leaf exudates. Although cultivated tomatoes lack type IV glandular trichomes, which are the leading producer of acylsugars (Lucini et al. 2015), the modest secretion of acylsugars in Moneymaker plants might be associated with acylsugars-producing type I glandular trichomes (McDowell et al. 2011; Fan et al. 2019). However, low levels of acylsugars produced by type I trichomes are insignificant in enhancing the resistance of pest-susceptible cultivated tomatoes to control herbivores (Rodríguez-López et al. 2012, 2020; Escobar-Bravo et al. 2016).

It is worth mentioning that BC_5S_2 tomato plants that were enclosed with *N. tenuis* at different growth ages (either at the 1- or 4-leaf growth stages) resulted in differentiated secretion of acylsugars. Namely, *N. tenuis* induction increased the content of acylsugars twice more in BC_5S_2 plants of 6-8 fully developed leaves that have been punctured since the 1-leaf growth stage, than in equivalent plants of 6-8 leaves that were exposed to *N. tenuis* at the 4-leaf growth stage. This point is reinforced by Rodríguez-López et al., (2011), who showed that the type IV trichomes-based BC_3S_2 introgression line (ABL 14-8) did not effectively express a high acylsugars level until the 10-leaf growth stage. These results highlight the benefits of establishing *N. tenuis* at early growth stages, in tomato nurseries, to strengthen plant defense during the most vulnerable vegetative juvenile stages after planting. The results of this work can partly explain the practical success of *N. tenuis* in tomato cultivation when its release is conducted from the nursery just at the moment when the plant is still in the 1-leaf growth stage, which is one of the vegetative stages used in this work. In southern Europe, it is common (in around 7.000 ha of protected tomatoes) to release *N. tenuis* in the nursery about seven days before transplanting to the field (Pérez-Hedo et al. 2020). This practice results in a better predator establishment and a more homogeneous distribution throughout the crop (Calvo et al. 2012). During their permanence in the nursery, *N.*

tenuis oviposits on the tiny seedlings so that they are already carriers of *N. tenuis* eggs when transplanted under field conditions. In addition, during this nursery phase, *N. tenuis* feeds on the plant and activates its immune system (Pérez-Hedo et al. 2022). As verified in this work, activating tomato seedlings could increase the number of type IV glandular trichomes and acylsugar secretion if type IV trichome-expressing plants were commercially used. Therefore, the tomato plant activated in the nursery after infestation with *N. tenuis* would have increased protection against whiteflies and, consequently, against whitefly-transmitted viruses when planted (Fortes et al. 2020; Escobar-Bravo et al. 2016; Rodríguez-López et al. 2020).

Many studies demonstrated that plant trichomes induction is a jasmonic acid-mediated resistance (Luo et al. 2009, 2012; Hamza et al. 2018). In this work, it was shown that *N. tenuis* phytophagy significantly induced the overexpression of JA signaling pathway-responsive gene *PIN2* (*proteinase inhibitor II*) and JA-biosynthesis-related gene *LoxD* (*lipoxygenaseD*) (**Figure** *4*). These results are supported by several studies (Pérez-Hedo et al. 2015a, 2015b; Naselli et al. 2016) that have demonstrated the activation of the JA metabolic pathway by *N. tenuis* upregulating the expression of *PIN2*. Besides, transgenic tomato cultivars expressing a barley proteinase inhibitor revealed induction of endogenous expression of the *PIN2* gene and increased density of glandular trichomes (Hamza et al. 2018). The genetic expression analysis conducted here also revealed significant overexpression of the *BCKD-E2* (*branched chain keto-acid dehydrogenase subunit 2)* gene in *N. tenuis*-punctured BC_5S_2 plants. A low quantity of acylsugars was also secreted by Moneymaker plants, and the BCKD-E2 primer was expressed in these plants. The Moneymaker genotype used in this work presents type I trichomes that produce acylsugars but are insignificant compared to BC5S2 plants with high expression of acylsugars-producing trichomes type IV. BCKD gene complex plays a role in the biosynthesis of acylsugars by conversion of keto acids to acyl-CoAs, involved in acylsugars production (Slocombe et al. 2008). Interestingly, the observed high upregulation of *BCKD-E2* correlated with the increased acylsugars content observed in activated tomato plants. Similarly, a JA-related defense elicitor has been proven to induce the expression of the *BCKD-E2* gene, increasing the production of acylsugars in glandular trichomes type IV after sequential MeJA treatment (Escobar-Bravo et al. 2016).

Several studies have described the effect of JA-mediated defense on herbivore behavior (Sun et al. 2017; Zhang et al. 2018; Riahi et al. 2022). Herein, it was demonstrated that the olfactory response of *B. tabaci* was altered, resulting in the repellence of *N. tenuis*-punctured BC_5S_2 plants to this key pest. Previous studies had already shown that *N. tenuis*-induced Moneymaker plants caused repellency to *B. tabaci* (Pérez-Hedo et al. 2015b). In this work we have observed that *N. tenuis*-induced BC_5S_2 plants were even more repellent than *N. tenuis*-induced Moneymaker plants (Figure 3B). This greater repellence might be explained by the higher density of type IV glandular trichomes present on the abaxial leaf surface of the BC5S2 induced plants when compared to Moneymaker.

Acylsugars-producing glandular trichomes have been largely demonstrated to confer resistance on whiteflies and other arthropod pests (Lucatti et al. 2013) mainly expressing antixenosis properties (Alba et al. 2009; Escobar-Bravo et al. 2016; Rodríguez-López et al. 2020). Leaf acylsugars are potentially associated with herbivore resistance. They have, thus, been reported to mainly contribute to broad-spectrum pest resistance in tomato genotypes with high acylsugar content (Maluf et al. 2010). Moreover, acylsugar-mediated resistance associated with type IV glandular trichomes presence effectively reduced preference, settlement, and feeding behavior of whiteflies [*B. tabaci*, *Trialeurodes vaporariorum* Westwood (Hemiptera, Aleyrodidae)], and *T. urticae* on *S. pimpinellifolium* and its derived inbred lines (Rodríguez-López et al. 2012; Rodriguez-Lopez et al. 2011; Rodríguez-López et al. 2020; Escobar-Bravo et al. 2016; Alba et al. 2009). The *B. tabaci* repellence observed in *N. tenuis*-punctured type IV trichome-expressing tomatoes might help to limit the spread of whitefly-transmitted viruses as already demonstrated (Rodríguez-López et al. 2012, 2020; Escobar-Bravo et al. 2016). Conversely, *N. tenuis* was thoroughly attracted to BC_5S_2 plants previously exposed to *N. tenuis*. Previous studies have reported that the JA-mediated defense positively affects mirid bugs' behavior. Silva et al. (2021) and Lins et al. (2014) have demonstrated, under laboratory conditions, that herbivore-induced plant volatiles (HIPVs), which are emitted from plants wounded by phytophagous arthropods, resulted into high attraction of *N. tenuis*. The JA signaling pathway is mainly the major metabolic mechanism underlying the induced release of these

HIPVs (Balmer et al. 2015; Pérez-Hedo et al. 2021). Besides, it has been shown that the induction of the JA signal transduction pathway and the resulting glandular trichomes density increase by expressing a protein inhibitor in some transgenic tomato plants, did not affect either *N. tenuis* development or viability (Hamza et al. 2018). Although a conclusion about the suitability of plants of the BC_5S_2-producing acylsugars line for *N. tenuis* development cannot be reached without carrying out further studies, it is worth noting the high population rate and the smooth mobility of *N. tenuis* individuals, mainly the first nymphal stages on these hairy induced plants during the 18 days of the experiment (C.R. personal observations). It should be considered that the production of allelochemicals, i.e., terpenes, mediated by the type VI glandular trichomes could also be a reason for the observed attractiveness of *N. tenuis* to BC_5S_2. The type VI trichomes were highly expressed in BC_5S_2 punctured plants (C.R and R.F personal observation). It is well documented that type VI glandular trichomes generally accumulate terpenes, mostly sesquiterpenes and monoterpenes in their glandular cells, and release these plant-specialized insecticides in the volatile form (Besser et al. 2009; Schilmiller et al. 2009; Xu et al. 2018). Ayelo et al. (2021) showed that four monoterpenes blended from HIPVs induced high attraction to the predatory bug *N. tenuis*. Further research addressing the implication of both type IV and type VI trichomes on the behavior, reproduction, and development of *N. tenuis* is mandatory when implementing the combined use of constitutive and inducible defense-based strategies.

Previous studies have shown that plants allocate a portion of their resources toward producing defensive compounds, such as trichomes, which can negatively impact growth and reproduction (Agrawal, 1999, 2001; Karban & Baldwin, 1997; Kursar et al., 2015). Therefore, the increase in producing trichomes by *N. tenuis* might reduce the resources available for growth. In this work, *N. tenuis* induced plant defenses and caused some plant damage in BC5S2 plants (i.e., the occurrence of necrotic rings on stems and petioles). However, the plants' growth could be considered standard throughout the experiment, and we did not observe any direct effect when comparing both treatments, with and without *N. tenuis*.

Overall, our study demonstrated that by using the biological control agent *N. tenuis,* early and high expression of type IV glandular trichomes has effectively been induced. Furthermore, *N. tenuis* phytophagy has increased the production of antiherbivore acylsugars, upregulating the JA-related gene expression, which will help to enhance resistance of tomato plants to whiteflies and consequently to control whitefly-transmitted viruses.

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Literature Cited

Agrawal, A. A. 1999. Induced responses to herbivory in wild radish: effects on several herbivores and plant fitness. Ecology. 80:1713-1723

Alba, J. M., Montserrat, M., and Fernández-Muñoz, R. 2009. Resistance to the two-spotted spider mite (*Tetranychus urticae*) by acylsucroses of wild tomato (*Solanum pimpinellifolium*) trichomes studied in a recombinant inbred line population. Exp. Appl. Acarol. 47:35–47.

Ayelo, P. M., Yusuf, A. A., Pirk, C. W. W., Chailleux, A., Mohamed, S. A., and Deletre, E. 2021. Terpenes from herbivore-induced tomato plant volatiles attract *Nesidiocoris tenuis* (Hemiptera: Miridae), a predator of major tomato pests. Pest Manag. Sci. 77:5255–5267.

Balmer, A., Pastor, V., Gamir, J., Flors, V., and Mauch-Mani, B. 2015. The "prime-ome": Towards a holistic approach to priming. Trends Plant Sci. 20:443–452.

Basak, J. 2016. Tomato Yellow Leaf Curl Virus: A Serious Threat to Tomato Plants World Wide. J. Plant Pathol. Microbiol. 07:04

Besser, K., Harper, A., Welsby, N., Schauvinhold, I., Slocombe, S., Li, Y., et al. 2009. Divergent regulation of terpenoid metabolism in the trichomes of wild and cultivated tomato species. Plant Physiol. 149:499–514.

Biondi, A., Alma, A., and Al-jboory, I. 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. 86:635–647.

Bouagga, S., Urbaneja, A., Depalo, L., Rubio, L., and Pérez-Hedo, M. 2020. Zoophytophagous predator-induced defences restrict accumulation of the tomato spotted wilt virus. Pest Manag. Sci. 76:561–567.

Bouagga, S., Urbaneja, A., Rambla, J. L., Flors, V., Granell, A., Jaques, J. A., et al. 2018. Zoophytophagous mirids provide pest control by inducing direct defences, antixenosis and attraction to parasitoids in sweet pepper plants. Pest Manag. Sci. 74:1286–1296.

Boughton, A. J., Hoover, K., and Felton, G. W. 2005. Methyl jasmonate application induces increased densities of glandular trichomes on tomato, *Lycopersicon esculentum* . J. Chem. Ecol. 31:2211–2216.

Brown, J. K., and Czosnek, H. 2002. Whitefly transmission of plant viruses p. 65-76. In *Advances in Botanical Research*, vol 36 Academic Press, Elsevier.

Byrne, D. N., and Miller, W. B. 1990. Carbohydrate and amino acid composition of phloem sap and honeydew produced by *Bemisia tabaci*. J. Insect Physiol. 36:433-439.

Calvo, F. J., Lorente, M. J., Stansly, P. A., and Belda, J. E. 2012. Preplant release of *Nesidiocoris tenuis* and supplementary tactics for control of *Tuta absoluta* and *Bemisa tabaci* in greenhouse tomato. Entomol. Exp. Appl. 143:111–119.

Calvo, J., Bolckmans, K., Stansly, P. A., and Urbaneja, A. 2009. Predation by *Nesidiocoris tenuis* on *Bemisia tabaci* and injury to tomato. BioControl. 54:237–246.

Chen, G., Escobar-Bravo, R., Kim, H. K., Leiss, K. A., and Klinkhamer, P. G. L.

2018. Induced resistance against western flower thrips by the *Pseudomonas syringae* -derived defense elicitors in tomato. Front. Plant Sci. 9:1-14.

Chinchilla-Ramírez, M., Garzo, E., Fereres, A., Gavara-Vidal, J., ten Broeke, C. J. ., van Loon, J. J. A., et al. 2021. Plant feeding by *Nesidiocoris tenuis*: Quantifying its behavioral and mechanical components. Biol. Control. 152:104402.

Dalin, P., Ågren, J., Björkman, C., Huttunen, P., and Kärkkäinen, K. 2008. Leaf trichome formation and plant resistance to herbivory. p. 89-105. In Schaller, A. (eds)*Induced plant resistance to herbivory*. Springer, Dordrecht

Desneux, N., Han, P., Mansour, R., Arnó, J., Brévault, T., Campos, M. R., et al. 2022. Integrated pest management of *Tuta absoluta*: practical implementations across different world regions. J. Pest Sci. 95:17-39

Dicke, M., and Hilker, M. 2003. Induced plant defences: from molecular biology to evolutionary ecology. Basic Appl. Ecol. 4:3-14.

Escobar-Bravo, R., Alba, J. M., Pons, C., Granell, A., Kant, M. R., Moriones, E., et al. 2016. A Jasmonate-Inducible Defense Trait Transferred from Wild into Cultivated Tomato Establishes Increased Whitefly Resistance and Reduced Viral Disease Incidence. Front. Plant Sci. 7, 1732

Escobar-Bravo, R., Klinkhamer, P. G. L., and Leiss, K. A. 2017. Induction of jasmonic acid-associated defenses by thrips alters host suitability for conspecifics and correlates with increased trichome densities in tomato. Plant Cell Physiol. 58:622-634.

Fan, P., Leong, B. J., and Last, R. L. 2019. Tip of the trichome: evolution of acylsugar metabolic diversity in Solanaceae. Curr Opin Plant Biol. 49:8-16.

Fereres, A., and Moreno, A. 2009. Behavioural aspects influencing plant virus transmission by homopteran insects. Virus Res. 141:158-168.

Firdaus, S., van Heusden, A. W., Hidayati, N., Supena, E. D. J., Mumm, R., de Vos, R. C. H., et al. 2013. Identification and QTL mapping of whitefly resistance components in *Solanum galapagense*. Theor. Appl. Genet. 126:1487-1501.

Firdaus, S., van Heusden, A. W., Hidayati, N., Supena, E. D. J., Visser, R. G. F., and Vosman, B. 2012. Resistance to *Bemisia tabaci* in tomato wild relatives. Euphytica. 187:31-45.

Fortes, I. M., Fernández-Muñoz, R., and Moriones, E. 2020. Host Plant Resistance to *Bemisia tabaci* to Control Damage Caused in Tomato Plants by the Emerging Crinivirus Tomato Chlorosis Virus. Front. Plant Sci. 11:1-9.

Gilbertson, R. L., Batuman, O., Webster, C. G., and Adkins, S. 2015. Role of the Insect Supervectors *Bemisia tabaci* and *Frankliniella occidentalis* in the Emergence and Global Spread of Plant Viruses. Annu. Rev. Virol. 2:67-93.

Glas, J. J., Schimmel, B. C. J., Alba, J. M., Escobar-Bravo, R., Schuurink, R. C., and Kant, M. R. 2012. Plant glandular trichomes as targets for breeding or engineering of resistance to herbivores. Int. J. Mol. Sci. 13:17077-17103.

Goffreda, J. C., Mutschler, M. A., and Tingey, W. M. 1988. Feeding behavior of potato aphid affected by glandular trichomes of wild tomato. Entomol. Exp. Appl. 48:101-107.

Gonzáles, W. L., Negritto, M. A., Suárez, L. H., and Gianoli, E. 2008. Induction of glandular and non-glandular trichomes by damage in leaves of *Madia sativa* under contrasting water regimes. Acta Oecologica. 33:128-132.

Hamza, R., Pérez-Hedo, M., Urbaneja, A., Rambla, J. L., Granell, A., Gaddour, K., et al. 2018. Expression of two barley proteinase inhibitors in tomato promotes endogenous defensive response and enhances resistance to *Tuta absoluta*. BMC Plant Biol. 18:1-14.

Hawthorne, D. J., Shapiro, J. A., Tingey, W. M., and Mutschler, M. A. 1992. Trichome-borne and artificially applied acylsugars of wild tomato deter feeding and oviposition of the leafminer *Liriomyza trifolii*. Entomol. Exp. Appl. 65:65-73.

Javed, K., and Qiu, D. 2020. Protein elicitor PebL1 of *Brevibacillus laterosporus* enhances resistance against *Myzus persicae* in tomato. Pathogens. 9:1-20.

Lapidot, M., Friedmann, M., Lachman, O., Yehezkel, A., Nahon, S., Cohen, S., et al. 1997. Comparison of resistance level to tomato yellow leaf curl virus among commercial cultivars and breeding lines. Plant Dis. 81:1425-1428.

Lefeuvre, P., Martin, D. P., Harkins, G., Lemey, P., Gray, A. J. A., Meredith, S., et al. 2010. The spread of tomato yellow leaf curl virus from the middle east to the world. PLoS Pathog. 6: e1001164

Liedl, B. E., Lawson, D. M., White, K. K., Shapiro, J. A., Cohen, D. E., Carson, W. G., et al. 1995. Acylglucoses of the wild tomato *Lycopersicon pennellii* alters settling and reduces oviposition of *Bemisia argentifolii* . J. Econ. Entomol. 88:742-748.

Lin, Y., and Wagner, G. J. 1994. Rapid and Simple Method for Estimation of Sugar Esters. J. Agric. Food Chem. 42:1709-1712.

Lins, J. C., van Loon, J. J. A., Bueno, V. H. P., Lucas-Barbosa, D., Dicke, M., and van Lenteren, J. C. 2014. Response of the zoophytophagous predators *Macrolophus pygmaeus* and *Nesidiocoris tenuis* to volatiles of uninfested plants and to plants infested by prey or conspecifics. BioControl. 59:707-718.

Lucatti, A. F., Van Heusden, A. W., De Vos, R. C. H., Visser, R. G. F., and Vosman, B. 2013. Differences in insect resistance between tomato species endemic to the Galapagos Islands. BMC Evol. Biol. 13.

Lucini, T., Faria, M. V., Rohde, C., Resende, J. T. V., and de Oliveira, J. R. F. 2015. Acylsugar and the role of trichomes in tomato genotypes resistance to *Tetranychus urticae*. Arthropod. Plant. Interact. 9:45-53.

Luo, M., Ding, L. W., Ge, Z. J., Wang, Z. Y., Hu, B. L., Yang, X. B., et al. 2012. The characterization of SaPIN2b, a plant trichome-localized proteinase inhibitor from *Solanum americanum*. Int. J. Mol. Sci. 13:15162–15176.

Luo, M., Wang, Z., Li, H., Xia, K. F., Cai, Y., and Xu, Z. F. 2009. Overexpression of a weed (Solanum americanum) proteinase inhibitor in transgenic tobacco results in increased glandular trichome density and enhanced resistance to *Helicoverpa armigera* and *Spodoptera litura*. Int. J. Mol. Sci. 10:1896–1910.

Maluf, W. R., Maciel, G. M., Gomes, L. A. A., Cardoso, M. das G., Gonçalves, L. D., da Silva, E. C., et al. 2010. Broad-spectrum arthropod resistance in hybrids between high-and low-acylsugar tomato lines. Crop Sci. 50:439–450.

Maruthi, M. N., Muniyappa, V., Green, S. K., Colvin, J., and Hanson, P. 2003. Resistance of tomato and sweet-pepper genotypes to Tomato leaf curl Bangalore virus and its vector *Bemisia tabaci* . Int. J. Pest Manag. 49:297-303.

McDowell, E. T., Kapteyn, J., Schmidt, A., Li, C., Kang, J. H., Descour, A., et al. 2011. Comparative functional genomic analysis of *solanum* glandular trichome types. Plant Physiol. 155:524–539.

Moriones, E., and Navas-Castillo, J. 2000. Tomato yellow leaf curl virus, an emerging virus complex causing epidemics worldwide. Virus Res. 71:123–134.

Mutschler, M. A., Doerge, R. W., Liu, S. C., Kuai, J. P., Liedl, B. E., and Shapiro, J. A. 1996. QTL analysis of pest resistance in the wild tomato *Lycopersicon pennellii* : QTLs controlling acylsugar level and composition. Theor. Appl. Genet. 92:709-718.

Naselli, M., Urbaneja, A., Siscaro, G., Jaques, J., Zappalà, L., Flors, V., et al. 2016. Stage-Related defense response Inductiion in tomato plants by *Nesidiocoris tenuis*. Int. J. Mol. Sci. 17:1210

Paspati, A., Rambla, J. L., López Gresa, M. P., Arbona, V., Gómez-Cadenas, A., Granell, A., et al. 2021. Tomato trichomes are deadly hurdles limiting the establishment of *Amblyseius swirskii* Athias-Henriot (Acari: Phytoseiidae). Biol. Control. 157:104572

Pérez-Hedo, M., Alonso-Valiente, M., Vacas, S., Gallego, C., Rambla, J. L., Navarro-Llopis, V., et al. 2021. Eliciting tomato plant defenses by exposure to herbivore induced plant volatiles. Entomol. Gen. 41:209–218.

Pérez-Hedo, M., Bouagga, S., Jaques, J. A., Flors, V., and Urbaneja, A. 2015a. Tomato plant responses to feeding behavior of three zoophytophagous predators (Hemiptera: Miridae). Biol. Control. 86:46–51.

Pérez-Hedo, M., Bouagga, S., Zhang, N. X., Moerkens, R., Messelink, G., Jaques, J. A., et al. 2022. Induction of plant defenses: the added value of zoophytophagous predators. J. Pest Sci. 95:1510-1517.

Pérez-Hedo, M., Riahi, C., and Urbaneja, A. 2020. Use of zoophytophagous mirid bugs in horticultural crops: Current challenges and future perspectives. Pest

Manag. Sci. 77:33-42

Pérez-Hedo, M., Urbaneja-Bernat, P., Jaques, J. A., Flors, V., and Urbaneja, A. 2015b. Defensive plant responses induced by *Nesidiocoris tenuis* (Hemiptera: Miridae) on tomato plants. J. Pest Sci. 88:543-554.

Pérez-Hedo, M., and Urbaneja, A. 2016. The zoophytophagous predator *Nesidiocoris tenuis*: a successful but controversial biocontrol agent in tomato crops. In *Advances in Insect Control and Resistance Management*, eds. A R Horowitz and I Ishaaya. Cham: Springer International Publishing, p. 121-138. Available at: http://link.springer.com/10.1007/978-3-319-31800-4_7.

Pico, B., Jo& Diez, M., and Nuez, F. 1996. Viral diseases causing the greatest economic losses to the tomato crop. II. The Tomato yellow leaf curl virus-a review. Sci. Hortic. (Amsterdam). 67:151-196.

Research and Markets World - Tomato. 2022. World - Tomato - Mark. Anal. Forecast. Size, Trends Insights.

Riahi, C., González-Rodríguez, J., Alonso-Valiente, M., Urbaneja, A., and Pérez-Hedo, M. 2022. Eliciting Plant Defenses Through Herbivore-Induced Plant Volatiles' Exposure in Sweet Peppers. Front. Ecol. Evol. 9 Available at: https://www.frontiersin.org/articles/10.3389/fevo.2021.776827/full.

Rodriguez-Lopez, M. J., Garzo, E., Bonani, J. P., Fereres, A., Fernandez-Munoz, R., and Moriones, E. 2011. Whitefly Resistance Traits Derived from the Wild Tomato Solanum pimpinellifolium Affect the Preference and Feeding Behavior of Bemisia tabaci and Reduce the Spread of Tomato yellow leaf curl virus. Phytopathology. 101:1191-1201.

Rodríguez-López, M. J., Garzo, E., Bonani, J. P., Fernández-Muñoz, R., Moriones, E., and Fereres, A. 2012. Acylsucrose-producing tomato plants forces *Bemisia tabaci* to shift its preferred settling and feeding site. PLoS One. 7.

Rodríguez-López, M. J., Moriones, E., and Fernández-Muñoz, R. 2020. An acylsucrose-producing tomato line derived from the wild species *Solanum pimpinellifolium* decreases fitness of the whitefly T*rialeurodes vaporariorum*. Insects. 11:616.

Schilmiller, A. L., Schauvinhold, I., Larson, M., Xu, R., Charbonneau, A. L., Schmidt, A., et al. 2009. Monoterpenes in the glandular trichomes of tomato are synthesized from a neryl diphosphate precursor rather than geranyl diphosphate. Proc. Natl. Acad. Sci. U. S. A. 106:10865-10870.

Silva, D. B., Urbaneja, A., and Pérez-Hedo, M. 2020. Response of mirid predators to synthetic herbivore-induced plant volatiles. Entomol. Exp. Appl. In press:EEA-2019-0220.

Simmons, A. T., and Gurr, G. M. 2005. Trichomes of *Lycopersicon* species and their hybrids: Effects on pests and natural enemies. Agric. For. Entomol. 7:265-276.

Slocombe, S. P., Schauvinhold, I., McQuinn, R. P., Besser, K., Welsby, N. A., Harper, A., et al. 2008. Transcriptomic and reverse genetic analyses of branched-chain fatty acid and acyl sugar production in *Solanum pennellii* and *Nicotiana benthamiana* . Plant Physiol. 148:1830–1846.

Stansly, P. A., Naranjo, S. E., Brown, J. K., Horowitz, A. R., Legg, J. P., Polston, J. E., et al. 2010. Bemisia*: Bionomics and management of a global pest*. Springer Netherlands.

Sun, Y. C., Pan, L. L., Ying, F. Z., Li, P., Wang, X. W., and Liu, S. S. 2017. Jasmonic acid-related resistance in tomato mediates interactions between whitefly and whitefly-transmitted virus. Sci. Rep. 7:1–7.

Tortorici, S., Biondi, A., Pérez-Hedo, M., Larbat, R., and Zappalà, L. 2022. Plant defences for enhanced integrated pest management in tomato. Ann. Appl. Biol. 180:328–337.

Toscano, L. C., Boiça Jr., A. L., and Maruyama, W. I. 2002. Non preference of whitefly for oviposition in tomato genotypes. Sci. Agric. 59:677–681.

Traw, M. B., and Dawson, T. E. 2002. Differential induction of trichomes by three herbivores of black mustard. Oecologia. 131:526–532.

Tsuchida, Y., Doi, M., Ishikawa, R., and Kageyama, C. 2017. Inhibitory effect of *Nesidiocoris tenuis* (Hemiptera: Miridae) on Tomato yellow leaf curl virus (TYLCV) transmission by *Bemisia tabaci* (Hemiptera: Aleyrodidae) in greenhouse tomato. Japanese J. Appl. Entomol. Zool. 61:215–222.

Urbaneja, A., Montón, H., and Mollá, O. 2009. Suitability of the tomato borer *Tuta absoluta* as prey for *Macrolophus pygmaeus* and *Nesidiocoris tenuis*. J. Appl. Entomol. 133:292–296.

van Lenteren, J. C. 2012. The state of commercial augmentative biological control: plenty of natural enemies, but a frustrating lack of uptake. Biol. Control. 57:1–20.

van Lenteren, J. C., Bolckmans, K., Köhl, J., Ravensberg, W. J., and Urbaneja, A. 2018. Biological control using invertebrates and microorganisms: plenty of new opportunities. BioControl. 63:39–59

Vendemiatti, E., Therezan, R., Vicente, M. H., Pinto, M. de S., Bergau, N., Yang, L., et al. 2022. The Genetic Complexity of Type-IV Trichome Development Reveals the Steps towards an Insect-Resistant Tomato. Plants. 11:1–20.

Wagner, G. J., Wang, E., and Shepherd, R. W. 2004. New approaches for studying and exploiting an old protuberance, the plant trichome. Ann. Bot. 93:3–11.

Xu, J., Van Herwijnen, Z. O., Dräger, D. B., Sui, C., Haring, M. A., and Schuurink, R. C. 2018. SlMYC1 regulates type VI glandular trichome formation and terpene biosynthesis in tomato glandular cells. Plant Cell. 30:2988–3005.

Yao, Q., Peng, Z., Tong, H., Yang, F., Xing, G., Wang, L., et al. 2019. Tomato Plant Flavonoids Increase Whitefly Resistance and Reduce Spread of Tomato yellow leaf curl virus. J. Econ. Entomol. 112:2790-2796.

Zhang, P. J., He, Y. C., Zhao, C., Ye, Z. H., and Yu, X. P. 2018. Jasmonic acid-dependent defenses play a key role in defending tomato against *Bemisia tabaci* nymphs, but not adults. Front. Plant Sci. 9:1-10.

Table 1: Primers used for quantification of expression of *Actin*, *EF1* (*elongator factor 1*) *BCKD-E2* (*branched chain keto-acid dehydrogenase subunit 2*), *PIN2* (*proteinase inhibitor II*), and *LoxD* (*lipoxygenaseD*) genes.

Gene	Primer forward (5’ → 3’)	Primer reverse (5’ → 3’)
Actin	5’-TTAGCACCTTCCAGCAGATGT-3’	5’-AACAGACAGGACACTCGCACT-3’
EF1	5’- GATTGGTGGTATTGGAACTGTC-3’	5’-AGCTTCGTGGTGCATCTC-3’
BCKD-E2	5’-TGTCGAAGAGATAAATTGTGATGC-3’	5’-TGAGGACGGGAAGGAAAGTG-3’
PIN2	5’-GAAAATCGTTAATTTATCCCAC-3’	5’-ACATACAAACTTTCCATCTTTA-3’
LoxD	5’-AACAGACAGGACACTCGCACT-3’	5’-ATGTGCTGCCAATATAAATGGTTCC-3’

FIGURE CAPTIONS

Figure 1. Type IV glandular trichomes density on abaxial leaflet surfaces of advanced backcross line BC_5S_2 intact plants and BC_5S_2 plants infested with *Nesidiocoris tenuis* at 12 and 18 days after infestation. (A) Plants infested at the 1-leaf growth stage. (B) Plants infested at the 4-leaf growth stage. Measurements were performed with a stereo microscope. Means of three replicates ± standard error are shown with significant differences between BC_5S_2 intact and infested plants tested by *t*-test at $P < 0.05$.

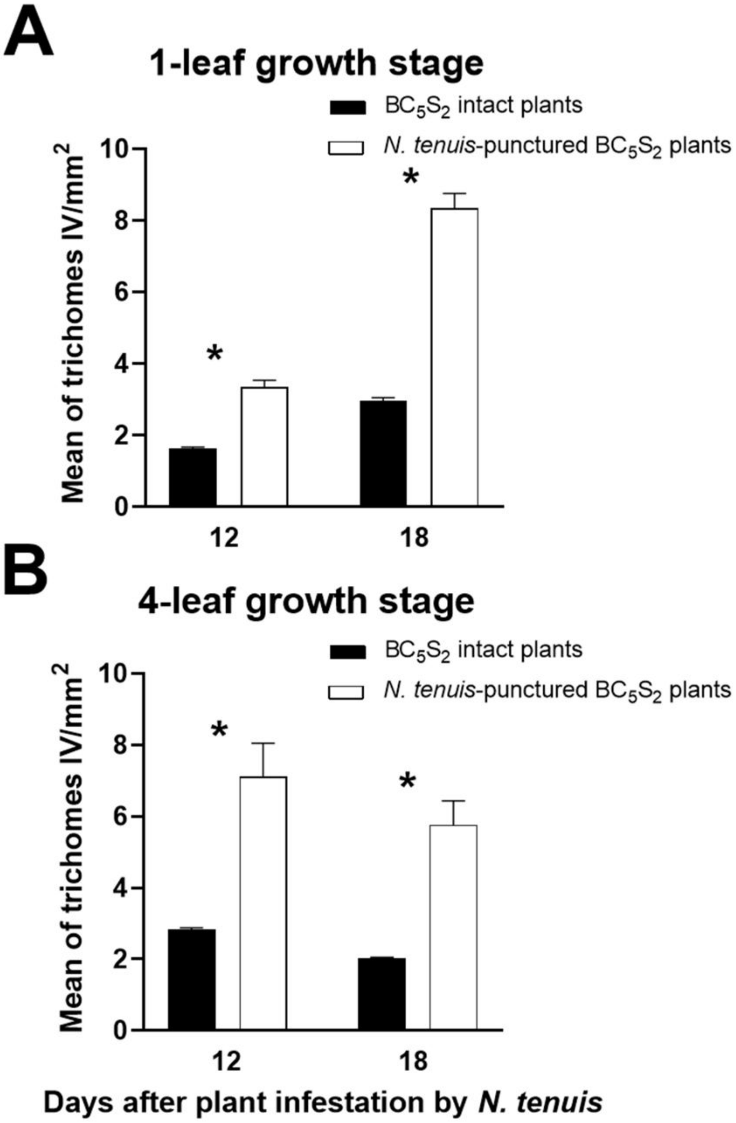
Figure 2. Acylsugars production in near-isogenic lines Moneymaker (MM) and BC_5S_2 intact plants and plants infested with *Nesidiocoris tenuis* at 12 and 18 days after infestation. (A) Plants infested at the 1-leaf growth stage. (B) Plants infested at the 4-leaf growth stage. Means of three replicates ± standard error are shown with significant differences between BC_5S_2 intact and infested plants tested by two-way ANOVA and mean comparison by Tukey's test at $P < 0.05$.

Figure 3. Response of *Nesidiocoris tenuis* and *Bemisia tabaci* females in a Y-tube olfactometer when exposed to two odor sources: (A) BC_5S_2 intact plants and BC_5S_2 plants infested with *Nesidiocoris tenuis* for 14 days. (B) Moneymaker (MM) and BC_5S_2 plants infested with *Nesidiocoris tenuis* for 14 days. Significant differences are based on χ^2 tests and are marked using asterisk * ($P < 0.05$); *n* is the total

number of responder females, nc (no choice) is the number of females that did not make a choice.

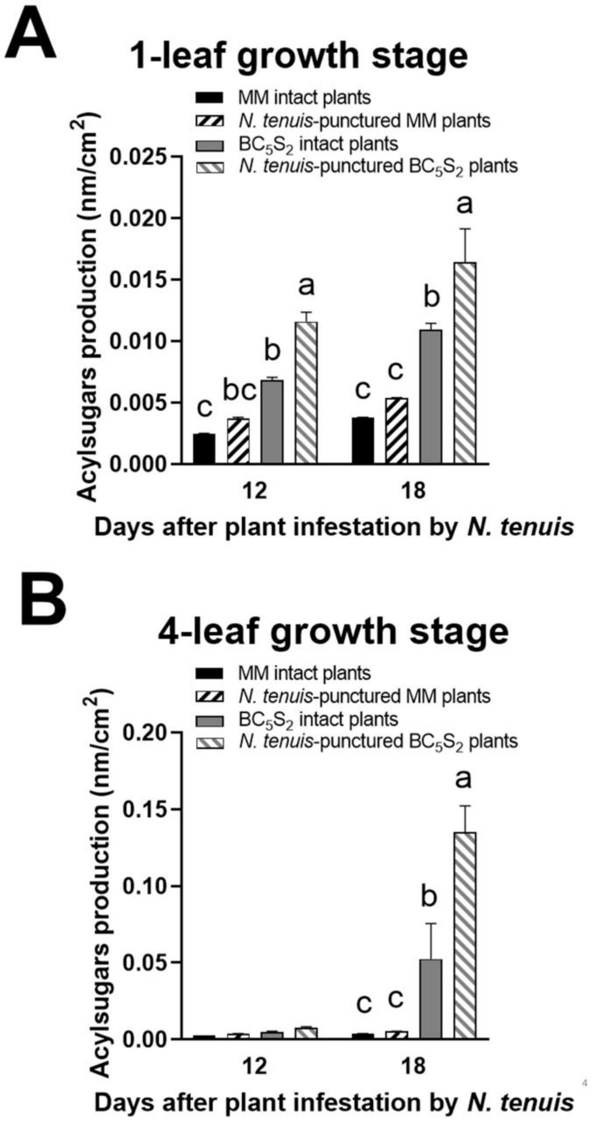
Figure 4. Relative expression of (A,B) *BCKD-E2 (branched chain keto-acid dehydrogenase subunit 2)* (acylsugars biosynthesis pathway), (C,D) *PIN2 (proteinase inhibitor II)* (JA signaling pathway) and (E,F) *LoxD (lipoxygenaseD)* (JA pathway) in Moneymaker (MM) and BC_5S_2 intact plants and plants infested with *Nesidiocoris tenuis* at 14 days after infestation. Gene expression levels were determined relative to *Actin* (A, C, E) and to *EF1* (*elongation factor 1*) (B, D, F) expression and displayed as the mean value of six biological replicated leaf samples, including standard error. Significant differences based on ANOVA and Tukey's multiple comparison test are represented in different letters ($P < 0.05$).

829 **Fig.1**

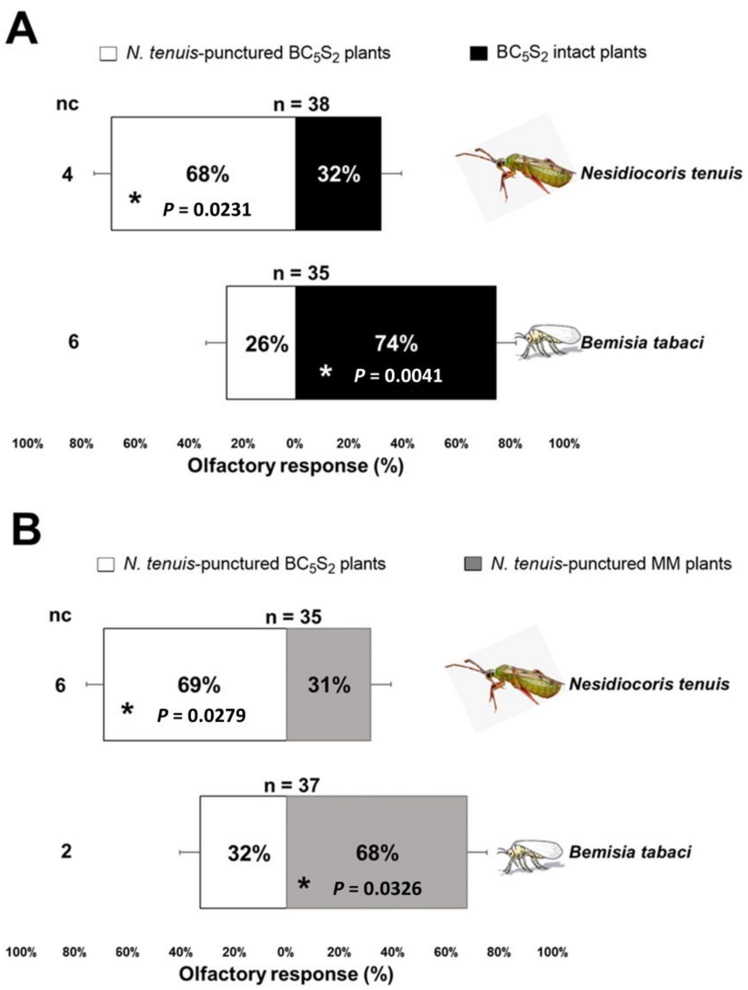

A
1-leaf growth stage
BC5S2 intact plants
N. tenuis-punctured BC5S2 plants
Mean of trichomes IV/mm2
10
8
6
4
2
0
*
*
12
18
B
4-leaf growth stage
BC5S2 intact plants
N. tenuis-punctured BC5S2 plants
Mean of trichomes IV/mm2
10
8
6
4
2
0
*
*
12
18
Days after plant infestation by N. tenuis

830

Fig. 2

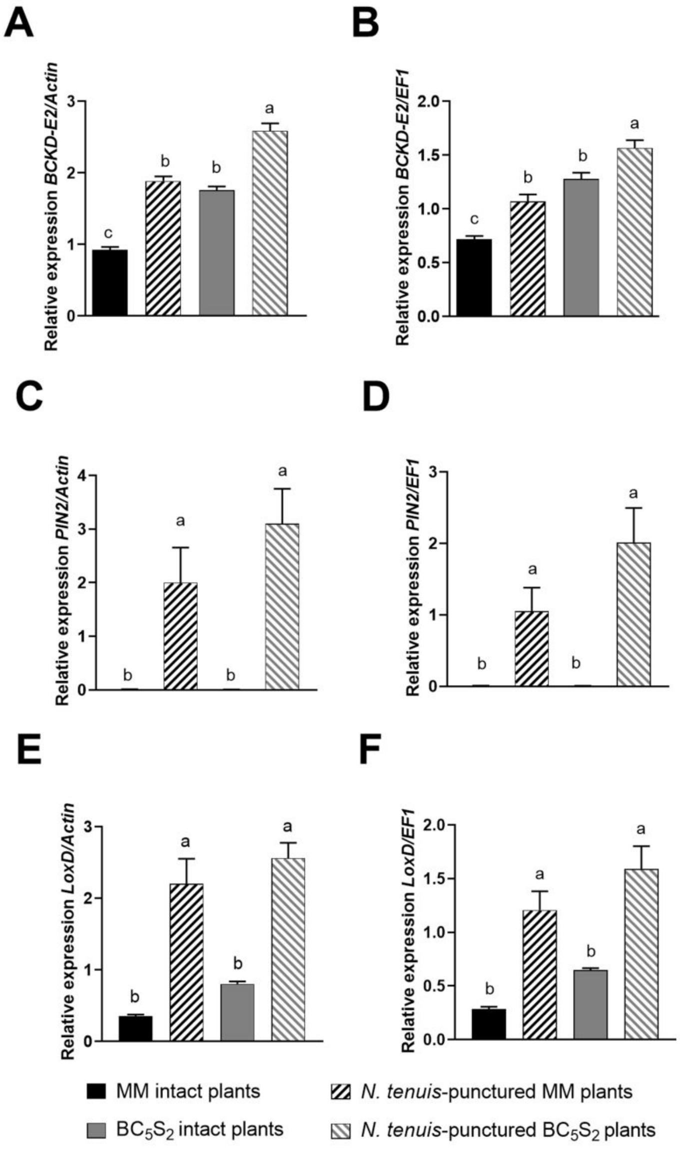

A
1-leaf growth stage
MM intact plants
N. tenuis-punctured MM plants
BC5S2 intact plants
N. tenuis-punctured BC5S2 plants
Acylsugars production (nm/cm2)
0.025
0.020
0.015
0.010
0.005
0.000
c
bc
b
a
c
c
b
a
12
18
Days after plant infestation by N. tenuis
B
4-leaf growth stage
MM intact plants
N. tenuis-punctured MM plants
BC5S2 intact plants
N. tenuis-punctured BC5S2 plants
Acylsugars production (nm/cm2)
0.20
0.15
0.10
0.05
0.00
c
c
b
a
12
18
Days after plant infestation by N. tenuis

835 **Fig. 3**


A
☐ N. tenuis-punctured BC5S2 plants
■ BC5S2 intact plants
nc
n = 38
4
68%
32%
*
P = 0.0231
Nesidiocoris tenuis
n = 35
6
26%
74%
*
P = 0.0041
Bemisia tabaci
100% 80% 60% 40% 20% 0% 20% 40% 60% 80% 100%
Olfactory response (%)
B
☐ N. tenuis-punctured BC5S2 plants
▨ N. tenuis-punctured MM plants
nc
n = 35
6
69%
31%
*
P = 0.0279
Nesidiocoris tenuis
n = 37
2
32%
68%
*
P = 0.0326
Bemisia tabaci
100% 80% 60% 40% 20% 0% 20% 40% 60% 80% 100%
Olfactory response (%)

836

Fig. 4


A
Relative expression BCKD-E2/Actin
0
1
2
3
c
b
b
a
B
Relative expression BCKD-E2/EF1
0.0
0.5
1.0
1.5
2.0
c
b
b
a
C
Relative expression PIN2/Actin
0
1
2
3
4
b
a
b
a
D
Relative expression PIN2/EF1
0
1
2
3
b
a
b
a
E
Relative expression LoxD/Actin
0
1
2
3
b
a
b
a
F
Relative expression LoxD/EF1
0.0
0.5
1.0
1.5
2.0
b
a
b
a
MM intact plants
N. tenuis-punctured MM plants
BC5S2 intact plants
N. tenuis-punctured BC5S2 plants