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Tomato Inoculation With the Endophytic Strain *Fusarium solani* K Results in Reduced Feeding Damage by the Zoophytophagous Predator *Nesidiocoris tenuis*

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Belowground symbiosis of plants with beneficial microbes is known to confer resistance to aboveground pests such as herbivorous arthropods and pathogens. Similarly, microbe-induced plant responses may also impact natural enemies of pests via the elicitation of plant defense responses and/or alteration of plant quality and growth. *Nesidiocoris tenuis* is a zoophytophagous predator and an efficient biological control agent of greenhouse pests. Its usefulness in plant protection is often hindered by its ability to damage plants at high predator population densities or when prey is scarce. In this study, we investigated the effect of *Fusarium solani* strain K (FsK), an endophytic fungal isolate that colonizes tomato root tissues, on the capability of *N. tenuis* to cause necrotic rings, an easily discernible symptom, on tomato stems and leaves. We found significantly less necrotic rings formed on FsK-inoculated plants for all tomato cultivars tested. FsK has been previously shown to confer ethylene-mediated tomato resistance to both foliar and root fungal pathogens; thus, the ethylene-insensitive *Never ripe* (*Nr*) and *epinastic* (*epi*) tomato plant mutant lines were included in our study to assess the role of ethylene in the recorded FsK-mediated plant damage reduction. The jasmonic acid (JA)-biosynthesis tomato mutant *def-1* was also used since JA is known to mediate major anti-herbivore plant responses. We show that ethylene and JA are required for FsK to efficiently protect tomato plants from *N. tenuis* feeding. No necrotic rings were recorded on FsK-inoculated *epi* plants suggesting that ethylene overproduction may be key to tomato resistance to *N. tenuis* feeding.

Keywords: biological control, endophyte, ethylene, jasmonic acid, plant damage, tomato, zoophytophagous predator

INTRODUCTION

Plants are associated with a vast diversity of microbes that exert beneficial effects on their performance. Soil-borne microbes in particular, such as endophytic fungi, plant growth promoting fungi and rhizobacteria as well as arbuscular mycorrhizae have long been recognized for their benefits to plant growth and nutrition (Smith and Smith, 2011; Hadar and Papadopoulou, 2012; Finkel et al., 2017). In addition, certain root colonizing microbes are known to antagonize soil-borne pathogens and/or prime plant defense against future attackers (Pineda et al., 2010; Pieterse et al., 2014).

Soil-borne beneficial microbes can affect aboveground herbivores both positively and negatively (Hartley and Gange, 2009; Shikano et al., 2017). Improved plant growth and/or nutrition by plant-growth promoting fungi and rhizobacteria have been shown to result in positive effects on herbivore performance (Pineda et al., 2010; Ahemad and Kibret, 2014). On the other hand, defense priming triggered by beneficial microbes, often referred to as Induced Systemic Resistance (ISR), can impact herbivores via direct or indirect defense elicitation (Pineda et al., 2010; Pieterse et al., 2014).

Microbe-ISR is mediated by phytohormones that also control plant defense against herbivores. In particular, ISR is mediated by priming of defense-related genes upon attack and involves an increased sensitivity to jasmonic acid (JA) and ethylene (ET) (Rosenblueth and Martínez-Romero, 2006; Van Wees et al., 2008). JA-mediated plant responses can be directly effective against chewing herbivores but also against phloem feeders, such as aphids and whiteflies, which normally activate the SA-signaling pathway to counteract JA-defenses via crosstalk (Walling, 2000; Schaller, 2008). Ethylene on the other hand is a modulator of the JA and SA signaling pathways in plant defense against pathogens and acts either by synergizing JA or by enabling SA antagonism with JA (Pieterse et al., 2012). To date, very little is known on the role of ET in plant responses against herbivores (Stahl et al., 2018).

Beneficial microbes can, also, impact the so-called plant's indirect defense. Upon herbivore attack plants normally emit a blend of volatiles that attract its natural enemies (Karban and Baldwin, 1997; Dicke and Baldwin, 2010). The JA signaling pathway is the key regulator of this process, suggesting that ISR in microbe-inoculated plants could modify the volatiles emitted in response to herbivory (Pineda et al., 2010). Indeed, selected soil beneficial microbes are capable of altering the composition or the emission rate of this blend and thus the attractiveness of the infested plant to certain predators and parasitoids (e.g., Fontana et al., 2009; Schausberger et al., 2012; Pineda et al., 2013). Nevertheless, besides behavior, plant-mediated effects of beneficial microbes on the performance of natural enemies have only been scarcely addressed so far (e.g., Battaglia et al., 2013; Prieto et al., 2017).

In this regard, zoophytophagous predators are of particular interest as they feed on both plant and prey. *Nesidiocoris tenuis* is one such predator and an efficient biocontrol agent of several plant pests. Nevertheless, it may also cause significant plant damage at high predator population densities or when prey is scarce (Sánchez and Lacasa, 2008; Sanchez, 2009; Arnó et al., 2010; Castañé Cristina et al., 2011) as it can feed on the plant i.e. shoots and petioles, specifically the phloem and neighboring parenchyma cells (Raman and Sanjayan, 1984). Necrotic rings are the externally visible symptoms around the stems and leaf petioles caused by the frequent stylet penetration and tissue sap feeding by *N. tenuis* along the stylet track, which result in wound response, cell necrosis and increased protein content at the feeding site. Besides necrotic rings on stems and leaves, flower abortion and punctures on fruits are the main symptoms related to *N. tenuis* feeding on tomato (Calvo et al., 2009; Arnó et al., 2010; Castañé Cristina et al., 2011).

In this study, we assessed the effects of *Fusarium solani* strain K (FsK) on *N. tenuis*, specifically its ability to cause necrotic rings on tomato plants. FsK is an endophytic fungus isolated from the roots of tomato plants grown on suppressive compost. It colonizes the roots, including vascular tissues but ingress ceases at the root crown area and fungal growth is not detected in aboveground tomato tissues (Kavroulakis et al., 2007, Skiada, unpublished data). FsK has been previously shown to confer resistance not only against root but also foliar plant pathogens in tomato. In addition, it was shown that an intact ethylene signaling pathway was necessary to confer resistance to foliar pathogens by FsK (Kavroulakis et al., 2007), indicating that FsK can induce systemic responses to the plant. We, thus, hypothesized that FsK mediates effectual tomato responses against arthropods that attack aboveground tissues of the plant, too. To explore putative defense mechanisms mediating the effects of FsK on the formation of necrotic rings by *N. tenuis*, ethylene and jasmonate plant mutant lines were used in parallel with their wild type progenitors.

MATERIALS AND METHODS

Fungal Strain

A *F. solani* strain FsK (Kavroulakis et al., 2007) routinely cultured on potato dextrose broth (PDB) at 25°C for 5 days in the dark was used in the experiments. Following removal of mycelium fragments by sieving, conidia were recovered by centrifugation at 4000 g, counted using a haemocytometer and suspended in an appropriate volume of 0.85% NaCl in order to achieve the desired inoculum concentration. Application of the inoculum of strain FsK with 10^4 conidia cm^{-3} of potting mix was performed as water drench 1 week after seed sowing.

Plants

Wild-type tomato (*Solanum lycopersicum*) cultivars Pearson, VFN8 and Castlemart and their mutant lines *Nr*, *epi*, and *def-1*, respectively as well as the commercial cultivar ACE55 were used in this study. The wild type cultivars are the progenitors of the mutant plant lines. *Nr* plants block ethylene perception (Lanahan et al., 1994) whereas *epi* is an ethylene overproducing tomato line (Fujino et al., 1988). *Def-1* plants are deficient in JA accumulation in response to wounding and systemin (Howe et al., 1996). Pearson, VFN8, *Nr* and *epi* seeds were obtained from the Tomato Genetics Resource Center (University of California, Davis). Castlemart and *def-1* seeds were kindly provided by Greg Howe (Michigan State University).

Seeds were surface-sterilized in 2.5% NaOCl and sown directly into 10 cm diameter pots, each containing approximately 300 $cm^3$of peat blended with an NPK fertilizer (20-20-20) to a total concentration of 0.8 g l^{-1} of potting mix. The pots were placed in a climate room with a temperature of 25 ± 1°C, 65 ± 5% relative humidity (RH) and a 16L:8D photoperiod. Plants were regularly watered and once a week fertilized with a balanced nutrient solution which consisted of the following macronutrients (mM): $Ca(NO_3)_2.4H_2O$ (11.1); $NaH_2PO_4.2H_2O$ (0.0094); $Na_2HPO_4.12H_2O$ (0.006); K_2SO_4 (6.410); $MgSO_4.7H_2O$ (3,840); $CaCl_2.2H_2O$ (2); and micronutrients (μM): H_3BO_3 (69); $MnSO_4.4H_2O$(10.4); $ZnSO_4.7H_2O$ (1.2); $CuSO_4.5H_2O$ (1.7); $NaMoO_4.2H_2O$ (0.13); and FeEDDHA (0.3).

Predator

Nesidiocoris tenuis was reared on *Nicotiana tabacum* plants, which can support *N. tenuis* feeding (Calvo et al., 2012; Bueno et al., 2013; Sukhoruchenko et al., 2015). The rearing was initiated with nymphs and adults collected from *Solanum nigrum* plants in the area of Ierapetra, eastern Crete in the summer of 2012, and kept in wooden-framed muslin cages (100 cm length × 50 cm width × 70 cm height) in a climate room with 25 ± 1°C, 65 ± 5% RH and 16L:8D. Eggs of *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) were provided *ad libitum* with a thin brush on the leaves of *N. tabacum* plants as supplemental food for the predator.

Plant Damage Assessment

Three to four weeks-old tomato plants of all plant cultivars were inoculated with FsK as described above and individually transferred in cylindrical net cages (30 cm length × 10 cm diameter). Control (uninoculated) plants received water only. A pair of *N. tenuis* adults (male and female, <1 week old) was introduced in each cage without food or prey so as to be forced to feed on the plant. Total number of necrotic rings on shoot and leaves as well as the number of live predators on each plant were recorded after 1 week. At this time period, no predator nymphs had hatched as anticipated (Martínez-García et al., 2016). All cages were kept in a climate room (25 ± 0.5°C, 65 ± 5% RH, and 16L:8D). Experiments with wild-type (WT) ($n = 10–18$) and mutant lines ($n = 14–17$) were carried out in parallel in two blocks in time.

Quantification of Fungal Colonization by qPCR

FsK colonization of root tissues was verified for all tomato genotypes both in control and *N. tenuis*-exposed plants. Root tissues were collected from four replicates of each treatment 1 week after exposure to *N. tenuis*. Samples were used for whole genomic DNA extraction using the "NuncleoSpin® Plant II genomic DNA extraction" kit (MACHEREY-NAGEL GmbH &Co.KG, Duren, Germany). FsK colonization of root tissues was assessed via qPCR, by using primers pair FFsITS (5′-TGGTCA TTTAGAGGAAGTAA-3′) and RFsITS (5′-GGTATGTTCACA GGGTTGATG−3′), specific for a ca 100 bp fragment of *F. solani* ITS region. An external standard curve was generated in order to quantify the copy number of ITS gene in total DNA extracted from root tissues of FsK-inoculated plants. The standard curve was generated as follows: ITS gene was amplified using FsK genomic DNA as template, the PCR product was purified and ligated into pGEM-T Easy vector (Promega, Madison, USA) and transformed to competent *Escherichia coli* DH5a cells. The recombinant plasmid was extracted again (NucleoSpin Plasmid, Macherey Nagel) and its concentration was determined via Qubit 3.0 Fluorometer. The copy numbers of the targeted gene were calculated from the concentration of the extracted plasmid DNA.

Serial 10-fold dilutions of the recombinant plasmid ranging from 5.9×10^0 to 5.9×10^8 copies/μl were subjected in triplicate to qPCR to construct the standard curve. qPCR amplification efficiencies for the under-study gene were 99.77%, with r^2 value of 0.998 and a slope of −3.327. Amplification occurred in a 10 μl reaction mixture containing Kapa SYBR FAST qPCR Master Mix (1x) Universal, 200 nM of each primer, and 1 μl of DNA, using the following thermocycling protocol: 3 min at 95°C; 45 cycles of 15 s at 95°C, 20 s at 58°C followed by a melting curve to check the specificity of the products. PCR products were furthermore analyzed on a 1.5% agarose gel in order to check for potential non-targeted amplifications.

Statistical Analysis

Two-way analysis of variance (ANOVA) was used to evaluate the effect of tomato cultivar and plant inoculation status (FsK inoculated/non-inoculated) and their interaction on the number of necrotic rings recorded on tomato plants when exposed to *N. tenuis*. Data were log(x+1) transformed to meet the criteria for parametric analysis. Pairwise comparisons by Student's *t*-test were used to compare the number of necrotic rings on wild-type tomato cultivars (ACE55, Castlemart, Pearson, and VFN8) and FsK inoculated or non-inoculated mutants (*def*-1, *Nr,* and *epi*) when exposed to *N. tenuis* as well as to compare FsK colonization levels between *N. tenuis* exposed wild-type plants and their mutants. In the cases homoscedasticity's assumption was not met, the non-parametric Mann-Whitney *U*-test was used. All statistics were performed in SPSS (SPSS, 2011).

RESULTS

Feeding Damage by *Nesidiocoris tenuis* Is Reduced on *Fs*K-Inoculated Plants Irrespectively of the Tomato Cultivar

Different tomato cultivars were used to assess putative cultivar-dependent effects of FsK on plant damage by *N. tenuis*. Plant feeding by the zoophytophagous predator for 1 week produced similar numbers of necrotic rings in all tomato cultivars [$F_{(3,105)} = 0.839$, $P = 0.475$] used in this study (**Figure 1**). Inoculating plants with FsK resulted in a significant reduction in the number of necrotic rings [$F_{(1,105)} = 82.128$, $P = 7.75E^{-15}$] in all cultivars compared to control (non-inoculated) plants (**Figure 1**). The interaction between cultivar and inoculation status (FsK-inoculated/non-inoculated) was not significant [$F_{(3,105)} = 0.013$,

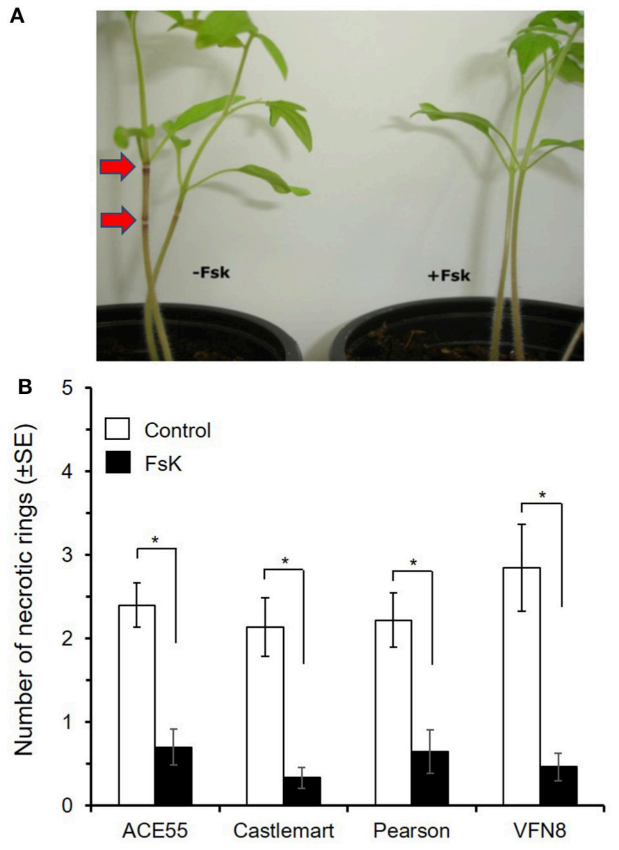

FIGURE 1 | Plant damage by *Nesidiocoris tenuis* is reduced on tomato plants inoculated with *Fusarium solani* strain K (FsK). Tomato FsK-inoculated (black bars, $N = 10–17$) or control (white bars, $N = 10–18$) plants were exposed to *N. tenuis* (one male, one female) for 1 week. **(A)** Necrotic rings (red arrows, left) caused by *N. tenuis* feeding on ACE55 control (-FsK) plants compared to FsK-inoculated (+FsK, right) plants where no symptoms are depicted **(B)** Mean (±SE) total number of necrotic rings on stems and leaves recorded for each tomato cultivar (ACE55, Castlemart, Pearson, VFN8) on day 7. Asterisks indicate significant differences within each cultivar after Student *t*-test ($P < 0.001$).

$P = 0.806$]. No effect was observed on the survival of the predators, which all remained alive at the end of the experiment (100% survival rate).

Ethylene and Jasmonic Acid Are Required for Plant Damage Reduction by *Nesidiocoris tenuis* on FsK-Inoculated Plants

We hypothesized that FsK-mediated tomato resistance to *N. tenuis* feeding may be linked to tomato JA-defenses since these constitute a major anti-herbivore defense (Howe et al., 1996; Karban and Baldwin, 1997; Walling, 2000). In addition, because FsK was previously shown to mediate tomato resistance against pathogens via the ethylene signaling pathway (Kavroulakis et al., 2007), we assumed ethylene might also be essential for FsK-mediated tomato resistance against *N. tenuis*. To test these, we investigated the effect of inoculating tomato mutant plant lines with FsK on the ability of *N. tenuis* to cause necrotic rings, to determine the involvement of the ethylene and jasmonic acid defense pathways in the FsK mode of action.

In the absence of FsK, we found that the numbers of necrotic rings recorded on both *Nr* and *def-1* plants were not significantly different compared to those on their wild-type relatives (Castlemart and Pearson, respectively) (**Figures 2A,B**), while significantly reduced number of rings were observed on *epi* mutant plants (**Figure 2C**). This indicates that although basal levels of ethylene or JA cannot protect tomato plants from the phytophagy, elevated ethylene levels may have a protective role against *N. tenuis* in tomato. In the presence of FsK, inoculated *Nr* and *def-1* plants displayed similar numbers of necrotic rings to non-inoculated mutants (**Figures 2A,B**). The plants of both mutant lines were not affected by the endophyte and the necrotic rings measured were significantly higher than those recorded on FsK-inoculated Castlemart ($t = -3.862$; $df = 28$; $P = 0.0006$) and Pearson ($t = 2.102$; $df = 32$; $P = 0.043$) wild-type plants, respectively (**Figures 2A,B**). In contrast, the presence of the endophyte further increased the response against *N. tenuis* feeding observed in *epi* mutant plants, resulting in significantly less necrotic rings on *epi* compared to wild-type VFN8 plants ($t = -2.744$; $df = 26$; $P = 0.011$). These results suggest that ethylene and jasmonate biosynthesis and signaling are essential for the expression of the FsK-mediated reduction of plant damage caused by *N. tenuis*.

To investigate the possibility that the differences observed in the activity of FsK in the various mutant plant lines could be attributed to a colonization efficiency of the FsK in these genotypes, we estimated by quantitative PCR the colonization levels of FsK in all tomato cultivars at the time of sampling. No significant differences were recorded in FsK colonization levels of tomato cultivars in all combinations [Pearson vs. *Nr*: $U = 7$, $P = 0.773$; VNF8 vs. *epi*: $t_{(5.96)} = -0.78$; $P = 0.465$; Castlemart vs. *def-1*: $t_{(3.74)} = 1.09$; $P = 0.341$]. Thus, the recorded reduction in the *N. tenuis*-caused plant damage could not be related to the colonization efficiency of FsK.

DISCUSSION

Microbes are considered capable of affecting plant-arthropod interactions (Hartley and Gange, 2009; Shikano et al., 2017). Induced plant responses by multiple biocontrol agents, such as zoophytophagous predators and soil-borne beneficial microbes may be mediated by interacting plant signaling pathways (Pappas et al., 2017). In this study, we report a mutualistic relationship between tomato and the fungal endophyte *F. solani* strain K (FsK), shown herein to mediate resistance to plant damage caused by the zoophytophagous predator *N. tenuis*. In addition, our data show that ethylene and jasmonic acid are required for the endophyte to effectively protect tomato, whereas ethylene overproduction results in null damage by *N. tenuis*.

Feeding intensity by *N. tenuis* is known to be affected by abiotic conditions (e.g., temperature) but also prey availability, with necrotic ring number increasing when prey is scarce, and vice versa (Arnó et al., 2006, 2010; Sanchez, 2008; Calvo et al.,

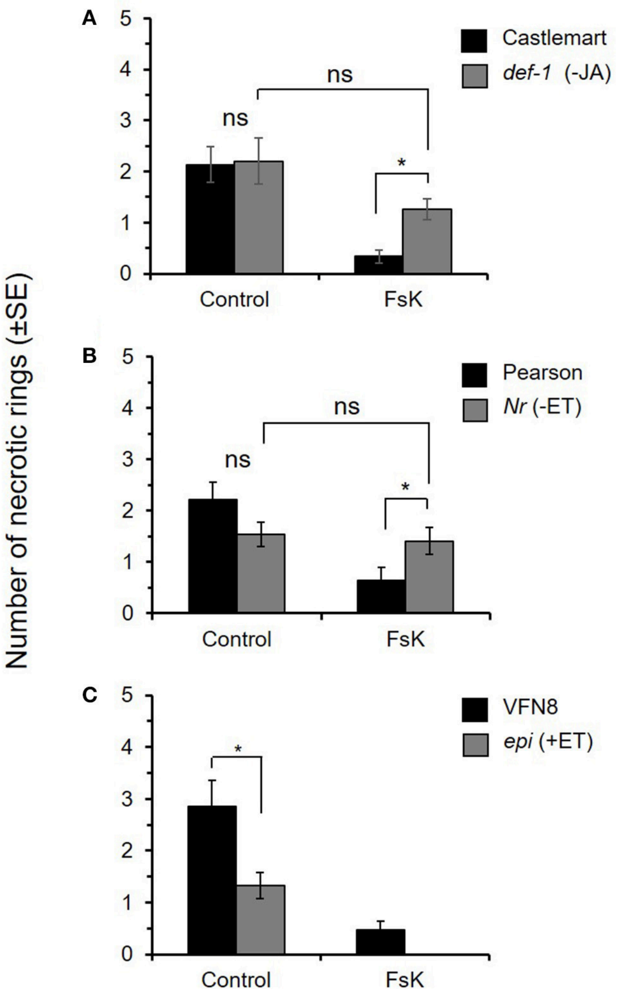

FIGURE 2 | Ethylene and jasmonate signaling is essential for the expression of *Fusarium solani* strain K (FsK) systemic effects on plant damage by *Nesidiocoris tenuis*. FsK-inoculated (FsK, $N = 14–17$) or non-inoculated (Control, $N = 13–18$) tomato plants were exposed to *N. tenuis* (one male, one female) for 1 week. Mean (±SE) total number of necrotic rings on stems and leaves on **(A)** Castlemart wild-type and JA-biosynthesis *def-1* mutant, **(B)** Pearson wild-type and ethylene-insensitive *Nr* mutant and **(C)** VFN8 wild-type and ethylene overproducing *epi* mutant. Bars depicting numbers of necrotic rings on WT plants (except ACE55) are the same as those shown in **Figure 1**. Asterisks indicate significant differences ($P < 0.05$); ns, not significant.

2009). In addition, specific tomato cultivars suffer more damage by *N. tenuis* than others (Pérez-Hedo and Urbaneja, 2016), suggesting that symptom intensity may also be related to plant traits. In our study, no cultivar-dependent difference on the symptoms developed was found and intensity of plant damage caused by *N. tenuis* was similar on all wild-type tomato cultivars tested. Moreover, FsK inoculation resulted in similar reduction in the number of necrotic rings across all wild-type tomato cultivars. In addition, no prey was available for the predators and experiments were conducted under controlled environmental conditions, suggesting that mainly plant-related factors should have affected *N. tenuis* ability to cause less necrotic rings. The fact that no differences were recorded between cultivars when FsK was present suggests the involvement of similar mechanisms mediating tomato resistance to *N. tenuis* feeding across all cultivars.

Reduction of feeding damage by *N. tenuis* on FsK-inoculated plants may be related with tomato resistance mechanisms. For example, antixenosis and/or antibiosis could be involved when *N. tenuis* is reluctant to feed on the plant due to the induction of plant defense-related responses or changes in plant nutritional quality by FsK. On the other hand, FsK-inoculated plants may display increased tolerance via accelerated healing of symptoms caused by *N. tenuis* feeding. The latter was shown for necrotic rings that completely disappeared after exposing tomato plants to *N. tenuis* only temporarily, for a few days (Arnó et al., 2006, 2010). Thus, antixenosis, antibiosis and/or tolerance may be involved in FsK-mediated tomato resistance to *N. tenuis* feeding but this needs to be further explored. In our study, all predators introduced to the control and the FsK-inoculated plants survived at the end of the experimental period, indicating that either the changes in plant response conferred by FsK have no direct impact on the predator or *N. tenuis* was not affected for the experimental period of this work. A more detailed investigation into the performance and feeding preferences of the predator will be needed to address the effects on the predator.

The prominent role of ethylene-mediated tomato responses in its interaction with *N. tenuis*, is clearly depicted by the significant reduction of necrotic rings in the ethylene overproducing *epi* mutant plants. This effect was evidently amplified by the presence of the endophyte and resulted in augmented tomato resistance against *N. tenuis,* since no rings were detected on FsK-inoculated *epi* plants. It is not known whether the endophyte is capable of inducing ethylene production in the plant and, thereby, further enhancing the positive impact of elevated levels of ethylene against *N. tenuis*. This putative mode of action resembles the reported induction of ethylene biosynthesis as a mechanism of plant protection against root-knot nematodes by *Trichoderma harzianum* (Leonetti et al., 2017). A focused study on the effect of FsK colonization on ethylene biosynthesis and signaling pathway, which would involve measurements of hormonal levels in plant tissues, will provide further insight on this mode of action.

On the other hand, both an intact ethylene and jasmonic acid pathway is shown to be essential for the expression of FsK-mediated resistance to *N. tenuis* feeding in this study. Our previous results show that FsK is able to colonize the root of tomato plants (Kavroulakis et al., 2007) and we have not been able to detect fungal ingress in the stems and leaves of the plant under our experimental conditions. Thus, a systemic effect of FsK on hormonal balance is anticipated. Hormone crosstalk is a well-established mechanism of plant resistance against pathogens and herbivores. Although for arthropods there is no general model that can describe the type of regulation exerted by the hormonal pathways and there is a strong influence of the feeding guild, JA signaling appears to be central to plant resistance against arthropods (Stahl et al., 2018). Ethylene, as a modulator of JA and SA signaling pathways has been shown to act by synergizing JA or enabling SA antagonism with JA and, thus, to variably impact arthropods studied so far (Pieterse et al., 2012; Stahl et al., 2018). FsK inoculation did not increase resistance against *N. tenuis* feeding neither in the ethylene perception-deficient *Nr* nor the jasmonic-deficient *def-1* mutant plants. This indicates towards a synergistic role between ethylene and JA in this case. In this regard, ethylene involvement in SA antagonism to JA cannot be concluded by the present study; further studies including the SA-deficient transgenic *nahG* tomato line are needed to study the putative involvement of SA in this tripartite interaction. Finally, we have not observed any differences in the capability of FsK to colonize the various mutant genotypes when compared with the progenitor plant lines. This suggests that the endophyte triggers a systemic response in the plant, which is not related to its colonization level or to its physical presence and interaction with *N. tenuis*.

Zoophytophagous predators such as *N. tenuis* and *Macrolophus pygmaeus* are known to induce JA defenses in response to their phytophagy on tomato (Pappas et al., 2015, 2016; Pérez-Hedo et al., 2015a,b). Nevertheless, relatively little is known about the effects of JA- or SA-mediated plant responses on their performance and behavior. The expression of proteinase inhibitors, known to be induced by wounding, was recently shown not to affect the development and survival of *N. tenuis* in barley (Hamza et al., 2018). On the other hand, *Podisus maculiventris* preferred jasmonate-insensitive plants and their survival was higher on these compared to jasmonate-overexpressing plants (Thaler et al., 2015). Finally, *M. pygmaeus* development was shown to be positively affected by tomato inoculation with *Trichoderma longibrachiatum* strain MK1, which also increased plants attractiveness to this predator possibly via the involvement of both the SA and JA signaling pathways (Battaglia et al., 2013). To date, no study has ever explored JA/SA-mediated responses on *N. tenuis* feeding behavior nor the underlying mechanisms involved in zoophytophagous predator-plant-microbe interactions.

We conclude that inoculating tomato plants with FsK results in significant reduction in plant damage caused by *N. tenuis* feeding. In addition, we show evidence for the involvement of the ethylene and JA signaling pathways in FsK-mediated tomato resistance to *N. tenuis*. The ecological implications of these results are highly relevant to biological control because tomato association with FsK is shown to provide substantial benefits to the plant by conferring resistance not only to pathogens but also against arthropods. Plant damage caused by *N. tenuis* feeding poses an important limitation in the use of an otherwise highly efficient biocontrol agent, when prey is scarce or at high predator populations. The fact that FsK negatively affects *N. tenuis* feeding damage on plants is promising but needs to be further explored by considering effects on performance and predation efficiency, also in the presence of prey. In this regard, it is important to understand the regulatory mechanisms involved in FsK-mediated resistance to *N. tenuis* in tomato.

AUTHOR CONTRIBUTIONS

NK, KV, and KP designed the study. NK, KV, NG, and VS performed experiments. NK, KV, MP, GB, and KP analyzed data. NK, MP, KV, and KP wrote the paper, with contribution from all authors.

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Conflict of Interest Statement: Fusarium solani FsK is patented (20070100563/1006119, issued by the Industrial Property Organization to NK, KP).

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.