JOSE JAHIR MORALES MURILLO

INTERACTION OF TOMATO PLANTS INFECTED BY THE NEMATODE *Meloidogyne javanica* ON ABOVEGROUND HERBIVORE *Tetranychus urticae* AND ON BEHAVIOUR OF ITS PREDATOR *Phytoseiulus macropilis*

Dissertation submitted to the Entomology Graduate Program of the Universidade Federal de Viçosa in partial fulfillment of therequirements for the degree of *Magister Scientiae*.

Adviser: Angelo Pallini Filho

VIÇOSA - MINAS GERAIS
2021

Ficha catalográfica elaborada pela Biblioteca Central da Universidade Federal de Viçosa - Campus Viçosa

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M828i
2021

Morales Murillo, Jose Jahir, 1992-

Interaction of tomato plants infected by the nematode *Meloidogyne javanica* on aboveground herbivore *Tetranychus urticae* and on behaviour of its predator *Phytoseiulus macropilis* / Jose Jahir Morales Murillo. – Vicosa, MG, 2021.

1 dissertação eletrônica (52 f.): il. (algumas color.).

Orientador: Ângelo Pallini Filho.

Dissertação (mestrado) - Universidade Federal de Viçosa, Departamento de Entomologia, 2021.

Inclui bibliografia.

DOI: https://doi.org/10.47328/ufvbbt.2022.155

Modo de acesso: World Wide Web.

1. Nematoda em plantas. 2. Relação hospedeiro-parasito. 3. Herbivoria. 4. Relação inseto-planta. 5. Tomate - Doenças e pragas . I. Filho, Ângelo Pallini, 1965-. II. Universidade Federal de Viçosa. Departamento de Entomologia. Programa de Pós-Graduação em Entomologia. III. Título.

CDD 22. ed. 632.6257

Bibliotecário(a) responsável: Alice Regina Pinto CRB6 2523

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APPROVED: October 15, 2021.

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ACKNOWLEDGEMENTS

Quero agradecer a Deus por me permitir chegar até este momento e pela sabedoriaque adquiri.

Agradeço ao meu pai pelos conselhos de todos os dias e sua típica frase "resignación y paciencia" sem ele essa grande conquista não seria a mesma. Minha mãe pelas orações, ternura e todo aquele carinho que me fazem uma pessoa melhora cada dia. Ao meu irmão por seu apoio incondicional e por ser aquele grande amigo verdadeiro que sempre está para mim em qualquer momento. Amo vocês.

Ao Professor Angelo pela confiança desde que escrevi o primeiro e-mail solicitandoo estágio e por todos os conhecimentos adquiridos.

Ao Professor Arne por todos os ensinamentos.

Ao Coorientador Douglas Ferreira Parreira pela paciência e ensinamento. Ao professor Leandro Grassi pela disposição do laboratório de Nematologia.

À Cassia pela ajuda na inoculação dos nematoides e todos os protocolos para desenvolver os experimentos. Ao pessoal do laboratório de Nematologia pelo espaço e ajuda na inoculação dos nematoides.

Ao Ítalo por ser minha mão direita na montagem e avaliação dos experimentos e por se tornar meu irmão adotivo e me dar o sobrinho mais lindo do mundo. A Vanessa e o Leo pela ajuda na montagem e avaliações dos experimentos, trabalharsozinho é bem complexo.

Ao Vinicius por ser meu amigo nos roles e por sempre estar disposto a me escutar quando precisava.

A todos os companheiros do Laboratório de Acarologia: Adriana, André, Milena, Júlia e o Pedro por tantos bons momentos. Também a Paolita e o Aldo pelos risos e brincadeiras.

Minha família Viçosense-colombiana: Kathe e Deysi minhas irmãs; Isa, Jhon, Daniele Mancho. Sem o Rastaman eu não tinha vivido toda esta experiência.

Ao Jhon e o Dayvson pelas noites de ajuda fazendo a discussão a parte mais difícilpara mim. Ao Weslei e a Thay pela forca, bons momentos e bons roles.

O Walysson, o Pablo, a Maribel e a Vivi por serem essa amizade para toda avida.

Ao melhor grupo de estudos Insectum por todos os aprendizados.

Aos professores e alunos do programa de Pós-Graduação em Entomologia por todos ensinamentos, em especial a minha turma 2019-1 a melhor e mais unida quejá passou pela Entomologia.

À Universidade Federal de Viçosa e ao Programa de Pós-Graduação em Entomologia pela oportunidade e pela qualidade institucional.

Ao Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), a Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) e a Fundação de Amparo à Pesquisa de Minas Gerais (FAPEMIG) pela bolsa de estudoe o financiamento do projeto.

“Aquele que fala mais não é o mais esperto, mas sim
aquele que é mais quieto”
Proverbio Chinês

ABSTRACT

MURILLO, Jose Jahir Morales, M.Sc., Universidade Federal de Viçosa, October, 2021. **Interaction of tomato plants infected by the nematode *Meloidogyne javanica* on aboveground herbivore *Tetranychus urticae* and on behaviour of its predator *Phytoseiulus macropilis***. Adviser: Angelo Pallini Filho.

Tritrophic interactions play an important role in the dynamics of arthropods with their hosts. By inducing defenses, plants respond to herbivore attacks, may affect your development. The general objective of this research was to investigate the effect of the nematode *Meloidogyne javanica* on the performance of the herbivore *Tetranychus urticae* in tomato plants and, subsequently, whether the nematode infestation affectedthe behavior of its predator *Phytoseiulus macropilis*. In the first experiment with the herbivore, tomato plants were infested with 2,000 eggs of the nematode *M. javanica* for a period of 40 days. The immature development, oviposition and preference for choosing plants with or without the nematode were evaluated. A second experiment was carried out with different levels of nematode eggs' infestation (1,000 and 6,000) atdifferent exposure times (15, 30, and 45 days after infestation). In this experiment, onlyimmature development and survival were evaluated. The spider mite in the first experiment did not show significant differences on immature development and survival,oviposition and preference. For the second experiment, the development of theherbivore *T. urticae* was affected, with high mortality compared to the control at 30 and45 days after nematode infestation. For the predatory mite, preference experiments evaluated plants attacked simultaneously by the herbivore mite and the nematode or only by the herbivore mite. The predatory mite showed no preference for plants inoculated with the nematode, however, more eggs were found in these plants. The results of this study indicated that with high initial infestation and over time of exposureto the pathogen, the quality of the plant is affected and, consequently, the performanceof aboveground herbivores. The attraction of more predators to plants with nematode infestation increased after 24 hours, which may have varied the number of females that oviposited in this treatment. Thus, it is important to understand the effect on plants when they are attacked by spatially separated organisms above and below ground. In the same way, knowing the importance of direct and indirect interactions in ecological communities it is possible to develop new management techniques andstrategies for crop protection.

Keywords: Tritrophic interactions. Root system. Aboveground herbivory. Induced response.

RESUMO

MURILLO, Jose Jahir Morales, M.Sc., Universidade Federal de Viçosa, outubro de 2021. **Interação de plantas de tomates infectadas pelo nematoide *Meloidogyne javanica* sobre o herbívoro *Tetranychus urticae* e o comportamento de seu predador *Phytoseiulus macropilis***. Orientador: Angelo Pallini Filho.

As interações tritróficas desempenham um papel importante na dinâmica de artrópodes com seus hospedeiros. Através da indução de defesas, as plantas respondem aos ataques de herbívoros podendo afetar seu desenvolvimento. O objetivo geral desta pesquisa foi investigar o efeito do nematoide *Meloidogyne javanica* sobre o desempenho do ácaro herbívoro *Tetranychus urticae* em plantas detomate e, posteriormente, observar se a infestação do nematoide é capaz de afetar o comportamento do ácaro predador *Phytoseiulus macropilis*. No primeiro experimento com o herbívoro foi feita a infestação de plantas de tomate com 2,000 ovos do nematoide *Meloidogyne javanica* por um período de 40 dias. Foram avaliados o desenvolvimento dos imaturos, a oviposição e a preferência de escolha por plantas com ou sem o nematoide. Um segundo experimento foi realizado com diferentes níveis de infestação de ovos do nematoide (1,000 e 6.000) em diferentes tempos de exposição (15, 30, e 45 dias após a infestação). Nesse experimento foi avaliado o desenvolvimento imaturo e sobrevivência. No primeiro experimento não foram observadas diferenças significativas no desenvolvimento dos imaturos e sobrevivência, oviposição e preferência de *T. urticae*. Para o segundo experimento odesenvolvimento do herbívoro *T. urticae* foi afetado, apresentando alta mortalidade em relação ao controle com 30 e 45 dias após a infestação do nematoide. Para o ácaro predador, foram avaliadas as preferências entre plantas atacadas simultaneamente pelo ácaro rajado e o nematoide ou apenas pelo ácaro rajado. O ácaro predador não mostrou preferência por plantas com infestação do nematoide, entretanto, uma maior quantidade de ovos foi encontrada nestas plantas. Os resultados deste estudo indicam que, com alta infestação inicial e ao longo do tempode exposição ao patógeno, a qualidade da planta é afetada e consequentemente a performance dos herbívoros. A atração de mais predadores nas plantas com infestação do nematoide aumentou após 24 horas, o que pode ter variado o númerode fêmeas que ovipositaram neste tratamento. Assim, é importante compreender o Efeito nas plantas quando são atacadas por organismos separados espacialmente acima e abaixo do solo, e com isso escrutinar a importância das interações diretas e indiretas nas comunidades ecológicas que possam contribuir ao desenvolvimento detécnicas de manejo e proteção de plantas.

Palavras-chave: Interações tritróficas. Sistema radicular. Herbívoria. Respostas induzidas.

SUMMARY

GENERAL INTRODUCTION

The natural ecosystems are composed of three trophic levels that interact: plants, herbivores and natural enemies of herbivores (Price, et al 1980). This interaction notonly has an effect on plants, but also on natural enemies. These effects involve the defense of plants against attack by herbivores. (Holtz, 2019). The attack of arthropods can affect the plant primary metabolism and resource allocation (Biere &Goverse, 2016) and at the same time can trigger different types of defenses againstherbivores (Freeman & Beattie, 2008a)

Thus, plants after being attacked by herbivores, show changes in their secondary metabolism (Schoonhoven et al., 2005a). These changes are mainly therelease of terpenoids compounds and derivatives of fatty acids, among other secondary metabolites (Mumm & Dicke, 2010). Secondary metabolites are called herbivore-induced plant volatiles (HIPVS) and these chemicals attract beneficial predators and can also repel harmful insects (Freeman & Beattie, 2008b).

The preference and performance of phytophagous invertebrates for plant hosts is affected by the nutrition provided by the plants, as well as by chemical toxins and morphology (Gong et al., 2018). For predators, the outcome of reproductive success will depend on variation in prey selection behavior, which results in quantitative and/or qualitative differences in food intake (Dicke et al., 1988).

For example, when comparing the olfactory responses of volatiles from strawberry (*Fragaria sp.*) cultivars infested with the two spotted-spider mite

Tetranychus urticae Koch (Acari: Tetranychidae) and non-infested plants, it was found that predators prefer infested plants (Rezaie & Baniameri, 2018). Other authors like (Sabelis & Van De Baan, 1983) revealed that volatiles, Kairomones, emitted from apple (*Malus domestica*) leaves infested by two- spotted mites *T. urticae* attracted the predator *Phytoseiulus persimilis (*Acari: Phytoseiidae)*.*

The preference and selection for host plants for herbivores will influence cropyield, in this case the production of tomato (*Solanum lycopersicum L.*) is limited by abiotic and biotic factors. Among the biotic factors, there are pests and diseases that lead to low yields (Najjuuko, 2021). The two-spotted spider mite *T. urticae* is one of the most important agricultural pests in the world with a wide range of host plants, including the tomato crop (Tak & Isman, 2017). This phytophagous mite pierces the parenchymal cells of the plants and suck the nutrients using their stylets, achieving severe chlorosis in the plant which results in the reduction of the total yield of the plant (Park & Lee, 2002).

In addition to the phytophagous mite attacks in the aerial part, the tomato's rootis also affected by root-knot nematodes (*Meloidogyne* spp.). The root-knot nematodeis a harmful tomato pathogen that causes more than 27% of yield losses (Saad et al., 2017). The primary symptoms are related to the formation of protuberances, nodules or galls on the small roots, which restrict the movement of water and nutrients to the plant (Cardona et al., 2016). Plant parasitic nematodes and root- shoot interactions are mediated by plant-induced chemicals and can have profound effects on the performance of different herbivores (Van Dam et al., 2005a).

Root-feeding nematodes can also influence aboveground insects through theireffects on the shared plant host (Bezemer et al., 2005; Kaplan et al., 2011). It is relevant to mention that leaf-root interactions have important ecological consequences on herbivores above and belowground (Kaplan et al., 2008)*.* In the rhizosphere a variety of interactions produce changes in the physical and chemical properties of soil. It has been observed that underground root herbivory by nematodes in tomatoes allows the increase of leaves' defenses and reduces oviposition and the performance of the insects that damage the leave like leaf-miners(Arce et al., 2017).

However, the root-shoot interactions produced by changes in induced defenses also occur between above and belowground organisms (Papadopoulos &van Dam, 2017). An example is the interaction system between *Meloidogyne incognita* and *Myzus persicae*. This nematode caused 55 and 72 % decrease in aphid *Myzus persicae* growth and fecundity, respectively. On the other hand, aphidsdid not affect nematode performance (Kaplan, et al., 2011).

Other studies have shown reduced larval development time and adult body weight of the tobacco cutworm *Spodoptera litura* when feeding on plants inoculated with the soybean root cyst nematode *Heterodera glycines* (Li & Meng,2017). Theseworks confirm the existence of interactions between pests that attack the plant simultaneously, causing changes in its performance.

The organisms in the third (arthropod predators and parasitoids) and higher trophic levels eg hyperparasitoids may be affected by Plants (Dicke, 1999; Han et al.2019). The reason is that some traits of plants affect the quality (nutritional) of the herbivore that is finally consumed by natural enemies (Schoonhoven et al., 2005b).The *Phytoseiulus macropilis* Banks, 1904 (Acari:

Phytoseiidae) is a natural enemyand biological control agent able to reduce *T. urticae* populations in tomato crops under greenhouse conditions (Gigon et al., 2016), on strawberry plants under greenhouse conditions, (Vacacela Ajila et al., 2019, Costa et al., 2012), sweet potato (Castro et al., 2014), sweet pepper and citrus (Reis et al., 2000), among others.

In tomato plants was found that the behavior of *P. macropilis* was similar to that of *Phytoseiulus longipes* Evans and that the trichomes hindered locomotion as well as the consumption of prey and the oviposition rate of both predators (Sato et al., 2011). According to the literature, *P. macropilis* has a higher predation rate than *Phytoseiulus persimilis* Athias-Henriot. (Charles and White, 1988) (Oliveira et al., 2007). These results suggest that *P. macropilis* is a promising candidate to control two-spotted spider mites, which is why it is used in our experiment.

Thus, the general objective of this study was to evaluate whether tomato plantsinfested at the root by the nematode *M. javanica* affect the performance and choice preference of the herbivorous mite *T. urticae* and the predator *P. macropilis,* respectively. Therefore, In the first chapter, we evaluated whether the herbivorous mite

T. urticae prefers plants infested or not with different doses of eggs of *M. javanica* at different exposure times and if your immature development, oviposition rate and choice preference would be affected. In the second chapter, we evaluated whether the inoculation of *M. javanica* has any effect on the choice preference of the predator *P. macropilis*. The results of this study help to improve our knowledge base on the possible secondary effects of infestation by two spatially separated pests in systems where there is a tritrophic interaction.

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CHAPTER 1

THE EFFECT OF ROOT-KNOT NEMATODE *Meloidogyne javanica* IN THEPERFORMANCE OF THE SPIDER MITE *Tetranychus urticae*

ABSTRACT

The phytophagous mite *Tetranychus urticae* is an important cosmopolitan pest that causes great annual losses in crops and there are several methods used for its control including the use of predatory mites. To better understand the tritrophic interaction, weevaluated the immature development and survival, oviposition rate and choice preference of *T. urticae* when feeding on tomato leaf discs from plants previously infested by the root-knot nematode *Meloidogyne javanica* or plants without infestation. When the mite fed on leaf discs of tomato plants inoculated with differentamounts of infestation of the nematode (6,000 and 1,000eggs) at different time of exposure (15,30 and 45 days), the immature development presented a lower cumulative proportion of adults for days 30 and 45. There was no significant difference in the choice of herbivores for plants infested or not by the nematode. Thisresult showed that when a plant is infested with nematode at different exposure time,the immature development of aboveground *T. urticae* is affected.

Keywords: Host choice. Juvenile development and survival. Oviposition rate.

Introduction

The co-evolution between plants and herbivores has been ongoing for 400 million years (Labandeira, 2013) and plants developed strategies to avoid the attack of herbivores by combination of constitutive and inducible defenses. Nevertheless, herbivore pests employ different morphological and biochemical defensive strategiesto overcome the defenses of the plant (War et al., 2018).

Plants are attacked by pathogens and herbivores with a wide range of lifestyles, both on above and belowground. These indirect interactions may involve changes in the primary and secondary metabolites within the host plant if it isshared by various pathogens (Castagneyrol et al., 2018). The two-spotted spider mite *Tetranychus urticae* Koch (Acari: Tetranychidae) is the most important aboveground pest that feeds on thousands of host plants (Helle &Sabelis, 1985; Migeon et al., 2010). Hence, the great adaptability of this pest to different host plantshas already been demonstrated (Fry, 1989; Sousa et al.,2019), which leads to presenting itself as a serious problem in agricultural crops.

In the belowground level more than 2,000 species of plants are used as hosts by species of the genus *Meloidogyne* spp. known as root-knot nematode (Bernardo et al., 2011). These nematodes cause damage to the roots of the host, which leads to invasion of secondary pathogens (Caboni et al., 2016, 2015). Root attack forces responses on the plant in terms of changes in biomass, gene expression and the production of secondary metabolites (Johnson et al., 2016). Root–shoot interactionsmediated by induced plant chemistry may have profound effects on the performanceof herbivores (Van Dam, Raaijmakers, & Van Der Putten, 2005a).

Root-induced responses affect the effectiveness of induced defense responses and alter multitrophic interactions on the leaves (Bezemer & Van Dam, 2005). It has been documented in the literature how the interactions of species in the rhizosphere can influence various processes that improve the resistance of plants tobiotic and abiotic stress (Bais et al., 2006). Interactions on the aboveground cannot be observed in the same way as at the root level where the interactions occur on a smaller scale (Van Der Putten, 2001). The multiple interactions among insects,pathogens, and their host plants can increase the incidence and severity of host injury,influencing plant yield and mortality.

For example, one study showed that a root-eating flea beetle *Longitarsus bethae* performed better on *Lantana camara* plants infected with *M. javanica* than onplants with healthy roots (Musedeli, 2020). On the other hand, other study showed that soybean cyst root nematode *Heterodera glycine* negatively affects the above- ground leaf-chewing *Spodoptera litura* by increasing the developmental time and decreasing adult body weight (Li & Meng, 2017).

The interactions between aerial and subterranean pathogens identified the following two deficiencies that require further investigation: (1) Not only study the simpleinteractions of species but to incorporate more complexity to the communities with interactions of more than three organisms in the same system, and (2) make the transition from laboratory studies to research based on field experiments (Kaplan, Sardanelli, & Denno, 2009a). To know if there is any effect on the plant-root-herbivorerelationship and the effect caused by the root infection by the nematode on the behavior and host choice of aboveground herbivores, the objective of the present study was to evaluate whether the infection of the nematode *M. javanica* on tomato plants would affect the performance of the herbivorous spider mite *T. urticae.*

Material and Methods

Plant Material

Tomato seeds (*Solanum lycopersicum* var. Santa Clara I-5300) were sown in polystyrene trays with 100 cells with commercial substrate (Mecplant®). After 15 days, the plants were transplanted to sterilized soil and transferred to 2-liter pot with 1:1 ratio of soil and sand. Plants were watered daily and fertilized weekly with a mixture of 50 g of N-P-K (20-05-20) and 100 g of simple superphosphate dissolved in 20 L of water (Lemos et al., 2015). The plants were left in the early stages of growth in a cage (1 m x 1 m x 0.70 m). When the plants reached a height of approximately 1m,they were taken to an acclimatized room at 28 ± 2 ° C, 60 ± 10% RH. The seeds of Jack bean plants were cultivated in plastic pots (2 L) filled with commercial substrate(Mecplant®, Brazil) in greenhouse by 21 days old. When the plants had two fully developed leaves, they were used for the rearing of mites in the experiments.

***Tetranychus urticae* rearing**

The two-spotted spider mite was reared on jack bean plants (*Canavalia ensiformis* L.) DC; Sementes Caiçara®, Brazil). The cultures of spider mites were started with individuals obtained from a commercial company (Ecotrix Biodefensivos Pesquisa &Consultoria, Viçosa, MG, Brazil). Plants were replacing every two weeks. Fifty femaleswere separated and placed on detached tomato leaves, of which the leaf petiole wasinserted into a PVC tube with water to maintain leaf turgor. The mites were left for approximately 30 generations. New tomato leaves were placed in the PVC tubes every two days a week. The rearings was kept in a room under controlled conditions(25 ± 2 ºC, 70 ± 10% RH, 12 hours of light).

Nematode Rearing

The initial inoculum for the nematode rearings was provided by the Laboratory for Biological Control of Phytonematodes (Federal University of Viçosa, Viçosa, Brazil).The nematodes were reared on tomato roots of the cultivar Santa Clara I-5300. Usingthe technique adapted by Boneti & Ferraz (1981), roots were harvested and the eggsproduced were washed with 1% sodium hypochlorite. The resulting egg suspensionwas passed through a 20 µm mesh sieve, subsequently washed with abundant tap water. Eggs were suspended in water and used in all experiments.

Experimental unit

For the immature development and oviposition experiments, the experimental units used consisted of black plastic Petri dishes (Ø = 5 cm, h = 1.5 cm). Tomato leaf discswere placed on a water-saturated cotton disc in the experimental units. The leaf discwas placed with the abaxial side facing up. Previous tests were carried out to assessthe durability of the discs. On the fourth day of evaluation, all discs were replaced. Each experimental unit was placed on a tray and kept in a climate-controlled cabinet(25 ± 2 ºC, 70 ± 10% RH, 12 hours of photophase).

Primary Soil infestation

Tomato plants were infested with approximately 2,000 eggs of *M. javanica* applied in the root zone after 5 days of plant transplantation. The infestation was carried out in holes (2 cm) around each tomato plant. After forty days, when root galls are usuallyvisible to the naked eye, the plants were used for the experiments. Control plants received only water and fertilization.

Immature development and survival

To evaluate the suitability of the host plant for tetranychid mites, it is common to estimate the intrinsic growth rate (r_m), performing a complete life table study (Krips et al., 1998, Ferrero et al., 2007, Razmjou et al., 2009). There are, however, other different methods to the life table study such as the assessment of oviposition rate during peak of oviposition and immature survival to adulthood (Janssen & Sabelis, 1992, Sarmento et al., 2011).

Newly emerged *T. urticae* larvae were removed from the colonies rearings andindividually placed in the experimental units. Each treatment (plants with and without inoculation of nematodes) consisted of 20 replicates with 3 pseudo-replicates. Experimental units were left in plastic trays and stored in a climate cabinet (25 ± 1°C, 70 ± 10% RH, 12:12 L: D). Immature development rate and survival were monitored daily until the individual reached adulthood or had died. Data on the effectof infected plants by nematodes on developmental and survival time were analyzed with cox proportional Hazards model of the "survival" package (Therneau, 2013) in R v.4.0.5 (R Core Team 2021).

Oviposition

For each replicate, a single 10-12 days old (from egg wave) adult female of *T. urticae* was taken from the rearing and placed on an experimental unit. During five days, theoviposition was recorded daily. But oviposition on the first day was not included in theanalysis due to the effect of the diet of the previous days (Sabelis, 1990). Eggs wereremoved daily to avoid counting the eggs laid the day before. Leaf discs were checkedevery day for survival of the mites and oviposition rates were corrected for mortality.The experimental units were kept in a climatic cabinet (25 ± 1 °C, 70 ± 10% RH, 12:12 L: D). Using © RStudio (https://www.rstudio.com) trial version, the effect of nematode infected plants on *T. urticae* oviposition was evaluated with a linear model of mixed, effects with treatment and time as fixed factors and individuals as a randomfactor to correct for repeated measures. The contrasts between treatments were evaluated with Tukey's method with the lsmeans package.

Host plant choice by two-spotted spider mites

In this experiment, female mites were given a choice between leaf discs of plants infested with 2,000 eggs of nematode *M. javanica* for 40 days or plants without infestation of nematodes (control). The experiment was carried out in arenas formedby tomato leaf discs (diameter = 3 cm) changing leaf discs around the fourth day when deterioration started. Leaf discs were fixed with the abaxial side up, each one placed in an agar / water solution (1 g of agar in 10 mL of water) in a Petri dish (6.0 x 1.5 cm) (Vacacela Ajila et al., 2019), which was joined by another disc of PVC sheets (diameter = 3 cm and 0.5 cm wide). Immediately after the solution solidifies and dries in the Petri dish, the excess solution was removed leaving only the outline of the tomato leaf disc floating in the center and the space was filled with distilled water to prevent the mites from escaping and keep the leaf disc hydrated and turgid. In each treatment, fifteen replications were used. Adult females were released in themiddle of the bridge and if after 5 minutes the mite did not respond by going to the right or left side, it was changed to another. Two blocks of experiments were carriedout, with an average of two independent replicate with 60 and 45 females. The dataon the effect of the different treatments were analyzed using the generalized linear model (GLM) with quasi-Poisson error distribution to correct for over-dispersion (Crawley, 2007).

Secondary soil infestation

Based on the primary soil infestation, a secondary soil infestation was carried out to verify if the plant response is presented differently in terms of inoculum quantity and exposure time. Different levels of infestation by *M. javanica* eggs (1,000 and6,000) on different days after the infestation (15, 30 and 45) and control were evaluated. The plants at time 1 (15 days after the infestation) did not have sufficient vegetativedevelopment and for this reason a different diameter than that used in primary soil infestation was used for each leaf disc. In this experiment, immature development and survival evaluations were carried out until the moment of the individuals reachedadult or die. Oviposition experiments are not carried out due to the high mortality of adults that occurs in the experimental units of the control, so there can be no comparisons between the different treatments.

Results

Primary soil infestation

Immature Development and Survival

There was no effect of infestation with 2,000 eggs of *M. javanica* in tomato roots in the *T. urticae* immature development (Fig.1, Cox proportional hazards: likelihood ratio = 39.14, df = 2, P = 0.9). Moreover, there was no significant effect of the infestation with the nematode on immature survival (Fig. 1, Cox proportional hazards:likelihood ratio = 1.07, df = 1, P = 0.3).

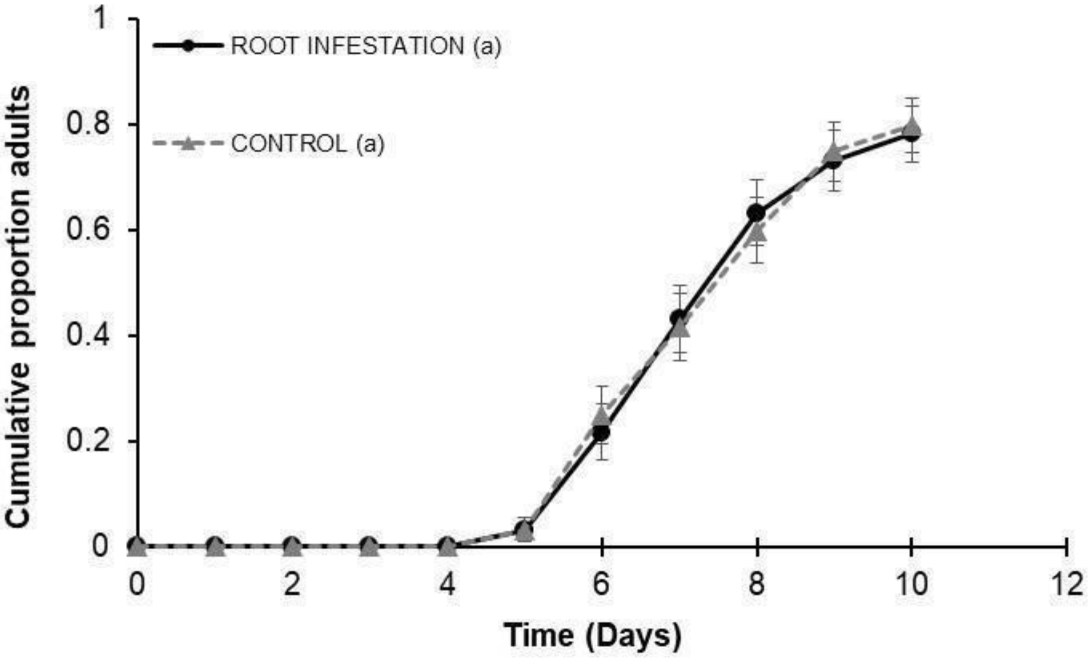

Fig.1. Tomato plants infested with 2,000 *M. javanica* eggs after 40 days (continuous line with circular symbol) and control (dotted line with triangular symbol). Lines represent the rate of individuals of *Tetranychus urticae* that reached adulthood as a function of time. Treatments with equal letters were not significantly different inthe development analysis (P= 0.9). The cumulative proportion of adults refers to thenumber of individuals of *Tetranychus urticae* that have reached adulthood.

Oviposition

Infestation with 2,000 eggs of *M. javanica* in tomato roots did not affect the ovipositionrate of *T. urticae* (Fig. 2, LME: X^2=1.659, P= 0.197). There was a small drop betweendays 3 and 4 in oviposition rate in both treatments, however this difference stabilizedin the last day.

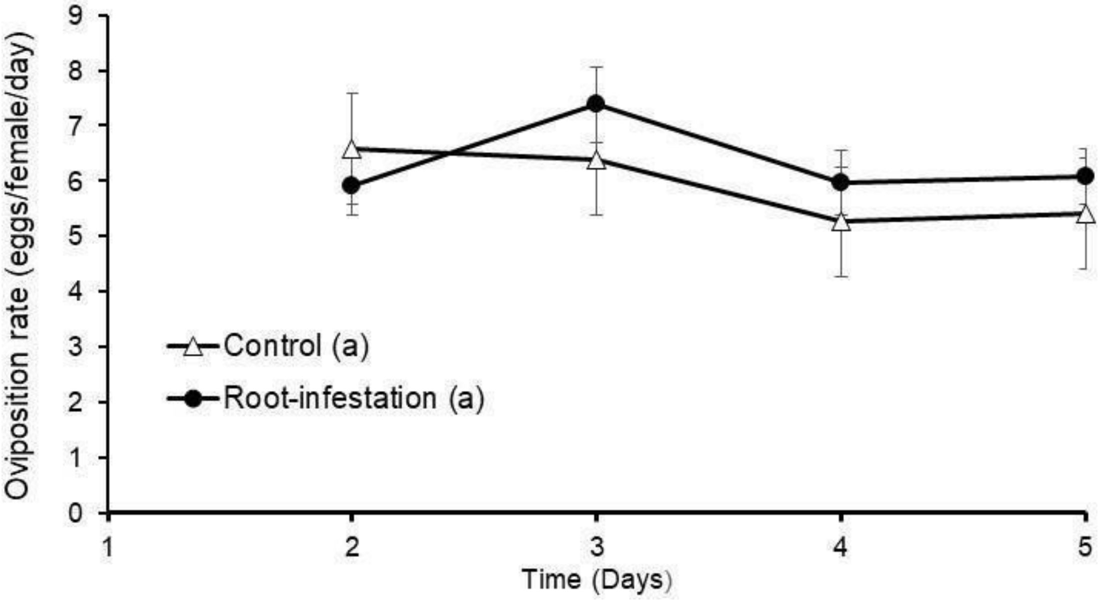

Fig. 2. Average oviposition rate (±SE) of *Tetranychus urticae* on the 2-5th day on leafdiscs of tomato. Root infestation (20 repetitions) is represented by solid line with circular symbol and control without infestation (20 repetitions) solid line with triangularsymbol. Treatments with equal letters were not significantly different in the oviposition analysis. The contrasts between treatments were evaluated with Tukey's method with the lsmeans package.

Host plant choice by the two-spotted spider mite

Tetranychus urticae did not show preference between leaf disc of tomato plants withinfestation or not by the nematode *M. javanica* (Fig. 3. GLM: X^2=0.5309, df= 1, P=0.9093). These results reinforce what was found above, that the infestation of2,000 eggs of *M. javanica* for 40 days does not affect the performance or the choice preference of *T. urticae*. The percentage of herbivores that did not choose was 18.3%for plants infested with nematodes and 37.8% for the treatment control.

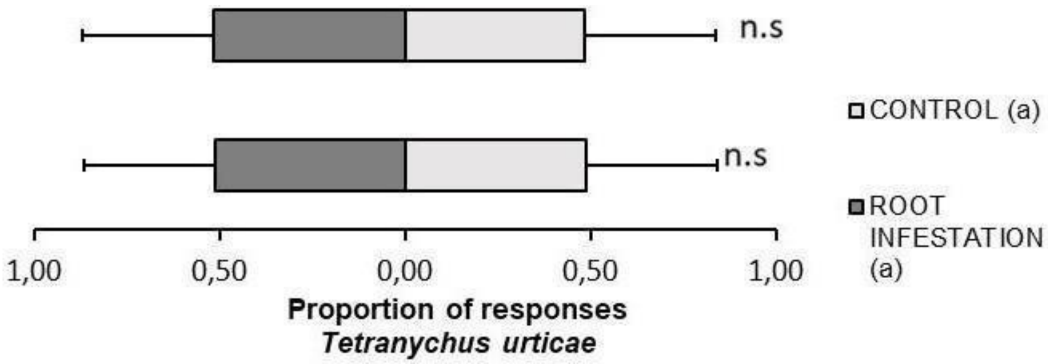

Fig. 3. Response ratio for choosing discs of plants infested with nematodes (dark) and control leaf discs (light) after 24 hours of herbivore release. Each bar is the average of two independent replicates with 60 and 45 females making a choice in each replicate, respectively.

Secondary soil infestation

Immature Development and Survival

There was not effect of infestation with 1,000 and 6,000 eggs of *M. javanica* in tomato roots regarding *T. urticae* immature development (Fig. 4, Likelihood ratio test= 4.76,df= 2, P =0.09). There was also no effect on survival with infestation of *M. javanica* (Fig. 4, Likelihood ratio test= 2.04, df= 2, P =0.4). From the sixth day, a high mortalityrate is evidenced both in treatments and in the control due to nematode infection.

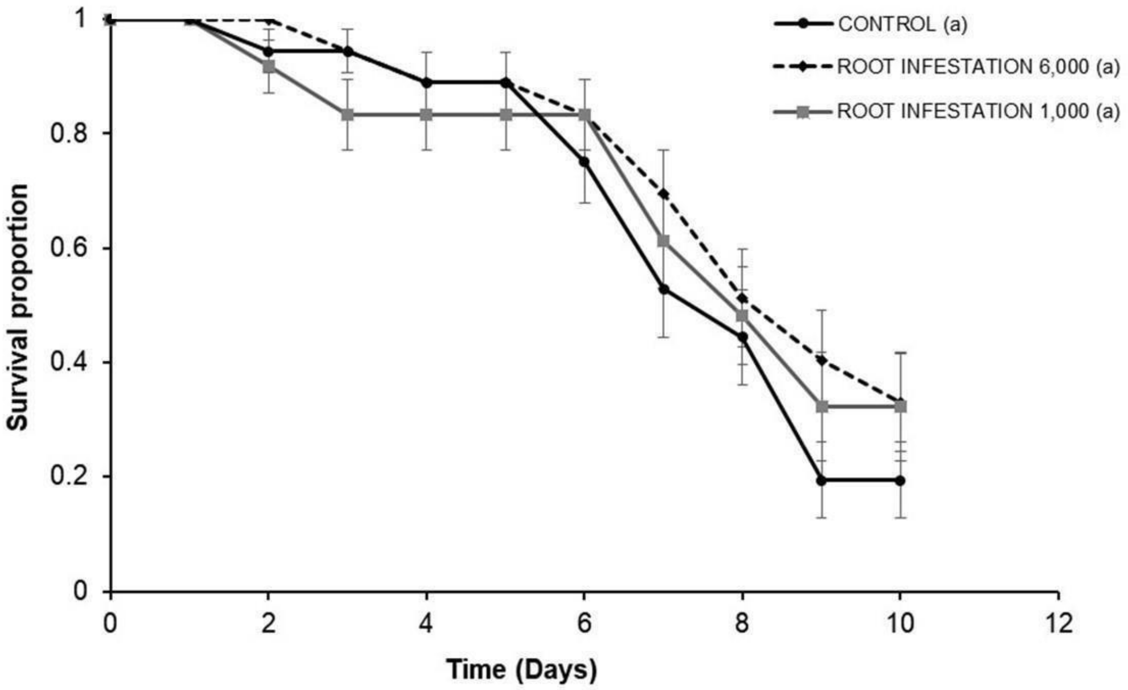

Fig.4. Development and survival of *Tetranychus urticae* feeding on tomato leaf discsof plants infested with *Meloidogyne javanica* for a period of 15 days. The final survivalis the mean cumulative proportion of individuals that reached adulthood as a functionof time. Treatments with the same letters do not present significant difference.

Immature development and survival

Treatments with infestation in the root by nematode *M. javanica* showed a significantdifference in the immature development of *T. urticae* (Likelihood ratio test = 32.36, df = 2, P <0.001). There was a significant effect of nematode presence on the survival of mites in relation to control (Likelihood ratio test= 39.4, df=2, P < 0.001).

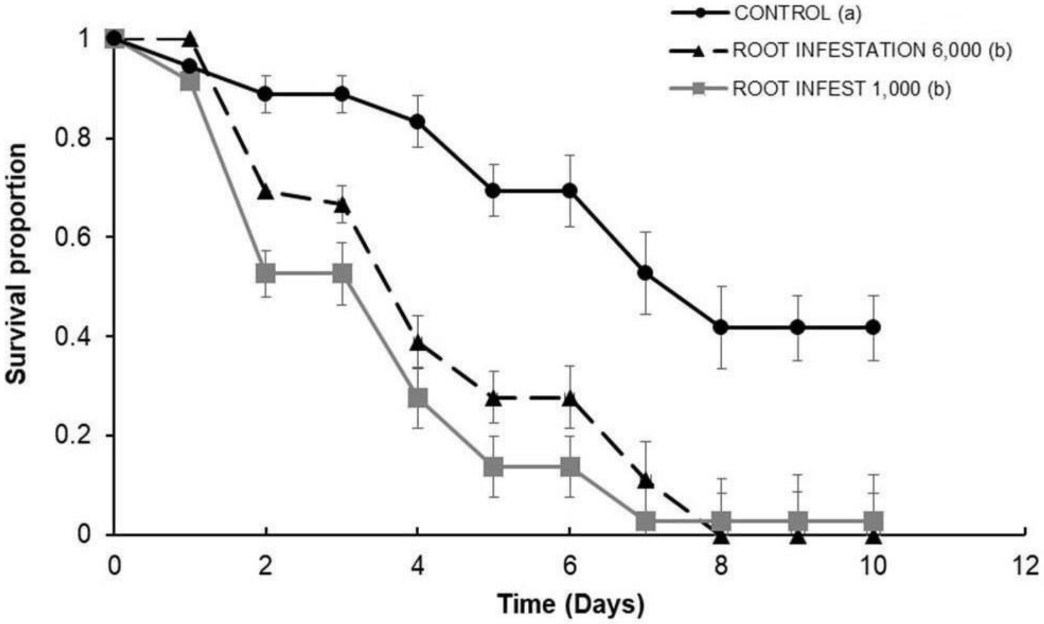

Fig.5.Survival proportion of *Tetranychus urticae* when feeding on tomato plant leaf discs with 30 days of nematode *M. javanica* infestation. Survival is the final adult ratio. Treatments with different letters were significantly different in developmental analysis (P < 0.001).

Immature development and survival

Root parasitism had a significant effect on the immature survival of the herbivore *T. urticae*. The control was different from the treatments with a higher survival proportion (Likelihood ratio test = 33.75, df = 2; P <0.001). In the control treatment, the proportion of adult female mortality was lower than in the treatments where was infested by the nematode *M. javanica* (Likelihood ratio test = 39.14, df = 2, P <0.001).

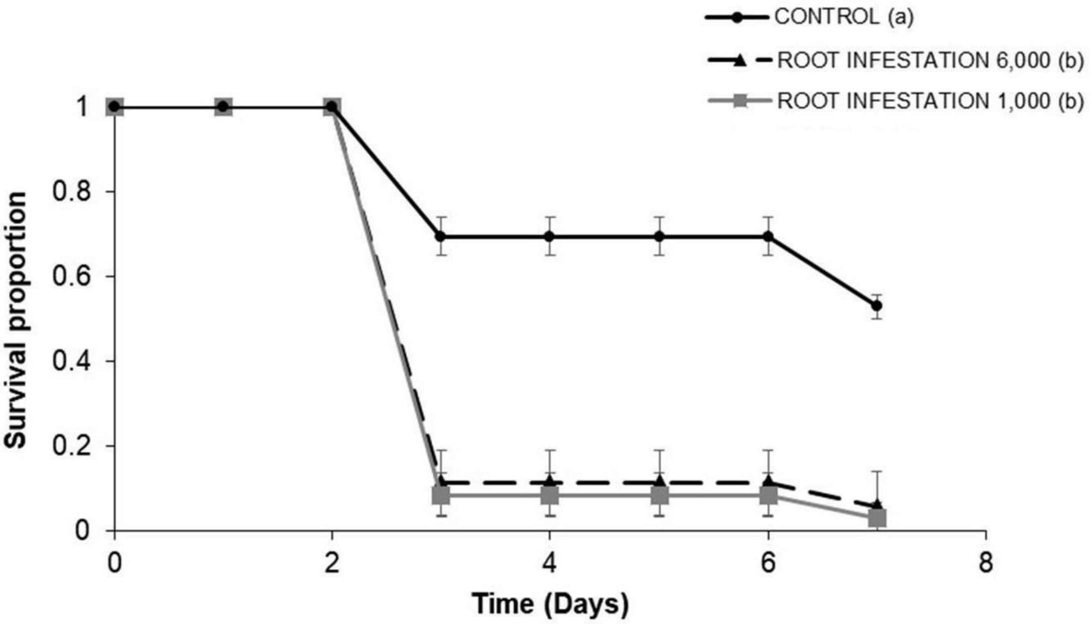

Fig.6. Survival proportion of *Tetranychus urticae* when feeding on tomato plant leaf discs with 45 days of nematode infestation. Survival is the final adult ratio. Treatments with different letters were significantly different in developmental analysis(P < 0.001).

Discussion

We show that the performance and preference of the phytophagous mite *T. urticae* was not affected by the presence of the gall nematode *M. javanica*. The same rate of immature development of *T. urticae* in clean plants and in plants that were previously attacked by the nematode *M. javanica* for 40 days is observed in the experiments. The oviposition rate also was not different between the treatment with 2,000 eggs during 40 days of infestation and the control. Kaplan et al (2009) reported that the abundance of *Manduca sexta* eggs and larvae was not affected by presence of the nematode *Meloidogyne incognita* corroborating with what was found in our study where the survival of the herbivorous mite was not affected by the presence ofthe attack on the root.

In the primary soil infestation, the damage produced in the root by the nematode *M. javanica* does not have effect the performance of the herbivorous mite.However, the high mortality is presented in the experiments with treatments of 1,000and 6,000 eggs at 30 and 45 days of exposure, decreasing the survival of *T. urticae*.It is assumed that only in these two inoculation experiments there was a significant infestation because the quality of the plant and the choice of the herbivorous mite persite for feeding were affected. It is believed that there was no infestation in the experiment with inoculation of 2000 eggs.

Our results showed a significant difference in the rate of oviposition of *T. urticae* females and in immature development, due to the high mortality of herbivoreswhen the plants presented different infestation rates of the nematode *M. javanica*. Li& Meng, (2017) demonstrated the reduction in larval development time and adult body weight of the tobacco cutworm *Spodoptera litura* that feeds on plants inoculated with the soybean cyst root nematode *Heterodera glycines*.

According to these studies, the responses of tritrophic interactions vary according to the arthropod involved. Mukhart et al. (2018) reports that there is a direct relationship between the initial density of nematodes and the reduction in growth and performance of host plants. However, from the experiments carried out in this research, we demonstrate that plants infested in the root region with nematodes do not change therate development and survival of herbivorous mites.

Pallini et al. (1997) showed that mites have a significant preference for plants infested with their conspecifics, but they strongly avoid plants with thrips. However, it is evidenced in our study that the preference and choice of herbivorous mites is not influenced when the plant is affected by a belowground organism.

Van Dam et al. (2005) reported that the growth and survival of the specialized herbivore *Pieris rapae* on leaves of *Brassica nigra* is significantly decreased when the generalist migratory endoparasitic nematode *Pratylenchus penetrans* is presentin the roots. Studies with different arthropods show that when a plant is affected in the root, growth and survival can be impaired, thus when there is a high infestation in the root, as in our study, the mortality of herbivorous mites was affected, showingthat the interaction root-plant-herbivore plays an important role in the ecosystem.

Healthy hosts present high concentrations of nitrogen and amino acids fora normal development and fitness of herbivores (Mattson, 1980). In this context, plantscan have mediated interactions between root feeding nematodes and the aboveground herbivory (Kaplan et al., 2009).

We suggest in this study that the results found here on the immature development of the herbivore from the secondary infestation of the soil are related to areduction in the quality of the plant due to a high initial infestation of the nematode

(6,000 eggs), or due to its exposure time. Herbivore behaviour is affected by plant quality dueto root damage, which causes loss of water and fertilizer absorption (Mai, 1985).

The applied studies on the interactions between spatially separated organisms at the above and belowground levels would be a potential tool in the management of economically important pest herbivores.

Likewise, future research would be necessary on how the quality of leaf tissueaffects the survival of herbivores. In experiments with pest nematodes, it would be essential to include the gall counting methodology, once through this approach we can safely identify whether or not the roots were affected. It is recommended to identify what type of compounds are released by the roots when they present galls ofthe nematode *M. javanica* and if these are involved in plant defenses and their potential use in integrated pest management.

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CHAPTER 2

THE EFFECT OF ROOT- KNOT NEMATODE Meloidogyne javanica on *Phytoseiulus macropilis* BEHAVIOUR IN TOMATO PLANTS

ABSTRACT

Phytoseiulus macropilis is one of the biological control agent of *Tetranychus urticae* and it is attracted by the volatiles released by the plants in response to an herbivore attack. At the root, various nematodes affect plants and trigger interactions betweenspatially separated organisms that influence the third trophic level where predators and parasitoids are found. The behaviour of predators and parasitoids are affected when the damage by a pathogen is presented in the root. For this reason, we investigated whether the infestation of the nematode *M. javanica* has any effect on the behaviour of the predator and on its choice preference for oviposition. Tomato plants infested by the aboveground herbivore *T. urticae* were also infested with 2,000eggs of the gall nematode *Meloidogyne javanica* for 40 days. The results showed that the predator does not have a preference for plants inoculated or not by the nematode. However, more eggs of predators were found in plants that were parasitized by *M. javanica*, which implies that the attraction of the predator to plants under the parasitism of the nematode is not affected. These results show that in the event of the presence of *T. urticae* and *M. javanica* together in tomato crops, the predatory mite release program will not be affected.

Key words: Choice preference. Oviposition site. Predator rate

INTRODUCTION

In the rhizosphere, species interactions can influence various processes, both above and belowground, affecting plant chemistry and impacting food webs (Tejesvi et al.,2019). When the soybean cyst root nematode *Heterodera glycines* is present, negative effects are seen on the parasitoid *Meteorus pulchricornis* regarding its development and reproductive potential (Li et al., 2017). On the contrary, beneficial organisms such as *Arbuscular mycorrhizae* show interactions favorable at the aboveground level in the plant. The mycorrhizal symbiosis with plants changed the emission of herbivore-induced plant volatile (HIPV). For example, for the predatory mite *Phytoseiulus persimilis,* HIPVs from mycorrhizal plants were more attractive than HIPVs from non-mycorrhizal plants (Schausberger et al., 2012). The ovipositionpreference of the predatory mite *P. persimilis* increases in bean plants (*Phaseolus vulgaris*) with mycorrhizae (Hoffmann et al., 2010).

Pests and beneficial organisms trigger root exudates containing specific metabolites with ecological impacts on the macro and microbiota of the soil, as well ason the entire plant (Bertin & Weston, 2003). When soil biota is negatively altered, the chemical composition of the soil changes and the absorption of mineral nutrients affect the yield of plants and communities at higher trophic levels. Guerrieri et al. (2004) in a host plant selection experiment, observed that adults of *Macrolophus pygmaeus* have a preference for the leaves of mycorrhizal plants compared to leavesthat were not inoculated with mycorrhizal, which influenced the acceptance of the host plant by the predator for food and oviposition.

The sedentary root knot nematode *Meloidogyne* spp. activates a complex hostdefense response: oxilipins and lipophilic signaling molecules. These are part of a complex that plays a key role during invasion and migration in soil biota (Fitoussi, 2021). It was found that the effects at higher trophic levels are caused by changes inthe quantity or quality of volatile emissions induced by herbivores (Van Dam & Heil, 2011). For instance, it has been shown that VOCs (Volatile Organic Compounds) produced by Arbuscular mycorrhizal fungi (AMF) on tomato plants attract the aphid parasitoid *Aphidius ervi*, even in the absence of aphids (Prieto et al., 2017). Parasitoids and predators are attracted to VOCs emitted by infested plants (Agrawalet al., 2002, Inbar & Gerling, 2008). Similarly, predatory insects follow the movementof prey by detecting the volatiles generated by each individual prey (Obrycki & Kring,1998, Hare, 2011).

The predatory mite *Phytoseiulus macropilis* (Banks) (Acari: Phytoseiidae) is aspecialized predator of tetranychid mites (Elbasheir et al., 1967, Souza- Pimentel etal., 2018). Each female oviposits on average 2.5 eggs/day for approximately 30 days(Souza-Pimentel et al., 2018). A shorter life cycle compared to *T. urticae* (Fadini et al., 2004) increases the ability to present high predation rates when the pest occursat high population density. Currently, no study investigated whether the attack on theroot affects in any way the behavior of *P. macropilis*, when the spider mite is presentin the aerial part.

The heterogeneity of the soil and the plant is positively related to the diversity of species in the root system presenting interactions between herbivores and naturalenemies at surface level (Kowalchuk et al., 2002). To develop sustainable strategiesin pest management, it is necessary to understand these interactions as an essentialissue in the ecology and coevolution of the ecosystem. Therefore, it is important to understand the aerial interactions caused by the inoculation of *M. javanica* and if thedamage in tomato roots caused any effect on the behavior of the predator *P.macropilis* for the choice preference to plants infested or not by the nematode and ifthis choice has an impact on its oviposition.

Materials and Methods

Plant material

Tomato seeds (*Solanum lycopersicum* var. Santa Clara I-5300) were sown in polystyrene trays with 100 cells with commercial substrate (Mecplant®). After 15 days, the plants were transplanted to a sterilized soil and transferred to 2-liter pot with 1:1 ratio of soil and sand. Plants were watered daily and fertilized weekly with a mixture of 50 g of N-P-K (20-05-20) and 100 g of simple superphosphate dissolved in 20 l of water (Lemos et al., 2015). The plants were left in the early stages of growthin a cage (1 m x 1 m x 0.70 m). When the plants reached a height of approximately 1m, they were taken to an acclimatized room at 28 ± 2 °C, 60 ± 10% RH. The seedsof Jack bean plants were cultivated in plastic pots (2 L) filled with commercial substrate (Mecplant®, Brazil) in greenhouse by 21 day sold. When the plants had twofully developed leaves, they were used for the rearing of mites in the experiments.

Phytoseiulus macropilis rearing

Colonies of the predator *Phytoseiulus macropilis* were obtained from an established stock culture held in the Laboratory of Acarology at the Federal University of Viçosa, Minas Gerais, Brazil. Predators were reared on datached leaves using Jack bean plants (*Canavalia ensiformis* L.) infested with *T. urticae*. The leaves were placed inside plastic trays (44 × 30 × 8 cm) and then placed in larger trays (60 × 40 × 10 cm). The space between the trays was then filled with

5% hypochlorite with water tocreate a barrier to prevent contamination with other arthropods. The trays with the specie of *P. macropilis* were kept in an air-conditioned room (25 ± 2 ° C, 70 ± 10% RH and 12 hours of photophase). New bean leaves were placed with *T. urticae* twice aweek.

Choice of *Phytoseiulus macropilis* between tomato plants infested by the nematode *Meloidogyne javanica* with previous infestation of *T. urticae* and plants only infested by *T. urticae.*

The preference of adult females of the predatory mites *Phytoseiulus macropilis* was tested in plants with infestation of the nematode *M. javanica* (Treub, 1885) (Chitwood,1949) (Heteroderidae) against plants without root infestation as a control. Tomato plants were infested with eggs of the root-knot nematode *M. javanica* after 5 days of transplantation. Around each tomato plant, the infestation was carried out with a holeof 2 cm deep. The nematode rearing procedures were provided by the PhytonematodeBiological Control Laboratory (Federal University of Viçosa, Viçosa, Brazil). When thetomato plants were approximately 40 days old and the leaves fully developed, one ofthem from each plant was infested with50 adult female of *T. urticae*. The plants werejoined with a satin ribbon (Merita®, 0.5 cm thick) to make a bridge between the leaves (Fonseca et al., 2020).

Another satin ribbon was tied to the center of this bridge, the other end of this tape was attached vertically to a Petri dish (5 cm diameter, 1 cm deep). So that the predatory mites could walk vertically on the belt to the bridge, and from there to the two plants and choose between plants with or without infestation of the nematode *M.javanica*. Subsequently, 50 adult females of the predatory mites were randomly collected from the culture and placed in two 1.5 ml Eppendorf safe-lock tubes. These Eppendorf tubes were placed in the Petri dish and the predatory mites were released,so that they could walk up the vertical belt to the bridge, and from there to the two plants. After 24 hours, the plants were destroyed and with the help of a stereoscope the counting of predatory mite that responded to the treatments was performed, as well as the place of preference for oviposition and the number of eggs oviposited. Twelve pairs of plants were used for each treatment. Each pair of plants was placed ina separate tray (55 × 40 × 10 cm) and the position of the trays was alternated to correctfor any possible influence of directionality on the response of predatory mites (Zhang, Van, Messelink, & Janssen, 2019). The numbers of predatory mites arriving on the plants with and without infestation of the nematode were compared with a linear mixedeffects model (LME) with treatment as fixed factor and replicate as random factor (Fig.1).

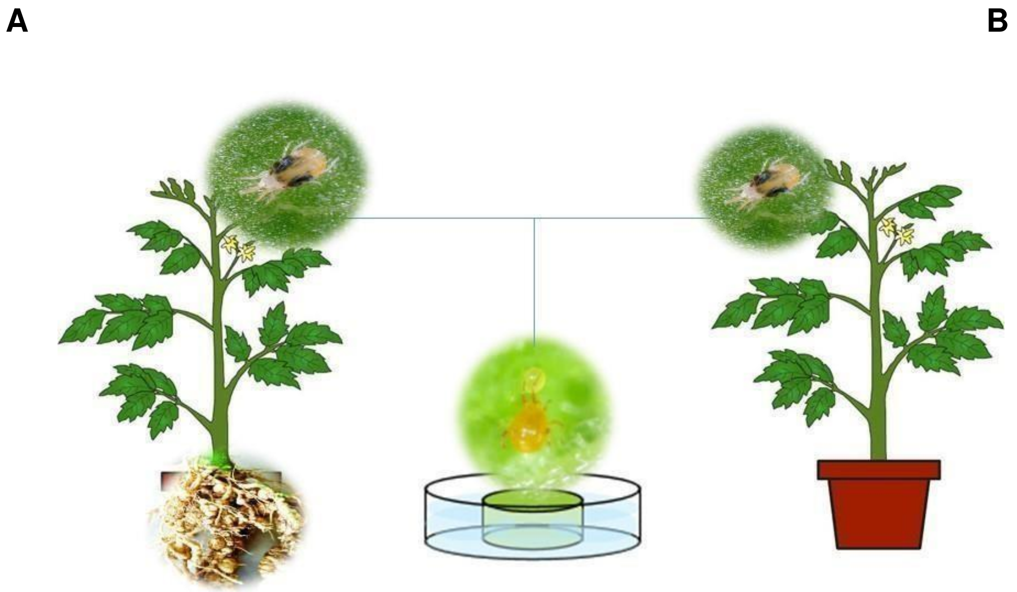

Fig.1. Choice preference experiment of the predator *Phytoseiulus macropilis* between tomato plants infested by the nematode *M. javanica* and by *Tetranychus urticae* (A) and plants infested only by *Tetranychus urticae* (B).

Results

Oviposition of *Phytoseiulus macropilis* in tomato plants with infestation of *T. urticae* or plants with infestation of the nematode *Meloidogyne javanica* +infestation of *T. urticae*

Phytoseiulus macropilis did not show a significant preference for plants infested by *T. urticae* only or by the combination of plants both infested by the nematode *M. javanica* and *T. urticae* (Fig.1. GLM: X^2 = 4.68, df. = 1, P= 0.4).

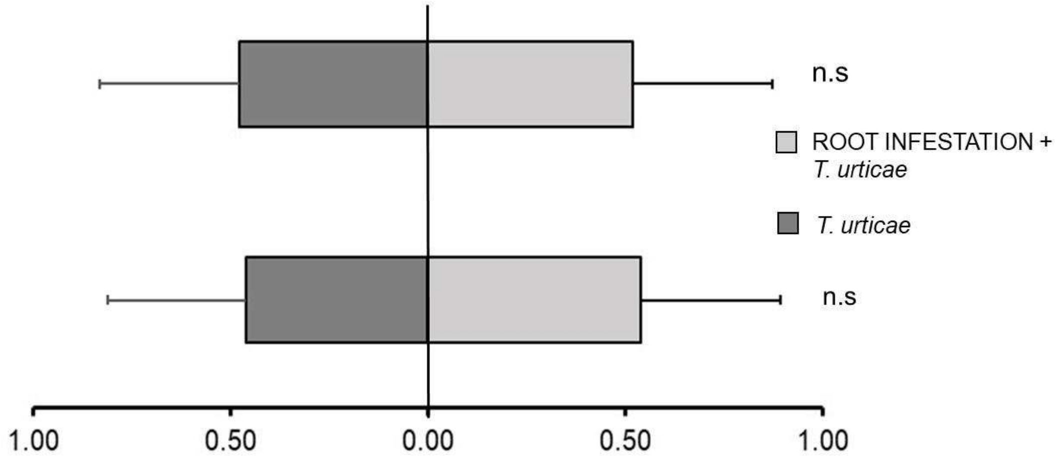

Fig.2. The choice response of *Phytoseiulus macropilis* between plants infested with spider mites *Tetranychus urticae* only (dark) and with nematode and *Tetranychus urticae* (light). Bars are averages of 8 and 12 replicates.

Oviposition of *Phytoseiulus macropilis* in tomato plants with infestation of *T. urticae* or plants with infestation of the nematode *Meloidogyne javanica*

Phytoseiulus macropilis had a significant preference to oviposit in plants with inoculation of the nematode *M. javanica,* finding more eggs than in control plants (**Fig.2**. GLM: X^2 = 25.365, df. = 1, P= 0.04). It was observed that the predator *P. macropilis* oviposited a greater number of eggs in tomato plants infested with 2,000 eggs of the nematode *M. javanica* for 40 days.

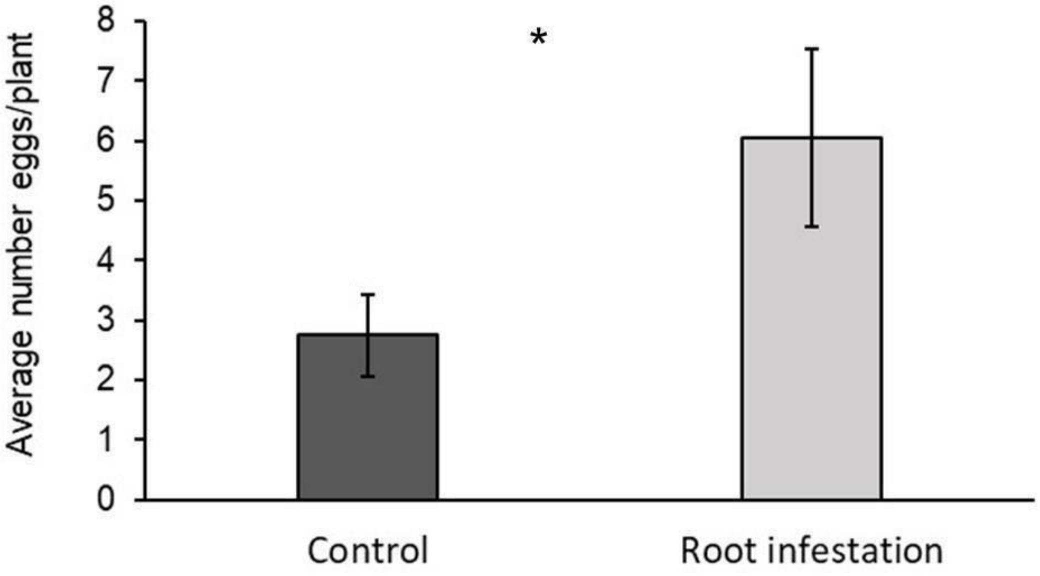

Fig.3. Average number of eggs oviposited by *Phytoseiulus macropilis* females in plants with or without nematode infestation and infestation of *T. urticae*, dark gray bars (control) and lighter gray (2,000 *Meloidogyne javanica* eggs at 40 days of infestation). * P = 0.04.

Discussion

In our study, there was no choice preference of the predator for plants infested or not by the nematode. However, more eggs oviposited by the predatory mite *P.macropilis* were found in plants infested with the nematode *M. javanica.*

In olfactometer experiment, Oliveira et al (2009) showed that during the search for prey *P. macropilis* is strongly attracted by the presence of a previous *T. urticae* infestation. Here we showed that the attack on roots does not cause difference in the choice of the predator when the host was previously attacked by theaboveground herbivore *T. urticae*. Consequently, the non-preference by the predatory mite for plants infested in the root can be attributed to a lower production of attracting compounds for the predator.

The oviposition increased when the predator *P. macropilis* chose for plants that were infested with the nematode *M. javanica*. Soler et al (2007) documented that *Cotesia glomerata* prefers ovipositing in hosts of *Brassica nigra* with root-undamagedby the herbivore *Delia radicum.* In our study, a possible effect on increased oviposition could be due to different mixtures of plant volatiles depending on whetherthe plant was exposed to root herbivory and leaf herbivory together or whether eachdamage occurs individually. Soler et al (2010) also found that root herbivory can affect the distribution of eggs of aboveground herbivores. There are studies that showed predatory mites avoiding oviposition sites where conspecific or heterospecific are present, which was reported by Walzer et al (2006) when the predatory mite *Phytoseiulus persimilis* prevented oviposition in mite patches with *Neuseiulus californicus* (Acari: Phytoseiidae) eggs.

Other types of biological parameters in natural enemies can be affected by herbivory in the root. Soler et al. (2005) reported that the herbivory of the root *Delia radicum* affects the development and adult size of the parasitoid *Cotesia glomerata* and the hyperparasitoid *Lysibia nana*. Our result showed that the nematode *M.javanica* does not affect the behavior of the predatory mite in search for the pest.

Our results indicated that in the eventual presence of two pests on the same plant, root damage can attract more predators to oviposit, which would benefit the biological control of the herbivorous pest. However, further research is needed to know how the interactions in the rhizosphere could contribute to the biological controlof species of economic importance without affecting the quality of the plant.

This results emphasize the need to understand and integrate the constitutivedefenses of the tomato plant and those induced by root nematodes and thus find a better understanding of the interactions of organisms above and below ground. Thiswould allow obtaining information on the improvement of tomato integrated pest management programs.

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GENERAL CONCLUSIONS

The amount of damage and the permanence of the pathogen on tomato plants determine the preference of herbivorous mites for oviposition and feeding patches. The results of this study are the basis for considering that the presence of different organisms in the same host causes different responses above and belowground. Our study showed that when the tomato plant is subjected for 40 days to a single infestation dose of the nematode *M. javanica* (2,000 eggs), the immature development and the oviposition rate of the herbivore *T. urticae* were not affected. However, when heavier soil infestation occurs, the different doses of 1,000 and 6,000eggs of the nematode at 30 and 45 days of exposure, a reduction in the normal immature development of the herbivore with high mortality was achieved. A possibledifference in the immature development of herbivores in the experiments withinfestation of 1,000 and 2,000 eggs of *M. javanica* could be by the different time of the year in which the experiments were carried out. Perhaps in the experiment where2000 eggs of *M. javanica* were used, there was not enough infestation in the root toaffect the choice of the herbivorous mite.

In this study, it was identified that when tomato plants are subjected to the infestation of eggs of the nematode *M. javanica*, the searching behavior of the predator *P. macropilis* for its prey is not affected. On the contrary, more eggs were oviposited onplants inoculated with the nematode. Knowledge of symbiotic relationships and interactions with organisms from different ecological niches could improve plant performance, since beneficial organisms are important keys increasing crop yields.