# Scientific Note

Effect of Methyl Jasmonate on the Performance of *Tetranychus evansi* Baker & Pritchard, 1960 (Acari: Tetranychidae) and *Phytoseiulus longipes* Evans, 1968 (Acari: Phytoseiidae) on Tomato Plants

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Abstract. Inducible anti-herbivore defenses in plants are predominantly regulated by jasmonic acid (JA). The red spider mite *Tetranychus evansi* Baker & Pritchard, 1960 (Acari: Tetranychidae) is an invasive pest known for its detrimental impact on tomato plants and other Solanaceae crops. Here, we investigated the extent to which *T. evansi* and the predatory mite *Phytoseiulus longipes* Evans, 1968 (Acari: Phytoseiidae) are affected by induced JA-defenses. Initially, we artificially induced the JA-response in tomato plants using exogenous methyl jasmonate (MeJA) and subsequently assessed the effect of JA defenses on spider mite by evaluating mortality and oviposition rates. Our findings revealed a higher mortality and lower oviposition rates on plants treated with MeJA compared to non-treated control plants. Furthermore, we examined the predatory mite's predation rates on spider mite eggs produced on MeJA-treated and non-treated tomato plants. The results showed a reduced predation on *T. evansi* eggs derived from MeJA-treated plants, indicating a potential negative impact of JA-induced defenses on the predator's performance. Finally, we released five predatory females on *T. evansi*-infested tomato plants treated and non-treated with MeJA, monitoring the predator population density for three generations. Predator population was not affected, as the abundance of larvae and adults was not significantly different between treatments. These findings underscore the negative impact of JA defenses on herbivores and highlight the trade-off it may pose on natural enemies.

Keywords: Acari, biological control, plant defense, population dynamics, methyl jasmonate (MeJA).

Plants have developed various defense mechanisms to protect themselves against herbivore attacks and ensure their survival. As part of plant defense mechanisms, inducible anti-herbivore defenses regulated by signaling molecules like jasmonic acid (JA) play a significant role in plant-arthropod interactions (Kant et al. 2015). The red spider mite *Tetranychus evansi* Baker & Pritchard, 1960 (Acari: Tetranychidae), an invasive mite pest that poses a significant threat to economically important crops, particularly tomato plants and other Solanaceae (Navajas et al. 2013). Interestingly, certain genotypes of *T. evansi* have evolved the ability to downregulate JA-dependent defenses in their host plants. This allows them to overcome the plant's natural immune responses and increase their performance on tomato plants (Sarmento et al. 2011). This unique adaptation is hypothesized to be the key factor driving the success of *T. evansi* as a pest species. Therefore, it is essential to understanding the relationship between inducible plant defenses, herbivore suppression, and the broader ecological implications of such interactions is of fundamental importance for developing effective strategies to mitigate the impact of *T. evansi* and enhance the success of pest management in agricultural settings.

Phytoseiulus longipes Evans, 1958 (Acari: Phytoseiidae) is seemingly the only known predatory mite able to consume eggs and other stages of *T. evansi* (Silva et al. 2010; Ataide et al. 2016) and has been considered a potential biological control agent of this pest in several crops, especially tomatoes (Silva et al. 2010; Savi et al. 2021). By actively feeding on *T. evansi*, *P. longipes* can contribute to the control of this pest, reducing plant damage and economic losses. However, the success of *P. longipes* is intricately linked to the fitness of its spider mite prey and the defensive strategies employed by attacked host plant. The relationship between JA-induced defenses, *T. evansi* performance,

and its interaction with *P. longipes* has not been well studied. A study by Ataide et al. (2016) showed that artificially restoring of JA-defenses in mutant tomato plants negatively impacted the oviposition performance of both mite species. In the present study, we further investigated the dynamics of this plant-herbivore-carnivore interaction by artificially inducing JA-responses in a commercial tomato plant using exogenous methyl jasmonate (MeJA). The study aimed (*i*) to elucidate the impact of exogenous applications of MeJA on *T. evansi* mortality and oviposition, (*ii*) the subsequent predation of its eggs by *P. longipes* and (*iii*) the short-term consequences of JA-treated plant defenses on the population density of *P. longipes*.

The experiments were carried out using *Solanum lycopersicum* cv. Santa Clara in laboratory and greenhouse conditions. Tomato plants were grown in a greenhouse with day/night temperatures of 18-25 °C, a 12:12 h (light:dark) regime and 50-70% relative humidity (RH). The strains of *T. evansi* and *P. longipes* used in this study were obtained from a natural population collected in Viçosa, Minas Gerais, Brazil, and have been maintained in the laboratory for over a year. *Tetranychus evansi* was fed on tomato leaves cv. Santa Clara, while *P. longipes* was fed on tomato leaves infested with *T. evansi*, obtained from the *T. evansi* colony. In all experiments, only adult female mites (3 ± 1 days old) obtained from a cohort were used to infest the tomato leaves. 40-50 days old tomato plants were treated with 150 µg MeJA (Sigma-Aldrich 95%) mixed with 20 µL lanolin (Sigma-Aldrich), following the methodology described in Baldwin et al. (1998) to induce JA plant defenses. One drop of this mixture (~10 µL) was directly applied to the petiole of the first newly formed tomato leaf containing 5 leaflets, using a 1 mL plastic syringe (Fisherbrand™). Only one tomato leaf per plant received the lanolin + MeJA treatment. Tomato plants treated

with lanolin + MeJA (*N*= 35) were individually transferred to mite-proof screen cages and placed in a greenhouse. Control plants (*N*= 30) received only lanolin without MeJA and were also placed in cages, but in a different greenhouse; hereafter referred to as non-treated plants. After twenty-four hours we infested the leaflet that received the lanolin + MeJA mixture with 12 *T. evansi* females, repeating the same process for non-treated plants. In all instances, a new barrier of lanolin only was applied to retain the mites in the infested leaflet. After 48h, infested leaflets were detached and oviposition and mortality of *T. evansi* were assessed. Spider mites were removed from the leaflet and the petiole of detached leaflet was inserted in an Eppendorf tube filled with water and cotton wool. Subsequently, one *P. longipes* female (3 ± 1 days old) obtained from a cohort, was added in each leaflet containing the spider mite eggs. Egg predation rate was assessed after another 48h.

In a subsequent experiment, we investigated changes in the population density of *P. longipes* feeding upon *T. evansi* on JA-treated and non-treated tomato plants. Plants were treated as described above (*N*= 15 for each treatment). Twenty-four hours after MeJA treatment we infested the tomato leaf that received the lanolin mixture with 100 *T. evansi* females and 5 *P. longipes* females. We monitored fluctuations in the number of predator larvae and adults weekly for 21 days (~three predator generations). Every other day we reapplied one drop of the lanolin mixture (with or without MeJA) on the same tomato leaf. Weekly applications were performed to ensure that the JA-defense response remained activated throughout the entire experimental period. All statistical analyses were performed using R program version 4.2.1 (R Developer Core Team 2022). Differences in the oviposition, mortality of spider mites, predation rate, number of larvae and adult predatory mites among treatments were tested using generalized linear models (GLM) with quasipoisson error distribution (Crawley 2012).

Defense induction by MeJA negatively affected the performance of *T. evansi* in tomato plants (Fig. 1). Mortality of *T. evansi* was higher on MeJA-treated leaflets compared to non-treated control leaflets ($F_{[1, 64]}$ = 16.20, $P \leq 0.001$; Fig. 1A). Similarly, the oviposition rate of *T. evansi* was significantly lower on leaves previously treated with MeJA than on non-treated ones ($F_{[1, 60]}$ = 9.60, $P = 0.002$; Fig. 1B). Predation rate of *P. longipes* was lower on the *T. evansi* fed on MeJA-treated leaves compared to the *T. evansi* fed on control leaves ($F_{[1, 40]}$ = 5.70; $P = 0.02$, Fig. 2A). The number of predators increased over time ($F_{[1, 68]}$ = 37.31; $P \leq 0.001$, Fig. 2b), but overall, it was not influenced by MeJA treatment ($F_{[1, 69]}$ = 1.17; $P = 0.28$, Fig. 2B). No significant interactions between day and treatment were observed ($F_{[1, 67]}$ = 0.59; $P = 0.44$, Fig. 2B).
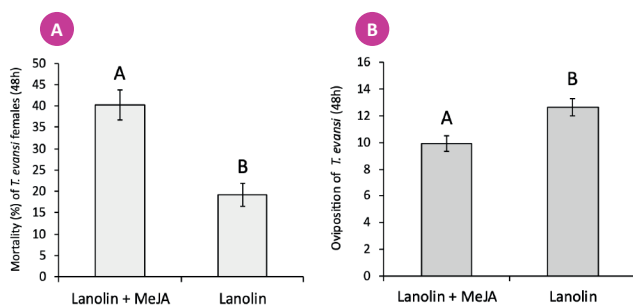

Figure 1. Effect of MeJA-treated tomato plants on *T. evansi* mortality (%) and number of eggs. Panel a) mortality of *T. evansi* females on tomato leaflets that were previously treated with lanolin + MeJA and on clean leaflets (lanolin). Panel b) *Tetranychus evansi* oviposition on tomato leaflets previously treated with MeJA and on clean leaflets (lanolin) over 48 hours. Bars represent the average (± SE). Different letters denote significant differences between treatments (GLM, P ≤ 0.05).

Previous studies have shown that plants treated with JA negatively affect the performance of several herbivores (Kant et al. 2004; Ament et al. 2004; Ament et al. 2006). MeJA induction activated defenses mechanisms in plants such as protease inhibitors (PIs), antioxidative enzymes, synthesis of alkaloids, volatile compounds, and formation of trichomes, all of which have harmful effects on arthropods (War et al. 2011). The decrease in *T. evansi* performance is likely due to the increase in plant defenses (Kant et al. 2015), suggesting that MeJA treatment

induces efficient defense mechanisms in plants. MeJA elicitation in tomato plants triggers various transcriptional responses and secondary metabolites involved in plant defenses (Kant et al. 2015; Ament et al. 2006). Thus, the reduction in the oviposition rate of *T. evansi* on MeJA treated plants was probably caused by the activation of tomato defense routes, as previously shown by Ataide et al. (2016). A lower feeding rate may partially explain the higher mortality in plants treated with MeJA (Fig. 1A), which can also lead to a lower rate of oviposition (Fig. 1B) and a decreased population growth over time. In a recent study, Zhifeng (2023) revealed a novel mode of action of JA defenses in mite reproduction. They demonstrated that mite feeding induced JA content in eggplants, resulting in increased coumarin levels and the inhibition of genes linked to mite reproduction. Consequently, this cascade of events led to a reduction in oviposition and hatchability of mite eggs in eggplants. This study reveals new venues regarding the role of JA defenses and their implications on spider mite performance and presents an exciting opportunity to further explore this phenomenon in tomato plants.
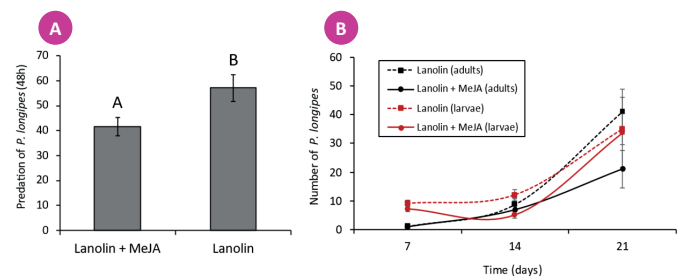

Figure 2. Effect of MeJA-treated tomato plants on *P. longipes* predation rate and on its population density. Panel a) predation rate of a *P. longipes* female on leaflets with *T. evansi* eggs treated (lanolin + MeJA) and non-treated with MeJA for 48h (lanolin). Panel b) the abundance of *P. longipes* feeding on *T. evansi* on tomato plants treated and non-treated by MeJA over 21 days. Bars represent the average (± SE). Different letters denote significant differences between treatments (GLM, P ≤ 0.05).

In addition to herbivore performance, plant defense can also mediate indirect interactions between the plant and the natural enemies they host (Kennedy 2003). These interactions are expected to benefit natural enemies; however, some herbivores have adapted to plant defenses and can use them against their natural enemies and parasitoids (Chaplin-Kramer et al. 2011). Studies report that herbivores, can sequester toxins produced by the later as a form of defense, hampering their predation by other organisms (Opitz & Müller 2009; Beran & Petschenka 2022). This suggest that the ingested MeJA by *T. evansi* may be passed to their eggs, thus reducing their suitability, i.e., reduced palatability and/or nutritional value (Tooker & De Morais 2005). This would explain why *P. longipes* fed on fewer *T. evansi* eggs produced on leaves treated with MeJA compared to those produced on untreated leaves (Fig. 2A). Low predation rates are usually expected to hinder population growth and development due to insufficient nutrients for predators' development. However, our findings contradict this hypothesis, as MeJA did not affect the population of the predator (Fig. 2B). One possible explanation is that, while the predation rate was influenced by MeJA, predator oviposition remained unaffected, as previously demonstrated by Ataide et al. (2016). As result, predators produced equal offspring in both treatments, mitigating the impact of the low predation rate on *T. evansi* eggs produced on MeJA-treated leaves. Although not evaluated in this study, it is worth noting that the application of MeJA could potentially increase tomato leaf trichome densities, known to be part of the plant defense mechanism (Li et al. 2018). Previous research has shown that natural enemies perform better on tomatoes lacking trichomes, suppressing herbivore densities better and faster on these plants (Legarrea et al. 2022). In fact, it has been previously shown that trichomes can significantly impact the performance of *T. evansi* (Savi et al. 2022) and reduce predation by *P. longipes* (Savi et al. 2021). Despite the documented low presence of both glandular and non-glandular trichome in the tomato cultivar used in our study (Oriani & Vendramim 2010), it is yet to be determined

whether the short duration of our experiments had an impact on trichome production in tomato plants.

In conclusion, our study reveals that a herbivore, which typically enhances its performance by manipulating plant defenses, loses the ability to do so after the exogenous induction of MeJA. As a result, its performance is impaired, indicating that MeJA treatment restores the manipulated plant defense, which proves to be efficient in protecting the tomato plant against *T. evansi*. *Tetranychus evansi* in turn, may have adapted to use these defensive substances to defend themselves against their natural enemies, such as *P. longipes*. However, since our data did not indicate that this effect was sufficient to reduce predator population growth, further investigations into this complex web of interactions become increasingly interesting and relevant. Understanding the interplay between inducible plant defenses and herbivore-predator interactions is crucial for developing effective strategies to manage invasive mite pests and enhance pest control measures in agricultural systems. Additionally, the potential collateral impact of exogenous JA application on plant productivity (Bhavanam & Stout 2021) warrants further evaluation.

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Authors' Contributions

Conceptualization, LMSA and CCMA; methodology, LMSA, CCMA, PAS; formal analysis, LMSA and PAS; investigation, LMSA, CCMA, PAS; resources, AP; writing-original draft preparation, LMSA, PAS; writing-review and editing, all authors; supervision, LMSA; project administration, AP; funding acquisition, AP. All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest Statement

The authors declare that there are no conflicts of interest over the ownership of the data presented in this manuscript.

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