UNIVERSIDADE FEDERAL DE VIÇOSA

Volatile Modulation by *Pochonia chlamydosporia* (Hypocreales: Clavicipitaceae) affects behavior of *Phthorimaea absoluta ni* (Lepidoptera: Gelechiidae) and development in tomato plants

Karenn Christiny Pereira Santos
Doctor Scientiae

VIÇOSA - MINAS GERAIS
2025

KARENN CHRISTINY PEREIRA SANTOS

Volatile Modulation by *Pochonia chlamydosporia* (Hypocreales: Clavicipitaceae) affects behavior of *Phthorimaea absoluta ni* (Lepidoptera: Gelechiidae) and development in tomato plants

Thesis submitted to the Entomology Graduate Program of the Universidade Federal de Viçosa in partial fulfillment of the requirements for the degree of *Doctor Scientiae*.

Adviser: Eraldo Rodrigues de Lima

Co-adviser: Natália de Souza Ribas

VIÇOSA - MINAS GERAIS
2025

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

T

Santos, Karenn Christiny Pereira, 1996-

S237e
2025

Volatile modulation by Pochonia chlamydosporia (Hypocreales: Clavicipitaceae) affects behavior of Phthorimaea absoluta ni (Lepidoptera: Gelechiidae) and development in tomato plants / Karenn Christiny Pereira Santos. – Viçosa, MG, 2025.
1 tese eletrônica (70 f.): il. (algumas color.).

Texto em inglês.
Orientador: Eraldo Rodrigues de Lima.
Tese (doutorado) - Universidade Federal de Viçosa, Departamento de Entomologia, 2025.
Referências bibliográficas: f. 23-25.
DOI: https://doi.org/10.47328/ufvbbt.2025.706
Modo de acesso: World Wide Web.

1. *Phthorimaea absoluta* . 2. *Pochonia chlamydosporia*. 3. Relação inseto-micro-organismo. 4. Semio-químicos. 5. Tomate - Doenças e pragas. I. Lima, Eraldo Rodrigues de, 1960-. II. Universidade Federal de Viçosa. Departamento de Entomologia. Programa de Pós-Graduação em Entomologia. III. Título.

CDD 22. ed. 632.78

Bibliotecário(a) responsável: Alice Regina Pinto Pires CRB-6/2523

KARENN CHRISTINY PEREIRA SANTOS

Volatile Modulation by *Pochonia chlamydosporia* (Hypocreales: Clavicipitaceae) affects behavior of *Phthorimaea absoluta ni* (Lepidoptera: Gelechiidae) and development in tomato plants

Thesis submitted to the Entomology Graduate Program of the Universidade Federal de Viçosa in partial fulfillment of the requirements for the degree of *Doctor Scientiae*.

APPROVED: July 31, 2025.

Assent:

Karenn Christiny Pereira Santos
Author

Eraldo Rodrigues de Lima
Adviser

To all women in science.

ACKNOWLEDGMENTS

To God and my spiritual guides.

To the Federal University of Viçosa (UFV) and the Graduate Program in Entomology for the opportunity to pursue my master’s degree.

To the National Council for Scientific and Technological Development (CNPq) for granting the scholarship.

To the Research Support Foundation of the State of Minas Gerais (FAPEMIG) for granting the scholarship.

To the Coordination for the Improvement of Higher Education Personnel (CAPES) for granting the scholarship.

To my adviser for all the teachings, friendship, guidance, and for trusting me throughout my academic journey in graduate school.

To Natália for her teachings, support, friendship, and co-supervision.

To all the professors of the department who played a fundamental role in building my theoretical foundation across various fields of knowledge.

To the students of the Nematode Biocontrol Laboratory (BIONEMA), especially Angélica, Huarlen, and Professor Leandro, for all their support.

To Professor Paulo Zarbin from the Semiochemical Laboratory at UFPR, along with his students, for contributing to my learning and to the development of part of my experiments.

To my mother, Edna, for always supporting me, believing in me, and teaching me never to give up on what we aim for.

To my father, Wellington, for his love, support, and encouragement.

To my siblings, Kátia and Luiz, for all their love and support in every moment, and to my brother Fellipe, for making my days lighter and happier, even from afar.

To all my family for their unconditional love and support, regardless of physical distance.

To Maria Alice, for being my partner in every moment, my safe harbor, and an example of resilience.

To all my friends, for always reminding me of the lightness of life and teaching me that moments of difficulty are just that: moments.

To Ane, Ana, Elaine, Juan, and Mateus, for all the help, technical and emotional support, and for being there for me through all moments in Viçosa. I love you all.

To my friends from the Department of Entomology Waly, Douglas, Samuel, Jéssica, Lorene, and Mellis. Thank you!

To my friends Yago, Isabela, Yuri, Gabi, Jão, Josi, Letícia, and Nicole. You were part of my best moments in Viçosa.

To Paulo and Silvinha, for welcoming me into your home, embracing me, and taking care of me with so much love and affection.

To my friends from the Laboratory of Semiochemicals and Insect Behavior, for the good times and the laughter we shared.

This work has been sponsored by the following Brazilian research agencies: Coordination for the Improvement of Higher Education Personnel (CAPES; Financing code 001), Minas Gerais State Foundation for Research Aid (FAPEMIG) and National Council of Scientific and Technological Development (CNPq).

"Those who have faith have everything. Those who do not have faith have nothing."

ABSTRACT

SANTOS, Karenn Christiny Pereira, D.Sc., Universidade Federal de Viçosa, July, 2025. **Volatile Modulation by *Pochonia chlamydosporia* (Hypocreales: Clavicipitaceae) affects behavior of *Phthorimaea absoluta ni* (Lepidoptera: Gelechiidae) and development in tomato plants**. Adviser: Eraldo Rodrigues de Lima. Co-adviser: Natália de Souza Ribas.

Phthorimaea absoluta is a key pest in tomato crops, and its females locate host plants through the detection of plant-emitted volatile organic compounds (VOCs). Changes in VOC profiles, such as those induced by microorganisms associated with the plant, can influence insect attraction and performance. In this study, we evaluated the impact of the endophytic fungus *Pochonia chlamydosporia* (isolate Pc10), associated with the roots of tomato plants (*Solanum lycopersicum* var. Santa Cruz), on VOC emission, attraction to *P. absoluta* females, and offspring development. Volatile compounds were collected from the aerial parts of control and fungus-inoculated plants and analyzed using gas chromatography coupled with electroantennographic detection (GC-EAD). Antennal responses were quantified, and bioactive compounds were identified using the Kovats retention index and reference standards. Additionally, oviposition preference assays and insect development tests were conducted under controlled conditions. We identified 23 VOCs in control plants and 17 in inoculated ones, with higher concentrations of p-cymene and salicylic acid in fungus-treated plants. Compounds such as a-pinene, ß-myrcene, and p-cymene elicited antennal responses in *P. absoluta*, with a-pinene being the most intense in the synthetic blend tests. Females preferred to oviposit on control plants, and the presence of the fungus reduced the development time of the egg and larval stages. Our results indicate that *P. chlamydosporia* can modulate multitrophic interactions between plants and herbivores by altering the composition of bioactive volatiles, reducing host attractiveness, and affecting pest development. These findings highlight the potential of this endophytic fungus as a complementary tool for sustainable management strategies against the tomato leafminer.

Keywords: VOCs; Semiochemicals; endophytism; IPM

RESUMO

SANTOS, Karenn Christiny Pereira, D.Sc., Universidade Federal de Viçosa, julho de 2025. **Modulação de voláteis por *Pochonia chlamydosporia* (Hypocreales: Clavicipitaceae) em plantas de tomate afeta comportamento e desenvolvimento de Phthorimaea absoluta (Lepidoptera: Gelechiidae)**. Orientador: Eraldo Rodrigues de Lima. Coorientadora: Natália de Souza Ribas.

Phthorimaea absoluta é uma praga-chave dos cultivos de tomate, cujas fêmeas localizam o hospedeiro por meio da detecção de compostos orgânicos voláteis (COVs) emitidos pelas plantas. Alterações no perfil desses compostos, por exemplo induzidas por microrganismos associados à planta, podem modificar a atratividade e o desempenho da praga. Neste estudo, avaliamos como a presença do fungo endofítico *Pochonia chlamydosporia* (isolado Pc10), associado às raízes de plantas de tomate *Solanum lycopersicum* (var. Santa Cruz), afeta a emissão de voláteis, a atratividade para fêmeas de P. absoluta e o desenvolvimento da prole. Foram realizadas coletas, identificação e quantificação de voláteis da parte aérea de plantas controle e plantas inoculadas com o fungo, seguidas de análises por cromatografia gasosa acoplada à eletroantenografia (GC-EAD). As respostas eletrofisiológicas das antenas foram quantificadas, e os compostos bioativos identificados por índice de retenção de Kovats e padrões de referência. Adicionalmente, conduziram-se bioensaios de preferência para oviposição e testes de desenvolvimento em condições controladas. Identificamos 23 COVs nas plantas controle e 17 nas plantas inoculadas, com maior concentração de p-cimeno e ácido salicílico nas plantas com o fungo. Compostos como a-pineno, ß-mirceno e p-cimeno induziram resposta nas antenas de *P. absoluta,* sendo o a-pineno o mais intenso no teste com mistura sintética. As fêmeas preferiram ovipositar em plantas controle, e o fungo reduziu o tempo de desenvolvimento dos estágios imaturos. Os resultados indicam que *P. chlamydosporia* pode modular a interação multitrófica entre planta e inseto via alteração dos voláteis emitidos, reduzindo a atratividade e afetando o desenvolvimento do inseto. Esses achados destacam o potencial do fungo como agente complementar em estratégias de manejo integrado da traça-do-tomateiro.

Palavras-chave: VOCs; Semioquímicos; endofitismo; MIP.

SUMÁRIO

General Introduction

The tomato leafminer *Phthorimaea absoluta* is a major pest of tomato crops worldwide (Urbaneja et al. 2009; Santana et al. 2019; Idriss et al. 2020; EPPO, 2024; Desneux et al. 2021; Yuan et al. 2024). The tomato leafminer causes significant losses in tomato crop productivity by creating mines in the leaves, which compromises photosynthesis, and by making galleries in the fruit, rendering it unsuitable for sale (Urbaneja et al. 2009; Santana et al. 2019; Idriss et al. 2020).

Volatile organic compounds (VOCs) emitted by plants modulate the behavior of herbivorous insects, natural enemies, and even neighboring plants, through a sensitization effect (Dudareva et al., 2013; Brilli et al., 2019). When attacked by insects, plants can release a mixture of volatile compounds, which can serve as olfactory signals for parasitoids or inhibit oviposition or insect development (Hilker & Fatouros, 2015). The VOCs emitted by tomato plants are the main mediators in *P. absoluta* decision-making when choosing its host (Proffit et al., 2010; Arce et al., 2017; Miano et al., 2022).

Microorganisms that modulate plant metabolism, causing alterations in volatile organic compounds, can be produced (Schulz-Bohm et al., 2017). These interactions that occur between organic and aerial organisms are mediated by volatile organic compounds (VOCs), essential for ecosystem functioning (Turlings & Erb, 2018). The analysis of these compounds is fundamental for understanding how plants and insects interact chemically and, consequently, in the development of sustainable management planning strategies (Bruce & Pickett, 2011).

Pochonia chlamydosporia is a fungus used as a biological control agent against nematode pests in soybean and tomato crops. Recent research has reported its role in inducing systemic resistance and modulating the profiles of volatile organic compounds (VOCs) produced by plants (Escudero et al., 2016; Ghahremani et al., 2019). *Pochonia chlamydosporia* is a root-dwelling endophytic fungus that, in association with roots, can alter the emission of bioactive VOCs. This will potentially affect interactions with herbivores and their natural enemies in the aerial parts of the plant. These characteristics give *P. chlamydosporia* the potential to act as an agent in integrated pest management.

The interaction between *P. chlamydosporia*, tomato plants, and *P. absoluta* represents a model system for studying the ecological consequences of microorganism-induced alterations in plant volatile compounds. The study of these multitrophic interactions allows for an understanding of chemical ecology and the development of innovative and sustainable strategies for pest control (Fatouros et al., 2016; Ghahremani et al., 2019). Thus, the objective of this work was to identify the variables that modulate the interaction involving an endophytic fungus, a tomato plant, and a specialist herbivore, as well as the potential applications of this association.

References

Arce C.C., Machado R.A., Ribas N.S, Cristaldo P.F, Ataíde L.M., Pallini A., Carmo F.M., Freitas L.G, Lima E. (2017). Nematode root herbivory in tomato increases leaf defenses and reduces leaf miner oviposition and performance. Journal of Chemical Ecology, 43: 120-128. DOI: 10.1007/s10886-016-0810-z

Brilli, F., Loreto, F., Baccelli, I. (2019). Exploiting plant volatile organic compounds (VOCs) in agriculture to improve sustainable defense strategies and the productivity of crops. Frontiers in Plant Science, 10: 264. DOI: 10.3389/fpls.2019.00264

Bruce, T. J. A., Pickett, J. A. (2011). Perception of plant volatile blends by herbivorous insects – Finding the right mix. Phytochemistry, 72(13): 1605–1611. DOI:10.1016/j.phytochem.2011.04.011

Desneux, N., Wajnberg, E., Wyckhuys, K. A. G., Burgio, G., Arpaia, S., Narváez-Vasquez, C. A., González-Cabrera, J., Ruescas, D. C., Tabone, E., Frandon, J., Pizzol, J., Poncet, C., Cabello, T., Urbaneja, A. (2010). Biological invasion of European tomato crops by *Tuta absoluta*: Ecology, geographic expansion and prospects for biological control. Journal of Pest Science, 83: 197–215. DOI: 10.1007/s10340-010-0321-6

Dudareva, N., Klempien, A., Muhlemann, J. K., Kaplan, I. (2013). Biosynthesis, function and metabolic engineering of plant volatile organic compounds. New Phytologist, 198(1): 16–32. DOI: 10.1111/nph.12145

Desneux, N., Han, P., Mansour, R., Arnó, J., Brévault, T., Campos, M. R., Chailleux, A., Guedes, R. N. C., Karimi, J., Konan, K. A. J., Lavoir, A. V., Luna, M. G., Perez-Hedo, M., Urbaneja, A., Verheggen, F. J., Zappalà, L., Abbes, K., Ali, A., Bayram, Y., Cantor, F., Cuthbertson, A. G. S., De Vis, R., Erler, F., Firake, D. M., Haddi, K., Hajjar, M. J., Ismoilov, K., Jaworski, C. C., Kenis, M., Liu, H. T., Madadi, H., Martin, T., Mazih, A., Messelink, G. J., Mohamed, S. A., Nofemela, R. S., Oke, A., Ramos, C., Ricupero, M., Roditakis, E., Shashank, P. R., Wan, F. H., Wang, M. H., Wang, S., Zhang, Y. B., Biondi, A. (2022). Integrated pest management of *Tuta absoluta*: practical implementations across different world regions. Journal of Pest Science, 95: 17–39. DOI: 10.1007/s10340-021-01442-8

Escudero, N., Lopez-Moya, F., Nombela, G., Sanchez-Campillo, M., Lopez-Llorca, L. V. (2016). *Pochonia chlamydosporia* induces plant-dependent systemic resistance to root-knot nematodes and modulates volatile organic compounds in tomato. Frontiers in Plant Science, 7: 1609. DOI: 10.3389/fpls.2016.01609

EPPO - European and Mediterranean Plant Protection Organization. (2023). *Global Database*. Available online at: https://gd.eppo.int/taxon/GNORAB/distribution (accessed June, 2024).

Fatouros, N. E., Cusumano, A., Danchin, E. G. J., Colazza, S. (2016). Prospects of herbivore egg-killing plant defenses for sustainable crop protection. Ecology and Evolution, 6(19): 6906–6918. DOI: 10.1002/ece3.2354

Ghahremani, Z., Escudero, N., Saus, E., Gabaldón, T., Sorribas, F. J., Lopez-Llorca, L. V. (2019). *Pochonia chlamydosporia* induces plant-dependent systemic resistance to root-knot nematodes and modulates volatile organic compounds in tomato. Frontiers in Plant Science, 10: 945. DOI: 10.3389/fpls.2019.00945

Hilker, M., & Fatouros, N. E. (2015). Plant responses to insect egg deposition. Annual Review of Entomology, 60: 493–515. DOI: 10.1146/annurev-ento-010814-021013

Idriss, G.E.A., Du Plessis, H., Khamis, F.M., Ekesi, S., Tanga, C.M., Mohamed, S.A. (2020). Host range and effects of plant species on preference and fitness of *Tuta absoluta* (Lepidoptera: Gelechiidae). Journal of Economic Entomology, 113: 1279–1289. DOI: 10.1093/jee/toaa002

Miano, J. L., Campos, M. R., Silva, W. M., Siqueira, H. A. A. (2022). Tomato volatile organic compounds and their role in the behavior of *Tuta absoluta* (Lepidoptera: Gelechiidae). Journal of Chemical Ecology, 48: 1–13. DOI: 10.1007/s10886-022-01341-3

Pieterse, C. M. J., Zamioudis, C., Berendsen, R. L., Weller, D. M., van Wees, S. C. M., Bakker, P. A. H. M. (2014). Induced systemic resistance by beneficial microbes. Annual Review of Phytopathology, 52: 347–375. DOI: 10.1146/annurev-phyto-082712-102340

Proffit, M., Birgersson, G., Bengtsson, M., Reis, Jr. R., Witzgall, P., Lima, E. (2011). Attraction and oviposition of *Tuta absoluta* females in response to tomato leaf volatiles. Journal of Chemical Ecology, 37: 565–574. DOI: 10.1007/s10886-011-9961-0

Santana, Jr, P.A., Kumar, L., Da Silva, R.S., Picanço, M.C. (2019). Global geographic distribution of *Tuta absoluta* as affected by climate change. Journal of Pest Science, 92: 1373-1385. DOI: 10.1007/s10340-018-1057-y

Schulz-Bohm, K., Martín-Sánchez, L., Garbeva, P. (2017). Microbial volatile organic compounds: Role in intra- and inter-kingdom interactions. Frontiers in Microbiology, 8: 2552. DOI: 10.3389/fmicb.2017.02552

Turlings, T. C. J., Erb, M. (2018). Tritrophic interactions mediated by herbivore-induced plant volatiles: Mechanisms, ecological relevance, and application potential. Annual Review of Entomology, 63: 433–452. DOI: 10.1146/annurev-ento-020117-043507

Urbaneja, A., Montón, H., Mollá, O. (2009). Suitability of the tomato borer *Tuta absoluta* as prey for *Macrolophus pygmaeus* and *Nesidiocoris tenuis*. Journal of Applied Entomology, 133: 292–296. DOI: 10.1111/j.1439-0418.2008.01319.x

Yuan, X., Zhang, Y., Hu L., Sang W., Yang, Z. (2024). Investigating the effects of species niche shifts on the potential distribution of *Tuta absoluta* (Lepidoptera: Gelechiidae) by using global occurrence data. Journal of Insect Science, 24(3): 8. DOI:10.1093/jisesa/ieae059

Chapter 1

Does inoculation of *Pochonia chlamydosporia* on *Solanum lycopersicum* tomato plants improve the resistance against the tomato borer *Phthorimaea absoluta*?

SANTOS, Karenn Christiny Pereira, D.Sc., Federal University of Viçosa, july de 2025. **Influence of the inoculation of *Pochonia chlamydosporia* (Hypocreales: Clavicipitaceae) on *Solanum lycopersicum* tomato plants in resistance against the tomato borer *Phthorimaea absoluta* (Lepidoptera: Gelechiidae).** Adviser: Eraldo Lima. Co-adviser: Natália de Souza Ribas

Abstract

Pochonia chlamydosporia is a root endophytic fungus that promotes plant growth without causing damage to the host plant. The presence of this fungus in plant roots can induce changes in plant metabolism, influencing both the oviposition choices of herbivorous insect females and their subsequent development. Therefore, our objective was to study how the presence of the endophytic fungus *P. chlamydosporia* in the roots of tomato plants (*Solanum lycopersicum* L.) affects the production of volatiles released by these plants, the attractiveness to the specialist herbivore (*Phthorimaea absoluta*), and its development. For this, we collected the headspace volatiles from the aerial parts of control plants and plants inoculated with *P. chlamydosporia*. Additionally, we evaluated the effect of the fungus on the oviposition choice of *P. absoluta* females. We also assessed the effect of oviposition and inoculation of *P. chlamydosporia* on the development of *P. absoluta* offspring at all stages (egg, larva, pupa, and adult). As a result, we identified 23 volatile organic compounds in control plants and 17 compounds in plants inoculated with *P. chlamydosporia*. Among them, the compounds p-cymene and salicylic acid, which are important for defense against herbivorous insects, had higher average concentrations in plants infected with *P. chlamydosporia*. We observed that *P. absoluta* females prefer to oviposit on clean plants, without the presence of the fungus. The presence of the fungus also reduced the development time of the egg stage and the total larval time. Therefore, tomato plants infected with the endophytic fungus *P. chlamydosporia* can influence the host choice of *P. absoluta* females, as well as the development of their offspring. The change in the composition of tomato volatiles and the decrease in *P. absoluta* oviposition, as demonstrated in this work, may be important for the development of new control techniques for the tomato leafminer, particularly *in association* with integrated pest management of this pest.

Keywords: Endophytism; insect-microorganism interaction; tritrophic interaction; plant volatiles.

1. Introduction

Plants use extremely specialized defense mechanisms to withstand biotic and abiotic stressors. These defense mechanisms can be chemical, involving the production of volatile compounds and secondary metabolites, or physical, such as the presence and density of trichomes (Karban & Myers, 1989; Schoonhoven et al. 2005; Agrawal, 2010; Arimura, 2021). One of the main stimuli for triggering these defensive reactions is contact with herbivorous insects, like pest insects (Mahanta et al. 2025).

One of the defense strategies has become the establishment of symbiotic and beneficial relationships with the microbiome in which the plant is embedded. In addition to potentially exhibiting pesticidal activity, the microorganisms act as antagonistic agents (Kumar et al. 2008; Zappalà et al. 2013; Tolba et al. 2021). The chemical compounds present in this system can be employed as tools in various fields, including agriculture (Mattoo et al. 2021). Endophytic fungi are a successful example of this symbiotic interaction. These soil microorganisms can promote plant growth by providing nutrients and assisting in pest and disease control (Kumar & Kaushik, 2013; Mattoo et al. 2021).

By promoting allelochemicals like secondary metabolites, boosting the synthesis of compounds like methyl salicylate and (Z)-jasmonate, and generating compounds that inhibit or eradicate phytopathogens, the bioactive compounds involved in the establishment of endophytes are produced (Kobayashi, 2015; Eng et al., 2018). The volatile organic compounds (VOCs) released by plants can also be altered by soil microorganisms, creating what are known as microbe-induced plant volatiles (MIPVs) (Sharifi et al., 2018). Therefore, interactions among herbivorous insects, plants, and endophytes can affect their performance and preference (Oki et al., 2009; Arce et al., 2017).

Because of its high nematode biocontrol (e.g., of the genus Meloidogyne) and ability to promote plant growth without endangering the host plant, *Pochonia chlamydosporia* is a root endophytic fungus that is frequently used in tomato and soybean crops (Escudero & Lopez-Llorca, 2012; Kozyrovska, 2013; Larriba et al., 2015; R.R. Coutinho et al., 2021). For instance, using certain isolates of *P. chlamydosporia* (Pc-3, Pc-10, and Pc-19) increased leaf mass by 100% to 330% in a study by Dallemole-Giaretta et al. (2015) using tomato and lettuce cultivars.

Additionally, *P. chlamydosporia*'s endophytism in tomato plants allowed for the expression of gene regulators linked to plant defense (Pentimone et al., 2018; Rosso et al., 2023), as well as inducing changes in metabolism and defense response (Ludwig-Muller et al., 2015; Gouveia et al., 2023).

Insect females use volatile organic compounds (VOCs) emitted by plants to navigate and locate the host where they will lay their eggs (Proffit et al. 2011; Arce et al. 2017). This decision is crucial for herbivorous insect females because it will be the food source for their offspring and, as a result, will influence the growth and survival of their descendants (Jaenike, 1978). Therefore, the interaction between endophyte, plant, and insect may have potential for controlling pest herbivores, both by influencing the choice of host plant and by affecting the development of the offspring.

The tomato leafminer, *Phthorimaea absoluta* (Meyrick, 1917) (Lepidoptera: Gelechiidae), is one of the main pests of tomato plants worldwide, with records in 50 countries across the Americas, Africa, Europe, and Asia (Urbaneja et al. 2009; Santana et al. 2019; Idriss et al. 2020; EPPO, 2024; Desneux et al. 2021; Yuan et al. 2024). The larvae of *P. absoluta* primarily feed on the leaves of the plants, which can result in production losses of up to 100% (Biondi et al. 2018; Desneux et al. 2021). Chemical control is one of the most commonly used methods for its management, but the excessive use of insecticides has led to resistance in the pest and environmental impacts (Guedes et al. 2019). Therefore, there is a need to study and develop other methods.

Due to physiological changes brought on by the fungus's endophytism, *P. chlamydosporia*'s interaction with tomato plant roots, for instance, may change the profile of volatile organic compounds (VOCs) that the plant releases and the attractiveness of P. absoluta females. The volatiles released during this interaction between the fungus and the tomato plant, as well as the consequences for a specialized herbivore like the tomato leafminer, are still unknown. Induced defense mechanisms expressed in the released volatile organic compounds may be discovered through a more thorough prospecting of the compounds involved in these interactions.

Given that soil microorganisms, such as endophytic fungus, can cause chances on plants metabolism and defense response, we hypothesize that: i) the presence of an endophytic fungus alters the VOC profile in plants; and consequently, ii) females of an specialist herbivore will prefer to oviposit on plants without the presence of this fungus. Thus, our objective was to study this trophic interaction, using the system: tomato plants *Solanum lycopersicum*, the fungus *Pochonia chlamydosporia* (isolated Pc10), and the specialist herbivore *Phthorimaea absoluta*, to determine possible compounds involved in this interaction and how these compounds can affect the behavior and development of this herbivore.

2. Methodology

2.1 Rearing of *Phthorimaea absoluta*

Leaves containing larvae and leaves containing eggs of *Phthorimaea absoluta* were obtained from the rearing colony of the Integrated Pest Management Laboratory at the Federal University of Viçosa (UFV), MG - Brazil, and placed in wooden cages (46x46x46cm) covered with organza to start the colony. Subsequently, the establishment and maintenance of the *P. absoluta* colony were carried out at the Laboratory of Semiochemicals and Insect Behavior at UFV. Fresh tomato leaves were placed in the cages to feed the larvae, allowing them to complete the larval cycle until the pupal stage. The pupae were manually removed from the leaves, placed in Petri dishes, and transferred to acrylic cages (30 x 30 x 30 cm) for adult emergence. Adults were fed ad libitum with a 10% sugar solution soaked in cotton, and fresh tomato leaves were provided in the cages for females to oviposit. The *P. absoluta* population is maintained in a rearing room at 25 ± 2 °C, with a photoperiod of 12:12 (light: dark) and 70 ± 5% relative humidity (RH).

2.2 Tomato Plants

Tomato plants (*Solanum lycopersicum* L.), var. Santa Cruz, where both the rearing of *P. absoluta* and the aeration and oviposition preference experiments were conducted. For the plants used in the rearing of *P. absoluta*, seedlings were obtained by sowing in plastic trays containing vegetable substrate (MecPlant®). The trays were placed in a germination chamber (TE-4000/1) with a photoperiod of 12h:12h (light:dark), at a temperature of 25°C and relative humidity of 75% ± 5%. After 10–15 days, seedlings with two true leaves (the second pair of expanding leaves, in addition to the cotyledonary leaves) were transplanted into 3L pots containing vegetable substrate (MecPlant). These were grown in a greenhouse at 25 ± 2°C, 70

± 5% relative humidity, and supplemented with a fertilizer solution containing NPK 4:14:8 (~16 g/pot) and single superphosphate (~8 g/pot) supplied every 7 days.

For the oviposition preference, development, and volatile organic compound collection experiments, 10 to 15 day old tomato seedlings were transplanted into 300 mL pots containing soil formed by mixing sand and red latosol (1:1 ratio). The soil, previously sterilized at 120°C for 2 hours (Zauza et al. 2007) to isolate the effect of the fungus on the plant, was moistened daily for at least 3 days before transplanting to make it suitable for receiving the plants. After transplanting, the plants were divided into two treatments: (I) plants with roots inoculated with the Pc10 isolate of *P. chlamydosporia* (inoculated plants) and (II) plants without fungus in the roots (control plants). For root inoculation, two holes (Ø ~1.5 cm and 2 cm deep) were made around the plant stem, adding 5,000 chlamydospores of *P. chlamydosporia* per gram of soil (Dallemole-Giaretta, 2008). Both inoculated and control plants were kept in a Mammoth Elite 120 grow tent (120x120x215 cm), where internal conditions were controlled, maintaining a temperature of 25 ± 2°C, 70 ± 5% relative humidity, and a photoperiod of 12:12 (light: dark). The plants were watered as needed to keep the soil moist and supplemented with NPK 4:14:8 (~0.4 g/pot) every 7 days. The experiments began 40 days after fungal inoculation, a period during which the fungus induces changes in plant defense-related phytohormones (Gouveia et al., 2022).

2.3 Weight standardization of *P. absoluta* individuals and the obtaining of mated females

The weight of *P. absoluta* individuals was determined based on pupal mass (Muller et al., 2016). Pupae were sexed using external morphological characteristics (Coelho & França, 1987; Genç, 2016) and weighed on an analytical balance (Shimadzu AUW220D/Calibration: max. 220 g and min. 1 mg). The weight of the pupae used ranged from 1.89 to 2.66 mg for males and from 2.10 to 3.43 mg for females (Gonçalves et. al. 2024). The weight range was established using 200 pupae of each sex to eliminate the effect of individual weight on the experiments. Each pupa was individually placed in a 70 mL plastic cage (properly labeled), with one end covered with organza to allow air circulation. The pupae were kept in a rearing room under the same conditions described previously.

Pairs of adults, placed in a 70 mL plastic cage, containing one female and one male (both 2 days old), were formed, and mated females were used in the oviposition tests. Mating occurred at the beginning of the photophase, which is the calling (receptivity) period for females (Hickel et. al, 1992), lasting between 200 and 280 minutes.

2.4 Chlamydospores of *Pochonia chlamydosporia*

The *P. chlamydosporia* fungus (Pc-10 isolate) used in this study was obtained from the fungal collection of the Laboratory of Biological Control of Phytonematodes at UFV. To obtain chlamydospores of *P. chlamydosporia*, a solid medium composed of 100 g of polished rice and 33 mL of distilled water (w/v) was used. The rice and water were added to bags with a biological filter, which were then autoclaved for 20 minutes at 120°C. After reaching room temperature, the bags were carefully opened in a laminar flow hood, and three discs (Ø = 9 mm) of potato dextrose agar (PDA) medium containing structures of the *P. chlamydosporia* fungus were added. Immediately after, the bags with the biological filter were closed and stored in a growth chamber for 21 days at 28°C in the dark. After this period, the rice colonized by the fungus was moistened with autoclaved water and manually macerated to release the chlamydospores. The resulting material was poured over a container with layers of gauze to separate the chlamydospores from the rice hulls, thus obtaining a suspension containing *P. chlamydosporia*. Finally, with the aid of a Neubauer chamber, the chlamydospores were quantified and the suspension was calibrated so that the fungal concentration was adjusted to 5,000 chlamydospores per gram of soil, a quantity sufficient for the good establishment of the fungus in the plant and, once established, for it to be actively efficient (Dallemole-Giaretta, 2008).

2.5 Soil Plating with *P. chlamydosporia*

To confirm the establishment of the fungus in the soil after inoculation, the number of colony-forming units (CFU) of the fungus was determined from the soil of each pot (experimental unit) (g of soil per mL of diluent) (Monteiro et al. 2020). Thus, 1 g of soil was collected from each experimental plot and added to a 50 mL Falcon tube containing 9 mL of water, previously autoclaved for 20 minutes at 120°C. This dilution was designated as 10^{-1}. Immediately after, the Falcon tubes containing the 10^{-1} dilution were shaken in a Vortex Mixer (KASVI basic K45-2820) for 60 seconds, and a 100 µL aliquot was transferred to a 2 mL Eppendorf tube containing 900 µL of autoclaved water. This dilution was called 10^{-2} and was used to estimate the number of CFUs of *P. chlamydosporia* in each experimental plot. After shaking the Eppendorf tubes for 30 seconds, a 100 µL aliquot of the dilution was deposited and spread with a Drigalski spatula on a Petri dish containing semi-selective medium for *P. chlamydosporia* (Gaspard et al. 1990). For each soil sample, 3 plates were used, i.e., three replicates. Finally, the plates were kept in a growth chamber at 28°C for 7 days, after which the CFUs were counted. The count was performed by direct observation of the plates, using a hand magnifying glass.

2.6 Headspace Volatile Collection and Chromatographic Analysis

Volatiles from the aerial parts of control plants (N = 6) and plants inoculated with the fungus *P. chlamydosporia* (N = 6) were collected using the headspace technique over a 24hour period. The plants were individually placed in cylindrical glass chambers (37 cm high × 14 cm in diameter) with the soil surface covered by aluminum foil (Arce et al. 2017). An air filter containing activated charcoal (1 L/min) was introduced into the chamber, and volatiles were drawn out (0.5 L/min) and trapped on 20 mg of HayeSep Q (80–100 mesh) (Analytical Research Systems, Florida, USA). Subsequently, the volatiles were eluted in 200 µL of hexane. The same procedure was used for all aeration tests. All collected samples were stored at -18°C for later analysis using GC-MS.

For the analysis of the collected volatiles, a 24 µL aliquot was taken from each sample, and 1 µL of 1000 ng n-heptyl acetate standard was added, resulting in a sample with 40 ng/µL of the internal standard. An aliquot was taken solely for the quantification and identification of the compounds. The remaining sample was stored for use in other experiments.

The volatile profile was obtained by injecting 1 µL of this sample into a gas chromatograph coupled with a mass spectrometer (Shimadzu GCMS – QP2010 SE), in splitless mode, Rtx-5MS (Restek chromatography products), mode into a DB-5 column (30 m × 0.25 mm; df = 0.25 µm) using helium as carrier gas (170 kPA). The temperature program used was the temperature starts at 35°C (held for 1 min), followed by an increase of 8°C/min until reaching 280°C, which is held for 5 min. The injector temperature was 250 °C. The identification and quantification of compounds were performed using comparisons to the mass spectra of NIST 14s library spectra and confirmed with the spectra of commercial standards.

2.7 Oviposition Preference of *P. absoluta*

Plants from both treatments were placed in a wooden cage (56 × 56 × 56 cm) covered with organza, separated diagonally by approximately 15 cm, and four mated *P. absoluta* females were released into each cage for 24 hours. Afterwards, the number of eggs was counted. Oviposition occurred in a room under controlled conditions of temperature (25 ± 2 °C), photoperiod (12:12, light: dark), and relative humidity (70 ± 5%). The females were released during the scotophase, which is the oviposition period of *P. absoluta* (Proffit et al., 2011), in a room with the same controlled conditions as previously described. The plants were acclimated in the test room 24 hours before the experiments. In total, 15 control plants, 15 inoculated plants, and 60 mated females were used.

2.8 Development of *P. absoluta*

Following the oviposition preference test, six plants from each treatment (inoculated and control) were separated and placed in individual cages. The number of eggs per plant was standardized to 5. The remaining eggs were removed with the aid of a fine brush (no. 00). The standardization aimed to: (I) ensure sufficient food for all larvae to complete their life cycle, (II) avoid competition for food among individuals, and (III) achieve similar levels of damage in all plants (Arce et al., 2017). Each plant was examined daily until the end of development. For each individual of *P. absoluta*, the duration (in days) of each stage (egg, larva, pupa, and adult) was recorded. The external morphology of the larval instars was previously observed to identify the instars (L1, L2, L3, and L4) (Figure 1). After the larval stage, the pupae were weighed on an analytical balance and individually placed in 70 mL cages to monitor the time to adult emergence and subsequently observe adult longevity. Thirty individuals were evaluated for each treatment (inoculated and control).

Figure 1: Larval instar of the *Phthorimaea absoluta.* From left to right: L1, L2, L3, and L4.

2.9 Statistical Analysis

The oviposition preference data were analyzed using chi-square analysis. The development data were analyzed using GLMs with a Poisson distribution, and least squares averages (emmeans) were performed for paired comparison. The volatile organic compound data were analyzed using GLMs with a Gaussian distribution, and least squares averages (emmeans) were performed for paired comparison. All the statistical analysis was performed using the software R (v. 4.0.0; R Development Core Team, 2020).

3. Results

3.1 Soil Plating with *P. chlamydosporia*

The establishment of the fungus in the soil of the plants used was confirmed in both the *P. absoluta* development experiment (n=6) and the oviposition preference tests (n=15). The average number of colony-forming units (CFU) in the development experiment was 4.56×10^3 CFU/g of soil, and in the preference tests, it was 2.91×10^3 CFU/g of soil.

3.2 Volatile Profile in Plants

A total of 23 volatile organic compounds were identified in the control plants, and 17 compounds were found in the plants inoculated with *Pochonia chlamydosporia* (Pc10 isolate) (40 days after inoculation) (Table 3). Among these compounds, 13 were found in both treatments: α-pinene, β-myrcene, α-phellandrene, α-terpinene, p-cymene, β-phellandrene, β-ocimene, 2,4-dimethyldecane, nonanal, dodecane, trans-β-caryophyllene, 4,7-dimethylundecane, and salicylic acid. Additionally, nine compounds were found only in the control plants: 2-carene, hexanal, sabinene, pentane, γ-terpinene, α-terpinolene, acetic acid, decanal, and δ-elemene. Furthermore, only one compound was identified exclusively in the plants with *P. chlamydosporia*, which was terpinene (Table 1).

We identified differences in the mean concentrations of the compounds between the control plants and the plants inoculated with the fungus (F = 9.9; df = 11,115; $P < 0.001$) (Table 1). Among the compounds found in both treatments, only p-cymene and salicylic acid had lower mean concentrations in the control plants and higher concentrations in the plants containing *P. chlamydosporia* (Table 1). For all other shared compounds, the mean concentrations were higher in the control plants than in those containing the fungus (Table 1).

Table 1. Mean of concentration (ng/µl) of volatile organic compound identity of control plants and plants inoculated with *Pochonia chlamydosporia* (Isolate Pc10), after 40 days of inoculation.

N°	**Treatment**	**Compound**	**RI $_{exp}$**	**RI $_{lit}$**	**Reference (literature)**	**Chemical Class**	**Mean Concentration ± SD (ng/µ)**	**Supplier/ Provided by***	**Purity/ Concentration***
1	*Pochonia chlamydosporia*	Nonane	906	900	Adams. 2017	Hydrocarbon (alkane)	0.363 ± 1.01	-	-
2	*Pochonia chlamydosporia*	α-Pinene*	946	939	NIST WebBook, 2023	Monoterpene	2.257 ± 1.120	Esalq - Piracicaba	40ng/µL (diluted in hexane)
3	*Pochonia chlamydosporia*	β-Myrcene*	990	988	Adams, 2017	Monoterpene	0.134 ± 0.091	SIGMA - ALDERICH	90%
4	*Pochonia chlamydosporia*	4-Carene*	1017	1011	Adams, 2017	Monoterpene	21.828 ± 5.60	-	30ng/µL (diluted in hexane)
5	*Pochonia chlamydosporia*	α-Phellandrene	1021	1005	Adams, 2017	Monoterpene	5.097 ± 1.23	Max Plank - Holanda	40ng/µL (diluted in hexane)
6	*Pochonia chlamydosporia*	α-Terpinene	1035	1017	NIST WebBook, 2023	Monoterpene	1.973 ± 0.99	-	-
7	*Pochonia chlamydosporia*	p-Cymene*	1045	1026	Adams, 2017	Aromatic monoterpene	0.870 ± 0.55	SIGMA - ALDERICH	99%
8	*Pochonia chlamydosporia*	β-Phellandrene	1051	1033	Adams, 2017	Monoterpene	76.203 ± 21.07	Max Plank - Holanda	40ng/µL (diluted in hexane)

9	*Pochonia chlamydosporia*	β-Ocimene*	1069	1050	NIST WebBook, 2023	Monoterpene	0.333 ± 0.16	SIGMA - ALDERICH	90%
10	*Pochonia chlamydosporia*	2,4-Dimethyl decane	1077	–	–	Hydrocarbon (branched alkane)	0.711 ± 0.32	-	-
11	*Pochonia chlamydosporia*	4,7-Dimethyl undecane	1124	–	–	Hydrocarbon (branched alkane)	0.450 ± 0.36	-	-
12	*Pochonia chlamydosporia*	Nonanal*	1129	1100	Adams, 2017	Aldehyde	1.007 ± 0.69	Lab. Semioquímicos - UFV	20ng/µL (diluted in hexane)
13	*Pochonia chlamydosporia*	Dodecane*	1230	1200	NIST WebBook, 2023	Hydrocarbon (alkane)	1.208 ± 0.14	Lab. Semioquímicos - UFV	30ng/µL (diluted in hexane)
14	*Pochonia chlamydosporia*	Tridecane	1338	1300	Adams, 2017	Hydrocarbon (alkane)	5.689 ± 1.58	-	-
15	*Pochonia chlamydosporia*	β-Caryophyllene	1488	1418	NIST WebBook, 2023	Sesquiterpene	2.578 ± 1.03	Esalq - Piracicaba	40ng/µL (diluted in hexane)
16	*Pochonia chlamydosporia*	Salicylic acid	2007	2015	NIST WebBook, 2023	Aromatic acid	11.622 ± 3.84	-	-
1	Control	α-Pinene*	944	939	Adams, 2017	Monoterpene	9.575 ± 4.055	Esalq - Piracicaba	40ng/µL (diluted in hexane)
2	Control	Hexanal*	968	801	NIST WebBook, 2023	Aldehyde	0.396 ± 0.222	Esalq - Piracicaba	40ng/µL (diluted in hexane)

3	Control	Sabinene*	987	969	Adams, 2017	Monoterpene	0.389 ± 0.164	SIGMA - ALDERICH	75%
4	Control	β-Myrcene*	1004	988	Adams, 2017	Monoterpene	1.236 ± 0.426	SIGMA - ALDERICH	90%
5	Control	2-Carene*	1016	1012	Adams, 2017	Monoterpene	41.873 ± 17,646	-	30ng/µL (diluted in hexane)
6	Control	α-Phellandrene	1020	1005	Adams, 2017	Monoterpene	10.818 ± 3.535	Max Plank - Holanda	
7	Control	α-Terpinene	1034	1017	NIST WebBook, 2023	Monoterpene	16.841 ± 26.797	-	-
8	Control	p-Cymene*	1044	1026	Adams, 2017	Aromatic monoterpene	0.623 ± 0.468	SIGMA - ALDERICH	99%
9	Control	β-Phellandrene	1050	1033	Adams, 2017	Monoterpene	155.867 ± 43.979	Max Plank - Holanda	
10	Control	β-Ocimene	1068	1050	NIST WebBook, 2023	Monoterpene	0.551 ± 0.200	SIGMA - ALDERICH	90%
11	Control	2,4-Dimethyl decane	1076	-	-	Hydrocarbon (branched alkane)	0.807 ± 0.630	-	-
12	Control	γ-Terpinene*	1080	1062	Adams, 2017	Monoterpene	0,698 ± 0,168	SIGMA - ALDERICH	98,50%
13	Control	(*Z*)-Caren-ol	1120	1130	Pherobase	Monoterpene (alcohol)	1.828 ± 1.082	-	-
14	Control	Nonanal*	1128	1100	Adams, 2017	Aldehyde	2.103 ± 1.932	-	20ng/µL (diluted in hexane)
15	Control	Acetic acid*	1141	1450	NIST WebBook, 2023	Carboxylic acid	0.516 ± 0.442	Max Plank - Holanda	30ng/µL (diluted in hexane)

16	Control	Dodecane*	1229	1200	NIST WebBook, 2023	Hydrocarbon (alkane)	2.268 ± 2.203	-	30ng/µL (diluted in hexane)
17	Control	Decanal	1239	1208	Adams, 2017	Aldehyde	1.431 ± 1.194		10ng/µL (diluted in hexane)
18	Control	4,7-Dimethyl undecane	1313	-	-	Hydrocarbon (branched alkane)	1.335 ± 1.237	-	-
19	Control	Tridecane	1337	1300	Adams, 2017	Hydrocarbon (alkane)	13.780 ± 14.970	-	-
20	Control	δ-Elemene	1389	1339	Adams, 2017	Sesquiterpene	4.562 ± 2.828	-	-
21	Control	β-Caryophyllene	1487	1418	NIST WebBook, 2023	Sesquiterpene	3.659 ± 1.502	Esalq - Piracicaba	
22	Control	Salicylic acid	1405	2015	NIST WebBook, 2023	Aromatic acid	2.167 ± 1.752	-	-

3. Preference of oviposition *P. absoluta*

Phthorimaea absoluta females prefer to oviposit on control tomato plants (without *Pochonia chlamydosporia*, 40 days after inoculation) ($X^2 = 211.1$; df= 14; $p < 0.001$). On average, 41.2 eggs were laid on control plants (N=15) and 21.53 eggs on plants with *P. chlamydosporia* (Figura 2).

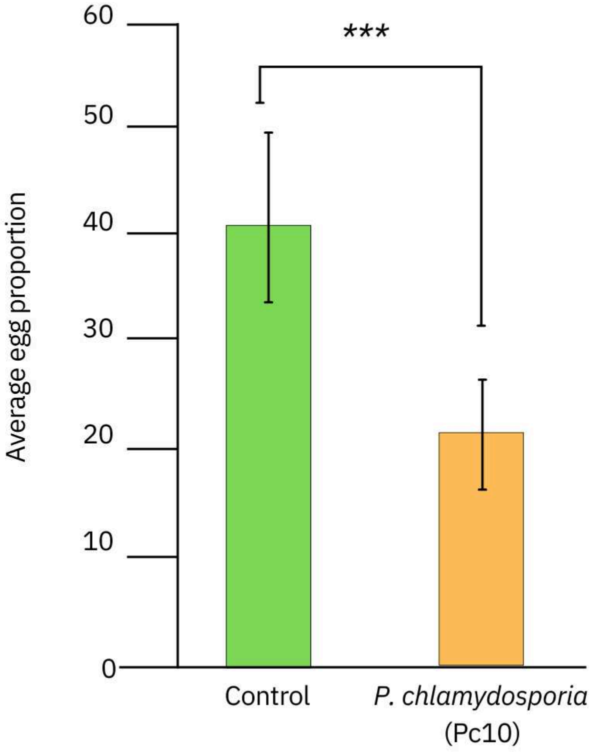

Figure 2. Average egg proportion of *Phthorimaea absoluta* in control plants (green bars) (N=15) and plants inoculated with *Pochonia chlamydosporia* (Isolate Pc10) (N=15) (orange bars), after 40 days of inoculation. The p-value was obtained through a Chi-square analysis. *** indicates a significant difference between treatments ($p < 0.001$).

3.4 Development of *P. absoluta*

Overall, the presence of *Pochonia chlamydosporia* in tomato plants had an impact on larval development (Figure 3). Larvae had a higher mean number of days in these stages on control plants than on plants with *P. chlamydosporia* L1 (F = 40.5; df = 1,58; $P < 0.001$) and L2 (F = 99.5; df = 1,58; $P < 0.001$). The opposite was observed for L3 larvae, which had a higher mean number of days in this stage on plants with *P. chlamydosporia* compared to control plants (F = 22.2; df = 1,58; $P < 0.001$). When analyzing the total larval period, we observed that the mean number of larval days was higher on control plants than on those with *P. chlamydosporia* (F = 22.5; df = 1,58; P < 0.001). Our treatments did not affect the duration of the L4 larval stage (F = 3.9; df = 2,58; P = 0.054).

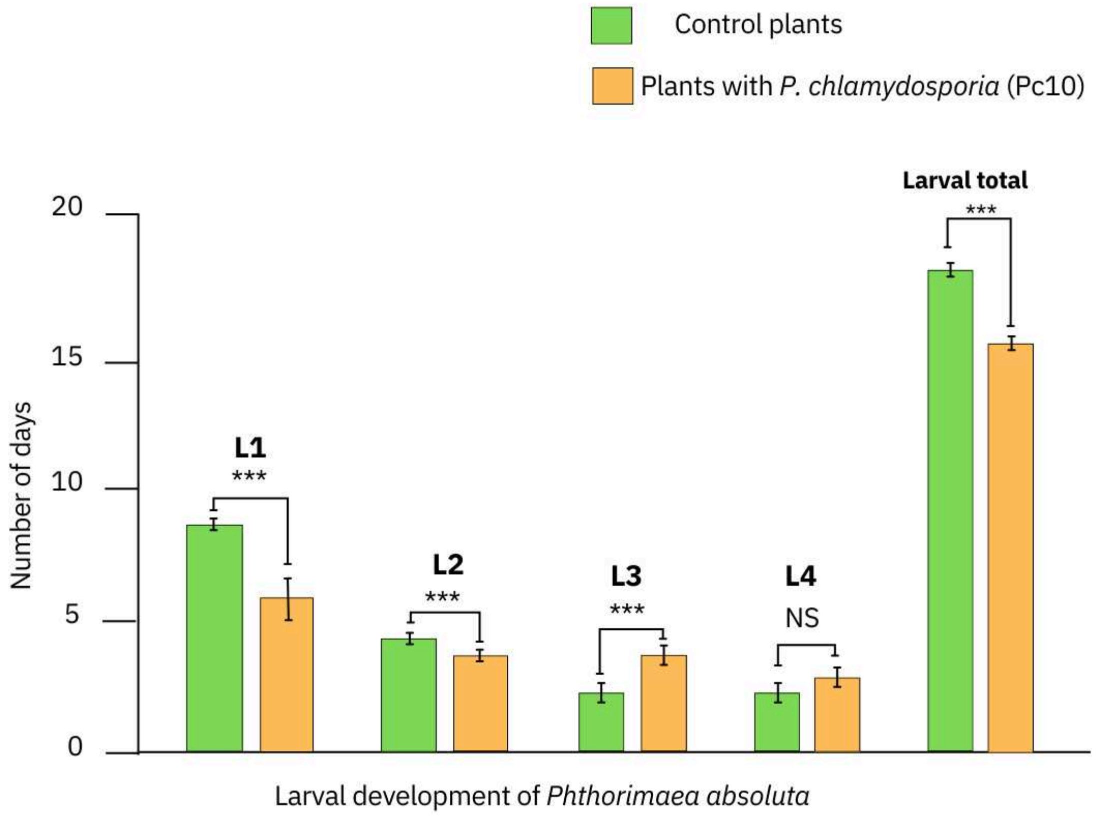

Figure 3. Average duration (in days) of larval development (L1, L2, L3, L4 stages, and total larval period) of *Phthorimaea absoluta* individuals (N=30) on control plants (green bars) and plants inoculated with *Pochonia chlamydosporia* (orange bars). P-values were generated through a generalized linear model (Gaussian family). *** indicates a significant difference between treatments (p< 0.001); NS indicates not significant.

The presence of *Pochonia chlamydosporia* in the plants also affected the stationary developmental stages, egg, and pupa. The mean number of days in the egg stage was higher in the control treatment than in the treatment with the fungus (F = 4.5; df = 1,58; P < 0.05) (Figure 4A). On the other hand, the mean number of days in the pupal stage was greater in insects that fed on plants with the fungus than in those on control plants (F = 19.8; df = 1,38; P < 0.001) (Figure 4B). The presence of the fungus did not affect pupal weight (F = 1.3; df = 1,37; P = 0.3). In addition, it did not affect adult longevity (F = 0.008; df = 2,53; P = 0.9) (Figure 4C), nor the total development time (in days) of the insects (F = 1.7; df = 2,53; P = 0.2) (Figure 4D).

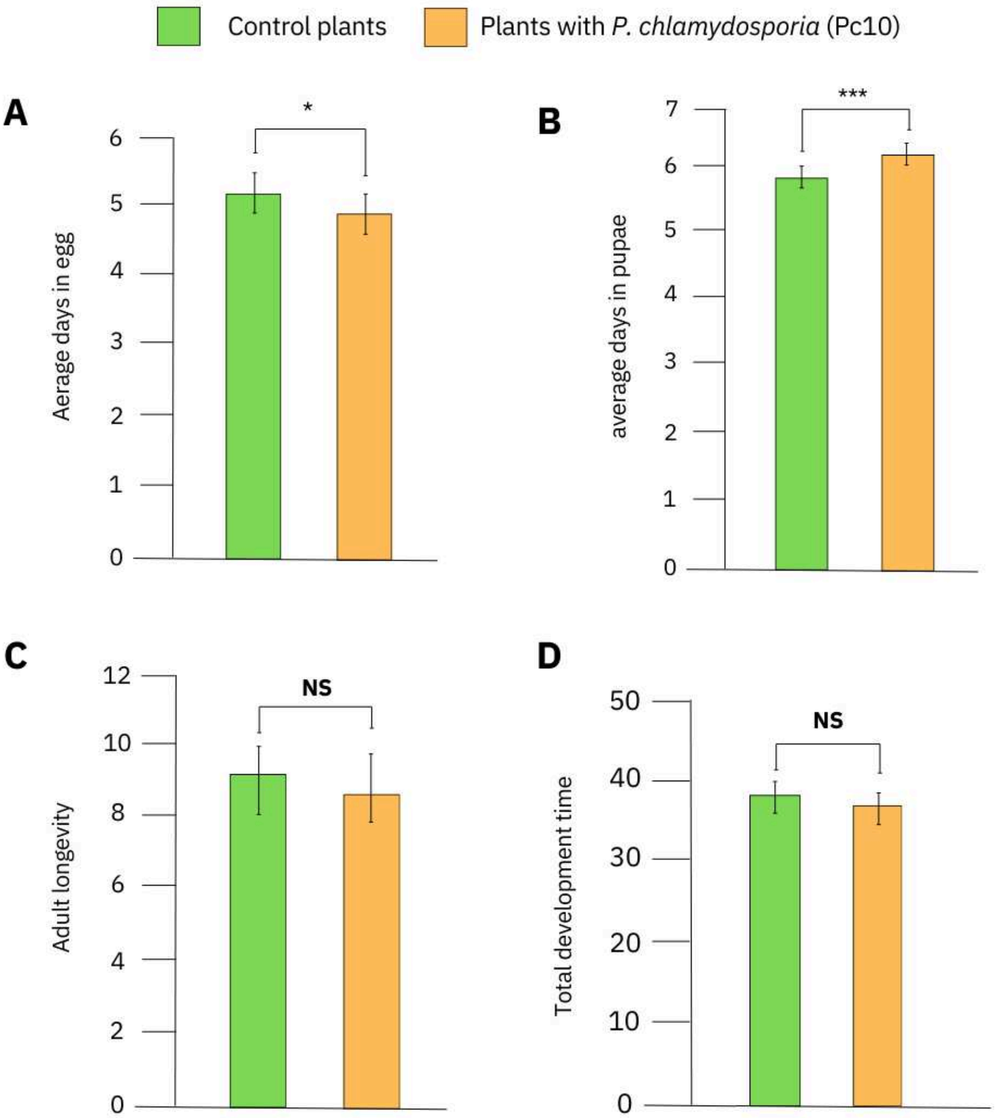

Figure 4. Average duration (in days) of stationary stages, egg (A) and pupa (B) of *Phthorimaea absoluta* individuals (N=30) on control plants (green bars) and plants inoculated with *Pochonia chlamydosporia* (orange bars); adult longevity (C) and total development time (D). P values were generated using a generalized linear model (Gaussian family). *indicates a difference between treatments ($p < 0.05$); *** indicates a difference between treatments ($p < 0.001$); NS indicates not significant.

4. Discussion

In this study, we observed that the presence of the fungus *Pochonia chlamydosporia*, after 40 days of inoculation in tomato plants, triggered modifications in the volatile compounds emitted, as well as in the oviposition performance of *Phthorimaea absoluta* females and in the developmental stages of this pest insect. These results are important for understanding the interaction between this fungus, tomato plants, and *P. absoluta*, since reports in the literature about this system are still scarce.

The female's choice of host plays a crucial role in the survival of her offspring. Among the groups of chemical compounds in tomato plants, the main host of *P. absoluta* (Silva et al., 2021; Subramani et al., 2021; Aparna et al., 2024), monoterpenes are important compounds for female attractiveness (Adams et al., 2023). Monoterpenes such as α-pinene, β-myrcene, γ-terpinene, β-ocimene, and p-cymene, found in this study, they are compounds that serve as cues that helps in the attractiveness of lepidopterans and other herbivorous insects when choosing a host for oviposition (Msisi et al., 2020).

One of the compounds that showed the highest average concentration in our study was the monoterpene p-cymene, but only in plants with *P. chlamydosporia*. It is well known that this compound plays a role in plant defense mechanisms and exhibits antimicrobial properties (Tian et al., 2018). Another compound found at a higher average concentration in plants with *P. chlamydosporia* compared to control plants was salicylic acid (SA). The presence of *P. chlamydosporia* in tomato plant roots induces the expression of gene regulators associated with plant defense (Pentimone et al., 2018). Its endophytism can activate or inhibit the expression of early defense genes in tomato leaves and also in banana plants, for example (Tolba et al., 2021). The change in the concentration of the phytohormone SA between the treatments with and without *P. chlamydosporia* may explain the preference of *P. absoluta* for control plants, since it is associated with plant defense pathways against herbivores. Microorganisms such as *P. chlamydosporia* can induce systemic changes in the plants they colonize, thereby influencing the behavior of herbivorous insects (Nishad et al., 2020).

Although we identified a greater number of VOCs in control plants, and most of the compounds found in both plant types had higher average concentrations in the controls, the small changes caused in the olfactory blend of tomato by the fungus had a significant influence on the oviposition performance of *P. absoluta* females. The presence of the endophyte *P. chlamydosporia* influenced the composition of the VOC *profile*. Most of the compounds found in both types of plants studied were monoterpenes, with β-phellandrene showing the highest mean concentration.

Pochonia chlamydosporia is capable of establishing itself in tomato plants without causing injury, while also promoting growth, reducing the flowering period, and providing beneficial hormonal regulation for the plant (Escudero et al., 2014; Pentimone et al., 2019; Gouveia et al., 2022). Moreover, this fungus can reduce some of the plant's chemical defenses during its establishment (Gouveia et al., 2022), particularly those related to secondary metabolites.

In view of this, we observed an influence on offspring development. The physiological condition of the plant is a critical factor in host plant selection by herbivorous insects, as females use sensory cues to locate and choose where to oviposit (Bernays & Chapman, 1994), which consequently affects the development of their offspring (Jaenike, 1978). The larval development stage of *P. absoluta* is of great economic importance, since the larvae feed on tomato plants, mining leaves and fruits, and causing significant yield losses (Biondi et al., 2018; Desneux et al., 2021). Our results showed that the first larval instars, L1 and L2, had a higher mean number of days in development on control plants than on plants with the fungus. These factors may explain the changes caused in the nutrition part of plants inoculated with the fungus, although this part needs future studies.

Some monoterpenes, such as 2-carene, sabinene, γ-terpinene, α-terpinene, as well as aldehydes like hexanal and decanal, and the sesquiterpene δ-elemene, were identified only in control plants. These compounds are related to plant resistance and defense (Nishida, 2014; Brilli et al., 2019), which may also explain the longer duration (in days) of the initial larval stages on control plants.

At one of the most critical stages for control, due to the larvae being more developed, the L3 larvae developed faster on control plants. We suggest that, since *P. absoluta* individuals in L1 and L2 developed more slowly on control plants, in the L3 and L4 stages, when larvae typically consume more leaf mass until reaching the pupal stage (Desneux et al., 2021), the consumption rate may have been higher. Consequently, the duration (in days) of these stages was shorter on these plants. However, when we examine the total larval development, *P. absoluta* individuals still develop in a shorter period on plants with *P. chlamydosporia*.

In this study, it was observed that the interaction between tomato plants and the endophytic fungus *P. chlamydosporia* has excellent potential in enhancing defenses against herbivores, such as the tomato leafminer, *P. absoluta*. The ability of the fungus to integrate with the plant and interact positively, even while causing changes in the composition of the volatiles emitted by the tomato plant, is an important characteristic for plant resistance. The increase in the concentration of certain plant defense-regulating compounds, associated with the presence of the fungus, suggests the activation of defense pathways against herbivores. This defensive response appears to make the plants less attractive for oviposition by insect females such as *P. absoluta*, as evidenced by the preference of this pest for control plants. Therefore, manipulating the levels of these compounds through inoculation with *P. chlamydosporia* may be a promising approach to enhance tomato plant defenses against herbivores. Future studies on the compounds found at higher concentrations in inoculated plants may also help in the development of pest control techniques. Additionally, it is necessary to assess the behavior (attraction or repulsion) of these insects towards each compound identified in this work and to determine which ones actually generate a bioactive response.

5. References

Adams B., Yusuf A.A; Torto, B.; Khamis F.M. (2023). Non-host plant odors influence the tritrophic interaction between tomato, its foliar herbivore *Tuta absoluta* and mirid predator *Nesidiocoris tenuis*. Frontier Plant Science, 14:1014865. DOI:10.3389/fpls.2023.1014865

Agrawal, A. A. (2010). Current trends in the evolutionary ecology of plant defense. Functional Ecology, 25: 420–432. DOI:10.1111/j.1365-2435.2010.01796.x

Arimura, G. (2021). Making Sense of the way plants sense herbivores. Trends in Plant Science. 26: 288–298. DOI:10.1016/j.tplants.2020.11.00

Arce C.C., Machado R.A., Ribas N.S, Cristaldo P.F, Ataíde L.M., Pallini A., Carmo F.M., Freitas L.G, Lima E. (2017). Nematode root herbivory in tomato increases leaf defenses and reduces leaf miner oviposition and performance. Journal of Chemical Ecology, 43: 120-128. DOI: 10.1007/s10886-016-0810-z

Agrawal, A. A. (2010). Current trends in the evolutionary ecology of plant defense. Functional Ecology, 25: 420–432. DOI:10.1111/j.1365-2435.2010.01796.x

Aparna, S.; Kumar, A.R.V; Sotelo-Cardona, P.; Srinivasan, R. (2024). Host plant selection is linked to performance in *Phthorimaea absoluta* (Lepidoptera: Gelechiidae). Environmental Entomology, 53(4): 665–676. DOI: 10.1093/ee/nvae044

Arimura, G. (2021). Making Sense of the way plants sense herbivores. Trends in Plant Science, 26: 288–298. DOI:10.1016/j.tplants.2020.11.001

Ayelo, P.M., Yusuf, A.A., Pirk, C.W.W., Mohamed, S.A., Chailleux, A., Deletre, E. (2021). The Role of *Trialeurodes vaporariorum*-Infested Tomato Plant Volatiles in the Attraction of *Encarsia formosa* (Hymenoptera: Aphelinidae). Journal of Chemical Ecology, 47: 192–203. DOI: 10.1007/s10886-021-01245-2

Bernays E.A., Chapman R.F. (1994). Host-plant selection by phytophagous insects. Springer Science & Business Media TS, Volume 2 ISBN: 978-0-412-03131-1

Biondi A., Guedes R.N.C, Wan F.H., Desneux N. (2018). Ecology, worldwide spread, and management of the invasive South American tomato pinworm, *Tuta absoluta*: past, present, and future. Annual Review of Entomology, 63: 239–258. DOI: 10.1146/annurev-ento-031616-034933

Brilli F.; Loreto, F.; Baccelli, I. (2019). Exploiting Plant Volatile Organic Compounds (VOCs) in Agriculture to Improve Sustainable Defense Strategies and Productivity of Crops. Frontier Plant Science, 10:264. DOI:10.3389/fpls.2019.00264

Bleeker, P.M.; Diergaarde, P.J.; Ament, K.; Guerra, J.; Weidner, M.; Schütz, S.; de Both, M.T.J.; Haring, M.A.; Schuurink, R.C. (2009). The role of specific tomato volatiles in tomato-whitefly interaction. Plant Physiology, 151: 925–935. DOI: 10.1104/pp.109.142661

Caparros Megido, R., De Backer, L., Ettaïb, R., Brostaux, Y., Fauconnier, M.L., Delaplace, P., Lognay, G., Belkadhi, M.S., Haubruge, E., Francis, F., Verheggen, F.J. (2014). Role of larval host plant experience and solanaceous plant volatile emissions in *Tuta absoluta* (Lepidoptera: Gelechiidae) host finding behavior. Arthropod-Plant Interactions, 8: 293–304. DOI:10.1007/s11829-014-9315-2

Chandrasekaran, M., Paramasivan, M., Sahayarayan, J.J. (2023). Microbial Volatile Organic Compounds: An Alternative for Chemical Fertilizers in Sustainable Agriculture Development. Microorganisms, 11(1): 42. DOI: 10.3390/microorganisms11010042

Coelho, M.D.C. & França, F. H. (1987). Biologia, quetotaxia da larva e descrição da pupa e adulto da traça-do-tomateiro. Pesquisa Agropecuária Brasileira 22: 129-135.

Dallemole-Giaretta, R., Freitas, L.G; Caixeta, L.B; Xavier, D.M; Ferraz, S., Fabry, C.F.S (2011). Produção de clamidósporos de *Pochonia chlamydosporia* em diferentes substratos. Ciências e agrotecnologia, Lavras, v. 35, n. 2, p. 314-321. DOI:10.1590/S1413-70542011000200012

Desneux, N., Han, P., Mansour, R., Arnó, J., Brévault, T., Campos, M. R., Chailleux, A., Guedes, R. N. C., Karimi, J., Konan, K. A. J., Lavoir, A. V., Luna, M. G., Perez-Hedo, M., Urbaneja, A., Verheggen, F. J., Zappalà, L., Abbes, K., Ali, A., Bayram, Y., Cantor, F., Cuthbertson, A. G. S., De Vis, R., Erler, F., Firake, D. M., Haddi, K., Hajjar, M. J., Ismoilov, K., Jaworski, C. C., Kenis, M., Liu, H. T., Madadi, H., Martin, T., Mazih, A., Messelink, G. J., Mohamed, S. A., Nofemela, R. S., Oke, A., Ramos, C., Ricupero, M., Roditakis, E., Shashank, P. R., Wan, F. H., Wang, M. H., Wang, S., Zhang, Y. B., Biondi, A. (2022). Integrated pest management of *Tuta absoluta*: practical implementations across different world regions. Journal of Pest Science, 95: 17–39. DOI: 10.1007/s10340-021-01442-8

Dicke, M.; Baldwin, I.T. (2010). The evolutionary context for herbivore-induced plant volatiles: Beyond the “cry for help”. Trends Plant Science, 15: 167–175. DOI: 10.1016/j.tplants.2009.12.002

EPPO - European and Mediterranean Plant Protection Organization. (2023). *Global Database*. Available online at: https://gd.eppo.int/taxon/GNORAB/distribution (accessed June, 2024).

Eng, F., Zienkiewicz, K., Gutiérrez-Rojas, M., Favela-Torres, E., Feussner, I. (2018). Jasmonic acid biosynthesis by microorganisms: Derivatives, first evidence on biochemical pathways and culture conditions for production. PeerJ Preprint, 6: e26655v1. DOI: 10.7287/peerj.preprints.26655v1

Escudero, N., Lopez-Llorca, N.V. (2012). Effects on plant growth and root-knot nematode infection of an endophytic GFP transformant of the nematophagous fungus *Pochonia chlamydosporia*. Symbiosis, 57: 33–42. DOI: 10.1007/s13199-012-0173-3

Gaspard, J.T., Jaffee, B.A., Ferris, H. (1990). Association of *Verticillium chlamydosporium* and *Paecilomyces lilacinus* with root-knot nematode infested soil. Journal of Nematology, 22(2): 207-213. PMID: 19287711; PMCID: PMC2619028.

Genç, H. (2016). The tomato leafminer, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae): pupal key characters for sexing individuals. Turkish Journal of Zoology, 40: 801-805. DOI:10.3906/zoo-1510-59

Guedes, R.N.C., Roditakis, E., Campos, M.R. (2019). Insecticide resistance in the tomato pinworm *Tuta absoluta*: patterns, spread, mechanisms, management and outlook. Journal of Pest Science, 92: 1329–1342. DOI: 10.1007/s10340-019-01086-9

Gonçalves, A.G.S, Haddi, K., Ribas, N.S., Santos, K.C.P., Tschoeke, L.F.P., Lima, E. (2024). Age, weight, and mating status of the male influence female choice and reproductive success in *Tuta absoluta.* Entomologia Generalis, 44(2): 443 - 450. DOI: 10.1127/entomologia/2024/2287

Gouveia, A.S., Avelar Monteiro, T.S., Balbino, H.M., Corbelli de Magalhães, F., Ramos, M.E.S., Moura, V.A.S., Luiz, P.H.D., Oliveira, M.G.A., Freitas, I.G., Ramos, H.J.O. (2023). Inoculation of *Pochonia chlamydosporia* triggers a defense response in tomato roots, affecting parasitism by *Meloidogyne javanica*. Microbiological Research, 266: 127242. DOI: 10.1016/j.micres.2022.127242

Hickel, E., Vilela, E., Lima, J., Lucia, T. (1992). Calling behavior of the tomato leafminer *Scrobipalpuloides absoluta* (Lepidoptera, Gelechiidae). Revista Brasileira de Entomologia, 36: 277-280.

Hilker, M., Fatouros, N.E. (2015) Plant responses to insect egg deposition. Annual Review Entomology, 60: 493–515. DOI: 10.1146/annurev-ento-010814-020620

Idriss, G.E.A., Du Plessis, H., Khamis, F.M., Ekesi, S., Tanga, C.M., Mohamed, S.A. (2020). Host range and effects of plant species on preference and fitness of *Tuta absoluta* (Lepidoptera: Gelechiidae). Journal of Economic Entomology, 113: 1279–1289. DOI: 10.1093/jee/toaa002

Jaenike, J. (1978). On optimal oviposition behavior in phytophagous insects*.* Theoretical Population Biology, 14: 350–356. DOI: 10.1016/0040-5809(78)90012-6

Karban, R., Myers, J.H (1989). Induced plant response to herbivory. Annual Review of Ecology and Systematics, 20: 331-348. DOI: 10.1146/annurev.es.20.110189.001555

Kessler, A., Baldwin, I.T. (2001). Defensive function of herbivore-induced plant volatile emissions in nature. Science, 291: 2141–2144. DOI: 10.1126/science.291.5511.2141

Kobayashi, K. (2015). Plant methyl salicylate induces defense responses in the rhizobacterium *Bacillus subtilis.* Environmental Microbiology, 7(4), 1365–1376. DOI: 10.1111/1462-2920.12613

Kozyrovska, N.O. (2013). Crosstalk between endophytes and a plant host within information processing networks. Biopolymers and Cell, 29: 234–243. DOI: 10.7124/bc.00081D

Kumar, S., Kaushik, N. (2013). Metabolites of endophytic fungi as novel source of biofungicide: a review. Phytochemistry Reviews. 11: 507-522. DOI: 10.1007/s11101-013-9271-y

Kumar, P., Whitten, M., Thoeming, G., Borgemeister, C., Poehling, H.M. (2008). Effects of bio-pesticides on *Eretmocerus warrae* (Hym., Aphelinidae), a parasitoid of *Bemisia tabaci* (Hom., Aleyrodidae). Journal of Applied Entomology, 132: 605–613. DOI: 10.1111/j.1439-0418.2008.01282.x

Larriba, E., Jaime, M.D., Nislow, C., Martín-Nieto, J., Lopez-Llorca, L.V. (2015). Endophytic colonization of barley (*Hordeum vulgare*) roots by the nematophagous fungus *Pochonia chlamydosporia* reveals plant growth promotion and a general defense and stress transcriptomic response. Journal of Plant Research, 128: 665-678. DOI: 10.1007/s10265-015-0731-x

Lemos, F. (2015). Indirect interactions in tomato attacked by *Tetranychus evansi.* Doctor's thesis, Universidade Federal de Viçosa (Brasil).

Ludwig-Müller, J. (2015). Plants and endophytes: equal partners in secondary metabolite production?. Biotechnology Letters, 37: 1325–1334. DOI: 10.1007/s10529-015-1814-4

Mahanta, D.K., Komal, J., Samal, I., Bhoi, T.K., Kumar, P.V.D., Mohapatra, S., Athulya, R., Majhi, P.K., Mastinu, A. (2025). Plant Defense Responses to Insect Herbivores Through Molecular Signaling, Secondary Metabolites, and Associated Epigenetic Regulation. Plant-Environment Interactions, 6: e70035. DOI: 10.1002/pei3.70035

Mattoo, A. J., & Nonzom, S. (2021). Endophytic fungi: understanding complex cross-talks. Symbiosis, 83(3): 237–264. DOI: 10.1007/s13199-020-00744-2

Meents, A. K & Mithöfer, A. (2020). Plant–Plant Communication: Is There a Role for Volatile Damage-Associated Molecular Patterns?. Frontier Plant Science, 11:583275. DOI: 10.3389/fpls.2020.583275

Milonas, P.G.; Anastasaki, E.; Partsinevelos, G. (2019). Oviposition-induced volatiles affect electrophysiological and behavioral responses of egg Parasitoids. Insects, 10: 437. DOI: 10.3390/insects10120437

Monteiro, H.S.A., Pacheco, P.V.M., Gouveia, A.S., Balbino, H.M., & Freitas, L.G. (2020). Pochonia. Beneficial Microbes in Agro-Ecology, 669–682. DOI:10.1016/B978-0-12-823414-3.00033-2

Muller, K., Thiéry, D., Motreuil, S., Moreau, J. (2016). What makes a good mate? Factors influencing male and female reproductive success in a polyphagous moth. Animal Behaviour, 120, 31–39. DOI: 10.1016/j.anbehav.2016.07.027

Msisi, D., Matojo, N.D.; Kimbokota, F. (2020). Attraction of female tomato leaf miner, *Tuta absoluta* (Meyrick, 1917) (Lepidoptera:Gelechiidae) to shared compounds from hosts. Phytoparasitica, 49: 153–162. DOI:10.1007/s12600-020-00848-x

Nishida, R. (2014). Chemical ecology of insect–plant interactions: ecological significance of plant secondary metabolite. Bioscience, Biotechnology, and Biochemistry, 78(1): 1–13. DOI:10.1080/09168451.2014.877836

Nishad, R.; Ahmed, T.; Rahman, V.J.; Kareem, A. (2020). Modulation of Plant Defense System in Response to Microbial Interactions. Frontier in Microbiology, 11:1298. DOI: 10.3389/fmicb.2020.01298

Oki, Y., Soares, N., Belmiro, M.S., Corrêa Junior, A., Fernandes, G.W. (2009). The influence of the endophytic fungi on the herbivores from *Baccharis dracunculifolia* (Asteraceae). Neotropical Biology and Conservation, 4(2): 83-88. DOI: 10.4013/5119

Peñaflor, M.F.G.V., Erb, M., Robert, C., A.M., Miranda, L.A., Werneburg, A.G., Dossi, F.C. A., Turlings, T.C.J. & Bento, J.M.S. (2011). Oviposition by a moth suppresses constitutive and herbivore-induced plant volatiles in maize. Planta, 234(1): 207-215. DOI: 10.1007/s00425-011-1409-9

Pentimone, I., Lébron, R., Hackenberg, M., Rosso, L.C., Colagiero, M., Nigro, F., Ciancio A. (2018). Identification of tomato miRNAs responsive to root colonization by endophytic *Pochonia chlamydosporia.* Applied Microbiology Biotechnology, 102: 907–919. DOI: 10.1007/s00253-017-8608-7

Proffit, M., Birgersson, G., Bengtsson, M., Reis, Jr. R., Witzgall, P., Lima, E. (2011). Attraction and oviposition of *Tuta absoluta* females in response to tomato leaf volatiles. Journal of Chemical Ecology, 37: 565–574. DOI: 10.1007/s10886-011-9961-0

Santana, Jr, P.A., Kumar, L., Da Silva, R.S., Picanço, M.C. (2019). Global geographic distribution of *Tuta absoluta* as affected by climate change. Journal of Pest Science, 92: 1373-1385. DOI: 10.1007/s10340-018-1057-y

Schoonhoven, L.M, Van Loon, J.J.A, Dicke, M. (2005). Insect-plant biology. Oxford University Press, Volume 2. ISBN 0-19-852595-8

Silva, G.A., Queiroz, E.A., Arcanjo, L.P. *et al.* (2021). Biological performance and oviposition preference of tomato pinworm *Tuta absoluta* when offered a range of Solanaceous host plants. Scientific Reports, 11: 1153 (2021). DOI: 10.1038/s41598-020-80434-7

Sharif, R., Lee, S.M., Ryu, C.M. (2018) Microbe-induced plant volatiles. New Phytologist, 220: 684–691. DOI: 10.1111/nph.14955

Subramani, V., Damodaram, K. J.P., Krishnegowda, R.G., Parepally, S. K., Kempraj, V., Thimmappa, R., Boregowda, L.H., (2021). Volatile chemical signals underlying the host plant preferences of *Tuta absoluta.* Entomologia Experimentalis et Applicata, 169: 997-1007. DOI:10.1111/eea.13099

Tolba, S.R.T., Moustafa, M.M.A,. Elshawaf I.I.S, Rosso, L.C., Pentimone, I., Colagiero, M., Bubici, G., Prigigallo, M.I., Ciancio, A. (2021). Root endophytism by *Pochonia chlamydosporia* affects defense-gene expression in leaves of monocot and dicot hosts under multiple biotic interactions. Plants, 10(4): 718. DOI: 10.3390/plants10040718

Tian, F., Young Woo, S., Yoo Lee, S., Sook Chun, H. (2018). P-Cymene and its derivatives exhibit antiaflatoxigenic activities against *Aspergillus flavus* through multiple modes of action. Applied Biological Chemistry, 61(5): 489–497. DOI:10.1007/s13765-018-0382-4

Urbaneja, A., Montón, H., Mollá, O. (2009). Suitability of the tomato borer *Tuta absoluta* as prey for *Macrolophus pygmaeus* and *Nesidiocoris tenuis*. Journal of Applied Entomology, 133: 292–296. DOI: 10.1111/j.1439-0418.2008.01319.x

Yactayo-Chang, Jessica, P., Hoang, V. Tang, Jorrel Mendoza, Shawn A. Christensen, and Anna K. Block (2020). Plant Defense Chemicals against Insect Pests. Agronomy, 10(8): 1156. DOI: 10.3390/agronomy10081156

Yuan, X., Zhang, Y., Hu L., Sang W., Yang, Z. (2024). Investigating the effects of species niche shifts on the potential distribution of *Tuta absoluta* (Lepidoptera: Gelechiidae) by using global occurrence data. Journal of Insect Science, 24(3): 8. DOI:10.1093/jisesa/ieae059

Zauza, E.A.V; Alfenas, A.C; Mafia, R.G. (2007). Esterilização, preparo de meios de cultura e fatores associados ao cultivo de fitopatógenos. Métodos em fitopatologia: p 23-51.

Zappalà, L., Biondi, A., Alma, A., Al-Jboory, I.J., Arnò, J., Bayram, A., Chailleux, A., El-Arnaouty, A., Gerling, D., Guenaoui, Y., Shaltiel-Harpaz, L., Siscaro, G., Stavrinides, M., Tavella, L., Vercher Aznar, R., Urbaneja, A., Desneux, N. (2013). Natural enemies of the South American moth, *Tuta absoluta*, in Europe, North Africa and Middle East, and their potential use in pest control strategies. Journal of Pest Science, 86: 635–647. DOI: 10.1007/s10340-013-0531-9

Zhang, P. Y., Chen, K. S., He, P.Q., Liu, S.H., Jiang, W.F. (2008). Effects of crop development on the emission of volatiles in leaves of *Lycopersicon esculentum* and its inhibitory activity to *Botrytis cinerea* and *Fusarium oxysporum*. Journal of Integrate Plant Biology, 50: 84–91. DOI: 10.1111/j.1744-7909.2007.00597.x

Zhu, H., Fu, J., Wang, H., Bidochka, M.J., Duan, M., Xu, W., Sui, L., Ren, B., Li. Q., Zhang, Z. (2023). Fitness consequences of oviposition choice by an herbivorous insect on a host plant colonized by an endophytic entomopathogenic fungus. Journal of Pest Science, 96: 745–758. DOI: 10.1007/s10340-022-01527-y

Chapter 2

Gas Chromatography–Electroantennographic Detection response of *Phthorimaea absoluta* (Lepidoptera: Gelechiidae) to volatile compounds from tomato plants *Solanum lycopersicum* with and without inoculation of *Pochonia chlamydosporia* (Hypocreales: Clavicipitaceae), analyzed by Gas Chromatography–Electroantennographic Detection (GC–EAD)

SANTOS, Karenn Christiny Pereira, D.Sc., Federal University of Viçosa, july de 2025. **Gas Chromatography–Electroantennographic Detection response of *Phthorimaea absoluta* (Lepidoptera: Gelechiidae) to volatile compounds from tomato plants *Solanum lycopersicum* with and without inoculation of *Pochonia chlamydosporia* (Hypocreales: Clavicipitaceae), analyzed by Gas Chromatography–Electroantennographic Detection (GC–EAD).** Adviser: Eraldo Lima. Co-adviser: Natália de Souza Ribas

Abstract

Phthorimaea absoluta is one of the primary pests of solanaceous crops, particularly tomatoes. Females locate the host plant by detecting volatile organic compounds (VOCs) emitted by the plants, and changes in the chemical profile of these compounds can influence this choice. Detection occurs through sensors located on the antennae, which capture electrophysiological signals and odors. Microorganisms, such as endophytic fungi, associated with the roots can induce changes in plant metabolism, thereby modifying the VOC profile emitted. This modulation can interfere with the plant's attractiveness to pest insects, acting as a form of indirect defense. This study investigated how VOCs from tomato plants with and without inoculation of the endophytic fungus *Pochonia chlamydosporia* (Pc10 isolate) influence the electrophysiological response of *P. absoluta* antennae. For this, one µL of volatile extracts from fungus-inoculated and control plants (var. Santa Cruz) was analysed into a gas chromatography system coupled with electroantennographic detection (GC-EAD). The antennal responses, recorded in microvolts, were processed using GC-EAD software (Syntech), and bioactive compounds were identified by Kovats retention index. In tests with plants without the fungus, α-pinene and β-myrcene elicited antennal responses; in plants with *P. chlamydosporia*, the detected compounds were β-myrcene and p-cymene. Although β-myrcene was found in both, there was no significant difference in response intensity. After identifying bioactive compounds, a synthetic blend containing α-pinene, β-myrcene, γ-terpinene, β-ocimene, and p-cymene was tested, and the highest mean antennal response was observed for α-pinene. We conclude that, although *P. chlamydosporia* did not significantly alter the intensity of perception of the compounds by *P. absoluta*, there were qualitative variations in the profile of bioactive volatiles. These changes may impact pest behavior, especially regarding β-myrcene. These results may support studies with attractive traps and sustainable management strategies.

Keywords: Oviposition, Semiochemicals, Chemoreception.

1. Introduction

Understanding the mechanisms that determine and modulate insect oviposition behavior becomes increasingly necessary, especially when dealing with those considered to cause economic damage to large-scale agricultural crops. Much of this behavior is guided by the perception of cues, chemical signals emitted either by other insects or by plants. There is a high sensitivity and specificity in the detection of the compounds involved in this interaction.

Phthorimaea absoluta, currently the main tomato pest (Urbaneja et al., 2009; Santana et al., 2019; Idriss et al., 2020; Desneux et al., 2021; Yuan et al., 2024; EPPO, 2025), exhibits behavior driven by volatile organic compounds, such as monoterpenes, which are detected by sensilla on its antennae, thereby modulating the response to a specific odor (Naturere et al., 2022). More specifically, compounds of the Green Leaf Volatiles (GLVs) type and terpenes, such as (E)-β-ocimene and α-pinene, are responsible for shaping the antennal response of *P. absoluta*, indicating their importance in pest orientation and attraction (Fiaboe et al., 2023).

Understanding the factors involved makes it possible to filter compounds of interest, in order to then use them as a tool for controlling insect oviposition and feeding behavior. Considering that female herbivorous insects are capable of detecting small chemical changes in plants, which can be decisive in their choice, factors such as the physiological condition of the plant have a considerable influence.

The soil microbiota, for example, can induce systemic resistance and qualitatively alter volatiles (Ghahremani et al., 2019; Escudero et al., 2016). An example is the fungus *Pochonia chlamydosporia*, capable of modifying the composition of bioactive compounds in our previous studies, suggesting an indirect influence on host choice by *P. absoluta*. The effect of microorganisms on VOC emission is also evidenced in studies with chitosan, an important agent in plant defense and nutrition (Reyes-Pérez et al., 2020); when applied to *P. chlamydosporia* cultures, it influences the production of volatile compounds that can be perceived by insect antennae (Mestre-Tomás et al., 2022).

In light of such facts regarding the beneficial symbiotic relationship of *P. chlamydopsoria* with tomato plants and its influence on the composition of volatile compounds emitted by tomato plants, it is possible to hypothesize that the perception by the antennae of female *P. absoluta*, the main tomato pest, may also be affected. Consequently, this could be an important factor in the host-locating behavior of this pest insect. The present study aimed to evaluate the behavioral responses of *P. absoluta* antennae using samples of VOCs from tomato plants with and without inoculation of the endophyte *P. chlamydosporia* as an attractant.

2. Methodology

2.1 Gas Chromatography–Electroantennographic Detection (GC-EAD)

The rearing of *P. absoluta*, the insects used, the conditions of the plants used on this experiment and the collection of VOCs was made is the same described previously on chapter 1. To evaluate the electrophysiological response in the antennae of *P. absoluta*, 1 µL of the volatile organic compound (VOC) extract from both types of plants (fungus-inoculated and control) was injected and processed by gas chromatography with a flame ionization detector (GC-FID) using a Shimadzu GC-2010 equipped with an Rtx-5 Crossbond capillary column (5% diphenyl–95% dimethyl polysiloxane; 30 m, 0.25 mm i.d., and 0.25 µm film thickness; Thames Restek UK Ltd). The injector was operated in splitless mode. The temperature program was adapted from the methodology developed by Lemos et al. (2015): the temperature started at 35 °C (held for 1 min), followed by an increase of 8 °C/min until reaching 260 °C, which was held for 5 min. The injector temperature was 230 °C.

The electrophysiological responses of the antenna, measured in microvolts (200 µV), were amplified by the IDAC-2 interface (Syntech) and processed with a data acquisition controller, with subsequent recording using GC-EAD software (Syntech GC-EAD 32, version 4.6). To identify VOCs that elicited significant antennal responses, the Kovats retention index (KI) was calculated for each compound using a C7–C30 hydrocarbon series (30 ng/µL, C12 60 ng/µL as the reference peak on chromatogram). The identification of compounds were performed using comparisons to the Retention Index (Kovats Index) and confirmed with the spectra of commercial standards.

Mated *Phthorimaea absoluta* females were placed in 70 mL containers and then anesthetized in a vertical freezer at -20 °C for 5 minutes to immobilize the insect. Subsequently, the female was held by the wings using entomological forceps (Biologika). Using another fine-tipped forceps (titanium alloy, B 0.15 mm, Seablue), the antenna was removed from the antenna escape region. The antenna was then placed in an electrode (microcapillary electrodes with conductive gel Syntech) for coupling. Approximately 30 antennae were tested in GC-EAD recordings for each type of attractant. Only compounds that triggered three consistent EAG responses, i.e., compounds that stimulated at least three antennae, were considered EAG-active and used in the results (Miano et al., 2022).

After identifying the most bioactive compounds, a blend of these compounds was created using synthetic standards from the Laboratory of Semiochemicals and Insect Behavior (UFV) stock, which were then tested to evaluate the electrophysiological response in the antennae of *P. absoluta* females, following the same methodological specifications described above.

2.2 Statistical analysis

The normality of the data was determined using the Shapiro-Wilk normality test. To analyze differences in the median intensity of antennal responses between treatments, a Kruskal-Wallis test was performed. To assess whether there were differences in the mean intensity of the identified compounds between treatments, an ANOVA was conducted. All the statistical analysis was performed by software R Studio.

3. Results

A total of 24 antennae were tested with control samples, of which 6 showed measurable bioactive responses. Among these, 3 antennae responded to the compound α-pinene and 3 to β-myrcene (Table 2.1). For the volatile samples from plants inoculated with *Pochonia chlamydosporia* (isolate Pc10), 28 antennae were tested, with 10 showing measurable bioactive responses. We observed 4 antennae responding to β-myrcene and 3 to p-cymene (Table 2.1).

In both treatments, β-myrcene was found to be bioactive in the antennae. However, there was no statistically significant difference in the mean response intensity of β-myrcene between the control and *Pochonia chlamydosporia* inoculated treatments (p-value = 0.777) (Fig. 2.1). Furthermore, when comparing the mean response intensity among the compounds themselves (α-pinene, β-myrcene, p-cymene) across both treatments, no significant difference was found (p-value = 0.1085).

Table 2.1: Response intensity of the antenna and identification of compounds for volatile samples from tomato control plants and inoculated with *Pochonia chlamydosporia* (isolate Pc10).

Treatment	Antenna	Compound	KI	Value (µV)	Qualitative GC-EAD Response
Control	1	α-pinene	1030	60	+
Control	1	α-pinene	1030	80	+
Control	2	α-pinene	1030	180	++
Control	3	α-pinene	1030	20	+
Control	3	α-pinene	1030	320	+++
Control	4	β-myrcene	902	40	+
Control	5	β-myrcene	902	280	+++
Control	6	β-myrcene	902	20	+
P. chlamydosporia	1	p-cimene	1045	20	+
P. chlamydosporia	2	p-cimene	1045	20	+
P. chlamydosporia	3	p-cimene	1045	20	+
P. chlamydosporia	4	β-myrcene	990	160	++
P. chlamydosporia	4	β-myrcene	990	60	+
P. chlamydosporia	5	β-myrcene	990	160	++
P. chlamydosporia	6	β-myrcene	990	60	+
P. chlamydosporia	7	β-myrcene	990	20	+

[k] Identification of compounds according to mass spectrum and Kovats retention index in comparison with references in libraries and comparison with KIs in similar columns;

"+" indicates a weak antennal response, "++" moderate, and "+++" strong.

Note: we used mean ± standard deviation to define the thresholds for low, medium, and high response.

After identifying the bioactive compounds, a blend sample (40 ng/µL) was prepared containing α-pinene, β-myrcene, γ-terpinene, β-ocimene, and p-cymene. In total, 33 antennae were tested, of which 12 showed measurable responses: 3 antennae responded to β-ocimene, 3 to α-pinene, 3 to γ-terpinene, and 6 to p-cymene. The highest mean antennal response intensity was observed for α-pinene (660 µV).

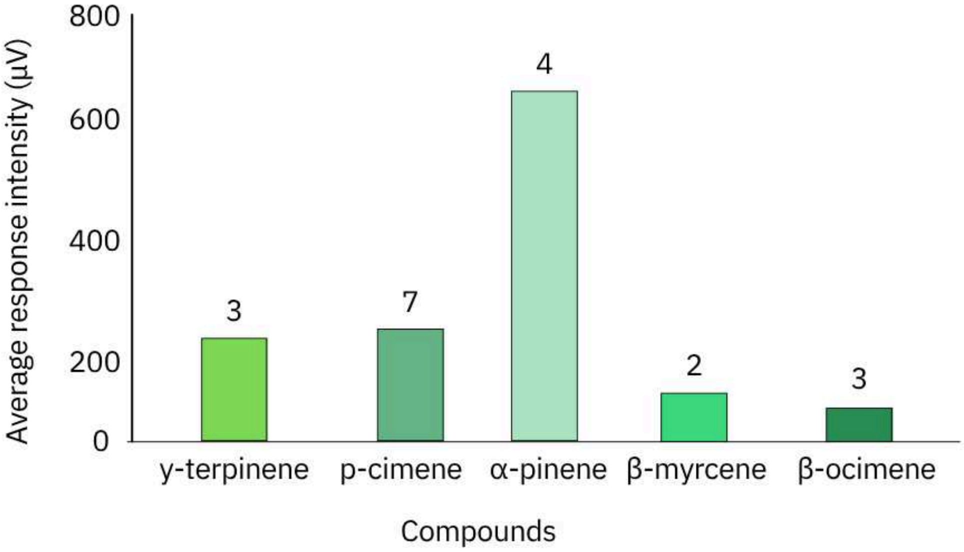

Figure 2.1: Average response intensity of the antennas of *Phthorimaea absoluta* in GC-FID-EAD, tested for a blend of synthetic compounds from tomato plants (y-terpinene, p-cymene, α-pinene, β-myrcene, and β-ocimene). Overlapping numbers indicate the number of responsive antenna.

4. Discussion

This study investigated the electrophysiological response of *Phthorimaea absoluta* antennae to volatile compounds from tomato plants with and without inoculation by *Pochonia chlamydosporia* (isolate Pc10). The GC-EAD technique (gas chromatography coupled with electroantennographic detection) enabled the identification of compounds with potential olfactory activity relevant to this insect, an important step in identifying semiochemicals applicable to pest management and understanding how the fungus influences herbivore host location.

The data obtained showed that, although the initial hypothesis was that antennae would respond more intensely to volatiles from control plants (without fungal inoculation), the compound β-myrcene was detected as bioactive in both treatments. However, no statistically significant difference was observed in the mean response intensity to this compound between the groups. This result suggests that β-myrcene is a crucial compound in tomato plants for mediating plant-insect interactions, regardless of the presence of *P. chlamydosporia*. This finding is consistent with other studies that have shown this monoterpene is involved in the attraction of herbivorous Lepidoptera, as well as other economically important insects such as *Bemisia tabaci* (Bruce et al., 2005; Bleeker et al., 2009; Liang et al., 2025).

There was no significant difference in the quantitative response, measured by intensity, to the compounds α-pinene, β-myrcene, and p-cymene between our treatments. The absence of statistical significance may reflect a low sensory activity of the antennae in distinguishing between these volatile structures in terms of intensity. Nevertheless, in qualitative terms, we previously observed that changes in these compounds influence the behavior of *Phthorimaea absoluta*. Furthermore, interindividual variability among antennae may have influenced the results, as also observed in other similar studies (Natale et al., 2003; Witzgall et al., 2010).

Our blend of the synthetic compounds (containing α-pinene, β-myrcene, γ-terpinene, β-ocimene, and p-cymene) showed a highlight of the compound α-pinene, indicating that this compound, although less frequently detected in individual samples, may have greater sensory efficacy when present in more complex blends. Blends of volatile compounds are responsible for the specificity in behavioral responses, compared to isolated compounds, as demonstrated in several studies with Lepidoptera and their hosts (Bruce & Pickett, 2011; Webster et al., 2010).

In our previous study, the compound p-cimene showed higher concentrations in plants inoculated with *Pochonia chlamydosporia.* Moreover, this compound induced a higher frequency of detection in samples from inoculated plants, suggesting a possible specific role in the context of plant-microorganism-insect interaction. The emission of p-cymene in tomato plants may modulate the attraction of herbivorous insects, potentially causing repellency (Bleeker et al., 2009; Bleeker et al., 2011; Pizzo et al., 2024). These factors underscore the importance of evaluating compounds like p-cymene in future behavioral assays.

Our results suggest that qualitative changes caused by inoculation of *P. chlamydosporia* (isolated Pc10) in tomato plants may influence the antennae behavior of *P. absoluta*. The applicability of these findings extends to subsequent experiments with olfactory traps and behavioral analyses, to verify, on a larger scale (such as in the field), and later integrate into the management of this pest.

5. References

Bleeker, P.M., Diergaarde, P.J., Ament, K., Guerra, J., Weidner, M., Schütz, S., de Both, M.T.J., Haring, M.A., Schuurink, R.C. (2009). The role of specific tomato volatiles in tomato–whitefly interaction. Plant Physiology, 151(2): 925–935. DOI: 10.1104/pp.109.142661

Bleeker, P.M., Diergaarde, P.J., Ament, K., Schütz, S., Johne, B., Dijkink, J., Hiemstra, H., De Gelder, R., De Both, M.T.J., Sabelis, M.W., Haring, M.A., Schuurink, R. C. (2011). Tomato-produced 7-epizingiberene and R-curcumene act as repellents to whiteflies. Phytochemistry, 72: 68–73. DOI: 10.1016/j.phytochem.2010.10.014

Bruce, T.J.A., Wadhams, L.J., Woodcock, C.M. (2005). Insect host location: a volatile situation. Trends in Plant Science, 10(6): 269–274. DOI: 10.1016/j.tplants.2005.04.003

Bruce, T.J.A., Pickett, J.A. (2011). Perception of plant volatile blends by herbivorous insects–finding the right mix. Phytochemistry, 72(13): 1605–1611. DOI: 10.1016/j.phytochem.2011.04.011

Coelho, M.D.C. & França, F. H. (1987). Biologia, quetotaxia da larva e descrição da pupa e adulto da traça-do-tomateiro. Pesquisa Agropecuária Brasileira 22: 129-135

Dallemole-Giaretta, R.; Freitas, L.G; Caixeta, L.B; Xavier, D.M; Ferraz, S.; Fabry, C.F.S (2011). Produção de clamidósporos de *Pochonia chlamydosporia* em diferentes substratos. Ciências e Agrotecnologia, Lavras, v. 35, n. 2, p. 314-321. DOI:10.1590/S1413-70542011000200012

Desneux, N., Han, P., Mansour, R., Arnó, J., Brévault, T., Campos, M. R., Chailleux, A., Guedes, R. N. C., Karimi, J., Konan, K. A. J., Lavoir, A. V., Luna, M. G., Perez-Hedo, M., Urbaneja, A., Verheggen, F. J., Zappalà, L., Abbes, K., Ali, A., Bayram, Y., Cantor, F., Cuthbertson, A. G. S., De Vis, R., Erler, F., Firake, D. M., Haddi, K., Hajjar, M. J., Ismoilov, K., Jaworski, C. C., Kenis, M., Liu, H. T., Madadi, H., Martin, T., Mazih, A., Messelink, G. J., Mohamed, S. A., Nofemela, R. S., Oke, A., Ramos, C., Ricupero, M., Roditakis, E., Shashank, P. R., Wan, F. H., Wang, M. H., Wang, S., Zhang, Y. B., Biondi, A. (2022). Integrated pest management of *Tuta absoluta*: practical implementations across different world regions. Journal of Pest Science, 95: 17–39. DOI: 10.1007/s10340-021-01442-8

EPPO - European and Mediterranean Plant Protection Organization. (2023). *Global Database*. Available online at: https://gd.eppo.int/taxon/GNORAB/distribution (accessed June, 2024).

Hickel, E., Vilela, E., Lima, J., Lucia, T. (1992). Calling behavior of the tomato leafminer *Scrobipalpuloides absoluta* (Lepidoptera, Gelechiidae). Revista Brasileira de Entomologia, 36: 277-280.

Idriss, G.E.A., Du Plessis, H., Khamis, F.M., Ekesi, S., Tanga, C.M., Mohamed, S.A. (2020). Host range and effects of plant species on preference and fitness of *Tuta absoluta* (Lepidoptera: Gelechiidae). Journal of Economic Entomology, 113: 1279–1289. DOI: 10.1093/jee/toaa002

Gaspard J.T., Jaffee B.A., Ferris H. (1990). Association of *Verticillium chlamydosporium* and *Paecilomyces lilacinus* with root-knot nematode infested soil. Journal of Nematology, 22(2): 207-213. PMID: 19287711; PMCID: PMC2619028

Genç, H. (2016). The tomato leafminer, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae): pupal key characters for sexing individuals. Turkish Journal of Zoology, 40: 801-805. DOI:10.3906/zoo-1510-59

Gonçalves, A.G.S, Haddi, K., Ribas, N.S., Santos, K.C.P., Tschoeke, L.F.P., Lima, E. (2024). Age, weight, and mating status of the male influence female choice and reproductive success in *Tuta absoluta.* Entomologia Generalis, 44(2): 443 - 450. DOI: 10.1127/entomologia/2024/2287

Gouveia, A.S., Avelar Monteiro, T.S., Balbino, H.M., Corbelli de Magalhães, F., Ramos, M.E.S., Moura, V.A.S., Luiz, P.H.D., Oliveira, M.G.A., Freitas, I.G., Ramos, H.J.O. (2023). Inoculation of *Pochonia chlamydosporia* triggers a defense response in tomato roots, affecting parasitism by *Meloidogyne javanica*. Microbiological Research, 266: 127242. DOI: 10.1016/j.micres.2022.127242

Liang, P., Zeng, y., Ning, J., Wu, X., Wang, W., Ren, J., Wu, Q., Yang, X., Wang, S., Guo, Z., Su, Q., Zhou, X., Turlings, T.C.J., Xie, W., and Zhang, Y. (2025). A plant virus manipulates both its host plant and the insect that facilitates its transmission. Science Advances, 11: 4563. DOI:10.1126/sciadv.adr4563

Miano, R., Pascal, A. Richard, M., Hassanali, A., Mohamed, S.A. (2022). Electroantennogram and machine learning reveal a volatile blend mediating avoidance behavior by *Tuta absoluta* females to a wild tomato plant. Scientific Reports, 12: 8965. 10.1038/s41598-022-13125-0

Monteiro, H.S.A., Pacheco, P.V.M., Gouveia, A.S., Balbino, H.M., & Freitas, L.G. (2020). Pochonia. Beneficial Microbes in Agro-Ecology, 669–682. DOI:10.1016/B978-0-12-823414-3.00033-2

Muller, K., Thiéry, D., Motreuil, S., Moreau, J. (2016). What makes a good mate? Factors influencing male and female reproductive success in a polyphagous moth. Animal Behaviour, 120, 31–39. DOI: 10.1016/j.anbehav.2016.07.027

Natale, D., Mattiacci, L., Hern, A., Pasqualini, E., Dorn, S. (2003). Response of female *Cydia molesta* (Lepidoptera: Tortricidae) to plant derived volatiles. Bulletin of Entomological Research, 93(4): 335–342. DOI: 10.1079/ber2003250

Pizzo, J.S., Rutz, T., Ojeda, A.S., Kartowikromo, K.Y., Hamid, A.M., Simmons, A., Da Silva, A.L.B.R., Rodrigues, C. (2024). Quantifying terpenes in tomato leaf extracts from different species using gas chromatography-mass spectrometry (GC-MS). Analytical Biochemistry, 689: 115503. DOI: 10.1016/j.ab.2024.115503

Reyes-Pérez, J.J., Enríquez-Acosta, E.A., Ramírez-Arrebato, M.A., Valenzuela, E.Z., Lara-Capistrán, L., Hernández-Montiel, L.G. (2020). Effect of chitosan on variables of tomato growth, yield and nutritional content. Revista mexicana de ciencias agrícolas, 11(3): 457-465. DOI: 10.29312/remexca.v11i3.2392

Santana, Jr, P.A., Kumar L., Da Silva R.S., Picanço M.C. (2019). Global geographic distribution of *Tuta absoluta* as affected by climate change. Journal of Pest Science, 92: 1373-1385. DOI: 10.1007/s10340-018-1057-y

Silva, S.S. (2008). Fatores da biologia reprodutiva que influenciam manejo comportamental de *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). Tese de Doutorado, Lavras, UFLA, 86p.

Urbaneja, A., Montón, H., Mollá, O. (2009). Suitability of the tomato borer *Tuta absoluta* as prey for *Macrolophus pygmaeus* and *Nesidiocoris tenuis*. Journal of Applied Entomology, 133: 292–296. DOI: 10.1111/j.1439-0418.2008.01319.x

Webster, B., Bruce, T., Pickett, J., Hardie, J. (2010). Volatiles functioning as host cues in a blend become nonhost cues when presented alone to the black bean aphid. Animal Behaviour, 79(2): 451–457. DOI: 10.1016/j.anbehav.2009.11.028

Witzgall, P., Kirsch, P., & Cork, A. (2010). Sex pheromones and their impact on pest management. Journal of Chemical Ecology, 36(1): 80–100. DOI: 10.1007/s10886-009-9737-y

Yuan, X., Zhang, Y., Hu L., Sang W., Yang, Z. (2024). Investigating the effects of species niche shifts on the potential distribution of *Tuta absoluta* (Lepidoptera:

Gelechiidae) by using global occurrence data. Journal of Insect Science, 24(3): 8. DOI:10.1093/jisesa/ieae059

Zauza, E.A.V; Alfenas, A.C; Mafia, R.G. (2007). Esterilização, preparo de meios de cultura e fatores associados ao cultivo de fitopatógenos. Métodos em fitopatologia: p 23-51

Chapter 3

How tomato plants *Solanum lycopersicum* inoculated with *Pochonia chlamydosporia* (Hypocreales: Clavicipitaceae) induce defense against oviposition by *Phthorimaea absoluta* (Lepidoptera: Gelechiidae)

SANTOS, Karenn Christiny Pereira, D.Sc., Federal University of Viçosa, july de 2025. **How tomato plants *Solanum lycopersicum* inoculated with *Pochonia chlamydosporia* (Hypocreales: Clavicipitaceae) induce defense against oviposition by *Phthorimaea absoluta* (Lepidoptera: Gelechiidae).** Adviser: Eraldo Lima. Co-adviser: Natália de Souza Ribas

Abstract

Plants have constitutive defense strategies against herbivore attacks, which can be innate and constantly produced. The presence of eggs on leaves influences the activation of these defenses, for example, and this activation serves as an important sensory cue for natural enemies, such as parasitoids. Microorganisms can also affect the release of volatiles by the plant, known as microbe-induced volatiles. In this study, we investigated changes in the emission of volatile organic compounds (VOCs) in tomato plants after insect oviposition, focusing on the temporal dynamics at 0, 24, and 48 hours after the deposition of *Phthorimaea absoluta* eggs and in the presence of *Pochonia chlamydosporia* in the roots. Using gas chromatography (GC-MS) and statistical modeling (GLM), we quantified the concentrations of the main VOCs and assessed the effects of compound identity, time, and their interaction on emission profiles. We observed significant quantitative and qualitative effects, indicating that both the type of compound and the timing after oviposition influence the VOC profile. The most affected compounds were 4-carene and β-felandrene, which showed significant changes in concentration over time, with 4-carene exhibiting a particular increase and β-phellandrene displaying strong temporal variation. Additionally, the compound α-terpinene also showed a significant increase within 24 hours. Our results suggest that oviposition causes a chemical adjustment in the plant, which consequently affects its ecosystem interactions. This work showed the importance of understanding how the injuries caused by oviposition, associated with the presence of microorganisms, affect plant defense signaling and provides a foundation for future studies on the ecological roles of specific VOCs in tomato-insect interactions.

Keywords: OIPVs, HIPVs, preference of oviposition, endophytic fungus.

1. Introduction

Scars left on the leaves after egg deposition by insects contain specific elicitors, such as egg-associated proteins, that plants can detect through surface receptors, triggering defense responses prior to larval feeding (Bruessow et al., 2010; Hilker et al., 2005), which in turn cause chemical changes in the plants. This process is called oviposition-induced plant volatiles (OIPVs), which can serve as early warning cues for egg parasitoids and predators (Hilker & Fatouros, 2015; Fatouros et al., 2008).

The deposition of insect eggs alone, in the absence of feeding, can induce significant physiological and chemical changes in plants. Volatile organic compounds (VOCs), are among the most studied defense-related metabolites involved in both direct deterrence and indirect defense strategies, such as attracting natural enemies of herbivores (Dudareva et al., 2006; Hilker and Fatouros, 2015).

In interactions with insects of the order Lepidoptera, for example, egg deposition usually represents the plant’s first contact with the herbivore rather than feeding damage (Fatouros et al., 2016). Upon perceiving these oviposition cues, plants activate early signaling cascades, including salicylic acid (SA) and jasmonic acid (JA) pathways and also, as a result of this response, starts the emission of defensive volatiles (Little et al., 2007; Bruessow & Reymond, 2007; Hilfiker et al., 2014). These responses help restrict larval development and enhance the recruitment of egg parasitoids, demonstrating that plants proactively adjust their physiology at the moment of oviposition, rather than waiting until herbivory occurs (Reymond, 2013; Fatouros et al., 2008).

Plants that have been exposed to insect oviposition often mount faster or stronger defense responses upon subsequent herbivore attack compared to plants without prior egg deposition (Hilker and Fatouros, 2015; Fatouros et al., 2016. This phenomenon, known as priming, allows the plant to respond more efficiently by activating relevant defense pathways in anticipation of damage (Bandoly et al., 2015). For example, in *Nicotiana attenuata* and *Arabidopsis thaliana*, oviposition triggers the early activation of JA and SA signaling, the expression of pathogenesis-related genes, and the release of defense volatiles even before larvae begin feeding (Bruessow and Reymond, 2007; Hilfiker et al., 2014).

Earlier responses reduce herbivore performance and can increase the recruitment of natural enemies. Transcriptomic studies have also shown that egg deposition upregulates genes associated with secondary metabolism and direct defense compounds (Singh et al., 2020), highlighting the idea that oviposition serves as a warning signal that reprograms the plant's physiology toward a heightened defensive state.

Understanding the role of root-associated microorganisms in modulating aboveground plant defenses enables understand how Microbial colonization, particularly by endophytic and rhizospheric fungi, can prime systemic plant defenses and alter VOC emission patterns in response to herbivore or oviposition stress (Guerrieri and Rasmann, 2019; Zehra et al., 2022). Beneficial fungi such as *Pochonia chlamydosporia* have been reported to influence tomato VOC profiles and potentially enhance the plant's responsiveness to insect attack (Monteiro et al., 2020).

Therefore, this study aims to test the hypothesis that VOC production will be higher in plants with both eggs and the fungus. For this, we will investigate how the tomato plants react defensively to the presence of eggs on leaves from *Phthorimaea absoluta*, an important pest for tomato cultivators, and by the association with nematophagus fungi *Pochonia chlamydosporia* in roots.

2. Methodology

2.1 Tomato leaves with eggs of *P. absoluta*

The rearing of *P. absoluta*, the insects used, the conditions of the plants used on this experiment was the same described previously on chapter 1. One plant from each treatment (control and inoculated) were placed in a wooden cage (56 × 56 × 56 cm) covered with organza and 4 mated *P. absoluta* females were released into each cage. The females were released during the scotophase, which is the oviposition period of *P. absoluta* (Proffit et al. 2011), in a room under controlled conditions of temperature (25 ± 2 °C), photoperiod (12:12, light:dark), and relative humidity (70 ± 5%). The number of eggs per plant was standardized to 12 (Milonas et. al, 2019), and the remaining eggs were removed with the aid of a fine brush (no. 00).

2.2 Headspace Volatile Collection and Chromatographic Analysis

Volatiles from the aerial parts of plants inoculated with the fungus *P. chlamydosporia* were collected using the headspace technique over 24h (N = 6) and 48h (N = 6) post-oviposition of *Phthorimaea absoluta*. The plants were individually placed in cylindrical glass chambers (37 cm high × 14 cm in diameter) with the soil surface covered by aluminum foil (Arce et al. 2017). An air filter containing activated charcoal (1 L/min) was introduced into the chamber, and volatiles were drawn out (0.5 L/min) and trapped on 20 mg of HayeSep Q (80–100 mesh) (Analytical Research Systems, Florida, USA). Subsequently, the volatiles were eluted in 200 µL of hexane. All collected samples were stored at -80°C for later analysis using GC-MS.

For the analysis of the collected volatiles, a 24 µL aliquot was taken from each sample, and 1 µL of 1000 ng n-heptyl acetate standard was added, resulting in a sample with 40 ng/µL of the internal standard. The volatile profile was obtained by injecting 1 µL of the sample into a gas chromatograph coupled with a mass spectrometer (Shimadzu GCMS – QP2010 SE), in splitless mode, Rtx-5MS (Restek chromatography products), mode onto a DB-5 column (30 m × 0.25 mm; df = 0.25 µm; J&W Scientific, Folsom, CA, USA), using helium as carrier gas (170 kPA). The temperature program used was temperature starts at 35°C (held for 1 min), followed by an increase of 8°C/min until reaching 280°C, which is held for 5 min. The identification and quantification of compounds were performed using comparisons to the mass spectra of NIST 14s library spectra and confirmed with the spectra of commercial standards.

2.3 Statistical analysis

The statistical analysis was performed using a generalized linear model (GLM) with a Gaussian distribution, aiming to evaluate the effects of time after oviposition (0h, 24h, and 48h) and the different volatile compounds emitted by the plants inoculated with *P. chlamydosporia*, as well as their interaction, on compound concentration.

3. Results

The presence of *Phthorimaea absoluta* eggs on tomato plants inoculated with *Pochonia chlamydosporia* (isolate Pc10) affected the expression of volatile organic compounds (VOCs). The time after oviposition (24h or 48h), when compared to non-oviposited plants, significantly influenced the concentration of these compounds (F = 955.22, p = 0.0229).

We identified 17 compounds on plants without eggs, 15 on plants with eggs for 24 hours and 19 compounds on plants with eggs for 48 hours (Table 3.1). Regardless of the presence of eggs, on plants inoculated with *P. chlamydosporia* (isolate Pc10), we identified 9 compounds, which were α-pinene, β-myrcene, α-phellandrene, α-terpinene, β-phellandrene, nonanal, dodecane, (Z)-β-caryophyllene, and salicylic acid (Table 3.1). Some alkane were identified only on plants post 24 hours of oviposition, such as 2-methyldecane and undecane. Other compounds, such as 5-methyl undecane and salicylate, were identified only in plants 48 hours post-oviposition (Table 3.1).

Table 3.1: List of compounds identified from tomato plants with *Phthorimamea absoluta* eggs and inoculated with *Pochonia chlamydosporia* (isolate Pc10) and respective means of concentration (ng/µl).

Compound	**Chemical class**	**RI $_{\text{exp}}$**	**RI $_{\text{Lit}}$**	**Reference (literature)**	**Mean Concentration ± SD (ng/µ) (0h)**	**Mean Concentration ± SD (ng/µ) (24h)**	**Mean Concentration ± SD (ng/µ) (48h)**	**Supplier/ Provided by***	**Purity/ Concentration***
Nonane	Aliphatic hydrocarbon	906	900	NIST 2024	0.36 ± 1.01	-	0.21 ± 0	-	-
α-Pinene*	Monoterpene hydrocarbon	944	939	NIST 2024	2.25 ± 1.120	2.03 ± 1.73	01.07 ± 0.44	Esalq - Piracicaba	40ng/µL (diluted in hexane)
Hexanal*	Aldehyde	968	801	NIST 2024	-	-	0.19 ± 0.19	Esalq - Piracicaba	40ng/µL (diluted in hexane)
β-Myrcene*	Monoterpene hydrocarbon	1004	991	NIST 2024	0.13 ± 0.091	1.20 ± 0.17	0.23 ± 0	SIGMA - ALDERICH	90%
4-Carene*	Monoterpene hydrocarbon	1017	1011	NIST 2024	**21.82 ± 5.60**	-	-	Lab. Semioquímicos - UFV	30ng/µL (diluted in hexane)
α-Phellandrene*	Monoterpene hydrocarbon	1020	1008	NIST 2024	5.09 ± 1.23	7.47 ± 43.53	12.28 ± 0.67	Max Plank - Holanda	40ng/µL (diluted in hexane)
2-Methyl decane	Aliphatic hydrocarbon	-	1015	NIST 2024	-	9.67 ± 0	4.88 ± 0	-	-
α-Terpinene	Monoterpene hydrocarbon	1035	1017	NIST 2024	**1.97 ± 0.99**	**19.23 ± 12.15**	**2.72 ± 5.93**	-	-
p-Cymene*	Monoterpene hydrocarbon	1045	1025	NIST 2024	0.87 ± 0.55	-	-	SIGMA - ALDERICH	99%
β-Phellandrene *	Monoterpene hydrocarbon	1051	1033	NIST 2024	**76.20 ± 21.07**	**116.49 ± 43.53**	**30.13 ± 11.26**	Max Plank - Holanda	40ng/µL (diluted in hexane)
β-Ocimene*	Monoterpene hydrocarbon	1069	1050	NIST 2024	0.33 ± 0.16	-	-	SIGMA - ALDERICH	90%
2,4-Dimethyl decane*	Aliphatic hydrocarbon	1077	1030	NIST 2024	0.71 ± 0.32	-	-	-	-
4,7-Dimethyl undecane	Aliphatic hydrocarbon	1124	1180	NIST 2024	0.45 ± 0.36	-	-	-	-

Nonanal*	Aldehyde	1129	1101	NIST 2024	1.00 ± 0.69	2.74 ± 0.51	1.70 ± 0.75	Lab. Semioquímicos - UFV	20ng/µL (diluted in hexane)
5-Methyl undecane	Aliphatic hydrocarbon	-	1155	NIST 2024	-	-	0.61 ± 0.33	-	-
2,6-Dimethyl undecane	Aliphatic hydrocarbon	-	1170	NIST 2024	-	5.81 ± 0	-	-	-
Dodecane*	Aliphatic hydrocarbon	1230	1200	NIST 2024	1.20 ± 0.14	1.37 ± 0.07	1.11 ± 0	Lab. Semioquímicos - UFV	30ng/µL (diluted in hexane)
Decanal	Aldehyde	1239	1200	NIST 2024	-	2.14 ± 0.80	1.45 ± 0.41	-	-
β-Caryophyllene*	Sesquiterpene hydrocarbon	1488	1418	NIST 2024	2.57 ± 1.03	2.56 ± 0.89	1.47 ± 0.92	Esalq - Piracicaba	40ng/µL (diluted in hexane)
Salicylic acid	Aromatic acid	2007	1325	NIST 2024	11.62 ± 3.84	-	1.45 ± 0.36	-	-
Salicylate	Aromatic ester/acid derivative	-		NIST 2024	-	-	0.21 ± 0	-	-

*Compounds identified by internal library and synthetic standards; RI = Retention Index calculated based on Kovats Index; Exp = RI based on this experiment; Lit = RI based on literature; and compounds named in **bold** significantly affected compounds (p<0.05) in interaction with time or main effects.

The concentration of β-phellandrene increased significantly 24 hours after oviposition ($p = 0.0184$); however, after 48 hours, its concentration decreased, becoming significantly lower compared to plants without oviposition or those evaluated after 24 hours ($p = 0.0150$) (Fig. 3.1A) (Table 3.1). The concentration of the compound changed significantly at 24 hours and 48 hours compared to the control plants without eggs. The compound α-terpinene showed a decrease in concentration in response to egg presence, particularly after 24 hours ($p = 0.0450$) (Fig. 3.1B) (Table 3.1).

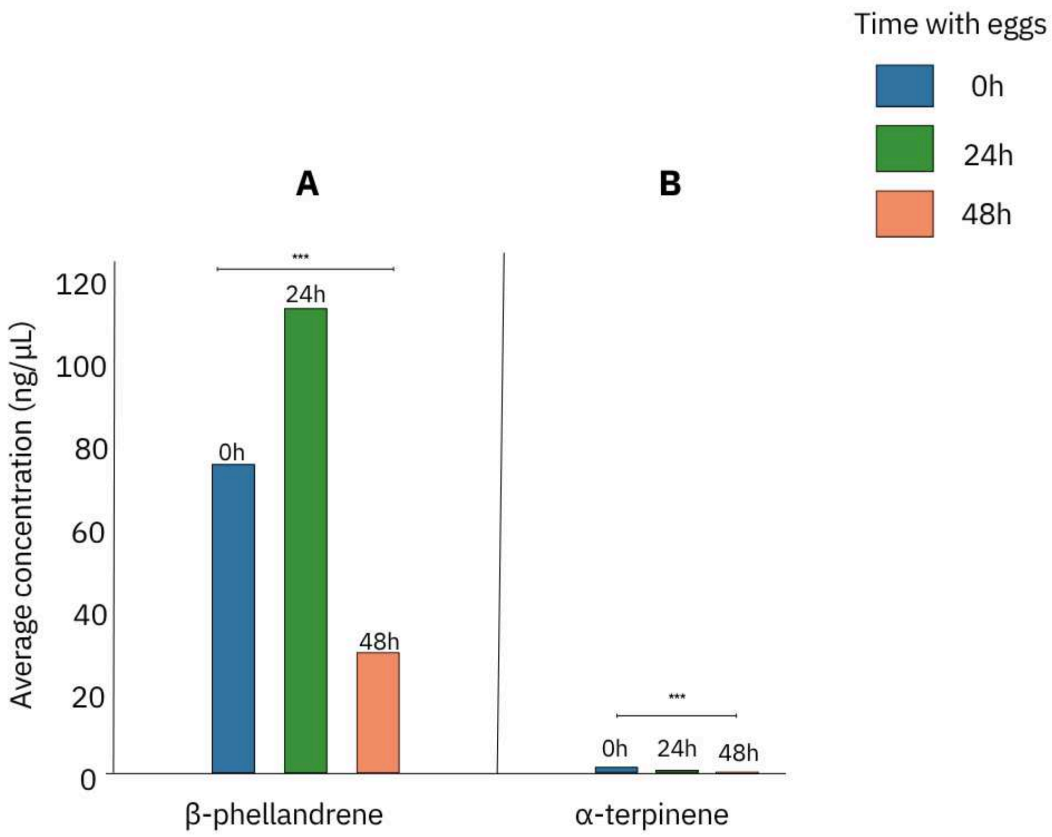

Figure 3.1: Average concentration of the compounds β-phellandrene (A), and α-terpinene (B) on tomato plants inoculated with *Pochonia chlamydosporia* (Isolate Pc10), over time post oviposition of *Phthorimaea absoluta.* Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

4. Discussion

In this study, we found that *Phthorimaea absoluta* eggs on tomato plants treated with the endophytic fungus *Pochonia chlamydosporia* (isolate Pc10) affected the volatile organic compounds (VOCs) both in quality and quantity. This change showed a direct link to how long the eggs are on the tomato plant, which relates to the endophytic fungus.

The presence of insect eggs prompts chemical defenses in the plant, known as oviposition-induced plant volatiles (OIPVs). These serve as key signals for natural enemies like parasitoids (Peñaflor et al., 2011; Hilker & Fatouros, 2015). When the plant starts to defend itself at this stage, it gains an edge over the organism during the damage phase, allowing it to react more quickly than plants without eggs (Pashalidou et al., 2020).

Our study identified important monoterpenes linked to OIPVs in tomato plants, including α-terpinene and β-phellandrene, influenced by oviposition. Understanding plant defense responses like OIPVs can help with combined pest management (IPM), as this knowledge can improve biological control of insects (Cusumano et al., 2020; Das & Barik, 2022).

Compounds such as α-terpinene and β-phellandrene mainly come from the trichomes of tomato leaves. They are linked to physical defenses against herbivores and attract natural enemies (Ayelo et al., 2021). The interaction between α-terpinene and β-phellandrene serves as an important tool for resisting pest insects (D'Esposito et al., 2023), including *P. absoluta*. Additionally, the absence of the compound 4-carene in plants with eggs may affect the oviposition preference of *P. absoluta* (Nishida, 2014).

We saw a rise in β-phellandrene concentration 24 hours after oviposition, indicating a quick plant response to this type of stimulus. This change can act as a warning signal and aid in attracting natural enemies like parasitoids or predators (Ayelo et al., 2021; Adams et al., 2023), including *Trichogramma pretiosum* and Nesidiocoris tenuis.

However, β-phellandrene levels dropped after 48 hours of oviposition. This suggests a time-dependent metabolic adjustment. Despite this change, it's important to mention that β-phellandrene's interaction with other compounds, particularly when mixed with other monoterpenes, supports communication across different species (Ayelo et al., 2021; Silva et al., 2023). Thus, the presence of *P. chlamydosporia* in oviposited plants acts as an added trigger for defense in tomato plants.

We noticed a decrease in α-terpinene concentration in response to oviposition, especially after 24 hours. This indicates a suppression of this compound, likely as a tactic to balance secondary metabolism, similar to the observation with β-phellandrene. Furthermore, α-terpinene is linked to direct defense against insects and regulates the release of other secondary volatiles (Bleeker et al., 2009), potentially in conjunction with compounds like β-phellandrene.

Alkanes found only in plants where *P. absoluta* laid eggs are commonly part of the volatile compound profile emitted by tomato plants, especially in reaction to herbivory or the presence of insect eggs (López et al., 2013; Nawrocka et al., 2023). These alkanes can impact insect host plant choice, whether by attracting or repelling them. They aid in forming physical or chemical barriers on the plant's surface and support communication between plants (Adams et al., 2023).

Based on our results, we can conclude that *P. chlamydosporia* might influence the metabolic adjustments of VOCs regarding the plant's response to oviposition. This could happen through priming for defensive metabolism or by releasing fungal-derived substances that change VOC profiles. This type of change has been noted in interactions involving endophytic or rhizospheric fungi that alter how attractive plants are to their natural enemies (Guerrieri et al., 2019; Zehra et al., 2022).

Overall, the presence of *Pochonia chlamydosporia* in tomato plants shows promise as an alternative for pest management, specifically for *Phthorimaea absoluta*. We previously noted that this pest prefers to lay eggs on plants without the fungus. However, when oviposition does happen, our results indicate that the plant quickly activates its defense mechanisms. Therefore, future studies with field applications could further clarify how this interaction could work on a larger scale.

5. References

Adams B., Yusuf A.A; Torto, B.; Khamis F.M. (2023). Non-host plant odors influence the tritrophic interaction between tomato, its foliar herbivore *Tuta absoluta* and mirid predator *Nesidiocoris tenuis*. Frontier Plant Science, 14:1014865. DOI:10.3389/fpls.2023.1014865

Arce C.C., Machado R.A., Ribas N.S, Cristaldo P.F, Ataíde L.M., Pallini A., Carmo F.M., Freitas L.G, Lima E. (2017). Nematode root herbivory in tomato increases leaf defenses and reduces leaf miner oviposition and performance. Journal of Chemical Ecology, 43: 120-128. DOI: 10.1007/s10886-016-0810-z

Ayelo, P.M., Yusuf, A.A., Pirk, C.W.W., Mohamed, S.A., Chailleux, A., Deletre, E. (2021). The Role of *Trialeurodes vaporariorum*-Infested Tomato Plant Volatiles in the Attraction of *Encarsia formosa* (Hymenoptera: Aphelinidae). Journal of Chemical Ecology, 47: 192–203. DOI: 10.1007/s10886-021-01245-2

Bandoly, M., Steppuhn, A., Baldwin, I.T. (2015). Priming of anti-herbivore defense in *Nicotiana attenuata* by insect oviposition: herbivore-specific effects. The Plant Journal, 83(4): 661–671. DOI: 10.1111/tpj.12919

Blenn, B., Bandoly, M., Kallenbach, M., Baldwin, I.T. (2012). Four diterpenoid glycosides from *Nicotiana attenuata* protect against attack from the mirid bug *Tupiocoris notatus*. Journal of Experimental Botany, 63(13): 469–482. DOI: 10.1093/jxb/err005

Bruessow, F., Reymond, P. (2007). Oviposition by *Pieris brassicae* triggers defense responses in *Arabidopsis*. Plant Signaling & Behavior, 2(3): 204–206. DOI: 10.4161/psb.2.3.4287

Coelho, M.D.C. & França, F. H. (1987). Biologia, quetotaxia da larva e descrição da pupa e adulto da traça-do-tomateiro. Pesquisa Agropecuária Brasileira 22: 129-135.

Dudareva, N., Negre, F., Nagegowda, D.A., Orlova, I. (2006). Plant volatiles: Recent advances and future perspectives. Trends in Plant Science, 11(8): 437–445. DOI: 10.1016/j.tplants.2006.07.003

Fatouros, N.E., Bukovinszkine-Kiss, G., Kalkers, L.A., Soler, R., van Loon, J.J.A., Dicke, M., Hilker, M. (2008). Oviposition-induced plant cues: Do they arrest *Trichogramma* wasps during host location? Journal of Chemical Ecology, 34: 805–813. DOI: 10.1007/s10886-008-9487-0

Fatouros, N.E., Cusumano, A., Danchin, E.G.J., Colazza, S. (2016). Prospects of herbivore egg-killing plant defenses for sustainable crop protection. Ecology and Evolution, 6(19): 6906–6918. DOI: 10.1002/ece3.2365

Cusumano A, Harvey J.A., Bourne M.E., Poelman E.H., de Boer J.G. (2020). Exploiting chemical ecology to manage hyperparasitoids in biological control of arthropod pests. Pest Management Science 76: 432–443. DOI: 10.1002/ps.5679

Das S. & Barik A. (2022). Microbe-induced plant volatiles and their role in plant disease management. Volume 1 ISBN: : 978-3-030-87511-4. DOI: 10.1007/978-3-030-87512-1_9

D'Esposito, D., Guadagno, A., Amoroso, C.G., Cascone, P., Cencetti, G., Michelozzi, M., Guerrieri, E., Ercolano, M.F. (2023). Genomic and metabolic profling of two tomato contrasting cultivars for tolerance to *Tuta absoluta.* Planta, 257:47. DOI:10.1007/s00425-023-04073-8

Gaspard J.T., Jaffee B.A., Ferris H. (1990). Association of *Verticillium chlamydosporium* and *Paecilomyces lilacinus* with root-knot nematode infested soil. Journal of Nematology, 22(2): 207-213. PMID: 19287711; PMCID: PMC2619028

Genç, H. (2016). The tomato leafminer, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae): pupal key characters for sexing individuals. Turkish Journal of Zoology, 40: 801-805. DOI:10.3906/zoo-1510-59

Gonçalves, A.G.S, Haddi, K., Ribas, N.S., Santos, K.C.P., Tschoeke, L.F.P., Lima, E. (2024). Age, weight, and mating status of the male influence female choice and reproductive success in *Tuta absoluta.* Entomologia Generalis, 44(2): 443 - 450. DOI: 10.1127/entomologia/2024/2287

Gouveia, A.S., Avelar Monteiro, T.S., Balbino, H.M., Corbelli de Magalhães, F., Ramos, M.E.S., Moura, V.A.S., Luiz, P.H.D., Oliveira, M.G.A., Freitas, I.G., Ramos, H.J.O. (2023). Inoculation of *Pochonia chlamydosporia* triggers a defense response in tomato roots, affecting parasitism by *Meloidogyne javanica*. Microbiological Research, 266: 127242. DOI: 10.1016/j.micres.2022.127242

Guerrieri, E., Rasmann, S. (2019). Belowground signaling to aboveground insect herbivores: the role of root-associated microorganisms. Frontiers in Plant Science, 10: 1327. DOI: 10.3389/fpls.2019.01327

Hickel, E., Vilela, E., Lima, J., Lucia, T. (1992). Calling behavior of the tomato leafminer *Scrobipalpuloides absoluta* (Lepidoptera, Gelechiidae). Revista Brasileira de Entomologia, 36: 277-280.

Hilfiker, O., Groux, R., Bruessow, F., Kiefer, K., Zeier, J., Reymond, P. (2014). Insect eggs induce a systemic acquired resistance in *Arabidopsis*. The Plant Journal, 80(6): 1085–1094. DOI: 10.1111/tpj.12703

Hilker, M., Fatouros, N.E. (2015) Plant responses to insect egg deposition. Annual Review Entomology, 60: 493–515. DOI: 10.1146/annurev-ento-010814-020620

Lemos, F. (2015). Indirect interactions in tomato attacked by *Tetranychus evansi.* Doctor's thesis, Universidade Federal de Viçosa (Brasil).

López, A.Y.I., Martínez-Gallardo, N.A., Ramírez-Romero, R., López, M.G., Sánchez-Hernández, C., Délano-Frier, J.P. (2012). Cross-kingdom effects of plant-plant signaling via volatile organic compounds emitted by tomato (*Solanum lycopersicum*) plants infested by the greenhouse whitefly (*Trialeurodes vaporariorum*). Journal of Chemical Ecology, 38(11): 1376-86. DOI: 10.1007/s10886-012-0201-z

Muller, K., Thiéry, D., Motreuil, S., Moreau, J. (2016). What makes a good mate? Factors influencing male and female reproductive success in a polyphagous moth. Animal Behaviour, 120, 31–39. DOI: 10.1016/j.anbehav.2016.07.027

Milonas, P.G.; Anastasaki, E.; Partsinevelos, G. (2019). Oviposition-induced volatiles affect electrophysiological and behavioral responses of egg Parasitoids. Insects, 10: 437. DOI: 10.3390/insects10120437

Monteiro, H.S.A., Pacheco, P.V.M., Gouveia, A.S., Balbino, H.M., & Freitas, L.G. (2020). Pochonia. Beneficial Microbes in Agro-Ecology, 669–682. DOI:10.1016/B978-0-12-823414-3.00033-2

Nawrocka, J., Szymczak, K., Skwarek-Fadecka, M., Małolepsza, U. (2023). Toward the Analysis of Volatile Organic Compounds from Tomato Plants (*Solanum lycopersicum* L.) Treated with *Trichoderma virens* or/and *Botrytis cinerea*. Cells, 12(9):1271. DOI: 10.3390/cells12091271

Nishida, R. (2014). Chemical ecology of insect–plant interactions: ecological significance of plant secondary metabolite. Bioscience, Biotechnology, and Biochemistry, 78(1): 1–13. DOI:10.1080/09168451.2014.877836

Peñaflor, M.F.G.V., Erb, M., Robert, C. , A.M., Miranda, L.A., Werneburg, A.G., Dossi, F.C. A., Turlings, T.C.J. & Bento, J.M.S (2011). Oviposition by a moth suppresses constitutive and herbivore-induced plant volatiles in maize. Planta, 234(1): 207-215. DOI: 10.1007/s00425-011-1409-9

Proffit, M., Birgersson, G., Bengtsson, M., Reis, Jr. R., Witzgall, P., Lima, E. (2011). Attraction and oviposition of *Tuta absoluta* females in response to tomato leaf volatiles. Journal of Chemical Ecology, 37: 565–574. DOI: 10.1007/s10886-011-9961-0

Pashalidou, F. G., Eyman, L., Sims, J., Buckley, J., Fatouros, N. E., De Moraes, C. M., Mescher, M. C. (2020). Plant volatiles induced by herbivore eggs prime defences and mediate shifts in the reproductive strategy of receiving plants. Ecology Letters. 23(7): 1097–1106. DOI: https://doi.org/10.1111/ele.13509

Zauza, E.A.V; Alfenas, A.C; Mafia, R.G. (2007). Esterilização, preparo de meios de cultura e fatores associados ao cultivo de fitopatógenos. Métodos em fitopatologia: p 23-51

Zehra, A., Naz, S., & Kamal, S. (2022). Rhizosphere microbes as bio-inducers of systemic resistance in plants. Microbiological Research, 257: 126982. DOI: 10.1016/j.micres.2021.126982

General Conclusions

Our studies have shown that the interaction between the endophytic fungus *Pochonia chlamydosporia* and tomato plants (*Solanum lycopersicum*) induces significant changes in the profile of volatile organic compounds (VOCs) emitted by the plants, which directly affect the oviposition behavior and development of the specialist herbivore *Phthorimaea absoluta*. As a consequence of this interaction, the presence of the fungus in the roots alters both quantitatively and qualitatively the emission of these volatiles, highlighting compounds such as p-cymene, salicylic acid, β-myrcene, and 4-carene, which play crucial roles in plant defense against herbivores and in modulating the attractiveness to the tomato leafminer.

The presence of *P. chlamydosporia* also influenced the behavioral responses of *P. absoluta* antennae, although it was more evident qualitatively .Although the fungus did not significantly change the antennal perception intensity of some bioactive compounds by the leafminer, the qualitative variations in the VOC profile appear to influence female oviposition preference, which tends to avoid plants inoculated with *P. chlamydosporia*. Additionally, the presence of the fungus accelerates the egg and larval development of the insect, which may have implications for the pest population dynamics.

When we observed the induction of post-oviposition defense of *P. absoluta*, there was a direct relationship related to the time the egg was on the plant, combined with the effect of the fungus on the plant. Specific compound concentrations vary over 24 and 48 hours after oviposition. This reconfiguration of VOCs suggests an indirect defense mechanism that may involve the recruitment of natural enemies, contributing to tritrophic interactions.

In light of our findings, it is possible to highlight the importance of studying and understanding the variables of the fungus-plant-insect interaction on systems in integrated pest management strategies. Moreover, how to use the potential of endophytic fungi as a tool to modify plant biochemistry and reduce the impact of specialist herbivores through manipulation of the chemical signals that regulate insect behavior.