UNIVERSIDADE ESTADUAL PAULISTA
FACULDADE DE CIÊNCIAS AGRÁRIAS E VETERINÁRIAS
CÂMPUS DE JABOTICABAL

RESISTANT GENOTYPES, BIOLOGICAL CONTROL AND SELECTIVE PESTICIDES FOR THE INTEGRATED MANAGEMENT OF *Tetranychus evansi* (ACARI: TETRANYCHIDAE) ON TOMATO

Patrice Jacob SAVI

Agronomist Engineer

Jaboticabal-SP
2022

UNIVERSIDADE ESTADUAL PAULISTA
FACULDADE DE CIÊNCIAS AGRÁRIAS E VETERINÁRIAS
CÂMPUS DE JABOTICABAL

RESISTANT GENOTYPES, BIOLOGICAL CONTROL AND SELECTIVE PESTICIDES FOR THE INTEGRATED MANAGEMENT OF *Tetranychus evansi* (ACARI: TETRANYCHIDAE) ON TOMATO

Advisor: Prof. Dr. Daniel Junior de Andrade
Co- Advisor: Prof. Dr. Gilberto José de Moraes

Thesis presented to the Faculdade de Ciências Agrárias e Veterinárias-Unesp, Câmpus de Jaboticabal, as part of requirements to obtain the Doctorate degree in Agronomy (Agricultural Entomology)

2022

S267r

Savi, Patrice Jacob

Resistant genotypes, biological control and selective pesticides for the integrated management of Tetranychus evansi (Acari: Tetranychidae) on tomato / Patrice Jacob Savi. -- Jaboticabal, 2022

167 p. : il., tabs., fotos

Tese (doutorado) - Universidade Estadual Paulista (Unesp), Faculdade de Ciências Agrárias e Veterinárias, Jaboticabal

Orientador: Daniel Júnior de Andrade

Coorientador: Gilberto José de Moraes

1. Tomato red spider mite. 2. Integrated Pest Management. 3. Resistant genotype. 4. Phytoseiidae. 5. Impact of pesticides on predator. I. Título.

Sistema de geração automática de fichas catalográficas da Unesp. Biblioteca da Faculdade de Ciências Agrárias e Veterinárias, Jaboticabal. Dados fornecidos pelo autor(a).

Essa ficha não pode ser modificada.

UNIVERSIDADE ESTADUAL PAULISTA

Câmpus de Jaboticabal

CERTIFICADO DE APROVAÇÃO

TÍTULO DA TESE: RESISTANT GENOTYPES, BIOLOGICAL CONTROL AND SELECTIVE PESTICIDES FOR THE INTEGRATED MANAGEMENT OF *Tetranychus evansi* (ACARI: TETRANYCHIDAE) ON TOMATO

AUTOR: PATRICE JACOB SAVI

ORIENTADOR: DANIEL JUNIOR DE ANDRADE

COORIENTADOR: GILBERTO JOSÉ DE MORAES

Aprovado como parte das exigências para obtenção do Título de Doutor em AGRONOMIA (ENTOMOLOGIA AGRÍCOLA), pela Comissão Examinadora:

Prof. Dr. DANIEL JUNIOR DE ANDRADE (Participaçao Virtual)
Departamento de Ciencias da Producao Agricola / FCAV UNESP Jaboticabal

p/ Prof. Dr. DANIEL CARRILLO (Participaçao Virtual)
Departamento de Entomologia e Nematologia - UF/IFAS / Gainesville/Flórida

p/ Prof. Dr. RAPHAEL DE CAMPOS CASTILHO (Participaçao Virtual)
Departamento de Entomologia e Acarologia / ESALQ/USP - Piracicaba/SP

p/ Prof. Dr. MARCELO COUTINHO PICANÇO (Participaçao Virtual)
Universidade Federal de Viçosa-UFV / Viçosa/MG

p/ Pesquisador Dr. MÁRIO EIDI SATO (Participaçao Virtual)
Instituto Biológico de Campinas / Campinas/SP

Jaboticabal, 04 de março de 2022

ABOUT THE AUTHOR

PATRICE JACOB SAVI was born in Ifangni (Republic of Benin) on March 17th, 1991. Son of Martin Savi and Beatrice Lokossou, he was an undergraduate in Agronomy at Université de Parakou (Republic of Benin), where he finished his degree in 2012. He did an undergraduate thesis under the supervision of Prof. Dr. Fabien C.C. Hountondji and worked with pest management approaches in vegetable crops at the Songhai center in Porto-Novo (Republic of Benin). After getting his degree, he got his first job as a Head Agriculturist at "Groupe Vision Plantain Hévea Cacao (GVPHeC)" in Ifangni (South of the Republic of Benin), mostly working with farmers on the control of the banana weevil, *Cosmopolites sordidus* (Coleoptera: Curculionidae), and farm maintenance. From there, he went for an internship of two-month at a non-governmental organization "FUDEL" in Ifangni (South Benin) specializing in vegetable crop production to gain more hands-on experience as a horticulturist. After completing this internship, he got a position as Head horticulturist for two years at Aganmandin Farm in charge of Formation, Research in Horticulture, and Animal Husbandry working with all aspects related to the maintenance, pest, and disease control strategies for vegetable crops. From 2014 to 2016, he was also a Professor at Benin Secondary School. In March 2016, he was awarded a scholarship by Conselho Nacional de Desenvolvimento Científico e Tecnológico-CNPq to join the Master Program in Agricultural Entomology Graduate Program (Agronomy) at São Paulo State University (UNESP) working under the supervision of Prof. Daniel Júnior de Andrade, and Gilberto José de Moraes. While in his Master's, he got an interest in selecting African tomato genotypes resistant to *Tetranychus evansi* (Acari: Tetranychidae). He was immediately accepted to continue as a Doctorate student, soon after the completion of his Master´s program, in early 2018 at the same University, under the supervision of the same advisors, and received a scholarship from Coordenação de Aperfeiçoamento de Pessoal de Nível Superior-CAPES.

e-mail: savipatricejacob@yahoo.fr

DEDICATION

Every challenging work needs self-efforts as well as the guidance of elders especially those who were very close to our hearts.

My humble effort I dedicate to my

Parents: Béatrice and Martin
Brothers: Simon, David, Élie, and Isaac
Sisters: Agnès and Elisabeth

Whose affection, love, encouragement and prayers of day and night make me able to get such success and honor.

Along with all hardworking and respected

Teachers

A keep to growing, as a teacher is to keep company mainly with teachers who uplift you, whose presence inspire you, and whose dedication drives you

Robert John Meehan

ACKNOWLEDGMENTS

First of all, to God Almighty for granting me this valuable opportunity of knowledge and intellectual aptitude to pursue my education life.

To my beloved advisor Prof Dr. Daniel Júnior de Andrade, for being so generous with me, for opening his lab up to me, and for his feedback and advice in all aspects of this thesis. I honestly could not have asked for a better advisor and I am truly privileged to have had the opportunity to work under him.

To my esteemed co-advisor Prof. Dr. Gilberto José de Moraes for all his constant support, professional orientation, and constructive comments over the years of my Ph.D. journey. I learned many things from him that reflected fruitfully in my academic career.

To my precious undergraduate advisor Prof. Dr. Fabien C.C. Hountondji, for his excellent suggestions in drafting an article part of this thesis.

To Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) - Finance Code 001 for giving me the scholarship during my PhD study.

To Universidade Estadual Paulista “Júlio de Mesquita Filho”, School of Agricultural and Veterinarian Sciences of Jaboticabal (UNESP/FCAV), and the Department of Agricultural Sciences and Graduation Program in Agronomy (Agricultural Entomology), for the opportunity to perform my Ph.D. course.

To the former and current Graduation Program in Agronomy (Agricultural Entomology) coordinators: Prof. Dr. Raphael de Campos Castilho, Prof. Dr. Guilherme Duarte Rossi, Prof. Dr. Ricardo Antonio Polanczyk for their assistance and contribution.

To the professors of the Graduate Program in Agronomy (Agricultural Entomology), for the knowledge, which significantly contributed to my academic and personal formation.

To Prof. Dr. Raphael de Campos Castilho and Prof. Dr. Arlindo Leal Boiça Junior, for their valuable participation and contribution during my Ph.D. qualifying examination.

To Prof. Dr. Rogério Falleiros Carvalho, for his collaboration in conducting this work.

To Prof. Dr. Tiago Gallina, Universidade Federal do Pampa, Uruguaiana campus, Rio Grande do Sul (Brazil), for valuable assistance in collection of *Phytoseiulus longipes*.

To Prof. Dr. Wesley Augusto Conde Godoy and Dr. José Bruno Malaquias from the Insect Ecology Laboratory, Department of Entomology and Acarology, Escola Superior de Agricultura “Luiz de Queiroz”, Universidade de São Paulo (ESALQ/USP) for the assistance in the preparation of a video using the EthoVision XT software.

To Institut National des Recherches Agricoles du Bénin (INRAB) for supplying the seeds of cultivated tomatoes used in this work.

To Prof. Dr. André Luiz Lourenção of Instituto Agronômico (IAC) for supplying the seeds of South American tomato genotypes used in this work.

To Wanderley Pintado Brasil for helping me with the soil sterilization every time this was necessary.

To Ana Beatriz Piai Kapp, Bruno Rafael, Claudiane Martins da Rocha, Daiana Paixão Nogueira Silva, Gabriel, Monteiro, Hágabo Honorato, Maiara Alexandre, Jaqueline Della Vechia, Leilane Martins Lacerda, Matheus Cardoso de Castro, and Sidneia Terezinha Soares de Matos, undergraduate and graduate colleagues of Laboratory of Acarology, for their help and continuous companionship.

To the employees of the Agricultural Production Sciences Department for their help throughout the great time I spent at UNESP/FCAV.

To all who were not mentioned here but helped directly or indirectly the conduction of this work.

Lastly and especially my gratitude goes to my wonderful family, my mom Béatrice, dad Martin, brothers Simon, David, Élie and Isaac, and sisters Agnès and Elisabeth for their patience when I was away from them over the past six years. Without their encouragement, support, and love, I would never have even thought to attempt this work, throughout my academic career.

This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001.

To all of you my muito obrigado!!!

SUMMARY

Page

GENÓTIPOS RESISTENTES, CONTROLE BIOLÓGICO E PESTICIDAS SELETIVOS PARA O MANEJO INTEGRADO DE *Tetranychus evansi* (ACARI: TETRANYCHIDAE) EM TOMATE

RESUMO - O ácaro vermelho do tomateiro, *Tetranychus evansi* Baker & Pritchard (Acari: Tetranychidae), é uma praga invasora do tomateiro em vários países, com potencial de reduzir a produtividade em até 90% na África. Devido ao alto potencial biótico da praga, o manejo focado no uso de defensivos sintéticos muitas vezes não é eficiente ou insustentável ao longo do tempo, sendo necessária a sua integração com outros métodos de controle. Estudos anteriores encontraram em genótipos selvagens fonte expressiva de resistência (tricomas glandulares) que poderiam ser exploradas para aumentar o nível de resistência de variedades de interesse a esta praga. Além disso, *Phytoseiulus longipes* Evans (Phytoseiidae), encontrado na América do Sul, mostrou-se um promissor ácaro predador de *T. evansi*. No entanto, a integração deste ácaro predador em programas de MIP onde *T. evansi* é um problema sério requer conhecimento detalhado das interações com outras práticas de manejo. Dessa forma, objetivou-se com este trabalho estabelecer um sistema de manejo integrado para *T. evansi* com a aquisição de genótipos de tomateiro resistentes, biopesticides eficientes a *T. evansi*, um genótipo adequado que pudesse otimizar o desempenho do ácaro predador *Phytoseiulus longipes* Evans (Phytoseiidae) e com a definição de agrotóxicos seletivos comumente usado em tomateiro a esse predador. Os estudos foram conduzidos em condições de laboratório e semi-campo. As progênies F1, SPJ-10-2017 e SPJ-05-2018 obtidas cruzando o genótipo selvagem resistente [*Solanum habrochaites*, acesso PI 134417] com *Solanum lycopersicum*, cv TLCV15 [importante genótipo cultivado amplamente cultivado no Benin] herdaram significativos tricomas glandulares tipos I, IV e VI de seu pai resistente (PI 134417). As densidades de tricomas glandulares herdados pelos genótipos da progênie foram capazes de reduzir ou suprimir as infestações causadas por *T. evansi*. No entanto, o genótipo de progênie causou atrasos importantes no crescimento populacional e reduziu significativamente a sobrevivência e o potencial de predação de *P. longipes*. Os genótipos cultivados com maior número de tricomas não glandulares mostraram-se adequados para a implementação do programa IPM que visa otimizar o uso de *P. longipes* como agente de biocontrole. Os resultados demonstraram que o uso de biopesticidas à base de azadirachtin e oxymatrine apresentaram alta atividade contra *T. evansi* e pode ser uma importante alternativa para uso no manejo de *T. evansi* em substituição ou rotação com acaricidas sintéticos. Além disso, azadiractina mostrou-se mais segura ao ácaro predador tanto no controle biológico aumentativo quanto na conservação, enquanto a oximatrina mostrou-se adequada apenas para o controle biológico aumentativo se 10 dias for observado após aplicação. Os agrotóxicos comumente usados no sistema de cultivo do tomateiro como abamectina, propargite, imidacloprid e o fungo entomopatogênico *Hirsutella thompsonii* (Fischer) (Deuteromycetes) são mais compatíveis com o controle

biológico aumentativo do que com a conservação se os prazos de segurança adequados forem respeitados antes da liberação. Os inseticidas piretróides (cipermetrina e deltametrina) e organofosforados (dimetoato, clorpirifós) não são compatíveis com o uso de *P. longipes* em programas de MIP. Esses resultados são importantes para o manejo sustentável dessa praga invasora e, ao mesmo tempo, fornecem diretrizes práticas que possibilitam uma melhor forma de uso de agrotóxicos em programas de MIP que visam conservar ou realizar liberações aumentativas do ácaro predador.

Palavras-chave: ácaro vermelho do tomateiro, Manejo Integrado de Pragas, genótipo resistente, Phytoseiidae, potencial de predação, biopesticidas, impacto de agrotóxicos sobre predador

RESISTANT GENOTYPES, BIOLOGICAL CONTROL AND SELECTIVE PESTICIDES FOR THE INTEGRATED MANAGEMENT OF *TETRANYCHUS EVANSI* (ACARI: TETRANYCHIDAE) ON TOMATO

ABSTRACT - The tomato red spider mite, *Tetranychus evansi* Baker & Pritchard (Acari: Tetranychidae), is an invasive tomato pest in several countries, with the potential to reduce yield by up to 90% in Africa. Due to the high biotic potential of the pest, the management focused on the use of synthetic pesticides is often not efficient or unsustainable over time, requiring the integration with other control methods. Previous studies found in wild genotypes expressive source of resistance (glandular trichomes) that could be explored to increase resistance level of varieties of interest to this pest. Furthermore, *Phytoseiulus longipes* Evans (Phytoseiidae), found in South America proved to be a promising predatory mite of *T. evansi*. However, the incorporation of this predatory mite into IPM programs requires detailed knowledge of the interactions with other management practices. Within this context, the objective of the present study was to establish an integrated management system with the acquisition of tomato genotypes resistant to *T. evansi*, a suitable genotype that could optimize the performance of predatory mite *P. longipes* and with the definition of selective pesticides to this predator.The studies were conducted under laboratory and semi-field conditions. Our results indicated that the progenies F1, SPJ-10-2017 and SPJ-05-2018 obtained by crossing the wild-resistant genotype [*Solanum habrochaites*, Knapp e Spooner var *glabratum* access PI 134417] with *Solanum lycopersicum* L., cv. TLCV15 [cultivated genotype widely grown in Benin] have inherited significant glandular trichomes types I, IV and VI from their resistant parent (PI 134417). The densities of these glandular trichomes inherited by progeny genotypes were able to reduce supress the infestation caused by *T. evansi*. However, the bred progeny genotype SPJ-05-2018 caused important delays population growth and reduced significantly a survival, and the predation potential of *P. longipes*. The cultivated genotypes with many non-glandular trichomes proved to be more suitable for the implementation of IPM program that aim to optimize the use of *P. longipes* as biocontrol agent. The results showed that the use of azadirachtin- and oxymatrine based biopesticides had high activity against *T. evansi* and may be an important alternative in the management of the mite in replacement or rotation with synthetic acaricides. Azadirachtin proved to be the safest against the predatory mite toward both augmentative biological control and conservation whereas oxymatrine proved to be suitable only toward augmentative biological control 10 days after application. Other pesticides used in tomato cropping system such as abamectin, propargite, imidacloprid and the enthomopathogenic fungus *Hirsutella thompsonii* (Fischer) (Deuteromycetes) are more compatible with augmentative biological control than conservation if appropriate safety deadlines are respected before release. The insecticides belonging to pyrethroid (cypermethrin and deltamethrin) and organophosphate (dimethoate, chlorpyrifos) groups are not compatible with the use of *P. longipes* in IPM programs. These results are important to sustainably manage this invasive mite pest, and at the same time, provide

practical guidelines to enable a better way of using pesticides in IPM programs that aim to conserve or increase the predatory mite *P. longipes*.

Keywords: tomato red spider mite, Integrated Pest Management, resistant genotype, Phytoseiidae, predation potential, biopesticides, impact of pesticides on predator

LIST OF TABLES

Page

LIST OF FIGURES

Page

CHAPTER 1- General Considerations

Introduction

Tomato (*Solanum lycopersicum* L.) is one of the most widely cultivated and consumed vegetable crops worldwide (Kimura and Sinha, 2008; Sibomana et al., 2016). In sub-Saharan Africa, this crop is commonly planted by small-scale farmers and represents an important source of income (Arah et al., 2015; Sibomana et al., 2016). However, efforts of farmers to increase tomato production often face various problems in the field, such as interference from intruder organisms (pests and diseases) that substantially affect tomato production (Arah et al., 2015; Wakil et al., 2018; Abera et al., 2020). Among pests, the spider mites should be highlighted, as some species are key pests of this crop, requiring considerable investments in their control every year in an attempt to prevent yield losses (Brust and Gotoh, 2018).

In this group, *Tetranychus evansi* Baker & Pritchard (Acari: Tetranychidae), also known as tomato red spider mite, has been considered as one of the most devastating pests for tomato and other solanaceous plants in the world, mainly in Africa (Navajas et al., 2013; Azandémè-Hounmalon et al., 2015; Savi et al., 2019a; Djossou et al., 2020). This mite is considered native to South America, probably from the northeastern part of Brazil, but over the past decades, it has gradually invaded and colonized several tropical and subtropical habitats worldwide (Navajas et al., 2013). Importantly, in most invaded regions, *T. evansi* is a recurrent pest responsible for massive crop losses and significant economic damages, especially to small-scale farmers (Ferragut et al., 2013; Azandémè-Hounmalon et al., 2015; Knegt et al., 2020).

It is presently found in 44 countries in Africa, the Americas, Europe, and Asia (Migeon and Dorkeld, 2006-2019; Fan et al., 2021). The rapidly changing climate, which will result in higher average temperature and lower rainfall in large parts of the world, probably facilitates the ongoing expansion of *T. evansi* into new territories, thus putting progressively larger areas at risk (Meynard et al., 2013; Ximénez-Embún et al., 2016; Migeon and Dorkeld, 2006-2019). *Tetranychus evansi* pierces cell walls to extract their contents. This feeding activity causes chlorotic spots, reducing the

photosynthetic capacity and often leading to leaf fall (de Moraes and Flechtmann, 2008; Bensoussan et al., 2018). In the absence of effective management methods, mite-feeding activity can cause yield losses ranging from 56 to 100%, mostly in African countries (Sarr et al., 2002; Boubou et al., 2010; Azandémè-Hounmalon et al., 2015).

In general, spraying acaricides or insecticides with acaricidal properties is the major management strategy for controlling *T. evansi* (Blair, 1989; Toroitich et al., 2014; Gotoh et al., 2011; Azandémè-Hounmalon et al., 2015; Bagaram, 2016). However, the rapid development and high reproductive capacity (Qureshi et al., 1969; de Moraes and Flechtmann, 2008) allow *T. evansi* to reach high population levels, leading producers to conduct several annual pesticide applications for its control (Savi et al., 2019a). Unfortunately, this excessive usage of pesticides usually becomes untenable in the long run because it leads inevitably to the development of resistance, negatively impacting the environment as well as human and livestock health, besides increasing crop production costs (Blair, 1989; Nyoni et al., 2011; Azandémè-Hounmalon et al., 2015). Therefore, the sustainable management of this invasive mite requires the development of ecologically friendly crop protection methods that suit the needs of small farmers. Within this context, the use of acaricides should be subordinated or integrated with other control methods, such as biological control and the use of resistant genotypes. The use of selective acaricides has also gained more credibility in the last decades (Hoy, 2011; Stenberg, 2017; Stout et al., 2018; Duso et al., 2020).

In the search for such methods, the resistance of five cultivated tomatoes widely grown by smallholder farmers in western Africa (Kekefo, TOML4, Akikon, Tounvi, and TLCV15) and two wild relatives [*Solanum habrochaites* Knapp & Spooner (accessions PI 134417 and PI 134418) and *Solanum pennellii* Correll (accession LA-716)] have been evaluated in a previous study. Unfortunately, the tomato varieties grown in Africa and other commercial tomato cultivars have proved to be susceptible to *T. evansi*. In contrast, the wild tomato genotypes have been experimentally shown highly resistant to *T. evansi* (Savi et al., 2019a, b). This difference has been attributed to the presence of a greater number of glandular

trichomes and their toxic compounds (as acyl-sugars, methyl ketones, and terpenoids) on wild genotypes, but their absence of great reduction on cultivated tomatoes (Resende et al., 2008; Bleeker et al., 2012; Lucini et al., 2015). These structures proved to entangle or kill phytophagous arthropods through the sticky or toxic exudates that the trichomes produce (Kang et al., 2010; Zhang et al., 2020). They can also serve as repellent barriers to small herbivores, preventing them from feeding freely on the surface of a plant (Zhang et al., 2020). Hence, the introgression of glandular trichomes from wild tomato relatives to cultivated tomatoes, through interspecific crossings, could conceivably be one of the ways to increase the resistance degree of cultivars of interest against *T. evansi* and consequently adapted to sustainable production systems (Savi et al., 2019b). This could also help small-scale farmers who are not well resourced to purchase effective acaricides. That accounts for the first goal of this dissertation.

Biological control using natural enemies is also a major component of any integrated pest management (IPM) program (Van Driesche et al., 2008; Almarinez et al., 2020). In the case of spider mites, predatory mites of the family Phytoseiidae are the most commonly studied and important group of natural enemies considered for their control (McMurtry et al., 2013). Species of this family have been commercialized to suppress pest mite populations (McMurtry et al., 2013). Their feeding preference for phytophagous mites, short life cycles, and feasible large-scale production make them good candidates for pest control (Abad-Moyano et al., 2009). The phytoseiid mite *Phytoseiulus longipes* Evans (Acari: Phytoseiidae) found naturally in association with *T. evansi* in the extreme south of Brazil and northern Argentina proved to be the only promising predator for red spider mite control (Furtado et al., 2007; Silva et al., 2010; Ferreiro et al., 2011). This predatory mite has the potential to control other *Tetranychus* species, given its adaptation to the type I-a lifestyle of McMurtry et al. (2013).

However, the incorporation of predatory mites into IPM programs requires detailed knowledge and understanding of the interactions of these mites with other crop management practices (Fountain and Meed, 2015). The defensive traits that crops exhibit to protect themselves from damage caused by pests and disease can

also strongly influence the survival and efficacy of their predators. In the case of tomato, several studies have demonstrated that the performance of most phytoseiid mites tends to be lower compared to other crops (Koller et al., 2007; Sato et al., 2011; Davidson et al., 2016; Paspati et al., 2021). This fact has been attributed to the impact of tomato defenses mediated by the glandular trichomes and their exudates, increasing phytoseiid mortality or phytoseiid prey-searching efficacy if non-glandular and glandular trichomes are above a certain threshold (Castagnoli et al., 1999; Koller et al., 2007; Sato et al., 2011; Paspati et al., 2021). Tomato genotypes can vary highly in trichome density and such variation could influence differently the performance of phytoseiid mites. However, although tomato has been found to affect predatory mites more than other crops, studies about this subject are scarce. Thus, the effect of tomato genotypes with varying levels of susceptibility or resistance to *T. evansi* on *P. longipes* should be investigated. Such knowledge will be fundamental in the choice of tomato genotypes to optimize the use of this phytoseiid mite as a biocontrol agent in the integrated management program of *T. evansi*. Accordingly, this was the second goal of this dissertation.

In the development of Integrated Pest Management Programs, it is also important for farmers to have available pesticides minimally harmful to the natural enemies, or that the effect of these two control measures taken together is greater than the sum of their separate effect (Schmidt-Jeffris and Beers, 2020; Bilbo and Walgenbach, 2020). Several studies on non-target effects on key phytoseiid species have been conducted to address this need. However, knowledge of pesticides non-target effects on *P. longipes* is still scarce. Therefore, investigations should be conducted to determine which pesticides are most selective against this predatory mite in the tomato cropping system, as pest control practices adopted by most Western African tomato producers are dominated by the intensive use of pesticides, with no attention on their possible effect on natural enemies of *T. evansi*.

Thus, this dissertation intends to establish an integrated management system for *T. evansi*, with the acquisition of tomato genotypes resistant to *T. evansi*, a suitable genotype that could optimize the performance of predatory mite *P. longipes*, and with the definition of pesticides with lower risk to this predator. The thesis is

organized as follows. Chapter 2 explores the bottom-up effects of progeny genotypes from interspecific crossings of wild and cultivated tomatoes on the behavioral responses and demographic parameters of *T. evansi* to increase resistance degree of a cultivar of interest in the Republic of Benin (West Africa) cropping system against *T. evansi*. Chapter 3 compares *P. longipes* population performance and predation capacity on tomato genotypes with different susceptibility levels to *T. evansi*, to identify the genotype, which should be included in the envisioned IPM program to optimize *P. longipes* as a biocontrol agent. Chapter 4 evaluates the effectiveness of two bio-acaricides (azadirachtin- and oxymatrine-based formulations), which are labeled as selective for bio-control agents for controlling *T. evansi* on tomato crop, explores also their synergistic effect with the predatory mite *P. longipes* to support effective integrated management of *T. evansi*. Chapter 5 assesses the short- and long-term effects of direct exposure as well as the persistence of residual activity of ten pesticides commonly used in the Western African tomato cropping system against *P. longipes* to screen those are suitable toward conservation and/ or preservation of this predatory mite in the field. Chapter 6 presents a summary of overall empirical findings, conclusion, and suggestions resulting from this thesis.

2. Literature Review

2.1. Importance of tomato production

Tomato (*S. lycopersicum* L.) is one of the most popular horticultural commodities grown in practically every country of the world in outdoor fields, greenhouses, and screen houses (Adenuga et al., 2013). In 2019, world tomato production was estimated at more than 180.7 million tons produced on 5.3 million hectares generating $ 86.06 billion annually (FAO, 2019). Together, Asia and America account for 75% of the world's total production. China is the world´s top tomato grower with 62.86 million tons, followed by India with 19 million tons (FAO, 2019). Turkey and USA are the other major tomato producing countries with an estimated above 10 million tons (FAO, 2019)

In Africa, the total tomato production for 2019 was estimated to be about 16.125 million tons with Egypt leading the continent with 6.7518 million tons. In the Western African countries, Nigeria recorded the highest production level, with 3.8 million tons produced from a total area of about 264,000 hectares, while the estimated tomato production in the Republic of Benin stood at 274.700 tons produced on 37.648 hectares giving an average of 7.2 tons per hectare (FAO, 2019).

Tomato is an important component of the daily diet used as fresh or as processed products in preparation of different delicacies (Adenunga et al., 2013). This is because tomato is rich in vitamins, minerals, sugars, essential amino acids, iron, dietary fibers, and phosphorus (Arah et al., 2015). Moreover, it contains higher amounts of lycopene, a type of carotenoid with anti-oxidant properties (Arab and Steck, 2000) which is beneficial in reducing the incidence of some chronic diseases, like cancer and many other cardiovascular disorders (Miller et al., 2002; Arah et al., 2015). Tomato production can serve as a source of income for most rural and peri-urban producers in most developing countries (Çetin and Vadar, 2008), representing more than 51% of the total production of vegetable crops in the Republic of Benin (Sikirou et al., 2015). In that county, this crop is cultivated continuously throughout the year because apart from the most important rainy season that normally spans between April and July in the southern part of the country, and May to October in the northern part, there is a secondary rainy season between September and November, mainly in the south (Brassica, 2019). The crop is also grown in the so-called off-season period, between October to January in the north and between November to February in the south (Brasica, 2019). Despite the importance of the tomato crop, production is still insufficient to meet the national demand in most West African countries, requiring importation from neighboring countries.

2.2. Mite pests in tomato production

Tomato production is severely constrained by diseases and several insect and mite pests (Wakil et al., 2018). The mites constitute a diverse group of the subclass Acari, of the class Arachnidae, belonging to the subphylum Chelicerata of

the phylum Arthropoda. Among the arachnids, Acari is the only group containing species adapted to feeding on plants (Jeppson et al., 1975). Plant feeding mites play an important role as agricultural pests of fruit, vegetable, forage, ornamental, and other crops (Hoy et al., 2011; Tehri, 2014).

Among mite pests, Tetranychidae, also known as spider mites, standing out as the main group of plant-feeding mites, for the number of species and the number of host plants attacked by them around the world (Helle and Sabelis, 1985). About 1321 species belonging to over 70 genera are known to feed on 3917 plants (Migeon and Dorkeld, 2006-2021).

The major spider mite species in tomato production include the two-spotted spider mite, *Tetanychus urticae* Koch (Acari: Tetranychidae) and red spider mite *T. evansi* (Moraes and Flechtmann, 2008; Brust and Gotoh, 2018). The broad mite *Polyphagotarsonemus latus* (Banks) (Acari: Tarsonemidae) and the tomato russet mite *Aculops lycopersici* (Massee) (Acari: Eriophyidae), are other pest mites of great importance in tomato production (Brust and Gotoh, 2018).

2.3. *Tetranychus evansi*

2.3.1. Geographical Distribution

Tetranychus evansi was first recorded in northeastern Brazil under the name of *Tetranychus marianae* McGregor (Silva, 1954), but correct identification was described in 1960 from Mauritius (Baker and Pritchard, 1960). It was later found in several other countries, reaching more recently countries in Sub-Saharan Africa, Mediterranean area and Asia (Silva 1954; Navajas et al., 2013; Migeon and Dorkeld, 2006–2019). It has been reported from 45 countries worldwide. It has been considered that the climatic changes facilitated its invasion to new territories [see http://www.ensam.inra.fr/CBGP/spmweb/ (Migeon and Dorkeld, 2006-2021 Fan et al. 2021) for detailed coverage of its distribution].

2.3.2. Identification

Tetranychus evansi belongs to the family Tetranychidae, of the superfamily Tetranychoidae, of the suborder Prostigmata, of the order Trombidiformes, of the subclass Acari, of the class Arachnida, of the subphylum Chelicerata of the phylum Arthropoda. The *e*ggs of this species are typically round, with about 120 µm in diameter, pale orange soon after oviposition but rusty close to larval hatching. The larvae have three pairs of legs and are 150 µm in length, pale green or pink in color (Helle and Sabelis, 1985).

The protonymph and the deutonymph have four pairs of legs and are respectively about 310 and 350 µm in length. Their color ranges from orange to brick red or dark red. As in other tetranychids, sexes are dimorphic, the males being rather triangular and the females broadly oval. Males are about half as long as females, the latter about 500 µm long. The color of females ranges from light orange to deep reddish-orange or brown with an indistinct dark blotch on each side of the body, whereas males are yellow-orange-colored, with pale legs.

The shape of the structure on the palpal tarsus used for spinning silk and the shape of the male aedeagus (terminal part of a male copulatory organ) and the chaetotaxy of the legs are important characters to separate the adults from other *Tetranychus* species (Baker and Pritchard, 1960; de Moraes et al., 1987). Each empodium (structure between the two claws at the end of the tarsi) of the females ends in three pairs of filaments overlaid by a tiny mediodorsal spur. All four proximal tactile setae on female tarsus I are nearly in line with the proximal set of duplex setae. The aedeagus is upturned distally and the distal tip (head) is inverted shoe-shaped (Baker and Pritchard, 1960).

2.3.3. Bioecological aspects

The life cycle of *T. evansi* includes the same developmental stages as all other tetranychid species (egg, larva, protonymph, deutonymph and adult male and female). There is always an inactive (quiescent) phase between each active stage, referred to as protochrysalis, deutochrysalis and teliochrysalis, respectively. The life cycle of *T. evansi* has been studied by several authors (de Moraes and McMurtry, 1987; Bonato, 1999, Gotoh et al., 2010; Murungi et al., 2010; Zriki et al., 2013; Savi

et al., 2019b; Djossou et al., 2020). These authors observed that the population growth parameters of *T. evansi* such as developmental rate, survival, reproduction, and longevity vary in response to changes in temperature, relative humidity and host plant species. The development is favored by hot and dry conditions (minimum temperature 10°C; optimum temperature 34°C). *Tetranychus evansi* full life cycle ranges from 41.0 to 45.1days at 15°C to 5.5–6.5 days at 40°C, and 9.7–10.5 days at 25°C (de Moraes and McMurtry, 1987; Bonato, 1999, Gotoh et al., 2010; Murungi et al., 2010; Zriki et al., 2013; Savi et al., 2019b; Djossou et al., 2020). Males develop slightly more rapidly than females.

Reproduction can be sexual or by arrhenotoky parthenogenesis (Sabelis, 1985). When the reproduction is sexual, the mites mate, the male inserting its aedeagus into the female to deposit sperm by bending the tip of its idiosoma up. Mating lasts 30–90 seconds, with an average of 75 seconds, occurring several times along the life of the female (Qureshi et al., 1969; Navajas et al., 2013). As in other tetranychids, a male will remain over a deutonymph waiting for adult emergence, mating occurring soon after that. Unfertilized females lay haploid eggs, which produce only males; fertilized females produces haploid and diploid eggs, with a predominance of males offspring early and late in the period of oviposition. The sex ratio of progeny produced by mated females is usually three diploid females to one haploid male (Qureshi et al.,1969; Moraes et al., 1987). Total fecundity also varies with environmental conditions, reaching up to 49.71-243 at 25 ^{o}C (Moraes and McMurtry,1987; Gotoh et al., 2010; Savi et al., 2019b).

Tetranychus evansi prefers to live on the lower side of the leaves, but it can also occupy the upper side when the population is too high (de Moraes and Flechtmann, 2008; Navajas et al., 2013). The oviposition site seems to reduce the effect of high temperature, rainfall, and pesticide sprays, thus making control difficult (Helle and Sabelis, 1985). In addition, the habitat preference for the underside of leaves is difficult for the initial detection of mite infestation, thus providing an appropriate time for increasing its population (de Moraes and Flechtmann, 2008). In tropical and subtropical areas, they may remain active year-round (Navajas et al., 2013). The term spider mite highlights the ability of the tetranychid mites of the

subfamily Tetranychinae to produce a variable amount of webbing (Helle and Sabelis, 1985). *Tetranychus* species produce profuse webbing, but *T. evansi* is aoutstanding in this regard (de Moraes and McMurtry, 1987).

2.3.4. Dispersion

When the plant is damaged, resulting in an unsuitable food supply, mites tend to disperse (Kennedy and Smitley, 1985; Pralavorio et al., 1989; Kungu et al., 2020), usually aggregating on the uppermost parts of the plants (Kungu et al., 2020). Spider mites may disperse individually by walking from one plant to another, or aerially by positioning their bodies in such a way as to catch the wind (de Moraes and Flechtmann, 2008; Santos et al., 2020). Under extreme conditions (overcrowding coinciding with food depletion), individuals gather at the plant apex to form a ball made by mites and silk threads (Gerson, 1985; Kungu et al., 2020). This phenomenon is called ballooning. Once formed, the balls are not firmly attached to the apex of the plant. In the field, the wind or a passing animal would be sufficient for dispersing individuals in that ball (Kennedy and Smitley, 1985; de Moraes and Flechtmann, 2008, Hoy et al., 2011).

2.3.5. Host Plants and Economic Importance

Just like every spider mite, *T. evansi* is cell content-feeders, piercing plant parenchyma cells with their stylets, sucking up the contents, and leaving behind empty cells that are visible as white feeding scars (Bensoussan et al., 2016). Damage first appears as stipples that later result in a silvery or yellowish appearance to the leaves, thereby inducing enormous yield losses and even plant death (Savi et al., 2019b; Djossou et al., 2020). *Tetranychus evansi* feeds preferentially on solanaceous plants including cultivated crops such as *S. lycopersicum*, tobacco (*Nicotiana tabacum* L.), potato (*Solanum tuberosum* L.), and eggplant (*Solanum melongena* L.) (Navajas et al., 2013; Migeon and Dorkeld, 2006-2019). Although it has also been reported associated with 136 host plants of 36 other families (Migeon and Dorkeld, 2006–2021), high population levels have only been encountered a few

times on the Cucurbitaceae and Fabaceae (Navajas et al., 2013; Migeon and Dorkeld, 2006-2021). In Brazil, the red spider mite is not a serious pest, probably for being this its native habitat. However, this pest mite causes severe losses sometimes reaching 100% on cultivated tomatoes (Figure 1) in invaded areas mostly in African countries due to favorable climate conditions (Saunyama and Knapp, 2003; Azandémè-Hounmalon et al., 2015). It also disrupts the community composition of *T. urticae* and other indigenous spider mite species becoming the dominant species in invaded areas mostly in African countries (Ferragut et al., 2013; Azandémè-Hounmalon et al., 2015).

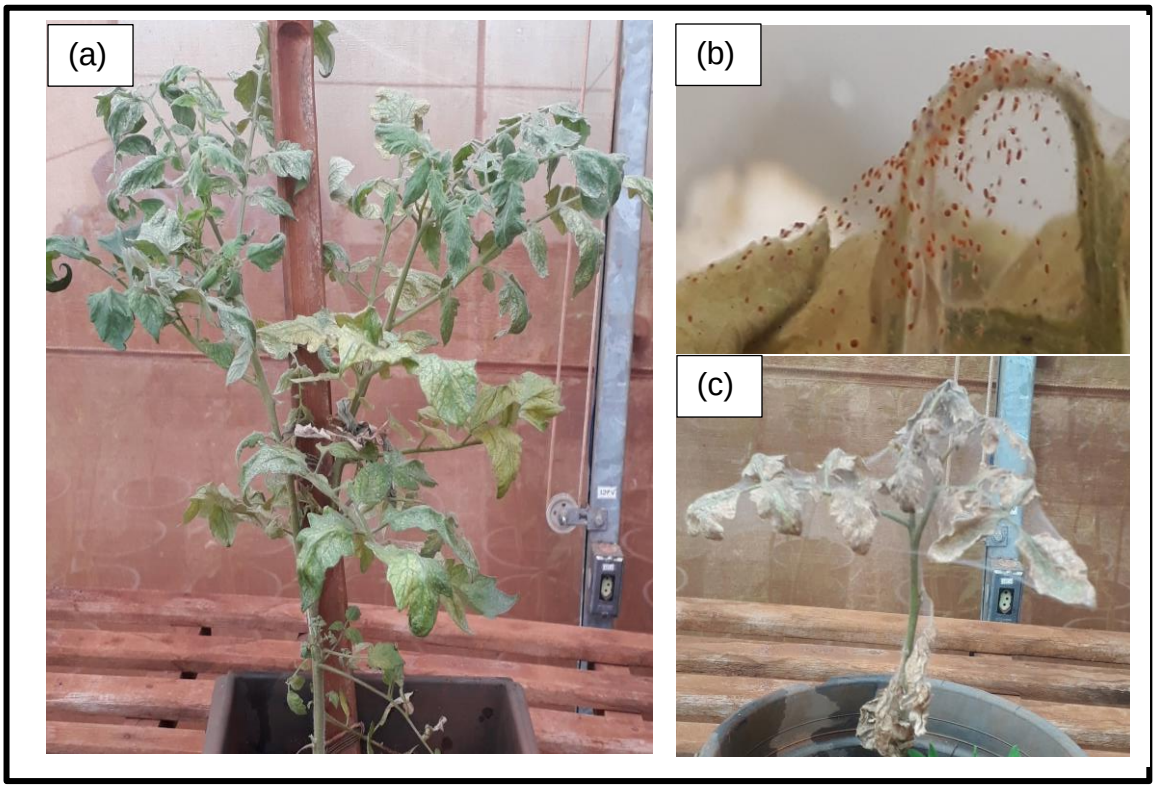

Figure 1. Symptoms (a), ballooning (b) caused by *Tetranychus evansi* on tomato plants. Death of tomato plant caused by *T. evansi* (c)

2.3.6. Management of *Tetranychus evansi*

2.3.6.1. Chemical control

Historically, chemical control is the major adopted practice in the management of *T. evansi*, as the discovery of other control strategies such as natural

enemies and plant resistance has been slow (Blair, 1989; Toroitich et al., 2014; Azandémè-Hounmalon et al., 2015; Bagaram, 2016). The older synthetic pesticides have a long history of use against *T. evansi*. For this reason, it is not surprising to find high resistance ratios of this mite to these pesticides. Early researches on effective pesticides for controlling red spider mite in Zimbabwe were conducted in the 1990s with 57 pesticides from a range of chemical groups under laboratory conditions (Blair, 1989). In that study, *T. evansi* was found to be tolerant to some organophosphates, such as thiophosphate. However, chemicals such as binapacryl, cyhexatin, and dicofol, evaluated in that study are no longer used in some countries because of their toxicity to humans and the environment.

Similarly, the survey conducted by Azandémè-Hounmalon et al. (2015) in the Republic of Benin revealed that older broad-spectrum insecticides such as organophosphates and pyrethroids used singly or in tandem by farmers in vegetable fields have not proved to be effective for controlling *T. evansi*. In Kenya, Toriotich et al. (2014) also indicated that the organophospate dimethoate should not be recommended in the management of *T. evansi*, but instead, suggesting that specific acaricides, such as propargite and abamectin, could produce high reductions in populations of *T. evansi* when applied with adequate application methods. Toroitich (2006) evaluated the effect of bifenthrin, lambda-cyhalothrin, dimethoate and profenofos + cypermethrin against *T. evansi* in the laboratory, but only bifenthrin and profenofos + cypermethrin proved to be effective.

Gotoh et al. (2011) examined the efficacy of acaricides recently developed in the management of *T. evansi* strains from Brazil, France, Kenya, Spain, Canary Island, Taiwan and Japan (Kagoshima, Osaka, and Tokyo). From 11 tested acaricides, bifenazate, cyenopyrafen, milbemectin, spirodiclofen and tebufenpyrad proved to be highly toxic to this mite. The authors suggested that these new products could be incorporated into acaricide rotations taking into consideration their modes of action. Four strains used by Nyoni et al. (2011) were collected from Malawi and France. The LC_{50} values of four chemicals to adult females were variable. Only abamectin was considered to be effective to all four strains tested (Nyoni et al., 2011). For bifenthrin, the LC_{50} values exceeded the recommended concentration in

all four strains tested, and the LC_{50} values of two Malawian strains (1858–3560mg/L) were 20 to 39-fold higher than those of the two French strains (92.0–134.6mg/L). For chlorpyriphos and fenpyroximate, LC_{50} values were similar among the four strains (Nyoni et al., 2011).

Some plant-based products such as neem (from *Azadirachta indica* A. Jussie) have been evaluated in the management of spider mites, including *T. evansi*. Soto et al. (2010) determined mortality of *T. evansi* females on tomato above 95% with Natuneem Agrícola® (Natural Rural, Araraquara, São Paulo, Brazil) and Organic Neem® (Dalquim Indústria e Comércio Ltda, Itajai, Santa Catarina, Brazil) (39.1 and 30.4 mg a.i. L^{-1}). In contrast, Santos et al. (2017) observed lower efficacy (5-15% mortality) of still other azadirachtin formulations (Organic® and Pironim®, Agroterra Insumos Agrotecnologia, São José do Rio Preto, São Paulo, Brazil) at concentrations of 2, 4, 6, 8, and 10% against *T. evansi* females.

The use of acaricide-treated nets, as traps to control phytophagous mites has been evaluated (Pralavorio et al., 1989; Martin et al., 2010). This technique has been used for controlling the broad mite, *Polyphagotarsonemus latus* (Banks), given its preference to the top leaves of plants, but it was hypothesized that it could also be used for the control of *T. evansi*, as this mite collectively migrates upwards towards the plant apex due to population pressure (Azandémè-Hounmalon et al. 2014; Kungu et al., 2020). While preventing the presence of acaricide residues on the plant and the environmental contamination, this technique would also lower mite population by preventing inter-plant dispersal by walking or aerially (Kungu et al., 2020).

2.3.6.2. Host plant resistance control

The concerns about the development of resistance of *T. evansi* to synthetic acaricides, the limited availability and high costs of effective acaricides, and worker safety issues have motivated the identification of novel, appropriate replacements for plant protection with fewer adverse impacts (Savi et al., 2019a, b). The cultivation of arthropod-resistant plants has been proposed as one of the main alternatives to

broad-spectrum acaricide use in pest control. The economic advantages that arthropod-resistant cultivars offer are genetically incorporated arthropod control for the cost of the seed alone (Smith, 2005).

Antixenosis (non-preference), antibiosis, and tolerance have been reported to be the three resistance categories through which plants defend themselves against attacks by herbivores, including pest mites (Maluf et al., 2010; Lucini et al., 2015; Savi et al., 2019b). Antixenosis refers to characteristics of the plant that prevent or reduce colonization by the pest. In other words, antixenosis cause adverse effects on mite behavior (turning the prospective host plant unattractive for feeding and reproduction). Antibiosis refers to characteristics of the plant that negatively affect its life cycle (development, reproduction, and survivorship of the pest), considered to be the most important category for mite management (Maluf et al., 2010; Lucini et al., 2015; Savi et al., 2019a,b). Tolerance refers to characteristics of the plant that enable them to withstand or recover from herbivore damage (Smith, 2005).

In the case of *T. evansi*, researchers have focused mainly on the two former resistance categories. Results obtained by these researchers showed that cultivated tomato (*S. lycopersicum*) genotypes are usually susceptible to *T. evansi* (Silva et al., 1992; Resende et al., 2008; Murungi et al., 2009; Onyambus et al., 2012; Musa et al., 2016; Savi et al., 2019; Djossou et al., 2020). Most plant resistance traits in relation to spider mites have been found in accessions of wild tomato relatives, particularly *S. habrochaites* Knapp and Spooner, *S. pennellii* Correll, *S. cheesmaniae* (L. Riley) Fosberg, and *S. galapagense* S. C. Darwin and Peralta (Silva et al., 1992; Resende et al., 2008; Murungi, et al., 2009; Onyambus et al., 2011; Lucini et al., 2015, Rakha et al., 2017; Savi et al., 2019a,b).

Leaf trichomes and the compounds produced by them have been considered to be the most important factor associated with the resistance mechanism of tomato genotypes (Simmons and Gurr, 2005; Rezende et al. 2008; Maluf et al., 2010; Rakha et al., 2017). Seven types of trichomes have been identified on tomato plants, grouped as glandular (types I, IV, VI, and VII) and non-glandular (types II, III, and V) (Luckwill, 1943; Simmons and Gurr, 2005). The glands of type I, IV, and VI on tomato

leaves play key anti-herbivory roles via chemical secretions (Kang et al., 2010; Zhang et al., 2020). They can also serve as repellent barriers to small herbivores and prevent them from feeding freely on the surface of a plant due to the high viscosity of allelochemical secretions (Simmons and Gurr, 2005; Zhang et al., 2020). Non-glandular trichomes may act as physical barriers, hampering micro-arthropods movement on the leaf surface when large and present in high densities (Baur et al., 1991; Aragão et al., 2000; Simmons and Gurr, 2005).

Leaves of cultivated tomatoes tend to have copious non-glandular whereas leaves of wild tomato accessions have usually abundant glandular trichomes (types I, IV, and VI) (Zhang et al., 2020). Three main chemical classes that have been identified in association with glandular trichomes are methyl-ketones (notably 2-tridecanone; Gonlçalves et al., 1998), sesquiterpenes (notably zingiberene; De Azevedo et al., 2003; Bleeker et al., 2012), and acyl sugars (Resende et al., 2008; Lucini et al., 2015). The former two are abundant in the wild tomato *S. habrochaites* and found in high concentrations in the glandular trichomes of types IV and VI, whereas the latter is present in high concentrations in type IV glandular trichomes of *S. pennellii*, *S. cheesmaniae,* and *S. galapagense* (Rahka et al., 2017).

The high defensive traits mediated by glandular trichomes and toxic compounds found on wild tomato relatives have been used to increase the level of resistance against mite pests, including *T. evansi*, in several cultivated tomatoes, through interspecific crosses (Rezende et al. 2008; Lucini et al., 2015; Maciel et al., 2018). The resistance level revealed by most of these interspecific hybrids was demonstrated to be generally comparable to that of wild tomato relatives (Resende et al. 2008; Alba et al., 2008; Lucini et al., 2015; Maciel et al., 2018; de Oliveira et al., 2018; AL-Bayati, 2019). This can constitute an important avenue to be further explored in an attempt to mitigate the use of synthetic pesticides.

2.3.6.3. Biological control

Biological control can be considered a powerful tool and one of the most important measures of pest control, providing environmentally safe and sustainable plant protection (Van Driesche et al., 2008; Almarinez et al., 2020). Arthropod

biocontrol agents and microbial pathogens have been successfully used in agricultural systems for many years (Van Driesche et al., 2008; Wang et al., 2014).

2.3.6.3.1. Arthropod biocontrol agents

Predatory mites of the family Phytoseiidae are important biocontrol agents of a variety of crop pests and their presence can negate the need for application of other control methods (McMurty et al., 2013; Fountain and Medd, 2015). The most common targets of predatory mite treatments are pest mites of the families Tetranychidae, Tarsonemidae, and Eriophyiidae (Gerson and Weintraub 2007; McMurty et al., 2013). However, predatory mites are also associated with the suppression of other small arthropod populations, including thrips (Thysanoptera: Thripidae) and whiteflies (Hemiptera: Aleyrodidae) (McMurty et al., 2013).

According to Demite et al. (2021), Phytoseiidae contained 2,522 described valid species, distributed in 91 genera and three subfamilies, Amblyseiinae, Phytoseiinae, and Typhlodrominae. Among the phytoseiids, *Phytoseiulus persimilis* Athias-Henriot and *Neoseiulus californicus* McGregor (Acari: Phytoseiidae) are some of the species that have been most extensively used for the protection of many crops against tetranychid pests worldwide (McMurty et al., 2013). These species proved to be particularly effective for controlling *T. urticae* (Gerson et al., 2003). However, attempts to control *T. evansi* using those phytoseiids have been unsuccessful (de Moraes and McMurtry, 1986; Escudero and Ferragut, 2005, Koller et al., 2007). The authors have observed that none of the tested predators could feed and grow properly on *T. evansi*. Moraes and McMurtry (1986) suggested that *T. evansi* contained a depressant, which hampered phytoseiids from consuming their eggs.

The fact that *T. evansi* has become a serious threat to Solanaceae crops in Africa and Europe, expanding its invasion in the 2000s (Migeon et al., 2009; Migeon and Dorkeld, 2019) has given new impetus to the search for effective natural enemies of this mite. Over that prospection, South America, mostly Brazil and Argentine were prioritized for being close to the region of origin of that pest (Rosa et

al., 2005; Fiaboe et al., 2007a,b; Furtado et al., 2006, 2007; Navajas et al., 2013). Predaceous mites and insect species most frequently found in association with *T. evansi* in these prospections were the following: Phytoseiidae: *Euseius concordis* (Chant) (Acari: Phytoseiidae), *N. californicus*, *Phytoseiulus fragariae* Denmark & Schicha (Acari: Phytoseiidae) and *P. longipes*; Insecta, Coccinellidae: the ladybird beetle *Stethorus tridens* Gordon(Coleoptera: Coccinelidae) (Rosa et al., 2005; Furtado et al., 2006, 2007a, b; Fiaboe et al. 2007a,b; Brito et al. 2008; da Silva et al., 2008). Upon further evaluation, only the predatory mite *P. longipes* (Figure 2) has been shown to be more promising for *T. evansi* control (Furtado et al., 2007).

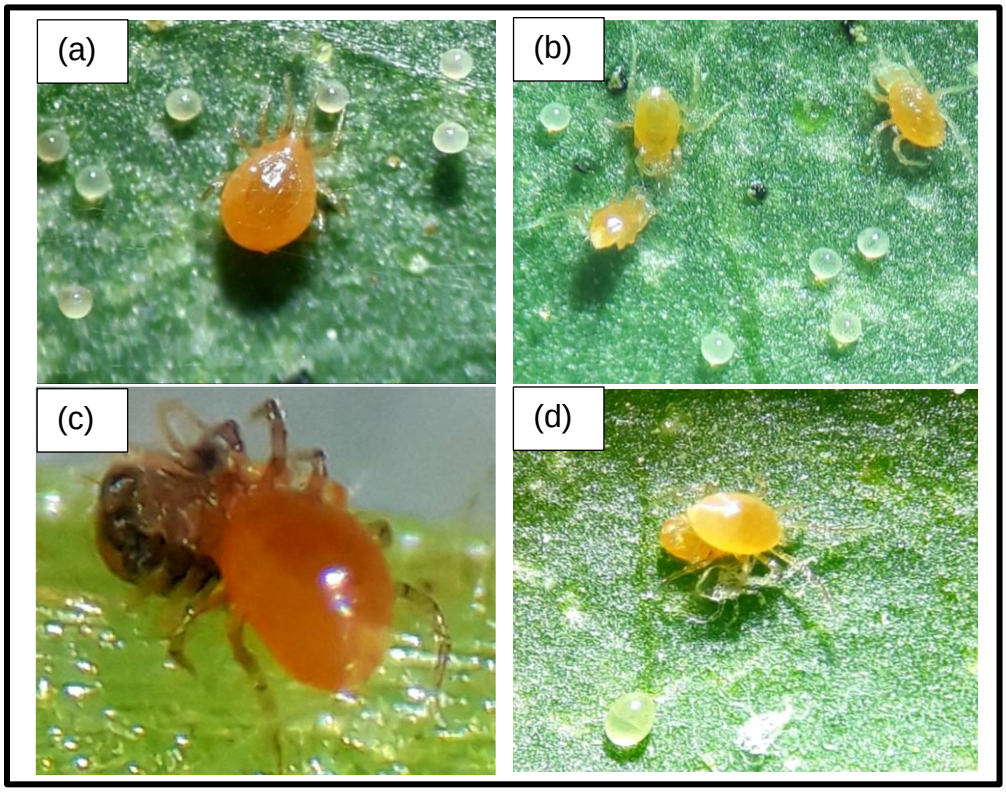

Figure 2. Female (a), nymphs (b) of *Phytoseiulus longipes* feeding on *Tetranychus evansi* eggs. (c) P. longipes female preying on *T. evansi* female. (d) Mating between male and female of *P. longipes* (Photos: Savi, P.J. & de Matos, S.T.S.)

2.3.6.3.2. Entomopathogenic fungi

Entomopathogenic fungi are also known to cause epizootics in populations of mites (der van Geest et al., 2000). In the case of *T. evansi*, *Neozygites floridana* (Weiser and Muma) Remaudiére and Keller (Entomophthorales: Neozygitaceae)] a pathogen of several spider mites species, has been observed associated with *T. evansi* (Humber et al., 1981). Isolates of *Metarhizium anisopliae* (Metschnikoff) Sorokin (Hypocreales: Clavicipitaceae) and *Beauveria bassiana* (Balsamo) (Hypocreales: Cordycipitaceae) have been tested against *T. evansi* and shown to be highly virulent, suggesting a potential for their use in the management of this pest (Wekesa et al. 2005; Bugeme et al., 2008; Maniania et al., 2008). The fungus *N. floridana* extensively found in Brazil infecting *T. evansi* has shown to be compatible with the phytoseiid mite *P. longipes* (Wekesa et al., 2007). However, Maniania et al.(2016) reported that there was no benefit in combining *M. anisopliae* and *P. longipes* for the control of *T. evansi* in tomato. Omukoko et al. (2020) reported under screen-house conditions that *B. bassiana* could colonize and persist on tomato varieties for 6 weeks and reduce adult *T. evansi* populations.

2.3.6.4. Cultural practices

Cultural control is a key first step in preventing initial infestations and the spread of *T. evansi*. Plants should be routinely examined for any evidence of infestation and all infested materials should be disposed of carefully (Wakil et al., 2018). Some weeds are better at sustaining mite pests than others and these weeds, such as plantains, black nightshade, or solanaceous weeds should be targeted (Brust and Gotoh, 2018). Once harvest is complete, crop residues should be destroyed thus removing a breeding ground for the mites. Pruning back the affected plants and removing infested leaves will reduce pest numbers (Brust and Gotoh, 2018).

Saunyama and Knapp (2003) pointed out that the pruning and trellising of tomato plants make the chemical control more effective and allow getting in better red spider mite management offering a positive effect on yields and quality of tomato fruits in Zimbabwe. Cantore et al. (2016) reported that tomato plants have high

sensitivity to water deficit. Therefore, water shortage caused by drought periods can lead to hydric stress, which can result in outbreaks of spider mites and considerable yield reduction (Cantore et al., 2016; Ximénez-Embún et al., 2016, 2018). For this reason, demand in the management of water application of tomato crops in dry regions or dry seasons should be stricter. Sprinkler irrigation has been used as an important practice to reduce the mite populations through mechanical actions that dislodge the mite, disrupting its life cycle (Chandler et al., 1979; Opit et al., 2001; Atakan et al., 2021

Alizade et al. (2016) reported that spider mites feeding on plants grown with low nitrogen inputs had reduced survival rates and delayed development. Thus, proper N content in fertilization input could avoid *T. evansi* population increase (Alizade et al., 2016; Brust and Gotoh, 2018). Although cultural practices have shown noticeable impact on mite population, these practices should be subordinated to other control strategies for more effective pest control.

2.3.7. Integrated pest management

Integrated pest management (IPM) is critical for effective and economically viable use (Duso et al., 2020). This approach involves the integration of multiple control tactics (cultural, biological, pesticide selective, and host plant resistance) to favor long-term stability of a cropping system, minimizing the disadvantages (mainly causing risk to human and environment) of chemical control programs (Kogan, 1998; Jonsson et al., 2008; Fathipour and Sedaratian, 2013). In such an approach, three types of interactions can occur between different control measures, namely additive, synergistic and antagonistic (Fathipour and Sedaratian, 2013). In additive interaction, the combined effect of two control measures is equal to the sum of the effect of the two measures applied separately (Fathipour and Sedaratian, 2013). In synergistic interaction, the resulting effect is greater than the sum of the separate effects. Finally, in antagonistic interaction the resulting effect is lower than the sum of the effects of measures applied independently of each other (Fathipour and Sedaratian, 2013). For this reason, it is essential to understand the compatibility

between different control tactics intended to be integrated for the control of a target pest in any crop, to optimize IPM planning.

2.3.7.1. Combination of biological control and pest mites- resistant plants

The defensive traits that help plants to protect themselves from damage caused by pests can vary qualitatively and quantitatively. Such variation may also affect positively or negatively other organisms, as predatory mites. Some studies have suggested gains in the concurrent use of resistant plants and phytoseiid mites in optimization of IPM (Bottrell et al., 1998; Khanamani et al., 2014; 2015; Fathipour et al., 2019). Fathipour et al. (2019) studied three cucumbers cultivars for their effects on the life table, and predation parameters of the phytoseiid *P. persimilis*. Their results suggested that the resistant cultivar supported more *P. persimilis* than other cultivars. Khanamani et al. (2015) found that a eggplant cultivar resistant to *T. urticae* concurs for better performance of the phytoseiid mite *Typhlodromus bagdasarjani* Wainstein & Arutunjan than susceptible cultivars.

However, several cases of negative effects between resistance traits and biological agents have been also mentioned in the literature (Heinz and Zalom, 1996; Drukker et al., 1997; Koller et al., 2007; Sato et al., 2011; Bottega et al., 2017; Han et al., 2019; Paspati et al., 2021). This seems to be the case with tomato crops in relation to the performance of some phytoseiid mites when compared to other crops. The defensive traits found in tomato crops and mediated by trichomes and toxic compounds are usually more deleterious to natural enemies than in other crops. For instance, the survival of the generalist predatory mite *Amblyseius swirskii* Athias-Henriot juveniles on tomato leaves was not different from that on sweet pepper, but adult survival was significantly lower on whole tomato plants (Paspati et al. 2021). *Amblyseius swirskii* walked slower on plant species with increasing trichome density and on tomato leaves, their walking speed was lower when compared to rose plants (Buitenhous et al., 2014). *Amblydromalus limonicus* Garman & McGregor preyed fewer *Bactericera cockerelli* (Sulc) (Hemiptera: Triozidae) psyllid nymphs per day on tomato than on sweet pepper, but the mite survival was similar on the leaves of both

plants (Davidson et al., 2016). The developmental time and sex ratio of *N. californicus* on *T. urticae* were similar on tomato and strawberry, but immature survival rate and oviposition were lower on tomatoes (Castagnoli et al., 1999). The oviposition rate of *N. californicus* was negatively affected on tomato leaves, both directly and indirectly through the prey *T. evansi*, when compared to bean leaves (Koller et al., 2007). Walking activity, predation, and oviposition rates of *Phytoseiulus macropilis* Banks and *P. longipes* fed with *T. uticae* were reduced on tomato leaves, when compared to strawberry (Sato et al., 2011). Although tomato crops have been shown in several studies to affect predatory mites, including *P. longipes*, more than other crops, studies that compare the performance of phytoseiid among different tomato genotypes are still scarce. Such knowledge is essential to choose the tomato genotype that may optimize the use of determined phytoseiid mites as a bio-control agents in IPM implementation.

2.3.7.2. Combination of Biological control and chemical control

Despite being effective in pest control, biological control is often not a stand-alone solution, requiring its incorporation into a larger pest management context, that frequently includes the application of chemical pesticides (Foutain and Medd, 2015; Bilbo and Walgenbach, 2020). For example, Bilbo and Walgenbach (2020) reported in staked tomatoes that the separate use of *P. persimilis* or the acaricide acramite labeled as selective did not reduce *T. urticae* pressure below the control, but the combination of both provided the most effective treatment in that regard.

Given that the management of non-target pests in the tomato cropping systems is dominated by the use of synthetic pesticides, which can range from broad-spectrum to selective (Nyoni et al., 2001; Gotoh et al., 2010; Azandémè-Houmalon et al., 2015), the combination of predatory mites with chemical control requires an in-depth knowledge of potential risks to the predatory mites. Thus, risk assessment should take into account both acute toxicity and sublethal effects on biological (immature-stage development, fecundity, fertility, longevity, and sex ratio) and behavioral (predation rate, mobility, orientation, and feeding activity) parameters

of the biological-control agents (Desneux et al., 2007; Guedes et al., 2016; Zanardi et al., 2017; Duso et al., 2020). Additionally, pesticides can act through multiple routes of exposure (direct or residual contact, food ingestion), and this should be considered in these studies. Applications of these concepts are essential to recognize among the pesticides used in agroecosystems those which could be safely combined with the predatory mite, allowing its conservation.

Several studies also reported that the compatibility of phytoseiid mites with chemical control could substantially vary between phytoseiid species and depending upon pesticide group (Fountain and Medd, 2015; Bergeron and Schmidt-Jeffris, 2020). For instance, bifenazate, labeled as selective for predatory mites, is minimally harmful to *P. persimilis* but can cause significant mortality of *Neoseiulus fallacis* (Garman) (Acari: Phytoseiidae), *N*. *californicus* and *Amblydromella caudiglans* (Schuster) (Schmidt-Jeffris and Beers, 2015, Schmidt-Jeffris et al., 2015, Bergeron and Schmidt-Jeffris, 2020). Similarly, hexythiazox, which is labeled as broad-spectrum acaricide proved to be more tolerated by *N. californicus* than by *P. persimilis* to the tested pesticides (Alzoubi and Çobanoglu, 2008). Chlorpyrifos, another broad-spectrum product showed no negative effect on the specialist phytoseiid *Galendromus occidentalis* Nesbitt, but lowered the population of the generalist phytoseiids *Amblyseius andersoni* Chant, *Galendromus flumenis* (Chant), *Metaseiulus citri* (Garman and McGregor), *M. pomi* (Parrott et al.), *Typhlodromus caudiglans* Schuster and *T. pyri* Scheuten] (Prischmann et al., 2005).

2.4. References

Abad-Moyano R, Pina T, Ferragut F, Urbaneja A (2009) Comparative life-history traits of three phytoseiid mites associated with *Tetranychus urticae* (Acari: Tetranychidae) colonies in clementine orchards in eastern Spain: implications for biological control. **Experimental and Applied Acarology** 47:121–132.

Abera G, Ibrahim AM, Forsido SF, Kuyu CG (2020) Assessment on post-harvest losses of tomato (*Lycopersicon esculentem* Mill.) in selected districts of East Shewa Zone of Ethiopia using a commodity system analysis methodology. **Heliyon**

6:e03749.

Adenuga AH, Muhammad-Lawal A, Rotimi OA, Adenuga AH, Muhammad-Lawal A, Rotimi OA (2013) Economics and technical efficiency of dry season tomato production in selected areas in Kwara state, Nigeria. **AgEcon Search** 5:11-19

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

AL-Bayati AS (2019) **Breeding for Tomato Resistance to Spider Mite *Tetranychus urticae* Koch (Acari: Tetranychidae)**. University of Kentucky Libraries

Alizade M, Hosseini M, Modarres awal M, Goldani M, Hosseini A (2016) Effects of nitrogen fertilization on population growth of two-spotted spider mite. **Systematic & Applied Acarology** 21:947-956.

Almarinez BJM, Barrion AT, Navasero MV, Navasero MM, Cayabyab BF, Carandang JSR, Legaspi JC, Watanabe K, Amalin DM (2020) Biological control: a major component of the pest management program for the invasive coconut scale insect, *Aspidiotus rigidus* Reyne, in the Philippines. **Insects** 11:745.

Alzoubi S, Cobanoglu S (2008) Toxicity of some pesticides against *Tetranychus urticae* and its predatory mites under laboratory conditions. **American-Eurasian Journal of Agricultural and Environmental** 3: 30-37.

Arab L, Steck S (2000) Lycopene and cardiovascular disease. The American **Journal of Clinical Nutrition** 71:1691S-1695S.

Arah IK, Kumah EK, Anku EK, Amaglo H (2015) An overview of post-harvest losses in tomato production in Africa: causes and possible prevention strategies. **Journal of Biology, Agriculture and Healthcare** 5: 78–88.

Atakan E, Sarıdaş MA, Pehlivan S, Achiri TD, Çeliktopuz E, Kapur B (2021) Influence of irrigation regimes on yield, pomological parameters and population development of *Tetranychus cinnabarinus* Boisd. (Acari: Tetranychidae) in

strawberry. **Systematic & Applied Acarology** 26: 1241-1253.

Azandémè-Hounmalon GY, Fellous S, Kreiter S, Fiaboe KKM, Subramanian S, Kungu M, Martin T (2014) Dispersal behavior of *Tetranychus evansi* and *T. urticae* on tomato at several spatial scales and densities: implications for integrated pest management. **PLoS ONE** 9:e95071.

Azandémè-Hounmalon GY, Affognon HD, Komlan FA, Tamò M, Fiaboe KKM, Kreiter S, Martin T (2015) Farmers' control practices against the invasive red spider mite, *Tetranychus evansi* Baker & Pritchard in Benin. **Crop Protection** 76:53–58.

Bagarama F (2016) Why is the red spider mite (*Tetranychus evansi*) a threat to dry season tomato growing in Tabora, Tanzania. Huria: **Journal of the Open University of Tanzania** 22: 1-10.

Baker EW, Pritchard AE (1960) The tetranychoid mites of Africa. **Hilgardia** 29:455–574.

Bensoussan N, Zhurov V, Yamakawa S, O'Neil CH, Suzuki T, Grbić M, Grbić V (2018) The digestive system of the two-spotted spider mite, *Tetranychus urticae* Koch, in the context of the mite-plant interaction. **Frontiers in Plant Science** 9:1206.

Bergeron PE, Schmidt-Jeffris RA (2020) Not all predators are equal: miticide non-target effects and differential selectivity. **Pest Management Science** 76:2170–2179.

Bilbo TR, Walgenbach JF (2020) Compatibility of Bifenazate and *Phytoseiulus persimilis* for management of twospotted spider mites in North Carolina staked tomatoes. **Journal of Economic Entomology** 113:2096–2103.

Blair BW (1989) Laboratory screening of acaricides against *Tetranychus evansi* Baker & Pritchard. **Crop Protection** 8:212–216.

Bleeker PM, Mirabella R, Diergaarde PJ, VanDoorn A, Tissier A, Kant MR, Prins M, de Vos M, Haring MA, Schuurink RC (2012) Improved herbivore resistance in cultivated tomato with the sesquiterpene biosynthetic pathway from a wild relative. **Proceedings of the National Academy of Sciences** 109:20124–20129.

Bonato O (1999) The effect of temperature on life history parameters of *Tetranychus evansi* (Acari: Tetranychidae). **Experimental & Applied Acarology** 23:11–19.

Bottega DB, Souza BHS de, Rodrigues NEL, Eduardo WI, Barbosa JC, Boiça Júnior AL (2017) Resistant and susceptible tomato genotypes have direct and indirect effects on *Podisus nigrispinus* preying on Tuta absoluta larvae. **Biological Control** 106:27–34.

Bottrell DG, Barbosa P, Gould F (1998) Manipulating natural enemies by plant variety selection and modification: a realistic strategy? **Annual Review of Entomology** 43:347–367.

Boubou A, Migeon A, Roderick GK, Navajas M (2011) Recent emergence and worldwide spread of the red tomato spider mite, *Tetranychus evansi*: genetic variation and multiple cryptic invasions: Recent emergence and multiple cryptic invasions of *Tetranychus evansi*. **Biological Invasions** 13:81–92.

Brassica (2019) Tomatoes in Benin**.** In: **World Vegetable Center**. https://avrdc.org/tomatoes-in-benin/. Accessed 28 Dec 2021

Britto EPJ, Gondim MGC, Torres JB, Fiaboe KKM, Moraes GJ, Knapp M (2009) Predation and reproductive output of the ladybird beetle *Stethorus tridens* preying on tomato red spider mite *Tetranychus evansi*. **BioControl** 54:363–368.

Brust GE, Gotoh T (2018) Mites. In: **Sustainable Management of Arthropod Pests of Tomato**. Elsevier, pp 111–130

Bugeme DM, Maniania NK, Knapp M, Boga HI (2009) Effect of temperature on virulence of *Beauveria bassiana* and *Metarhizium anisopliae* isolates to *Tetranychus evansi*. In: Bruin J, van der Geest LPS (eds) **Diseases of Mites and Ticks**. Springer Netherlands, Dordrecht, pp 275–285

Buitenhuis R, Shipp L, Scott-Dupree C, Brommit A, Lee W (2014) Host plant effects on the behaviour and performance of *Amblyseius swirskii* (Acari: Phytoseiidae). **Experimental & Applied Acarology** 62:171–180.

Cantore V, Lechkar O, Karabulut E, Sellami MH, Albrizio R, Boari F, Stellacci AM, Todorovic M (2016) Combined effect of deficit irrigation and strobilurin application on yield, fruit quality and water use efficiency of “cherry” tomato (*Solanum lycopersicum* L.). **Agricultural Water Management** 167:53–61.

Castagnoli M, Liguori M, Simoni S (1999) Effect of two different host plants on biological features of *Neoseiulus californicus* (Mcgregor). **International Journal of Acarology** 25:145–150.

Çetin B, Vardar A (2008) An economic analysis of energy requirements and input costs for tomato production in Turkey. **Renewable Energy** 33:428–433.

Chandler LD, Archer TL, Ward CR, Lyle WM (1979) Influences of irrigation practices on spider mite densities on field corn. **Environmental Entomology** 8:196–201.

da Silva CAD, Lourenção AL, Moraes GJ de (1992) Resistência de tomateiros ao ácaro vermelho *Tetranychus evansi* Baker & Pritchard (Acari: Tetranychidae). **Anais da Sociedade Entomológica do Brasil** 21:147–156.

da Silva FR, de Moraes GJ, Knapp M (2008) Distribution of *Tetranychus evansi* and its predator *Phytoseiulus longipes* (Acari: Tetranychidae, Phytoseiidae) in southern Brazil. **Experimental & Applied Acarology** 45:137–145.

Davidson MM, Nielsen M-C, Butler RC, Silberbauer RB (2016) Prey consumption and survival of the predatory mite, *Amblydromalus limonicus* , on different prey and host plants. **Biocontrol Science and Technology** 26:722–726.

de Azevedo SM, Ventura Faria M, Maluf WR, Barneche de Oliveira AC, de Freitas JA (2003) Zingiberene-mediated resistance to the South American tomato pinworm derived from *Lycopersicon hirsutum* var. *hirsutum*. **Euphytica** 134:347–351.

de Moraes GJ, McMurtry JA (1986) Suitability of the spider mite *Tetranychus evansi* as prey for *Phytoseiulus persimilis.* **Entomologia Experimentalis et Applicata** 40:109–115.

de Moraes GJ, McMurtry JA (1987) Effect of temperature and sperm supply on the reproductive potential of *Tetranychus evansi* (Acari: Tetranychidae). **Experimental & Applied Acarology** 3:95–107.

de Moraes GJ , McMURTRY JA, Baker EW (1987) Redescription and distribution of the spider mites *Tetranychus evansi* and *T. marianae*. **Acarologia** 28:333–343

de Moraes GJ, Flechtmann CHW (2008) **Manual de acarologia: acarologia básica e ácaros de plantas cultivadas no Brasi**l. Ribeirão Preto: Holos.

de Oliveira JRF, de Resende JTV, Maluf WR, Lucini T, de Lima Filho RB, de Lima IP, Nardi C (2018) Trichomes and allelochemicals in tomato genotypes have antagonistic effects upon behavior and biology of *Tetranychus urticae*. **Frontiers in Plant Science** 9:1132.

Demite PR, de Moraes G, McMurtry JA, Denmark HA, Castilho RC (2022) Phytoseiidae Database. Available from: www.lea.esalq.usp.br/phytoseiidae (accessed 06/01/2022)

der van Geest LPS (2000) Diseases of mites. **Experimental and Applied Acarology** 24:497–560.

Desneux N, Decourtye A, Delpuech J-M (2007) The sublethal effects of pesticides on beneficial arthropods. **Annual Review of Entomology** 52:81–106.

Djossou R, Azandémè-Hounmalon GY, Onzo A, Gnanvossou D, Assogba-Komlan F, Tamò M (2021) Susceptibility of ten tomato cultivars to attack by *Tetranychus evansi* Baker & Pritchard (Acari: Tetranychidae) under laboratory conditions. **International Journal of Tropical Insect Science** 41:425–431.

Drukker B, Bruin J, Jacobs G, Kroon A, Sabelis MW (2000) How predatory mites learn to cope with variability in volatile plant signals in the environment of their herbivorous prey. **Experimental & Applied Acarology** 24:881–895.

Duso C, Van Leeuwen T, Pozzebon A (2020) Improving the compatibility of pesticides and predatory mites: recent findings on physiological and ecological selectivity. **Current Opinion in Insect Science** 39:63–68.

Escudero LA, Ferragut F (2005) Life-history of predatory mites *Neoseiulus californicus* and *Phytoseiulus persimilis* (Acari: Phytoseiidae) on four spider mite species as prey, with special reference to *Tetranychus evansi* (Acari:

Tetranychidae). **Biological Control** 32:378–384.

Fan Q-H, Li D, Bennett S, Balan RK (2021) Diagnosis of a new to New Zealand spider mite, *Tetranychus evansi* Baker and Pritchard, 1960 (Acari: Tetranychidae). **New Zealand Entomologist** 44:59–70.

FAOSTAT (2019): Tomato production data in 2019. FAO, Rome, Italy. http://www.fao.org/faostat/ en/#home

Fathipour Y, Maleknia B, Bagheri A, Soufbaf M, Zalucki MP (2019) Spider mite host plant resistance traits improve the predatory performance of *Phytoseiulus persimilis* on cucumber, despite negative life history impacts. **Biological Control** 138:104064.

Fathipour Y, Sedaratian A (2013) **Integrated Management of *Helicoverpa armigera* in Soybean Cropping Systems**. IntechOpen

Ferragut F, Garzón-Luque E, Pekas A (2013) The invasive spider mite *Tetranychus evansi* (Acari: Tetranychidae) alters community composition and host-plant use of native relatives. **Experimental & Applied Acarology** 60:321–341.

Ferrero M, Calvo FJ, Atuahiva T, Tixier M-S, Kreiter S (2011) Biological control of *Tetranychus evansi* Baker & Pritchard and *Tetranychus urticae* Koch by *Phytoseiulus longipes* Evans in tomato greenhouses in Spain [Acari: Tetranychidae, Phytoseiidae]. **Biological Control** 58:30–35.

Fiaboe KKM, Gondim Jr MGC, De Moraes GJ, Ogol CKPO, Knapp M (2007a) Surveys for natural enemies of the tomato red spider mite *Tetranychus evansi* (Acari: Tetranychidae) in northeastern and southeastern Brazil. **Zootaxa** 1395:33–58.

Fiaboe KKM, Gondim MGC, de Moraes GJ, Ogol CKPO, Knapp M (2007b) Bionomics of the acarophagous ladybird beetle *Stethorus tridens* fed *Tetranychus evansi*. **Journal of Applied Entomology** 131:355–361.

Flechtmann CH, Knihinicki DK (2002) New species and new record of *Tetranychus* Dufour from Australia, with a key to the major groups in this genus based on females (Acari: Prostigmata: Tetranychidae). **Australian journal of entomology**

41:118–127.

Fountain MT, Medd N (2015) Integrating pesticides and predatory mites in soft fruit crops. **Phytoparasitica** 43:657–667.

Furtado IP, de Moraes GJ, Kreiter S, Knapp M (2006) Search for effective natural enemies of *Tetranychus evansi* in south and southeast Brazil. **Experimental & Applied Acarology** 40:157–174.

Furtado IP, Moraes GJ de, Kreiter S, Tixier M-S, Knapp M (2007a) Potential of a Brazilian population of the predatory mite *Phytoseiulus longipes* as a biological control agent of *Tetranychus evansi* (Acari: Phytoseiidae, Tetranychidae). **Biological Control** 42:139–147.

Furtado IP, Toledo S, de Moraes GJ, Kreiter S, Knapp M (2007b) Search for effective natural enemies of *Tetranychus evansi* (Acari: Tetranychidae) in northwest Argentina. **Experimental & Applied Acarology** 43:121–127.

Gerson U, Webbing (1985) In: **Spider mites, their biology, natural enemies and control**. (Helle W. and Sabelis M.W. eds). Elsevier, Amsterdam, The Netherlands, p.223-232.

Gerson U, Weintraub PG (2007) Mites for the control of pests in protected cultivation. **Pest Management Science** 63:658–676.

Gonçalves MIF, Maluf WR, Gomes LAA, Barbosa LV (1998) Variation of 2-Tridecanone level in tomato plant leaflets and resistance to two mite species (*Tetranychus* sp.). **Euphytica** 104:33–38.

Gotoh T, Fujiwara S, Kitashima Y (2011) Susceptibility to acaricides in nine strains of the tomato red spider mite *Tetranychus evansi* (Acari: Tetranychidae). **International Journal of Acarology** 37:93–102.

Gotoh T, Sugimoto N, Pallini A, Knapp M, Hernandez-Suarez E, Ferragut F, Ho C-C, Migeon A, Navajas M, Nachman G (2010) Reproductive performance of seven strains of the tomato red spider mite *Tetranychus evansi* (Acari: Tetranychidae) at five temperatures. **Experimental & Applied Acarology** 52:239–259.

Guedes RNC, Smagghe G, Stark JD, Desneux N (2016) Pesticide-induced stress in arthropod pests for optimized integrated pest management programs. **Annual Review of Entomology** 61:43–62.

Han P, Desneux N, Becker C, Larbat R, Le Bot J, Adamowicz S, Zhang J, Lavoir A-V (2019) Bottom-up effects of irrigation, fertilization and plant resistance on *Tuta absoluta*: implications for Integrated Pest Management. J Pest Sci 92:1359–1370.

Heinz KM, Zalom FG (1996) Performance of the predator *Delphastus pusillus* on *Bemisia* resistant and susceptible tomato lines. **Entomologia Experimentalis et Applicata** 81:345–352.

Helle W, Sabelis MW (eds) (1985) **Spider mites: their biology, natural enemies, and control**. Elsevier ; Distributors for the United States and Canada, Elsevier Science Pub. Co, Amsterdam ; New York : New York, NY

Hoy MA (2011) Agricultural acarology — introduction to integrated mite management. Boca Raton: CRC Press Taylor and Francis Group, p. 393.

Humber RA, Moraes GJ, Santos JM (1981) Natural infection of *Tetranychus evansi* [Acarina: Tetranychidae] by*Triplosporium* sp [Zygomycetes: Entomophthorales] in northeastern Brazil. **Entomophaga** 26:421–425.

Jeppson LR, Keifer HH, Baker EW (1975) **Mites injurious to economic plants**. University of California Press, Berkeley

Jonsson M, Wratten SD, Landis DA, Gurr GM (2008) Recent advances in conservation biological control of arthropods by arthropods. **Biological Control** 45:172–175.

Kang J-H, Liu G, Shi F, Jones AD, Beaudry RM, Howe GA (2010) The tomato *odorless-2* mutant is defective in trichome-based production of diverse specialized metabolites and broad-spectrum resistance to insect herbivores. **Plant Physiology** 154:262–272.

Kennedy GG, Smitley DR(1985) Dispersal. In: **Spider mites, their biology, natural enemies and contro**l. (Helle W. and Sabelis M.W. eds). Vol. 1A Elsevier,

Amsterdam, The Netherlands, p. 233-242.

Khanamani M, Fathipour Y, Hajiqanbar H (2015) Assessing compatibility of the predatory mite *Typhlodromus bagdasarjani* (Acari: Phytoseiidae) and resistant eggplant cultivar in a tritrophic system. **Annals of the Entomological Society of America** 108:501–512.

Khanamani M, Fathipour Y, Hajiqanbar H, Sedaratian A (2014) Two-spotted spider mite reared on resistant eggplant affects consumption rate and life table parameters of its predator, *Typhlodromus bagdasarjani* (Acari: Phytoseiidae). **Experimental & Applied Acarology** 63:241–252.

Kimura S, Sinha N (2008) **Tomato (*Solanum lycopersicum*): A Model Fruit-Bearing Crop**. Cold Spring Harb Protoc 2008:pdb.emo105.

Knegt B, Meijer TT, Kant MR, Kiers ET, Egas M (2020) *Tetranychus evansi* spider mite populations suppress tomato defenses to varying degrees. **Ecology and Evolution** 10:4375–4390.

Kogan M (1998) Integrated Pest Management: Historical Perspectives and Contemporary Developments. **Annual Review of Entomology** 43:243–270.

Koller M, Knapp M, Schausberger P (2007) Direct and indirect adverse effects of tomato on the predatory mite *Neoseiulus californicus* feeding on the spider mite *Tetranychus evansi*. **Entomologia Experimentalis et Applicata** 125:297–305.

Kungu M, Subramanian S, Salifu D, Fiaboe KKM, Azandémè-Hounmalon GY, Gitonga L, Onyambu GK, Deletre E, Martin T (2020) Influence of predatory mites, *Phytoseiulus longipes* Evans. on the within-plant diurnal migration and distribution of the red spider mite, *Tetranychus evansi*, Baker and Pritchard on African Nightshade, *Solanum scabrum*. In: Niassy S, Ekesi S, Migiro L, Otieno W (eds) **Sustainable Management of Invasive Pests in Africa**. Springer International Publishing, Cham, pp 267–282

Lucini T, Faria MV, Rohde C, Resende JTV, de Oliveira JRF (2015) Acylsugar and the role of trichomes in tomato genotypes resistance to *Tetranychus urticae*. **Arthropod-Plant Interactions** 9:45–53.

Maciel GM, Marquez GR, Silva EC da, Andaló V, Belloti IF (2018) Tomato genotypes with determinate growth and high acylsugar content presenting resistance to spider mite. **Crop Breed Appl Biotechnol** 18:1–8.

Maluf WR, Maciel GM, Gomes LAA, Cardoso M das G, Gonçalves LD, da Silva EC, Knapp M (2010) Broad-spectrum arthropod resistance in hybrids between high- and low-acylsugar tomato lines. **Crop Science** 50:439–450.

Maniania NK, Bugeme DM, Wekesa VW, Delalibera I, Knapp M (2008) Role of entomopathogenic fungi in the control of *Tetranychus evansi* and *Tetranychus urticae* (Acari: Tetranychidae), pests of horticultural crops. In: Bruin J, van der Geest LPS (eds) **Diseases of Mites and Ticks**. Springer Netherlands, Dordrecht, p. 259–274

Maniania NK, Ekesi S, Kungu MM, Salifu D, Srinivasan R (2016) The effect of combined application of the entomopathogenic fungus *Metarhizium anisopliae* and the release of predatory mite *Phytoseiulus longipes* for the control of the spider mite *Tetranychus evansi* on tomato. **Crop Protection** 90:49–53.

Martin T, Assogba-Komlan F, Sidick I, Ahle V, Chandre F (2010) An acaricide-treated net to control phytophagous mites. **Crop Protection** 29:470–475.

Mcmurtry JA, Moraes GJD, Sourassou NF (2013) Revision of the lifestyles of phytoseiid mites (Acari: Phytoseiidae) and implications for biological control strategies. **Systematic & Applied Acarology** 18:297-320.

Meynard CN, Migeon A, Navajas M (2013) Uncertainties in predicting species distributions under climate change: a case study using *Tetranychus evansi* (Acari: Tetranychidae), a widespread agricultural pest. **PLoS ONE** 8:e66445.

Migeon A, Dorkeld F (2006-2021) Spider Mites Web: a comprehensive database for the Tetranychidae. INRA, http://www.montpellier.inra.fr/CBGP/spmweb/

Miller EC, Giovannucci E, Erdman JW, Bahnson R, Schwartz SJ, Clinton SK (2002) Tomato products, lycopene, and prostate cancer risk. **Urologic Clinics of North America** 29:83–93.

Murungi LK, Nyende A, Wesonga J, Masinde P, Knapp M (2010) Effect of African

nightshade species (Solanaceae) on developmental time and life table parameters of *Tetranychus evansi* (Acari: Tetranychidae). **Experimental & Applied Acarology** 52:19–27.

Musah, SM, Kamau AW, Munene M (2016). Effects of tomato *Lycopersicon esculentum* (Mill) varieties on development time, fecundity, and longevity of red spider mite *Tetranychus evansi* (Baker and Pritchard). **International Journal of Agriculture Innovations and Research** 4: 972- 976.

Navajas M, de Moraes GJ, Auger P, Migeon A (2013) Review of the invasion of *Tetranychus evansi*: biology, colonization pathways, potential expansion and prospects for biological control. **Experimental & Applied Acarology** 59:43–65.

Nyoni BN, Gorman K, Mzilahowa T, Williamson MS, Navajas M, Field LM, Bass C (2011) Pyrethroid resistance in the tomato red spider mite, *Tetranychus evansi*, is associated with mutation of the para-type sodium channel. **Pest Management Science** 67:891–897.

Omukoko CA, Maniania NK, Wekesa VW, Turoop L (2020) Effects and persistence of endophytic *Beauveria bassiana* in tomato varieties on mite density *Tetranychus evansi* in the screenhouse. In: Niassy S, Ekesi S, Migiro L, Otieno W (eds) **Sustainable Management of Invasive Pests in Africa**. Springer International Publishing, Cham, pp 283–291

Opit GP, Jonas VM, Williams KA, Margolies DC, Nechols JR (2001) Effects of cultivar and irrigation management on population growth of the twospotted spider mite *Tetranychus urticae* on greenhouse ivy geranium. **Experimental & Applied Acarology** 25:849–857.

Paspati A, Rambla JL, López Gresa MP, Arbona V, Gómez-Cadenas A, Granell A, González-Cabrera J, Urbaneja A (2021) Tomato trichomes are deadly hurdles limiting the establishment of *Amblyseius swirskii* Athias-Henriot (Acari: Phytoseiidae). **Biological Control** 157:104572.

Pralavorio M, Fournier D, Millot P (1989) Activité migratoire des tétranyques: Mise en évidence d'un rythme. **Entomophaga** 34:129–134.

Prischmann DA, James DG, Wright LC, Teneyck RD, Snyder WE (2005) Effects of chlorpyrifos and sulfur on spider mites (Acari: Tetranychidae) and their natural

enemies. **Biological Control** 33:324–334.

Qureshi AH, Oatman ER, Fleschner CA (1969) Biology of the spider mite, *Tetranychus evansi*. **Annals of the Entomological Society of America** 62:898–903.

Rakha M, Bouba N, Ramasamy S, Regnard J-L, Hanson P (2017) Evaluation of wild tomato accessions (*Solanum* spp.) for resistance to two-spotted spider mite (*Tetranychus urticae* Koch) based on trichome type and acylsugar content. **Genetic Resources and Crop Evolution** 64:1011–1022.

Resende JTV de, Maluf WR, Cardoso M das G, Faria MV, Gonçalves LD, Nascimento IR do (2008) Resistance of tomato genotypes with high level of acylsugars to *Tetranychus evansi* Baker & Pritchard. **Scientia Agricola** 65:31–35.

Resende JTV, Maluf WR, Cardoso M das G, Nelson DL, Faria MV (2002) Inheritance of acylsugar contents in tomatoes derived from an interspecific cross with the wild tomato *Lycopersicon pennellii* and their effect on spider mite repellence. **Genetics and Molecular Research** 1:106–116

Rosa AA, Gondim Jr. MGC, Fiaboe KKM, Moraes GJ de, Knapp M (2005) Predatory mites associated with *Tetranychus evansi* Baker & Pritchard (Acari: Tetranychidae) on native solanaceous plants of coastal Pernambuco State, Brazil. **Neotropical Entomology** 34:689–692.

Santos MD dos, Maciel A da GS, Trindade RCP, Silva ES, Duarte AG (2017) Eficiência do óleo de nim e do extrato pironim sobre o ácaro vermelho do tomateiro *Tetranychus evansi* Baker & Pritchard (Acari: Tetranychidae). **Revista Brasileira de Ciências Agrárias** 15:53-59.

Santos FA, Rolim GS, Nachman GS, Andrade DJ (2020) Using mathematical models to describe aerial dispersal and silk ball formation of peanut red spider mite, *Tetranychus ogmophallos* (Acari: Tetranychidae). **Experimental & Applied Acarology** 81:85–102.

Sato MM, de Moraes GJ, Haddad ML, Wekesa VW (2011) Effect of trichomes on the predation of *Tetranychus urticae* (Acari: Tetranychidae) by *Phytoseiulus macropilis* (Acari: Phytoseiidae) on tomato, and the interference of webbing. **Experimental & Applied Acarology** 54:21–32.

Saunyama I, Knapp M (2004) Effect of pruning and trellising of tomatoes on red spider mite incidence and crop yield in Zimbabwe. **African Crop Science Journal** 11:269–277.

Sarr I, Knapp M, Ogol CKP, Baumgärtner J (2002) Impact of predators on *Tetranychus evansi* Baker and Pritchard populations and damage on tomatoes (*Lycopersicon esculentum* Mill.) in Kenya. Abstract retrieved from **Abstract book of the 11th International Congress of Acarology**, Merida, Mexico (No 271).

Savi PJ, De Moraes GJ, Boiça Junior AL, Melville CC, Carvalho RF, Lourenção AL, Andrade DJ (2019a) Impact of leaflet trichomes on settlement and oviposition of *Tetranychus evansi* (Acari: Tetranychidae) in African and South American tomatoes. **Systematic & Applied Acarology** 24:2559–2576.

Savi PJ, de Moraes GJ, Melville CC, Andrade DJ (2019b) Population performance of *Tetranychus evansi* (Acari: Tetranychidae) on African tomato varieties and wild tomato genotypes. **Experimental & Applied Acarology** 77:555–570.

Schmidt-Jeffris RA, Beers EH (2015) Comparative biology and pesticide susceptibility of *Amblydromella caudiglans* and *Galendromus occidentalis* as spider mite predators in apple orchards. **Experimental & Applied Acarology** 67:35–47.

Schmidt-Jeffris RA, Beers EH, Crowder DW (2015) Phytoseiids in Washington commercial apple orchards: biodiversity and factors affecting abundance. **Experimental & Applied Acarology** 67:21–34.

Schmidt-Jeffris RA, Coffey JL, Miller G, Farfan MA (2021) Residual Activity of Acaricides for Controlling Spider Mites in Watermelon and Their Impacts on Resident Predatory Mites. **Journal of Economic Entomology** 114:818–827.

Sibomana MS, Workneh TS, Audain K (2016) A review of postharvest handling and losses in the fresh tomato supply chain: a focus on Sub-Saharan Africa. **Food Security** 8:389–404.

Sikirou R (2015) Geographical distribution and prevalence of the main tomato fungal wilt diseases in Benin. **International Journal of Biological and Chemical**

Sciences 9:603-613.

Silva P (1954) Um novo ácaro nocivo ao tomateiro na Bahia. **Boletim do Instituto Biológico da Bahia** 1: 1–20.

Silva FR da, Moraes GJ de, Gondim Jr MGC, Knapp M, Rouam SL, Paes JLA, Oliveira GM de (2010) Efficiency of *Phytoseiulus longipes* Evans as a control agent of *Tetranychus evansi* Baker & Pritchard (Acari: Phytoseiidae: Tetranychidae) on screenhouse tomatoes. **Neotropical Entomology** 39:991–995.

Simmons AT, Gurr GM (2005) Trichomes of *Lycopersicon* species and their hybrids: effects on pests and natural enemies. **Agricultural & Forest Entomology** 7: 265–276.

Smith CM (2005) **Plant resistance to arthropods: molecular and conventional approaches**. Springer, Dordrecht, The Netherlands 423p.

Soto A, Venzon M, Oliveira RM, Oliveira HG, Pallini A (2010) Alternative control of *Tetranychus evansi* Baker & Pritchard (Acari: Tetranychidae) on tomato plants grown in greenhouses. **Neotropical Entomology** 39:638–644.

Stenberg JA (2017) A Conceptual Framework for Integrated **Pest Management. Trends in Plant Science** 22:759–769.

Stout MJ, Kurabchew H, Leite GLD (2018) Host-plant resistance in tomato. In: **Sustainable Management of Arthropod Pests of Tomato**. Elsevier, p. 217–236

Tehri K (2014) A review on reproductive strategies in twospotted spider mite, *Tetranychus urticae* Koch, 1836 (Acari: Tetranychidae). **Journal of Entomology and Zoology Studies** 2:35–39

Toroitich FJ, Knapp M, Nderitu JH, Olubayo FM, Obonyo M (2014) Susceptibility of geographically isolated populations of the tomato red spider mite (*Tetranychus evansi* Baker & Pritchard) to commonly used acaricides on tomato crops in Kenya. **Journal of Entomological and Acarological Research** 46:18-25.

Toroitich, F. J. (2006**) Effect of pesticides on the tobacco spider mite *Tetranychus evansi* Baker and Pritchard on tomatoes in Kenya**. 69f. Msc thesis, university of Nairobi, Kenya.

Van Driesche R, Hoddle M, Center TD (2008) **Control of pests and weeds by natural enemies: an introduction to biological control**, 1st ed. Blackwell Pub, Malden, MA, 484p.

Wakil W Brust, Gerald E, Perring T (2018) **Sustainable management of arthropod pests of tomato**. Academic Press, London 353p.

Wang C, Feng M-G (2014) Advances in fundamental and applied studies in China of fungal biocontrol agents for use against arthropod pests. **Biological Control** 68:129–135.

Wekesa VW, Moraes GJ, Knapp M, Delalibera I (2007) Interactions of two natural enemies of *Tetranychus evansi*, the fungal pathogen *Neozygites floridana* (Zygomycetes: Entomophthorales) and the predatory mite, *Phytoseiulus longipes* (Acari: Phytoseiidae). **Biological Control** 41:408–414.

Ximénez-Embún MG, Ortego F, Castañera P (2016) Drought-stressed tomato plants trigger bottom–up effects on the invasive *Tetranychus evansi*. **PLoS ONE** 11:e0145275.

Zanuzo Zanardi O, Pavan Bordini G, Aparecida Franco A, Jacob CRO, Takao Yamamoto P (2017) Sublethal effects of pyrethroid and neonicotinoid insecticides on *Iphiseiodes zuluagai* Denmark and Muma (Mesostigmata: Phytoseiidae). **Ecotoxicology** 26:1188–1198.

Zhang Y, Song H, Wang X, Zhou X, Zhang K, Chen X, Liu J, Han J, Wang A (2020) The roles of different types of trichomes in tomato resistance to cold, drought, whiteflies, and botrytis. **Agronomy** 10:411.

Zriki G, Saker I, Bouou A (2013) Life history parameters of the tomato red spider mite *Tetranychus evansi* (Acari: Tetranychidae), collected in syria, on two solanaceous plants. Acarines: **Journal of the Egyptian Society of Acarology** 7:71–76.

CHAPTER 2- Bottom-up effects of breeding tomato genotypes on behavioral responses and performance of *Tetranychus evansi* population

Patrice Jacob Savi[1*], Gilberto José de Moraes[2], Rogério Falleiros Carvalho[1], Daniel Júnior de Andrade[1]

[1] São Paulo State University (UNESP), College of Agricultural and Veterinary Sciences, Jaboticabal, São Paulo, Brazil.
[2] Luiz de Queiroz College of Agriculture, University of São Paulo (ESALQ/USP), Piracicaba, São Paulo, Brazil.

[*]Corresponding Author: savipatricejacob@yahoo.fr

Abstract

The tomato red spider mite, TRSM, *Tetranychus evansi* Baker & Pritchard (Acari: Tetranychidae), is an invasive tomato pest in several countries, with the potential to reduce yield by up to 90% in Africa. *Solanum habrochaites*, access PI 134417 is a wild tomato genotype resistant to several arthropod pests, including TRSM. There is an interest in increasing the resistance of a tomato genotype (*Solanum lycopersicum* cv. TLCV15) widely cultivated by smallholder western African farmers to TRSM, through interspecific crossings with that wild genotype. For this purpose, after obtaining the F1 progeny and as well as F2 (SPJ-10-2017) and BC1 back-crossed (SPJ-05-2018) genotypes selected for high glandular trichome densities, we characterized their resistance level to TRSM. We quantified the types and densities of trichomes on the abaxial surface of their leaflets and examined the subsequent bottom-up effects of these progeny plants' attributes on behavior and demographic parameters of the mite. Our results showed that the densities of glandular trichomes inherited from the resistant genotype (PI 134417) by the progenies were highly variable, with types I, IV, and VI being the most prevalent. The progeny SPJ-10-2017 was classified as resistant, while the progenies F1 and SPJ-05-2018 were classified as partially resistant. These findings constitute one of the first steps towards advancing breeding programs in African countries to obtain tomato genotypes resistant to TRSM, targeting more sustainable production.

Keywords: Life table parameters; trichomes; resistant genotype; tomato red spider mite; sustainable production.

Article published in the "Journal of Pest Science" (Impact factor: 5.918)

Introduction

Since the first report of the tomato red spider mite TRSM *Tetranychus evansi* Baker & Pritchard (Acari: Tetranychidae) in northeastern Brazil, it has emerged in recent decades as a severe invasive pest on numerous solanaceous crops, colonizing several tropical and subtropical habitats in the world (Silva 1954; Navajas et al. 2012). It is presently found in 44 countries in Africa, Americas, Europe, and Asia and studies have indicated that climate changes may have facilitated and accelerated its spread to new territories (Meynard et al. 2013; Ximénez-Embún et al. 2016; Migeon and Dorkeld 2021). TRSM feeds preferentially on leaves, causing chlorotic spots that substantially reduce plant photosynthetic capacity and affect transpiration, often resulting in leaf drought and fall. Consequently, fruits are more exposed to the sun, not acquiring the characteristic red color, depreciating their commercial value (de Moraes and Flechtmann 2008). In the African continent, TRSM damage can achieve 90% on tomato crop (Sarr et al. 2002; Boubou et al. 2010; Meynard et al. 2013).

Chemical control is the main method used for controlling TRSM. However, the efficacy of that method is not always acceptable, since this mite is armed with competitive biological characteristics such as the rapid development, high reproductive capacity, and the ability to withstand a wide range of temperatures which leads producers to intensify chemical sprays for controlling it (Bonato 1999; Nyoni et al. 2011; Azandémè-Hounmalon et al. 2015). As a consequence, the pest becomes more prone to evolving pesticide resistance, often leading to the occurrence of environmental pollution and poisoning risks to farmers and consumers (Handford et al. 2015). For this reason, ecological-based rather than chemical-based control options have been sought worldwide for pest management (Maluf et al. 2010; Han et al. 2019; Savi et al. 2019a, b).

Predatory mites of the family Phytoseiidae are important top-down regulators in terms of more sustainable approaches to phytophagous mite control (Furtado et al. 2007; Savi et al. 2021). However, the host plant may also have important effects on

the population dynamics of the arthropod herbivore community in the agroecosystems (van der Putten et al. 2001; Han et al. 2019). Herbivorous arthropods depend on the consumption of plant products to obtain water and nutrients for their survival and development, at the same time they must deal with plant defense strategies (Schoonhoven et al. 2005; Han et al. 2019). These defense strategies to herbivores can involve the synthesis of a multitude of compounds, such as allelochemicals, proteins, and various metabolites associated with morphological structures such as trichomes (Simons and Gurr 2005; Maluf et al. 2010; Lucini et al. 2015; Oliveira et al. 2020). Seven types of trichomes have been identified on tomato plants, grouped as glandular (types I, IV, VI, and VII) and non-glandular (types II, III, and V) (Luckwill 1943; Simmons and Gurr 2005). Glandular trichomes play an important role as a resistance mechanism to arthropod pests by releasing defense compounds such as acyl sugars, methyl-ketones, and sesquiterpenes (Simons and Gurr 2003; Lucini et al. 2015; Oliveira et al. 2020). Conversely, non-glandular trichomes can act only as physical barriers to mites (Savi et al. 2019a). Thus, the variation in the quality of the host plant traits, mostly glandular trichomes, can trigger cascade effects: the so-called “bottom-up effects” in organisms of higher trophic levels, including herbivorous arthropods and their natural enemies (Han et al. 2016, 2019; Savi et al. 2021). Such effects may cause antixenosis (changes in mite behavior leading to reduced host colonization) and/or antibiosis (impairment of pest fitness traits, as survival, oviposition, and development) (Maluf et al. 2010; Lucini et al. 2015; Savi et al. 2019a; Heidari et al. 2020). In the last decade, bottom-up control mechanisms have been investigated in integrated pest management (IPM) efforts against several arthropod pest populations, especially against invasive arthropod pests (see e.g. Hufbauer and Torchin 2007; Ximénez-Embún et al. 2016; Han et al. 2016, 2019; Heidari et al. 2020). Hence, understanding the bottom-up effects on TRSM can provide more insights into the planning of an effective IPM program.

Bottom-up effects have been investigated in cultivated tomato genotypes as well as in some wild tomato species, such as *Solanum habrochaites* Knapp & Spooner (Antonious and Snyder 2008; Bleeker et al. 2012), *Solanum pimpinellifolium* L (Alba et al. 2008), and *Solanum pennellii* Correll (Maciel et al. 2018). Resistance

mechanisms in these wild species have been associated mostly with the presence of glandular trichomes on leaves, usually less numerous on cultivated tomato genotypes (Bleeker et al. 2012). In a previous study, we compared the resistance to TRSM of five tomato genotypes commonly grown in West Africa (Benin) (Kekefo, TOML4, Akikon, Tounvi, and TLCV15) and three wild South American genotypes [*S. habrochaites* (PI 134417 and PI 134418) and *S. pennellii* (LA-716)]. The results showed that all cultivated tomato genotypes were more favorable to TRSM preference, development, and reproduction than the wild genotypes, which were highly resistant (Savi et al. 2019a, b). These results were related to the lower density or even absence of type I, IV, and VI glandular trichomes on the cultivated genotypes (Savi et al. 2019b).

Based on these results, the transfer of types I, IV, and VI glandular trichomes from wild tomato genotypes to high-yielding tomato cultivars, through interspecific crossings, could conceivably be one of the ways to obtain cultivars with high glandular trichome densities and consequently with characteristics of resistance to TRSM and adapted to sustainable production systems. Thus, the present study aimed to select progeny genotypes that conferred resistance against TRSM from interspecific crossing between *S. habrochaites* var. *hirsutum* access “PI 134417” and cultivated tomato *S. lycopersicum* cv.TLCV15, examining their subsequent bottom-up effects on the behavior responses and TRSM demographic parameters.

Materials and Methods

Selected tomato genotypes

The study included two parental tomato genotypes [*S. habrochaites* access PI 134417 (pollen donor) and *S. lycopersicum* cv. TLCV15 (female parent)] and three hybrid populations consisting of (a) F1 plants resulting from the crossings of the parental genotypes; (b) F2 segregating plants resulting from the self-fertilization of F1, from now on referred to as SPJ-10-2017; and (c) BC1 backcrossing segregating plants (F1 × TLCV15, the former used as pollen donor), from now on referred to as

SPJ-5-2018) (Fig. 1). For better understanding, these five populations are referred to in this paper as experimental populations.

Seeds of PI 134417 were supplied by “Instituto Agronômico de Campinas”, São Paulo, Brazil, while seeds of TLCV15 were provided by “Institut National des Recherches Agricoles du Bénin” (INRAB), imported to Brazil under authorization from the Brazilian Ministry of Agriculture, Livestock and Supply (Process #258/2016/SSV-SP), and approved under LQV report # 20170072. PI 134417 was chosen as pollen donor parent genotype by the facility of crossing with the cultivated tomato species (*S. lycopersicum*) and for having substantially high glandular trichome densities that confer resistance to several arthropod pests including TRSM (Savi et al. 2019b). TLCV15 was chosen as a female parent for having low glandular densities and for being susceptible to TRSM (Savi et al. 2019 b). Additionally, TLCV15 is relevant for smallholder farmers in Benin (West Africa) in terms of productivity when compared with other genotypes evaluated in previous studies (Savi et al. 2019a, b).

Seeds of the experimental populations were sown in Styrofoam cells filled with Bioplant® substrate (coconut fiber, pine bark, bovine manure, sawdust, vermiculite, rice husks, ashes, plaster calcium carbonate, magnesium, magnesium thermophosphate, and fertilizers) and kept in a screen house. Irrigation was done four times a day (8, 11, 14, and 17 h) by automatic sprinklers. Fertilizer (250 mL per tray of 10 g mono ammonium phosphate (MAP) diluted in 1 L of water) was applied two weeks after germination.

Seedlings were individually transplanted to 4-L pots 80% filled with a homogeneous mixture of soil, sand, and tanned bovine manure, which was previously sterilized in an autoclave at 120 ºC for 3 h. Plants were irrigated daily to field capacity and never sprayed with pesticides; each plant was fertilized every 15 days with 5 g of urea (44% N) and 5 g of potassium chloride (58% KCl). The plants were maintained in the screen house, with no control of environmental conditions. For the experiment, SPJ-10-2017 and SPJ-05-2018 populations were respectively taken only from F2 and BC1 segregating plants determined to have a minimum

density of four glandular trichomes/mm^2, based on the analysis of both surfaces of ten (10) randomly collected leaflets per plant, done as subsequently described.

Quantification of types and trichome densities

The density of each trichome type was evaluated on the abaxial surface of leaflets in 35 day-old plants of each genotype, given the mite preference for this surface. The leaflets were collected from twelve plants per genotype (5 leaflets per plant) located in the central third of each plant, totaling 60 leaflets per genotype. For the evaluation, sections of 4 × 4 mm were cut from the central part of each leaflet, gently washed with tap water, and fixed with 3% glutaraldehyde in 0.05 M potassium phosphate buffer for 72 h, at pH 7.4 and 8 °C. The sections were subsequently rinsed six times in pure buffer solution for 15 minutes each time and post-fixed in 2% osmium tetroxide in the same buffer for 24 h. Then, the samples were dehydrated in an ethanol series (30, 50, 70, 80, and three times in 100%), critical-point dried through liquid CO_2, mounted on metal stubs, and sputter-coated with 35 nm gold/ palladium in a Denton Vacuum Desk II metallizer (Campos et al. 2009). Afterward, trichomes were photographed under a scanning electron microscope (LEO 435 VP) at 20 kV, counting the number of each trichome type per mm^2 of leaf tissue, according to Luckwill (1943). Trichomes types V, VI, and VII were counted separately, but counts of type II and III non-glandular trichomes were pooled together for having lengths very close to each other. For the same reason, counts of types I and IV glandular trichomes were also pooled together. The design used was completely randomized.

TRSM stock colony

Mites of this species were obtained from a colony maintained on American black nightshade (*Solanum americanum* Mill) in the Acarology laboratory of the Department of Agricultural Sciences, College of Agricultural and Veterinary Sciences, São Paulo State University, Jaboticabal campus, in an air-conditioned

chamber at 25 ± 1ºC, 70 ± 10% RH, and 12 hours photoperiod. Subsequent colonies maintained on tomato plants as well as the experimental units used in laboratory tests were maintained under the same conditions.

Free-choice test

Two tests were conducted, one in a laboratory and another in a screen house, in randomized block designs, both to evaluate settlement and oviposition of TRSM females on each evaluated genotype, with the help of a stereomicroscope (Zeiss, Jena, Germany).

In the laboratory, each block was based on a Petri dish whose base was covered a 1-cm-thick piece of foam mat lined with a thin layer of hydrophilic cotton soaked in deionized water, topped by a pentagonal piece of polypropylene, removing a 3.5-cm-diameter semicircle from each of its sides, fitting in each semicircle a tomato leaf disc of one of the evaluated genotypes (Fig 1). The discs were cut from leaflets of the middle third of the canopy of 35-day-old plants of each genotype, and the distance between the inner edge of each disc and the center of the Petri dish was 1 cm. Once all pieces were in place, twenty 6-day-old adult females of TRSM were transferred with a brush from the nightshade stock colony to the center of the pentagon. A total of 20 blocks was used in the test, conducting the evaluations 24 and 48 h after the test was initiated.

In the screen house test, 40-day-old plants of the five genotypes were used and standardized in terms of height (30- cm) and a number of leaves (6). Each block consisted of a gray polypropylene disc (30 cm in diameter) fixed on top of a pole, at about 25 cm from the floor to serve as a horizontal surface onto which mites would be released, and from which they could move to plants in contact with it. The lower edge of the disc was smeared with insect-trap glue, to prevent the mites from escaping. One plant of each genotype was positioned around the disc, equidistant and randomly, tilted at about 60º about the floor, to have some of the leaflets touching the disc (Fig. 1). One hundred 6-day-old TRSM adult females were transferred from the nightshade stock colony to the polypropylene disc with a brush.

Twenty-four h later, the plants were taken to the laboratory, where all the leaves were excised to count the mites and eggs. A total of 12 blocks was used in the test, conducting the evaluations 24 h after the test was initiated. Environmental conditions in the screen-house were not controlled, ranging as follows: min<average<max: Temperature (º C) 17.1<25.5<34.7; Relative humidity (%) 27.4<59.0<91.9; Light: natural environment.

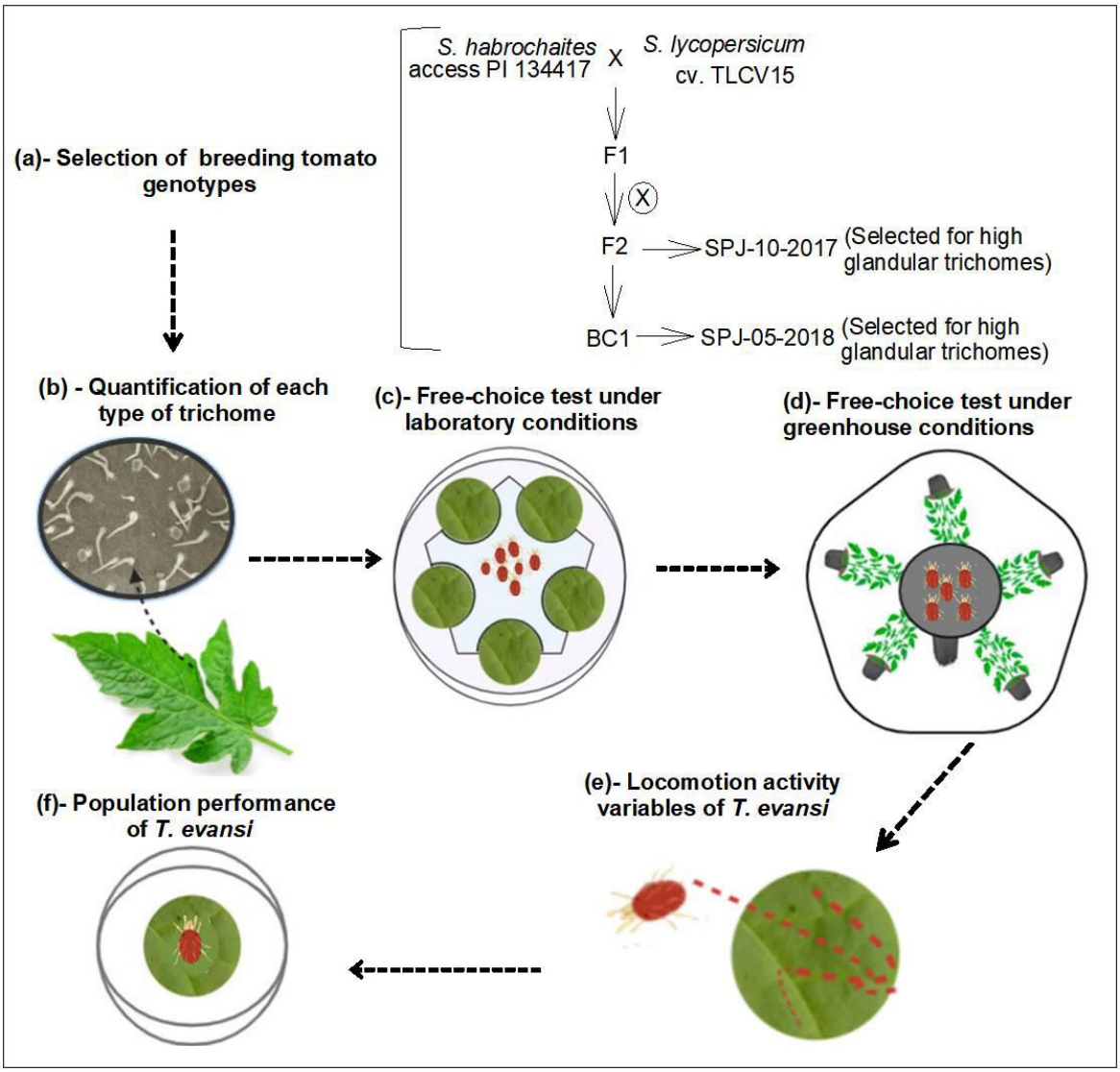

Fig. 1 Schematic representation of the experimental setups in different phases of this study.

Effect of genotypes on TRSM walking ability

For the evaluation of the walking behavior, each experimental unit consisted of a Petri dish (50 mm in diameter) covered with a piece of nylon foam layer (10mm thick) lined with a thin layer of cotton wool moistened with deionized water. A leaf disc (30 mm diameter) of a genotype, obtained as mentioned in the previous section, was placed onto the center of a dish with its abaxial surface up (Fig. 1), releasing five 6-day-old adult female TRSM at the center of the disc. The dish was positioned on the stage of a stereomicroscope with the light and a Galaxy J5 Prime® smartphone camera (a 13-megapixel rear camera, with f / 1.9 aperture, and to a five-megapixel front camera, with f / 2.2 aperture) was fixed to regular cellular telephone support (positioned slightly tilted, about 30º about the vertical, for better visualization of the mites, and about 20cm from the disc) to record the movement of the mites for 10 minutes. The procedure was repeated 12 times for each of the five genotypes, using a different experimental unit at each time. Subsequently, the videos were submitted for analysis using a computer equipped with video-tracking software (EthoVision XT, Noldus Information Technology Inc.®, Wageningen, Netherlands). Mean speed ($mm\ s^{-1}$), average traveled distance (mm), duration (s, representing the total period in which was motionless) and period(s) and frequency (n) of resting time and duration of high mobility (s) were evaluated.

Experimental procedure for evaluation of population performance

For adaptation, some TRSM were transferred from the stock colony to plants of each genotype to be tested 40 days before starting the experiments, onto which they were reared for three generations. In this period, plants with severely damaged or senescent leaves were replaced by new ones. These plants were maintained in an air-conditioned chamber under the same conditions mentioned for the stock colony. Experimental units used for this trial were of the same type described in the previous item. To obtain eggs of similar ages, one gravid TRSM female was transferred from

the colony maintained on a given genotype to leaf discs made with leaflets excised from the median third of the canopy of 35-60 day-old plants of the same genotype. Six hours later, females and extra eggs were removed, leaving a single egg per disc. For the treatments corresponding to genotypes TLCV15, F1, SPJ-10-2017 and SPJ-05-2018, a cohort of 120 eggs of TRSM was used, while for treatment of PI 134417 genotype, a cohort of 40 eggs was used, for the reduced number of available mites on this, because of their low survivorship on this genotype during the adaptation period. Upon reaching adulthood, mites were sexed and transferred in couples to new experimental units. For this purpose, some males (of unknown age) were taken from the stock colony of the corresponding genotype.

The units were examined every 24 h under a stereomicroscope to determine the duration of each developmental stage and survivorship. Mite size, moving ability, and presence of exuviae were considered to determine to molt. The following parameters were determined: pre-oviposition (APOP: the period between adult emergence and its first oviposition), total pre-oviposition (TPOP: the period from egg to first oviposition), oviposition days (number of days in which oviposition occurred), the longevity of each sex, sex ratio, and fecundity. Leaf discs were replenished with new ones every three days to maintain leaf discs' quality for mite development and reproduction. In units where males died before females, other males from the stock colony were used to replace them. Data on males that came from stock colonies and mites that died on the cotton wool layer while trying to escape were not used in statistical analysis. Eggs laid were removed at every observation period. The experiment was considered finished after all mites died.

Data analysis

The generalized linear model (GLM) with quasi-Poisson distribution was used to compare the number of trichomes between genotypes because they were counting data that did not follow a normal distribution. In both free-choice trials (laboratory and greenhouse), the number of mites attracted and of eggs laid on tomato genotypes were compared using a linear mixed-effects model with quasi-Poisson

distribution (http://www.poleto.com/funcoes.html) implemented in the R package “lme4” (Bates et al. 2015). The genotype was considered a fixed factor, while repetitive measures were considered a random factor. As repeated measures in free-choice performed under laboratory conditions were over time, the effect of genotype and time was evaluated by likelihood ratio tests (P <0.05), between a complete model and a reduced model. The same test was used to verify the significance of the interaction between genotype and time, comparing a model with the interaction and a model without the interaction. The best fit of the model for both parameters was assessed by semi-normal plots with simulation envelopes, using the "hnp" package (Demétrio et al. 2014). In case of significant differences between treatments, multiple comparisons for GLM models were performed using the post-hoc Tukey test (p <0.05) for free-choice trial data (number of mites attracted/ arrested and eggs laid), numbers of trichomes data between tomato genotypes, using the “glht” function in the “multcomp” package of R, with p value adjustments.

Walking ability data did not follow the homoscedasticity and normality assumptions for analysis of variance (ANOVA) even after transformations. Therefore, walking ability data were subjected to non-parametric analysis, comparing averages by the Kruskal-Wallis test using the statistical program R, version 3.5.3 (R Development Core Team, 2019).

The software TWOSEX-MSChart by Chi (2020a), available http://140.120.197.173/Ecology/prod02.htm was used to estimate development and reproduction raw data and to calculate population parameters (Chi et al. 2020b). The following parameters were estimated: age-stage–specific survival rate (s_{xj}), age-specific survival rate (l_x), age-specific fecundity (m_x), net reproduction rate (R_0), intrinsic rate of increase (r), stage-specific fecundity (f_{xj}), finite rate of increase (λ), and average generation time (T). Differences among genotypes were compared by the paired bootstrap test based on the confidence interval of differences (Efron and Tibshirani 1993).

Exploratory multivariate analyses were performed after the standardization of variables in which each one had mean = 0 and variance = 1. Then, the standardized data were processed using hierarchical cluster analysis considering as a measure

of similarity between pairs of lots the Euclidean distance and the Ward´s Method as the connection mode between the groups. The principal component analysis allows condensing as much of the original information contained in p variables (p = 15, this study) in two orthogonal latent variables called principal components, which are linear combinations of the original variables created with the two largest eigenvalues of the covariance matrix (Hair et al. 2006).

The adequacy of this analysis is verified by the amount of the total information of the original variables retained by the principal components showing eigenvalues greater than unity (Kaiser 1958). Eigenvalues lower than the unity do not preserve the relevant information. All exploratory multivariate analyses were performed in the Software STATISTICA version 11 (Statsoft 2012).

Results

Quantification of trichomes

All trichomes present on the parental genotypes PI 134417 and TLCV15 were found on progeny genotypes (F1, SPJ-10-2017 and SPJ-05-2018), and no significant differences were observed between genotypes for the densities of trichomes considering all types together ($F_{4, 55} = 1.171$, $P = 0.143$) (Fig. 2). For all non-glandular trichomes together (types II, III, and V) as well as for types II + III, densities on TLCV15 and SPJ-05-2018 were significantly higher than on other genotypes ($F_{4, 55} = 21.85$, $P < 0.001$ and $F_{4, 55} = 21.46$, $P < 0.001$, respectively). Type V was found in higher density on TLCV15 than on other genotypes ($F_{4, 55} = 17.96$, $P = 0.022$).

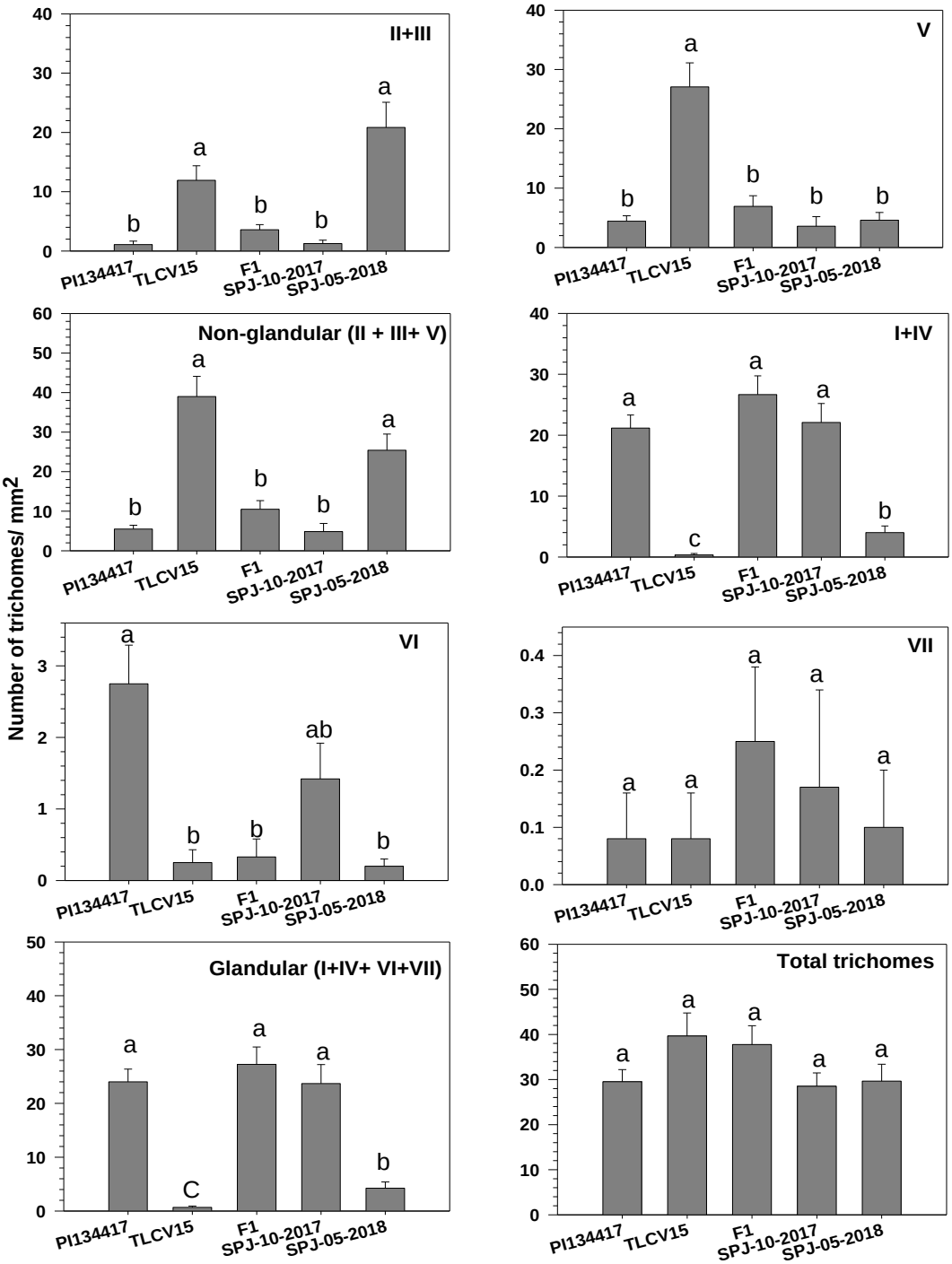

Fig. 2 Average trichome densities (±SE) on the abaxial leaf surfaces of evaluated tomato genotypes of 35- day-old plants, for each trichome type and their combinations. Above each bar, different letters indicate significant differences between treatments (GLM with quasi-Poisson distribution, followed by post hoc Tukey´s test; $P \leq 0.05$).

Among glandular trichomes, considering all types together (I, IV, VI, and VII) as well as for types I + IV, the densities were higher on PI 134417, F1, and SPJ-10-2017, intermediate on SPJ-05-2018 and lower on TLCV15 ($F_{4, 55} = 41.61$, $P < 0.001$ and $F_{4, 55} = 44.57$, $P < 0.001$, respectively). Type VI was found in higher densities on PI 134417 than on TCLV15, F1, and SPJ-05-2018, whereas density on SPJ-10-17 did not differ significantly from those two extremes ($F_{4, 55} = 29.86$, $P < 0.001$). Type VII was found in very low levels in all genotypes, and the variability in each genotype did not allow the detection of significant differences among them ($F_{4, 55} = 0.801$, $P = 0.531$).

Settlement and oviposition

A significant interaction was observed between evaluation time and the genotypes for the number of females settled on leaf discs in the free-choice in the laboratory test ($\chi2 = 16.72$, $df = 4$, $P = 0.022$) (Fig. 3a). A significant interaction was also observed between time and genotypes for the number of eggs laid by females settled on each disc ($\chi2 = 81.53$, $df = 4$, $P < 0.001$) (Fig. 3 b). The number of eggs laid on each disc by the settled females was significantly lower on PI 134417 and F1 at the 24 h and the 48 h evaluation ($F_{4, 95} = 17.84$, $P < 0.001$ and $F_{4, 95} = 37.58$, $P < 0.001$, respectively).

In the screen-house test, the number of females settled on PI 134417 plants was lower than on plants of other genotypes, except SPJ-10-2017 (on which the number settled was not statistically different); the number on TLCV15 was significantly higher than on PI 134417 and SPJ-10-2017 ($F_{4, 55} = 5.176$, $P < 0.001$) (Fig. 4a). Consequently, the number of eggs deposited was also significantly lower on PI 134417 and SPJ-10-2017 but no significant differences were observed between SPJ-10-2017 and other genotypes ($F_{4, 55} = 6.169$, $P < 0.001$) (Fig. 4b).

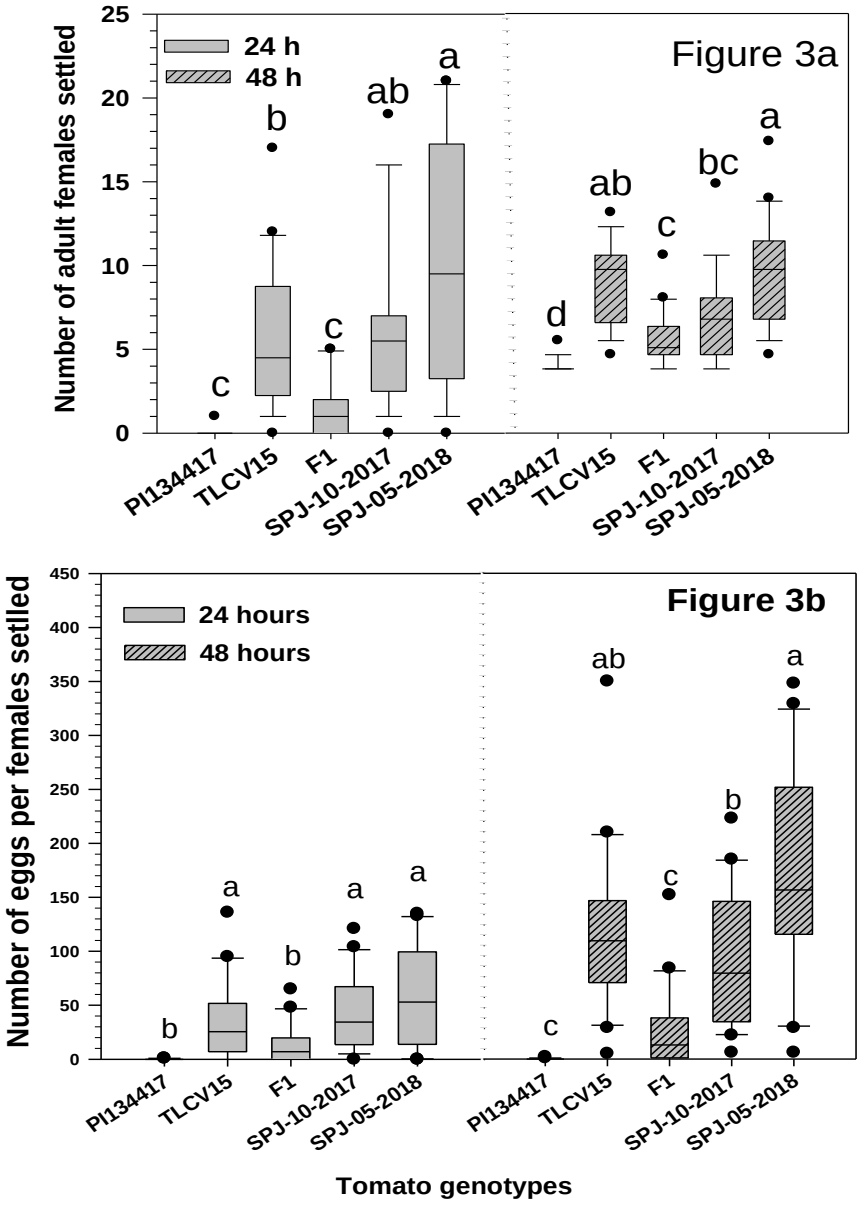

Fig. 3 Comparative box plot distribution of the numbers of adult females of *Tetranychus evansi* (a) or eggs laid by them (b) on leaflet discs of tomato plants, in a free-choice test under laboratory conditions, 24 and 48 h of mite release. Number of females released= 20; number of replicates =20. Box plots show the 25th and 75th percentile (boundary of the boxes) and respective median (transverse line within each box). Circles correspond to outliers. Above each box, different letters indicate significant differences between treatments (GLM with quasi-Poisson distribution, followed by post-hoc Tukey´s test; $p < 0.05$).

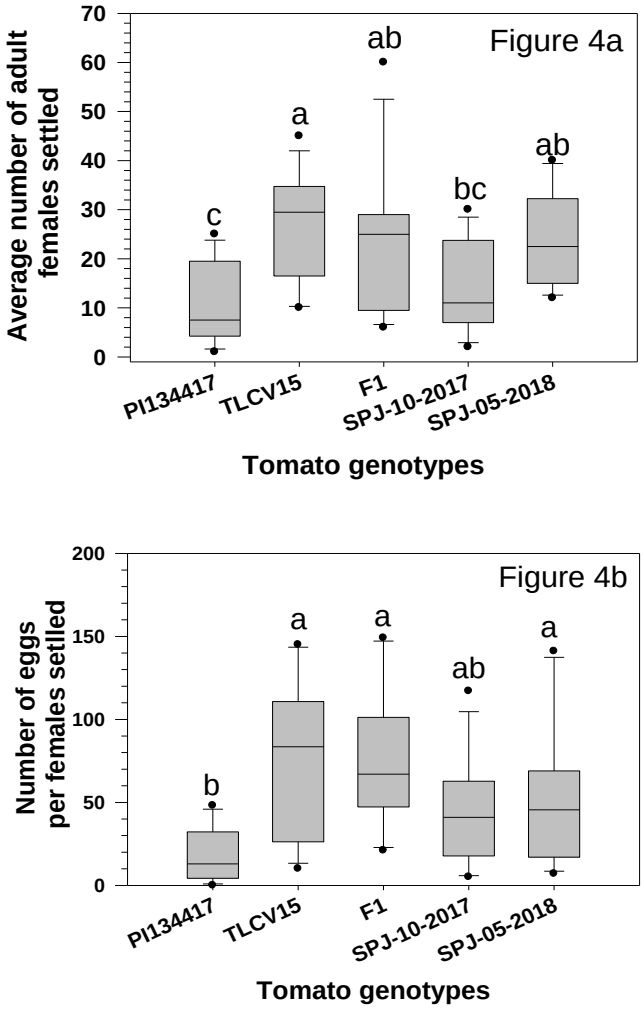

Fig. 4 Comparative box plot distribution of the number of adult females of *Tetranychus evansi* (**a**) or eggs laid by them (**b**) on tomato plants genotypes, in a free-choice test under greenhouse conditions 24 h of mite release. Number of females released= 100; the number of replicates =12 Box plots show 25th and 75th percentile (boundary of the boxes) and respective median (transverse line within each box). Circles correspond to outliers. Above each box, different letters indicate significant differences between treatments (GLM with quasi-Poisson distribution, followed by post-hoc Tukey´s test; $p < 0.05$).

Walking abilities

The results of the test showed that all progeny genotypes (F1, SPJ-10-2017, and SPJ-05-2018) caused major behavioral changes comparable to that of resistant parental genotype TLCV15 (Table 1). The speed of locomotion, mean distance travelled, frequency of resting time and high mobility rate were significantly higher on TLCV15 than on other genotypes, except that the mean rate of high mobility on F1 did not differ significantly from that on TLCV15 ($\chi2 = 42.85$, df = 4, $P < 0.001$, $\chi2 = 47.062$, df = 4, $P < 0.001$, $\chi2 = 47.53$, df = 4, $P < 0.001$ and $\chi2 = 13.81$; df = 4, $P < 0.001$, respectively). The pattern observed for resting time was exactly the reverse of what was observed for other parameters, that is, the lowest value of resting time was observed on TLCV15 genotype.

Table 1 Mean for locomotion activities (± SE) of *Tetranychus evansi* within an observation period of 10 min, on leaflets of tomato parental (PI 134417 and TLCV15) and progeny (F1, SPJ-10-2017 and SPJ-05-2018) genotypes.

Treatments	Locomotion		Not moving (rest time)		High mobility(s)
	Speed (mm s^{-1})	Mean distance traveled (mm)	Mean (s)	Frequency (n)	Mean (s)
PI 134417	0.053 ± 0.001b	0.053 ± 0.017c	595.5 ± 4.17a	1.0 ± 0.004b	1.6 ± 0.05b
TLCV15	0.406 ± 0.04a	36.143 ± 10.40a	29.7 ± 13.40b	79.5 ± 3.31a	2.5± 0.23a
F1	0.002 ± 0.0009b	0.117 ± 0.034b	528.1 ± 19.93a	1.3 ± 0.08b	2.1 ± 0.34ab
SPJ-10-2017	0.0006 ± 0.00003b	0.066 ± 0.039c	595.9 ± 0.53a	1.0 ± 0.00b	1.6 ± 0.18b
SPJ-05-2018	0.0006 ± 0.00003b	0.067 ± 0.015c	595.4 ± 1.46a	1.1± 0.06b	1.7 ± 0.10b
χ^2	47.0621	42.847	34.014	47.5313	13.8126
df	4	4	4	4	4
P	< 0.001	< 0.001	< 0.001	< 0.001	0.008

Within each column, means followed by different letters are significantly different (Kruskal-Wallis, $P < 0.05$).

Duration of developmental stages, reproduction, and life table parameters

TRSM did not complete immature development on genotype PI 134417, dying before reaching the deutonymphal stage (Table 2); consistently, the duration of the egg stage on that genotype was the longest. Conversely, TRSM completed immature development on all other genotypes. The duration of the female immature phase was longer on SPJ-10-2017 and F1 than SPJ-05-2018 and TLCV15, given the substantially longer duration of the larval, protonymphal, and deutonymphal stages on the former two genotypes. The duration of the male immature phase was longer on F1 and SPJ-10-2017, but the latter did not significantly differ from TLCV15 and SPJ-05-2018. Among progeny genotypes, the highest immature mortality occurred on SPJ-10-2017.

No significant differences were observed between TLCV15 and other progeny genotypes. Significant differences were observed for pre-oviposition periods (APOP and TPOP) between tomato genotypes. Females had a longer pre-oviposition period on F1 and SPJ-10-2017, starting oviposition about 1.0-1.5 days later on SPJ-05-2018 and TLCV15 genotypes (Table 2).

The number of days in oviposition, female longevity, male longevity, and fecundity of TRSM were substantially reduced on all progeny genotypes than observed on TLCV15.In addition, among progeny genotypes, SPJ-10-2017 differed significantly from other genotypes for female longevity and fecundity. The proportion of females (sex ratio) was significantly higher on F1 than SPJ-10-2017 and SPJ-05-2018, but these did not differ from the parental genotype TLCV15 (Table 2).

Net reproductive rate (R_0), intrinsic rate of increase (r), and finite rate of increase (λ) values were significantly higher on TLCV15, intermediate on F1 and SPJ-05-2018, and lower on SPJ-10-2017 (Table 2). Significant differences from one another were observed between genotypes for average generation time (T) being higher on TLCV15 and lower on SPJ-05-2018.

Table 2 Duration (mean ± SE) of different life stages, pre-adult mortality, pre-oviposition period (APOP), total pre-oviposition (TPOP), longevity oviposition days, sex ratio, fecundity, and life table parameters (R_0, r, λ, and T) of *Tetranychus evansi* on the parental (PI 134417 and TLCV15) and progeny (F1, SPJ-10-2017 and SPJ-05-2018) genotypes from interspecific crossing between PI 134417 and TLCV15. (Numbers in parentheses: n for the respective parameter).

Treatments	*Parental genotypes*		*Breeding tomato genotypes*		
	PI 134417	TLCV15	F1	SPJ-10-2017	SPJ-05-2018
Egg	6.6 ± 0.29a (22)	5.9± 0.06b(104)	5.6 ±0.08c (104)	5.5 ± 0.10c (99)	5.9 ± 0.10b (98)
Larva	2.6± 0.30b (7)	2.3± 0.07b (100)	3.2 ± 0.08a (98)	2.4 ± 0.13b (54)	2.4 ± 0.07b (88)
Protonymph	2.1 ± 0.00b (1)	2.4 ± 0.06ab (89)	2.7 ± 0.1a (94)	2.8 ± 0.26a (37)	2.3 ± 0.06b (85)
Deutonymph	-	2.2 ± 0.10a (82)	2.5 ± 0.11a (81)	2.7 ± 0.24a (21)	2.3 ± 0.11a (80)
Female pre-adult time	-	13.3± 0.17b(49)	14.1 ± 0.22a(58)	15.0 ± 1.15a (8)	13.1 ± 0.2b(41)
Male pre-adult time	-	12.2 ± 0.13b(33)	13.4 ± 0.29a(23)	12.6 ± 0.47ab(13)	12.4 ± 0.16b (39)
Pre-adult mortality (%)	100.0 ± 0.0a (40)	28.7±0.04c(115)	23.9±0.04c(106)	82.1±0.03b(117)	29.2±0.04c(113)
APOP	-	1.5 ± 0.08b(49)	2.4 ± 0.17 a (58)	3.0 ± 2.00a (8)	1.5± 0.14b(41)
TPOP	-	14.8 ± 0.19b(49)	16.5 ± 0.30a (58)	16.5 ± 0.5a (8)	14.7± 0.28b(41)
Oviposition days	-	15.2± 1.06a(49)	4.8 ±0.39b(58)	3.0 ± 1.00c(8)	4.7± 0.43bc(41)
Female adult longevity	-	18.5± 1.18a(49)	9.1 ± 0.58b(58)	4.3 ± 1.19d(8)	7.1± 0.43c(41)
Male adult longevity	-	21.5 ± 1.71a(33)	5.1 ± 0.61b(58)	5.0 ± 1.22b (13)	6.3± 0.47b(39)
Sex ratio(♀/♂+♀)	-	0.6 ±0,05ab (115)	0.7 ± 0.05a(106)	0.4 ±0.01b(117)	0.5 ±0.06b(113)
Fecundity(eggs/female)	-	83.0± 6.1a (49)	12.2 ± 1.82b(58)	2.0 ± 1.73c(8)	15.8 ± 2.23b(41)
R_0 (eggs/individual)	-	35.4± 4.58 a (104)	6.69± 1.15 b (104)	0.14 ± 0.12c(99)	5.73 ± 1.073b(98)
r (day^{-1})	-	0.16± 0.06a(104)	0.093±0.007b(104)	- 0.10± 0.05c(99)	0.099 ± 0.011b(98)
λ (day^{-1})	-	1.18± 0.007a(104)	1.097±0.008b(104)	0.90±0.046(99)c	1.104 ± 0.012b(98)
T (day)	-	21.89±0.3a(104)	20.37 ± 0.5b(104)	19.1 ± 0.03(99)c	17.56 ± 0.22d(98)

Means within a row followed by the same letter are not significantly different. The SEs were estimated by using 100,000 *bootstraps* and means were compared by using paired *bootstrap* test at 5% significance level. R_0 = net reproductive rate; r = intrinsic rate of increase; λ = finite rate of increase; T = mean length of a generation.

Age - survival rate and fecundity of the specific stage

The age-specific survival rate (s_{xj}) of TRSM expresses the probability of the eggs surviving until age x and stage j (Fig. 5a). The probability of a newly deposited egg to reach adulthood on the resistant parental genotype PI 134417 was 0.0%, whereas, on TLCV15, F1, SPJ-10-2017and SPJ-05-2018 the probability was, respectively, 42.6, 50.0, 4.3, and 33.6% for females and 43.5, 17.0, 6.8 and 32.7% for males. After adult emergence, the female survival curve stood out from that of males on TLCV15 and F1 during the life span of TRSM. However, this occurred only until the 39th day of adult emergence on TLCV15.

Age-specific survival rate (l_x: the probability of TRSM to survive up to age x considering all stages of development together), showed that 50.4% of individuals were still alive on TLCV15 on the 26th day of adult emergence, while on F1 only 12.3% were alive on that day; on SPJ-10-2017 and SPJ-05-2018, all individuals died before that date (Fig. 5b).

Age-stage specific fecundity (f_{xj}: the daily number of eggs produced per female of age x) and age-specific fecundity (m_x: average daily fecundity per individuals i.e, this number is divided by all individuals of age x) fluctuated throughout the oviposition period (Fig. 5b). The lowest daily values of f_{xj} and m_x occurred on progeny genotypes. The maximum peak of age-specific daily fecundity was 7.1 eggs and occurred on TLCV15 at 23 days adult emergence, while the lowest was 3.0, 3.9, and 4.3 eggs, respectively on SPJ-10-2017, F1, and SPJ-05-2018, at 24, 19, and 17 day-old TRSM respectively (Fig. 5b).

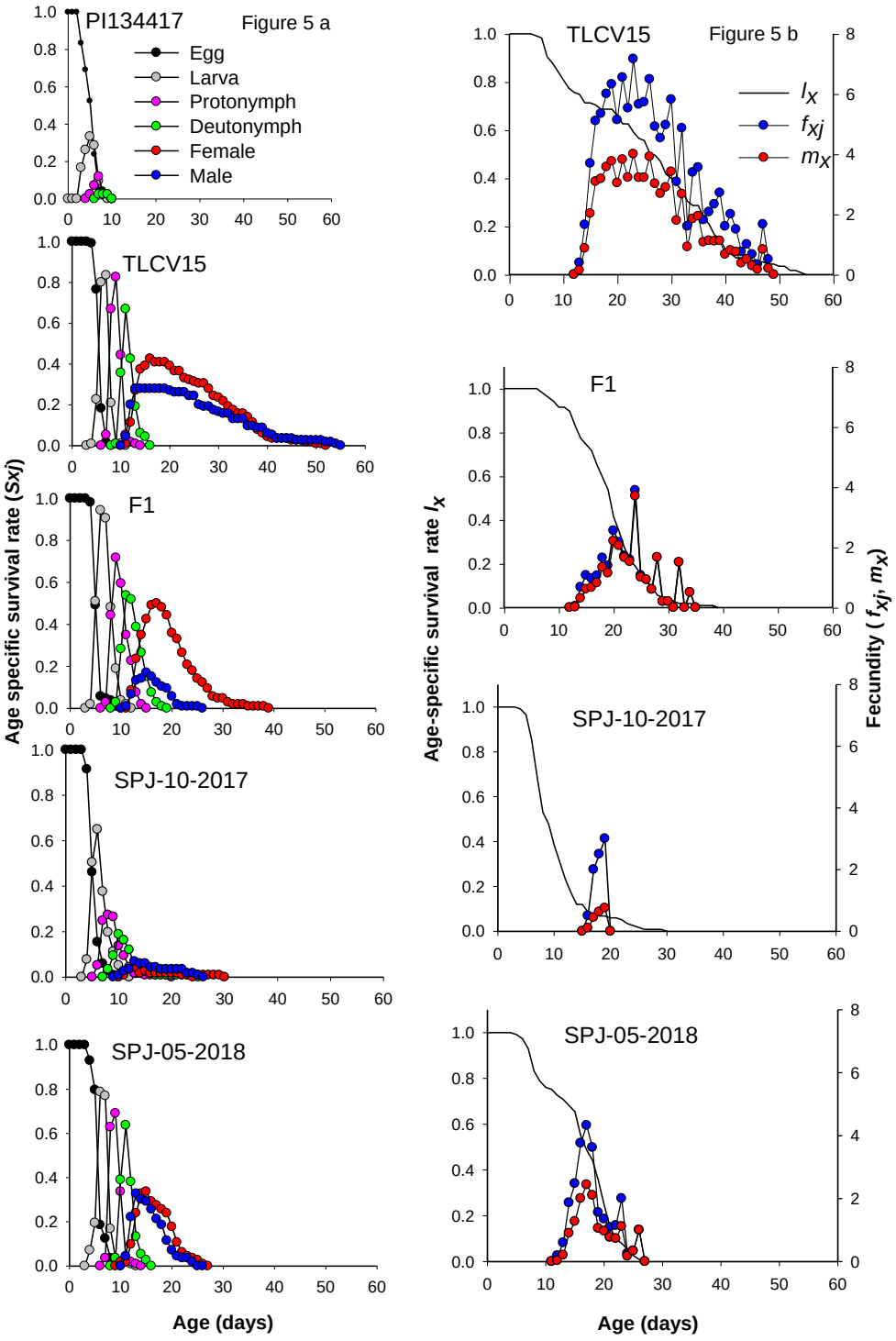

Fig. 5 Variation of survival rates and fecundity of *Tetranychus evansi* on different genotypes along life stages; (a) age-stage specific survival rates (s_{xj}); (b) age-specific survival rate (l_x), age-specific fecundity (m_x) and age-stage-specific fecundity (f_{xj}).

Cluster analysis

The cluster analysis clearly distinguished three genotype groups (Fig. 6). PI 134417 and SPJ-10-2017 constituted the first group (A), corresponding to the genotypes that most affected the behavioral responses and population performance of TRSM.

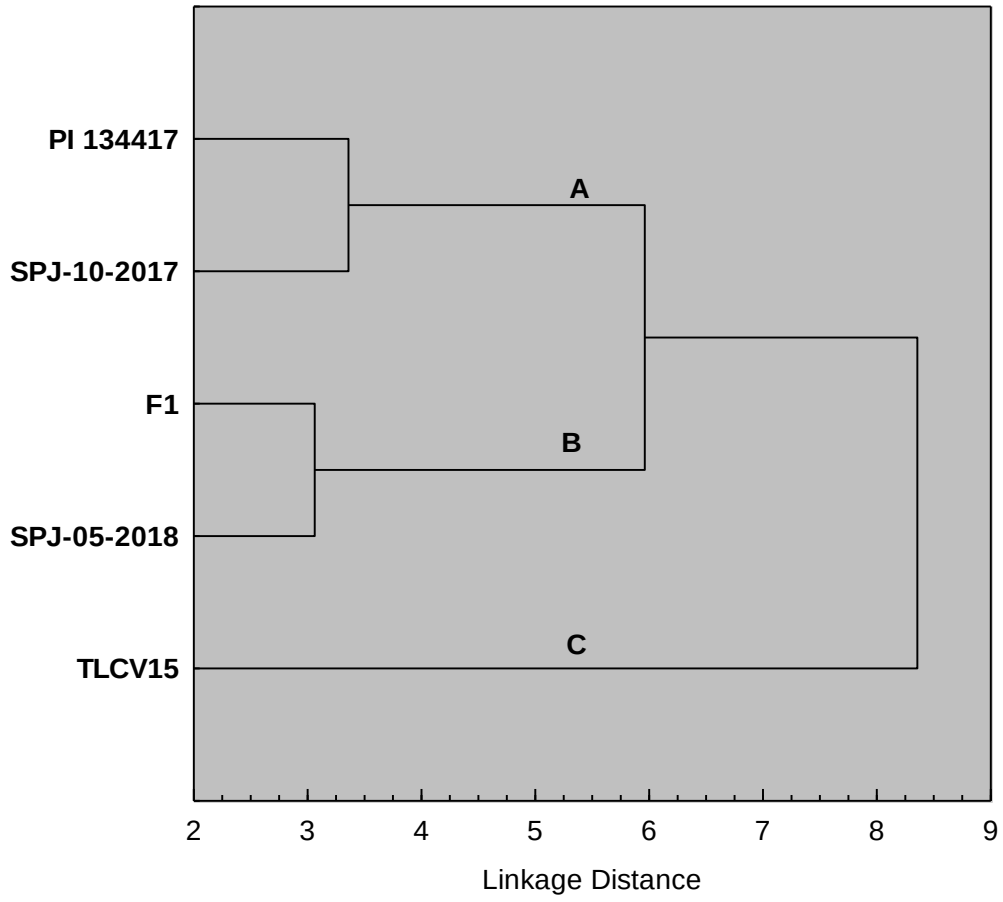

Fig. 6 Dendrogram of different tomato genotypes based on behavioral responses and performance of *Tetranychus evansi*, density, and type trichomes parameters.

F1 and SPJ-05-2018 constituted the second genotype group (B), congregating genotypes that affected moderately the behavior and population performance. TLCV15 constituted by itself group C, as the genotype most suitable for TRSM, isolated from the other two groups. Thus, genotypes PI 134417 and SPJ-

10-2017 were considered resistant, genotypes F1 and SPJ-05-2018, as partially resistant, and TLCV15, as susceptible.

The PC1 has expressed considerable action in the discrimination of groups, retaining most of the information contained in the variables of the experiment (69.54%) while PC2 retaining only 19.83% of the total variability of original variables (Fig. 7).

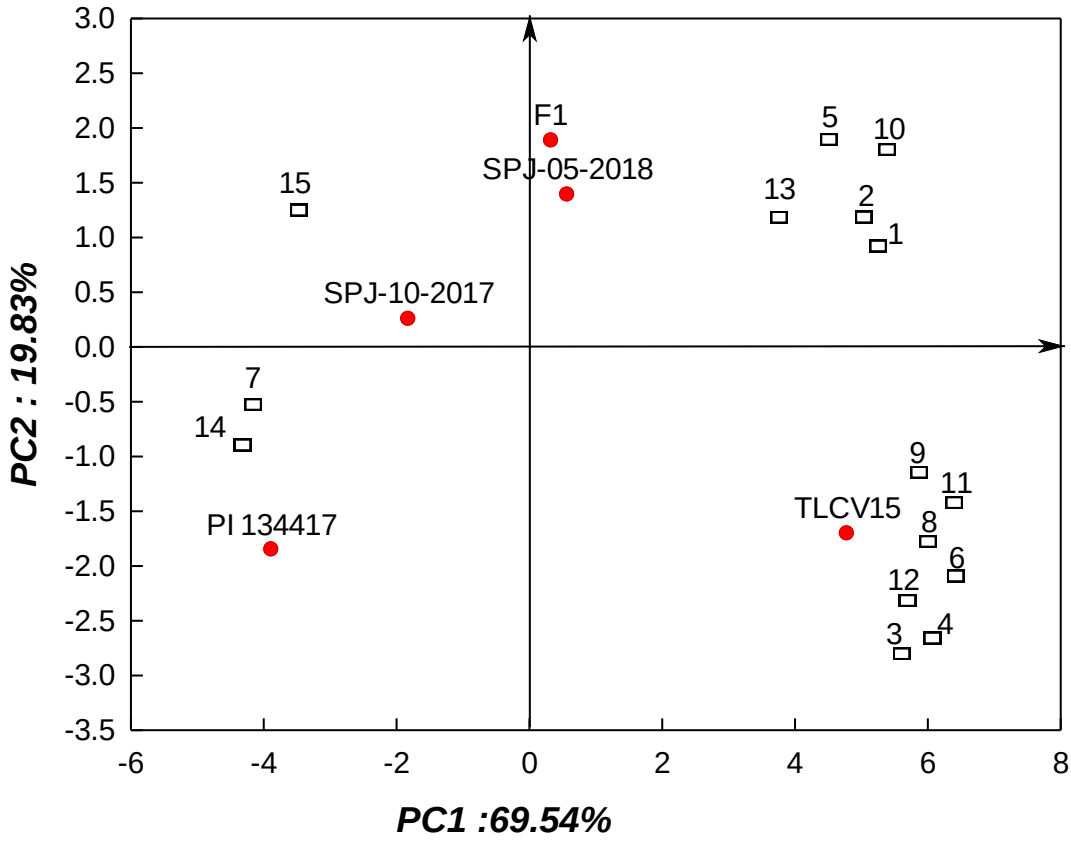

Fig. 7 Principal components analysis ordination diagram of preference and population performance parameters of *Tetranychus evansi*, density and types trichomes parameters on the parental genotypes (PI 134417and TLCV15), and progeny genotypes (F1, SPJ-10-2017 and SPJ-05-2018) from interspecific crossing between PI 134417 and TLCV15. 1- settlement mite; 2- oviposition in the free-choice trial; 3- average walking speed; 4- distance traveled; 5-Development period, 6-Fecundity, 7-Mortality rate; 8-Male longevity; 9- Female longevity; 10- Sex ratio; 11-Days in oviposition; 12- type V non-glandular trichomes; 13- type II+III non-glandular trichomes; 14- type VI glandular trichomes; 15- type I+IV glandular trichomes I+IV.

The significant and negative correlation of PC1 with type VI glandular trichomes (-0.83) and mortality rate (-0.83) variables were responsible for discrimination of group A (PI 134417 and SPJ-10-2017). F1 and SPJ-05-2018 had a negative and significant correlation with type I+IV glandular trichomes (-0.72). The strong contrast observed between genotypes of group B (F1 and SPJ-05-2018) was attributed to a negative and significant correlation between the type I+IV glandular trichomes (-0.72) variable and PC1. The positive and significant correlation of PC1 with settlement mite (0.92), oviposition in the free-choice trial (0.87), mean distance traveled (0.82), mean distance traveled (0.82) fecundity (0.92), male longevity (0.94), female longevity (0.99), days in oviposition (0.97) and type V non-glandular trichomes (0.86) was responsible by isolated TLCV15 (group C). The only relevant information associated with PC2 was the average speed (-0.74).

Discussion

More sustainable approaches are necessary to reduce losses caused by pests while aiming at reducing negative impacts on human health and environmental pollution related to pest control (Sharma and Ortiz 2002; Baker et al. 2020). In tomato, potential approaches include the development and use of genetically modified plants with desirable morphological characteristics leading to resistance to those pests, such as the presence of glandular trichomes, which negatively affect their behavioral responses and population growth (Maluf et al. 2001; Lucini et al. 2015; Savi et al. 2019b; Oliveira et al. 2020). In this study, a wild tomato genotype PI 134417 highly resistant to several pests, including TRSM, was used to increase through interspecific crosses the level of resistance of an important genotype, TLCV15, cultivated by smallholder farmers in West Africa(Benin), but susceptible to TRSM. In general, the types and densities of trichomes on progeny genotypes (F1, SPJ-10-2017 and SPJ-05-2018) were selected from the interspecific crossing of TLCV15 and PI 13447 varied significantly. The variants showed different bottom-up effects on the behavioral response and demographic characteristics of TRSM as

previously reported for other plant genotypes on several other pests (Han et al. 2019; Heidari et al. 2020).

All trichome types found on parental genotypes TLCV15 and PI 134417 occurred also on progeny genotype F1 and other segregating SPJ-10-2017 and SPJ-05-2018 genotypes, suggesting that the trichomes found on the parental genotypes were transferred to the progeny genotypes. However, only F1 and SPJ-10-2017 exhibited higher glandular trichome densities and lower non-glandular trichomes densities, for an average of 47% of the types I + IV + VI, similar to that on PI 134417. Types I and IV trichomes have been reported to be responsible for the main resistance mechanism on hybrid lines from crossing between *S. pennelli* (LA-716) and cultivated genotypes to pest mites (Resende et al. 2008; Lucini et al. 2015).

However, in this study, a considerable number of types I+IV glandular trichomes found on SPJ-10-2017 and F1 progenies led us to believe that the progenies resulting from crossings between PI 134417 and TLCV15 may also cause significant deleterious effects to the mites. A higher density of type VI glandular trichomes was found in SPJ-10-2017 progeny. That glandular trichome is known to be strongly involved in the resistance mechanisms of PI 134417 against several pest-mites (Resende et al. 2008; Oliveira et al. 2018; Savi et al. 2019b) suggesting that SPJ-10-2017 progeny could have a significant negative bottom-up effect on mites.

Although TLCV15 and SPJ-05-2018 selected from segregated population BC1 demonstrated high non-glandular trichome (II + III + V) densities, some glandular trichomes were also found on SP-05-2018, indicating that this genotype retained characters from the resistant parental PI 134417, even after backcrossing. These results can constitute a first step in the initiation of a genetic improvement program for promising resistant tomato genotypes adapted to sustainable production systems in which the use of pesticides could be reduced.

Progeny genotypes with higher glandular trichome densities (F1 and SPJ-10-2017) and parental genotype PI 134417 were the least preferred by adult female TRSM for initial colonization and oviposition under laboratory conditions. These results suggest the existence of antixenoxis resistance rates on progenies F1 and SPJ-10-2017 comparable to that of the parental genotype PI 134417.

However, these results were more conclusive in a free-choice trial performed in a screen-house, when lower settlement levels and initial colonization were noted only on PI 134417 and SPJ-10-2017, genotypes that had the highest type VI glandular trichome densities known for antixenosis resistance (Oliveira et al. 2018; Savi et al. 2019b). Other studies have also reported that wild genotype and their progenies are associated with types VI glandular trichomes, which reduced attractiveness and/ or arrestment as well as oviposition (Maluf et al. 2001; Oliveira et al. 2018; Al-Bayati 2019).

Variation in resistance traits of tomato plants can also drastically affect the behavior of arthropod-pests, including their pattern of walking abilities (Maluf et al. 2001). To date, studies assessing mite locomotion behavior to examine plant bottom-up effects have been conducted using very laborious and limited methods, for instance, the “thumbtack method” (Maluf et al. 2001; Resende et al. 2008; Al-Bayati 2019). This method consists of attaching a leaflet (with the abaxial surface up) to a piece of Styrofoam, pressing a metallic thumbtack through its center, transferring one or more mites onto the head of the thumbtack, and then evaluating traveled by the mite within a specific period (Maluf et al. 2001).

In the present study, a more sophisticated and practical method was used, in which locomotion behavior was recorded for later analyses with video-tracking software developed for that purpose. This method enabled us to accurately determine the locomotion and resting activities of the mites on each evaluated tomato genotype.

The progeny genotypes (F1, SPJ-10-2017, and SPJ-05-2018) considerably affected TRSM locomotion and resting, at a rate comparable to that found on resistant genotype PI 134417. On these genotypes, TRSM locomotion was impaired, resulting in average walking speed being 8 to 677 times lower and mean distance traveled 308 to 681 times shorter than recorded on the susceptible genotype TLCV15. Furthermore, resting periods was 18 to 20 times longer and resting frequency was 62 to 80 times lower (resting frequency very close to 1) on progeny genotypes and resistant parental genotype PI13447 than on TLCV15.

A possible explanation for the deleterious effects of progeny and parental genotype PI 134417 on the locomotion and resting activity of TRSM resides in the presence of sticky exudates of glandular trichomes I, IV and VI found in these genotypes that reduce the walking abilities of mite. Al Bayati (2019) also reported similar results about the strong arrestment of the hybrids derived from the interspecific crossing of wild genotype LA2329 with cultivated genotype Zaofen 2. That resistance is recognized as repellent antixenosis, which suggests that the progenies of our study inherited antixenosis genes for repellency against TRSM from PI 134417(Maluf et al. 2001; Al Bayati 2019).

Toxic compound profiles found in tomato genotypes can trigger cascade effects on the arthropod-pests demographic parameters including developmental time, survival rate, fecundity, and longevity (Lucini et al. 2015; Savi et al. 2019a; Oliveira et al. 2020). In this study, it was found that PI 134417 was not favorable for immature development of TRSM causing 100% mortality before the mites reached the deutonymphal stage, confirming the resistance of this genotype to TRSM (Savi et al. 2019a).

Notwithstanding, TRSM has completed the immature development phase on TLCV15 as well as on the progeny genotypes, the latter caused considerable deleterious effects on some TRSM demographic parameters. For instance, the duration of immature phase development was substantially longer on F1 and SPJ-10-2017, and mortality of immatures was higher on SPJ-10-2017 (82.0%). Furthermore, the days in oviposition, longevity, and fecundity were shorter/ lower on SPJ-10-2017, intermediate on F1, and SPJ-05-2018 compared to those of TLCV15. Part of these parameters obtained with progeny genotypes in this study is also much lower than those found in previous studies with the same mite on other cultivated tomato genotypes. For instance, values of fecundity and longevity of TRSM reported by Bonato (1999) and Savi et al. (2019a) respectively on Carioca-INRA tomato variety and African tomato varieties (Tounvi, Kekefo, Akikon, and TOML4) at 25 or 26^{o} C were substantially greater (74.8–111.1eggs/female and 16.1–20.4 days) than those obtained on progeny genotypes of this study.

The negative bottom-up effects of progeny genotypes on TRSM demographic parameters were possibly due to increased toxic substances, such as methyl ketones, 2-tridecanone, and 2-undecanone (Kennedy 2003; Keskin and Kumral 2015), found in large amounts in types I, IV, and VI glandular trichomes inherited by these progenies from their resistant parental genotype PI 134417. Oliveira et al. (2018) reported that genotypes selected for high density of glandular trichomes IV and VI, from the interspecific crossing between the Brazilian genotype *S. lycopersicum* cv. Redenção and the wild genotype *S. habrochaites* var. access to *hirsutum* PI-127826 were associated with a high content of toxic compounds such as zingiberene which provided an increase in the duration of the egg stage, total mortality of nymphs, and reduced fecundity in *T. urticae*.

Life table parameters are accurate and reliable tools to track the bottom-up effects of host plants on predator and prey populations (Khanamani et al. 2015; Fathipour et al. 2019; Savi et al. 2021). Among these, the intrinsic rate of increase (*r*) is the parameter that best reflects the combined effects of biological attributes such as survival, sex ratio, developmental duration, and fecundity (Janssen and Sabelis 1992). In the present study, the intrinsic rate of increase showed lower value on the progeny genotypes compared to that on TLCV15 as well as that found also on other cultivated tomato genotypes of previous studies under the same conditions (Bonato 1999; Savi et al. 2019a). These findings also suggest the existence of antibiosis resistance in the progeny genotypes. Actually, on the progeny genotype SPJ-10-2017, the negative rate of increase (*r*) obtained indicates that this genotype may lead to the suppression of the population of TRSM over time. Interestingly, it was in this progeny that the highest density of type VI glandular trichomes was found, further supporting the role of this trichome in the bottom-up effects control of TRSM in tomato plants. For advances in improving TLCV15 in the future, particular attention should be paid to SPJ-10-2017 by backcross hybrids advanced until completely inbred lines will be obtained for production in the field.

Although enlightening, the progeny genotypes obtained in this study are certainly preliminary in what concerns resistant genotypes and adequate for production and adaptation in the field. Had backcross hybrids advanced until

completely inbred lines had been obtained, it is uncertain whether these would still keep the same level of negative bottom-up effect on the pest mite population obtained in this study. For this, the bottom-up effect would need to be expanded on the advanced progeny hybrids to be obtained in the next step for field usefulness.

ACKNOWLEDGMENTS

To Coordination for the Improvement of Higher Education Personnel (CAPES), Brazil – Finance Code 001, for granting scholarships to PJS and the National Council for Scientific and Technological Development (CNPq) for granting the research productivity scholarship-Case No. 301492 / 2018-2 to DJA. We also thank Prof. Dr. Wesley Augusto Conde Godoy and Dr. José Bruno Malaquias (Insect Ecology Laboratory, Department of Entomology and Acarology, University of São Paulo) (ESALQ/USP), Piracicaba, São Paulo, Brazil for providing and assisting in the video tracking on EthoVision XT software.

REFERENCES

Alba JM, Montserrat M, Fernandez-Munoz R (2008) Resistance to the two-spotted spider mite (*Tetranychus urticae*) by acylsucroses of wild tomato (*Solanum pimpinellifolium*) trichomes studied in a recombinant inbred line population. Exp Appl Acarol 47:35-47. https://doi.org/10.1007/s10493-008-9192-4

Al-Bayati AS (2019) Breeding for tomato resistance to spider mite *Tetranychus urticae* Koch (Acari: Tetranychidae), Theses, University of Kentucky.

Antonious GF, Snyder JC (2008) Tomato leaf crude extracts for insects and spider mite control. In: V. R. Preedy, Watson RR (eds) Tomatoes, and tomato products: nutritional, medicinal and therapeutic properties. Science Publishers: Enfield: 269-297.

Azandémè-Hounmalon YG, Affognon HD, Assogba KF, Tamo M, Fiaboe KKM, Kreiter S, Martin T (2015) Farmers control practices against the invasive red spider mite, *Tetranychus evansi* Baker & Pritchard in Benin. Crop Prot 76: 53-58. https://doi.org/10.1016/j.cropro.2015.06.007

Baker BP, Green TA, Loker AJ (2020) Biological control and integrated pest management in organic and conventional systems Biol Control 140: 1040952. https://doi.org/10.1016/j.biocontrol.2019.104095

Bates D, Mächler M, Bolker B, Walker S (2015). Fitting linear mixed-effects models using lme4. J Stat Softw 67: 1–48.

Bleeker PM, Mirabella R, Diergaarde PJ, VanDoorn A, Tissier A Kant, MR Prins M, de Vos M, Haring MA, Schuurink RC (2012) Improved herbivore resistance in cultivated tomato with the sesquiterpene biosynthetic pathway from a wild relative Proc Natl Acad Sci 49: 20124-20129. doi:10.1073/pnas.1208756109

Bonato O (1999) The effect of temperature on life-history parameters of *Tetranychus evansi* (Acari: Tetranychidae). Exp Appl Acarol 23: 11–19.

Boubou A, Migeon A, Roderick G. Navajas M (2010) Recent emergence and worldwide spread of the red tomato spider mite, *Tetranychus evansi*: genetic variation and multiple cryptic invasions. *Biol Invasions* 13: 81–92.

Campos ML, de Almeida M, Rossi ML, Martinelli AP, Junior CGL, Figueira A, Rampelotti-Ferreira FT, Vendramim JD, Benedito VA, Peres LEP (2009) Brassinosteroids interact negatively with jasmonates in the formation of anti-herbivory traits in tomato. J Exp Bot 60: 4347- 4361. https://doi.org/10.1093/jxb/erp270

Chi H (2020a) TWOSEX-MSChart: a computer program for the age-stage, two-sex life table analysis. (http://140.120.197.173/Ecology/prod02.htm) (Accessed June 2020).

Chi H, You MS, Atlihan R, Smith CL, Kavousi A, Ozgokçe MS, Guncan A, Tuan SJ, Fu JW, Xu YY, Zheng FQ, Ye BH, Chu D, Yu Y, Gharekhani G, Saska P, Gotoh T, Schneider MI, Bussaman P, Gokçe A, Liu TX (2020b) Age-stage, two-sex life table: an introduction to theory, data analysis, and application. Entomol Gen https://doi.org/10.1127/entomologia/2020/0936

de Moraes GJ, Flechtmann CHW (2008) Manual de acarologia: acarologia básica e ácaros de plantas cultivadas no Brasil. Ribeirão Preto: Holos. 208p.

Demétrio CGB, Hinde J, Moral RA (2014) Models for overdispersed data in entomology. In: Ferreira CP, Godoy WAC (Eds.), Ecological Modeling Applied to Entomology. Springer, Switzerland, 219–259.

Efron B, Tibshirani RJ (1993) An introduction to the bootstrap. Chapman & Hall, New York

Fathipour Y, Maleknia B, Bagheri A, Soufbaf M, Zalucki MP (2019) Spider mite host plant resistance traits improve the predatory performance of *Phytoseiulus persimilis*

on cucumber, despite negative life history impacts. Biol Control 104064. https://doi.org/10.1016/j.biocontrol.2019.104064

Ferrero M, Calvo FJ, Atuahiva T, Tixier MS, Kreiter S (2011) Biological control of *Tetranychus evansi* Baker & Pritchard and *Tetranychus urticae* Koch by *Phytoseiulus longipes* Evans in tomato greenhouses in Spain—Acari: Tetranychidae, Phytoseiidae. Biol Control 58: 30–35. https://doi.org/10.1016/j.biocontrol.2011.03.012

Furtado IP, de Moraes GJ, Kreiter S, Tixier MS, Knapp M (2007) Potential of a Brazilian population of the predatory mite *Phytoseiulus longipes* as a biological control agent of *Tetranychus evansi* (Acari: Phytoselidae: Tetranychidae). Biol Control 42: 139–147.

Hair JF Jr, Black WC, Babin BJ, Anderson RE, Tatham RL (2006) Multivariate data analysis, 6th edn. Pearson Prentice Hall, New Jersey.

Han P, Desneux N, Amiens-Desneux E, Le Bot J, Bearez P, Lavoir AV (2016) Does plant cultivar difference modify the bottom-up effects of resource limitation on plant–herbivorous insect interactions? J Chem Ecol 42:1293–1303.

Han P, Desneux N, Becker C, Larbat R, Le Bot J, Adamowicz S, Zhang J, Lavoir AV (2019) Bottom-up effects of irrigation, fertilization and plant resistance on *Tuta absoluta*: implication for Integrated Pest Management. J Pest Sci 92:1359–1370.

Handford CE, Elliott CT, Campbell K (2015) Review of the global pesticide legislation and the scale of challenge in reaching the global harmonization of food safety standards. Integr Environ Asses 11:525–536.

Heidari N, Sedaratian-Jahromi A, Ghane-Jahromi M, Zalucki MP (2020) How bottom-up effects of different tomato cultivars affect population responses of *Tuta absoluta* (Lep.: Gelechiidae): a case study on host plant resistance. Arthropod-Plant Int 14:181–192.

Hufbauer RA, Torchin ME (2007) Integrating ecological and evolutionary theory of biological invasions. In: Nentwig W (ed) biological invasions. Springer, Berlin, pp 79–96

Janssen A, Sabelis MW (1992) Phytoseiid life-histories, local predator-prey dynamics, and strategies for control of tetranychid mites. Exp Appl Acarol 14: 233-250. https://doi.org/10.1007/bf01200566

Kaiser HF (1958) The varimax criterion for analytic rotation in factor analysis. Psychometrika 23:187-200

Kennedy GG (2003) Tomato, pests, parasitoids, and predators: tritrophic interactions involving the genus *Lycopersicon*. Ann Rev Entomol. 48: 51–72. https://doi.org/10.1146/annurev.ento.48.091801.112733

Keskin N, Kumral NA (2015) Screening tomato varietal resistance against the two-spotted spider mite [*Tetranychus urticae* (Koch)]. Int J Acarology 41: 300–309. https://doi.org/10.1080/01647954.2015.1028440

Khanamani M, Fathipour Y, Hajiqanbar H (2015) Assessing compatibility of the predatory mite *Typhlodromus bagdasarjani* (Acari: Phytoseiidae) and resistant eggplant cultivar in a tritrophic system. Ann Entomol Soc Am 108: 501-512. https://doi.org/10.1093/aesa/sav032

Lucini T, Faria MV, Rohde C, de Resende JTV (2015) Acylsugar and the role of trichomes in tomato genotypes resistance to *Tetranychus urticae*. Arthropod-Plant Int 9: 45-53.

Luckwill LC (1943) The genus *Lycopersicon*: an historical, biological, and taxonomic survey of the wild and cultivated tomatoes. Aberdeen University Press.

Maciel GM, Marquez GR, Silva ECD, Andaló V, Belloti IF (2018) Tomato genotypes with determinate growth and high acylsugar content presenting resistance to spider mite. Crop Breed Appl Biotechnol 18: 1-8.

Maluf WR, Campos GA, Cardoso MG (2001) Relationships between trichome types and spider mite (*Tetranychus evansi*) repellence in tomatoes with respect to foliar zingiberene contents. Euphytica 121: 73-80.

Maluf WR, Maciel GM, Gomes LAA, Cardoso MG, Gonçalves LD, Silva EC, Knapp M (2010) Broad-spectrum arthropod resistance in hybrids between high- and low-acylsugar tomato lines. Crop Sci 50: 439–450.

Meynard CN, Migeon A, Navajas M (2013) Uncertainties in predicting species distributions under climate change: a case study using *Tetranychus evansi* (Acari: Tetranychidae), a widespread agricultural pest. PLoS One 8: e66445.

Migeon A, Dorkeld F (2021) Spider Mites Web: a comprehensive database for the Tetranychidae. INRA, http://www.montpellier.inra.fr/CBGP/spmweb/

Navajas M, Moraes GJ, Pigeon PAA (2012) Revision of the invasion of *Tetranychus evansi*, biology, colonization pathways, potential expansion and prospective for biological control. Exp Appl Acarol 59: 43–65.

Nyoni BN, Gorman K, Mzilahowa T, Williamson MS, Navajas M, Field LM, Bass C (2011) Pyrethroid resistance in the tomato red spider mite, *Tetranychus evansi*, is associated with mutation of the para-type sodium channel Pest Manag Sci 67: 891–897. https://doi.org/10.1002/ps.2145

Oliveira JRF, Resende JTV, Filho RBL, Roberto SR, Silva PR, Rech C, Nardi C (2020) Tomato breeding for sustainable crop systems: high levels of zingiberene providing resistance to multiple arthropods. *Horticulturae* 6: 34-48. https://doi.org/10.3390/horticulturae6020034

Oliveira JRF, de Resende JTV, Maluf WR, Lucini T, de Lima Filho RBL, de Lima IP, Nardi C (2018) Trichomes and allelochemicals in tomato genotypes have antagonistic effects upon behavior and biology of *Tetranychus urticae*. Front Plant Sci 9. https://doi.org/10.3389/fpls.2018.01132

R Development Core Team (2019) R: A Language and Environment for Statistical Computing. Vienna.

Resende JTD, de Maluf WR, Cardoso MG, Faria MV, Gonçalves LD, Nascimento IR (2008) Resistance of tomato genotypes with high level of acyl sugars to *Tetranychus evansi* Baker & Pritchard. Sci Agric 65: 31–35

Sarr I, Knapp M, Ogol CKP, Baumgärtner J (2002) Impact of predators on *Tetranychus evansi* Baker and Pritchard populations and damage on tomatoes (*Lycopersicon esculentum* Mill.) in Kenya. Abstract retrieved from Abstract Abstract book of the 11th International Congress of Acarology, Merida, Mexico (No 271).

Savi PJ, de Moraes GJ, Boiça Junior AL, Melville CC, Carvalho RF, Lourenção AL, Andrade DJ (2019a) Impact of leaflet trichomes on settlement and oviposition of *Tetranychus evansi* (Acari: Tetranychidae) in African and South American tomatoes. Syst Appl Acarol 24: 2559–2576. https://doi.org/10.11158/saa.24.12.19

Savi PJ, de Moraes GJ, Melville CC, Andrade DJ (2019b) Population performance of *Tetranychus evansi* (Acari: Tetranychidae) on African tomato varieties and wild tomato genotypes. Exp Appl Acarol 77: 555–570.

Savi PJ, de Moraes GJ, Andrade DJ (2021) Effect of tomato genotypes with varying levels of susceptibility to *Tetranychus evansi* on performance and predation capacity of *Phytoseiulus longipes*. BioControl. https://doi.org/10.1007/s10526-021-10096-5

Schoonhoven LM, van Loon JJA, Dicke M (2005) Insect-plant biology. Oxford University Press, Oxford

Sharma HC, Ortiz R (2002) Host plant resistance to insects: an eco-friendly approach for pest management and environment conservation J Environ Biol 23: 111-135.

Silva P (1954) Um novo ácaro nocivo ao tomateiro na Bahia. Bol Inst Biol Bahia 1: 1–20.

Simmons AT, Gurr GM (2005) Trichomes of *Lycopersicon* species and their hybrids: effects on pests and natural enemies. J For Res 7: 265–276. https://doi.org/10.1111/j.1461-9563.2006.00271.x

Statsoft I (2012) STATISTICA (Data Analysis Software System); Version 11; Science Open: Berlin, German.

Van der Putten WH, Vet LEM, Harvey JA, Wäckers FL (2001) Linking above- and belowground multitrophic interactions of plants, herbivores, pathogens, and their antagonists. Trends Ecol Evol 16: 547–554

Ximénez-Embún MG, Ortego F, Castañera P (2016) Drought stressed tomato plants triggers bottom-up effects on the invasive *Tetranychus evansi*. PLoS One 11:e0145275.

CHAPTER 3 - Effect of tomato genotypes with varying levels of susceptibility to *Tetranychus evansi* on performance and predation capacity of *Phytoseiulus longipes*

Patrice Jacob Savi[1*], Gilberto José de Moraes[2], Daniel Júnior de Andrade[1]

[1] São Paulo State University (UNESP), College of Agricultural and Veterinary Sciences, Jaboticabal, São Paulo, Brazil.

[2] Luiz de Queiroz College of Agriculture, University of São Paulo (ESALQ/USP), Piracicaba, São Paulo, Brazil.

[*]Corresponding Author: savipatricejacob@yahoo.fr

Abstract

Tetranychus evansi Baker & Pritchard (Acari: Tetranychidae) is an invasive tomato pest in several African, American, Asian, and European countries, with the potential to reduce yield by up to 90%. Populations of the predaceous phytoseiid mite *Phytoseiulus longipes* Evans (Acari: Phytoseiidae) have been reported to have a high potential for controlling *T. evansi*. However, tomato genotypes can potentially affect the performance of *T. evansi* and the predation ability of *P. longipes*. We evaluated the performance and predation capacity of *P. longipes* feeding on *T. evansi* nymphs on the two cultivated genotypes widely grown in Western Africa and a breeding genotype. The two commercially evaluated genotypes were Tounvi (susceptible, with few trichomes), TLCV15 (susceptible, with many trichomes), and the breeding genotype was SPJ-05-2018 (partially resistant with many glandular trichomes) selected from interspecific crossing between and PI 13447(highly resistant wild tomato genotype). We hypothesized that the susceptible genotype (with fewer trichomes) would be more favorable for *P. longipes* when compared to the susceptible or partially resistant genotypes (with a higher number of non-glandular or glandular trichomes). The intrinsic rate of increase (*r*) and predation potential (ω) of *P. longipes* were significantly reduced on SPJ-05-2018, possibly due to a negative effect of its glandular trichomes. Although the intrinsic rate of increase was higher on both susceptible genotypes (TLCV15 and Tounvi), predation potential was higher on TLCV15. Our results refuted the hypothesis that *T. evansi*-susceptible genotype with fewer trichomes would be more favorable to *P. longipes.*

Keywords: two-sex life table, population, predation potential, phytoseiid mite, trichomes

Article published in "Biocontrol" (Impact factor: 3.571)

Introduction

The tomato red spider mite (TRSM), *Tetranychus evansi* Baker and Pritchard (Acari: Tetranychidae) is an invasive pest of tomato and other solanaceous crops in several African, American, Asian, and European countries, with the potential to cause up to 90% yield reduction (Sarr et al. 2002, Navajas et al. 2012). In the Republic of Benin (West Africa), tomato yield losses have been estimated to reach up to 56% (Azandémè-Hounmalon et al. 2015), as a result of damage caused by this mite to leaves, substantially reducing plant photosynthetic capacity. Additionally, defoliation exposes fruits to the sun and prevents them from developing their characteristic red color, depreciating commercial value (de Moraes and Flechtmann 2008).

Chemical control is the main method for controlling TRSM. However, this method is not always effective, since this mite develops rapidly and has a high reproductive capacity which leads producers to intensify chemical sprays for controlling it (de Moraes and Flechtmann 2008; Nyoni et al. 2011; Azandémè-Hounmalon et al. 2015). As consequence, the pest becomes more prone to developing pesticide resistance, often leading to the occurrence of environmental pollution, and poisoning risks to farmers and consumers (Handford et al. 2015). For this reason, alternative control measures have been sought worldwide (Furtado et al. 2007; Silva et al. 2010; Ferrero et al. 2007, 2013).

Predatory mites of the family Phytoseiidae are important agents for phytophagous mite control. Species of this family have been commercialized to suppress pest mite populations (McMurtry et al. 2013). Their feeding preference for phytophagous mites, short life-cycle, and feasible large-scale production make them good candidates for pest control (Abad-Moyano et al. 2009). After considerable search efforts in South America, possible place of origin of TRSM, the phytoseiid *Phytoseiulus longipes* Evans was the only promising predator found for the control of TRSM (Furtado et al. 2007; Silva et al. 2010; Ferreiro et al. 2011).

Pest populations are affected by several factors, including constitutive morphological and chemical traits of their host plants (Koller et al. 2007; Khanamani

et al. 2015; Fathipour et al. 2019). These factors can not only affect herbivores but also their predators. Trichomes are some of these factors that can interfere with these organisms (Koller et al. 2007; Sato et al. 2011). Seven trichome types have been identified on tomato plants (Simmons and Gurr 2005), grouped as non-glandular (II, III, and V) and glandular (I, IV, VI, and VII). Non-glandular trichomes interfere mostly physically with pests and predators, while glandular trichomes interfere physically and chemically, given their content of acyl sugars, methyl ketones, and sesquiterpenes, rendering plants less suitable to hosting pests and predators (Maluf et al. 2010; Khanamani et al. 2014; Lucini et al. 2015; Savi et al. 2019a).

In a previous study, we compared the resistance to TRSM of five tomato genotypes commonly grown in West Africa (Benin) (Kekefo, TOML4, Akikon, Tounvi, and TLCV15) and two wild South American genotypes (*Solanum habrochaites* Knapp & Spooner [PI134417 and PI134418]). The results showed that all cultivated tomato genotypes were more favorable to TRSM development and reproduction than the wild genotypes, which were highly resistant (Savi et al. 2019a, b). These results were related to the lower density or even absence of type I, IV, and VI glandular trichomes (Savi et al. 2019b) on the cultivated genotypes. We then selected one partially-resistant genotype (SPJ-05-2018) from the interspecific crossing between a highly susceptible genotype (TLCV15) and a highly resistant tomato genotype (PI134417) (Savi et al. unpublished).

The present study is a continuation of the preceding studies, intending to evaluate the hypothesis that a tomato genotype susceptible to TRSM (with few trichomes) is more favorable to *P. longipes* than the susceptible or partially resistant ones (high non-glandular or glandular trichome densities). Knowing the performance of a predator on different genotypes can be useful for practical purposes.

Life table parameters are important tools to track how predator and prey populations are affected by susceptible and resistant host plants (Khanamani et al. 2015; Fathipour et al. 2019). All recent studies on *P. longipes* population parameters have been based on a traditional life table analysis (Furtado et al. 2007; Ferrero et al. 2007, 2013). This method considers only females, disregarding the male

population and variation in developmental rates among individuals of a population. According to Chi and Liu (1985), this may result in errors in the estimation of life table parameters.

Given these limitations, Chi and Liu (1985) and Chi (1988) developed a theoretical model of life-table analysis. It considers ages or stages and development rates of both sexes. Such design allows deeper knowledge of arthropod biology and population growth parameters, which are fundamental for pest management efficiency. Additionally, the construction of life tables with this model allows a more accurate analysis of predator efficacy, which cannot be determined based solely on predation rate estimates (Yu et al. 2013). Instead, predation potential can be properly estimated by the predation rate and population growth of a given predator (Chi et al. 2011; Yu et al. 2013). This study compared *P. longipes* population performance and predation potential on tomato genotypes with different TRSM susceptibility levels, considered to be related by trichome types and densities.

Materials and Methods

Studied genotypes

Two susceptible (*Solanum lycopersicum* L. cv. Tounvi and TLCV15) and one partially resistant (SPJ-05-2018) genotypes (Savi et al. 2019a, b; Savi et al. unpublished) were selected. Tounvi was selected for having relatively low non-glandular (3.33/mm^2) and glandular (0.85/mm^2) trichome densities while TLCV15 was selected for having high non-glandular (39.0/mm^2) and low glandular (0.67/mm^2) trichome densities (Savi et al. 2019a, b). Both genotypes are relevant for smallholder farmers in Benin (Cabev 2016). Seeds were provided by “Institut National des Recherches Agricoles du Bénin” (INRAB), imported under authorization from Brazilian Ministry of Agriculture, Livestock and Supply (Process #258/2016/SSV-SP), and approved under LQV report # 20170072.

Compared to the previously mentioned genotypes, SPJ-05-2018 genotype was composed of plants that have much higher densities of glandular (4.25/mm^2)

and intermediate non-glandular trichomes (25.41/mm^2) selected from a segregating population referred to as BC1 (Savi et al unpublished). BC1 was obtained from crossing the genotype PI134417 (*Solanum habrochaites* Knapp & Spooner [seeds from pollen donor parent supplied by the “Instituto Agronômico de Campinas”, São Paulo, Brazil]) and the genotype TLCV15 (female parent) to produce F1 hybrid seeds. After that, F1 was used as pollen donor for crosses involving TLCV15 to obtain seeds that were used to produce plants population of BC1.

Tounvi, TLCV15, and BC1 seeds were sown in Styrofoam cells filled with Bioplant® substrate (coconut fiber, pine bark, bovine manure, sawdust, vermiculite, rice husks, ashes, plaster calcium carbonate, magnesium, magnesium thermophosphate, and fertilizers) and kept in a greenhouse. Irrigation was performed four times a day (8, 11, 14, and 17 h) by automatic sprinklers. Fertilizer (250 ml per tray of 10 g mono ammonium phosphate (MAP) diluted in 1 liter of water) was applied two weeks after germination and two weeks later.

Seedlings were individually transplanted to 4-l pots, 80% filled with a homogeneous mixture of soil, sand, and tanned bovine manure, which was previously sterilized in an autoclave at 120 ºC for 3 h. Plants were irrigated daily to field capacity and never sprayed with pesticides; each plant was fertilized every 15 days with 5 g of urea (44% N) and 5 g of potassium chloride (58% K_2O). The plants were maintained in a greenhouse with no control of environmental conditions. SPJ-05-2018 plants selected from the BC1 population had an average of at least four glandular trichomes/mm^2 by analyzing both surfaces of ten (10) leaflets per plant randomly collected.

Tetranychus evansi stock colony

Mites of this species were obtained from a colony maintained on American black nightshade (*Solanum americanum* Mill). Sixty days before starting the experiments, mites were transferred to plants of the respective genotypes to be tested, onto which they were reared for five generations, for adaptation. In this period, plants with severely damaged or senescent leaves were replaced with new

ones. The tomato plants were maintained in a climate-conditioned chamber at 25 ± 1ºC, 70 ± 10% relative humidity, and 12 hours photoperiod.

Phytoseiulus longipes stock colony

This colony was initiated with specimens from *S. lycopersicum*, *S. americamum*, and *Brugmansia suaveolensis* L. plants (Solanaceae) growing naturally in the urban area of Uruguaiana, Rio Grande do Sul, Brazil (29°49'48.0" S, 57°06'04.0" W and 29°45'12.0" S, 57°04'31.0" W). The mites were reared for three generations on excised leaflets of either Tounvi, TLCV15, or SPJ-05-2018 genotypes infested with TRSM and held onto a synthetic plate (Paviflex®; 22 x 15 cm) resting onto a piece of 3 cm thick foam mat in a plastic tray (25 x 17 x 9 cm). The mat was maintained permanently wet by the periodic addition of deionized water. The edge of the plate was covered with a strip of moistened cotton wool, which in contact with the mat prevented mites from escaping. To hold longer, the petioles of the leaflets were inserted in the strip of cotton wool. Infested leaflets were periodically replaced, and the trays were maintained in a climate-controlled chamber, under the same conditions mentioned in the previous section.

Experimental procedure

Each experimental unit consisted of a synthetic plate of the same type mentioned in the previous section (15 x 20 mm) resting onto a 1-cm-thick piece of foam mat inside a rectangular plastic dish (50 x 20 mm) and maintained wet by periodic addition of deionized water. To prevent mites from escaping, the plate edge was covered with a strip of cotton wool that contacted the mat. To obtain eggs of uniform age, 50 gravid *P. longipes* were transferred from the stock colony to a leaflet of each genotype infested by an unestimated but surplus number of TRSM, excised from the median third of the canopy of 35-50 day-old plants. Twelve hours later, 80 eggs were individualized in experimental units containing a leaflet selected at most as large as the synthetic plate onto which it rested. Leaflet petioles were inserted in

the wet cotton wool strip, to maintain turgidity. The experimental units were kept in a climate-conditioned chamber under the same climatic conditions mentioned for the stock colonies. Upon reaching adulthood, mites were sexed and transferred to new experimental units, to form couples. For this purpose, some males (of unknown age) were taken from the stock colony of the corresponding genotype.

At 6 AM and 6 PM, the units were examined under a stereomicroscope to determine the duration of each developmental stage and survivorship. Mite size, moving ability, and presence of exuviae were observed to determine moulting. The following parameters were determined: pre-oviposition (APOP: the period between adult emergence and its first oviposition), total pre-oviposition (TPOP: the period between first oviposition of parent and offspring generation), oviposition days (number of days in which oviposition occurred), the longevity of each sex, sex ratio, and fecundity.

In units where males died before females, other males from the stock colony were used to replace them. Data on males that came from stock colonies and mites that died on the strip of cotton wool while trying to escape were not used in statistical analysis. Eggs laid were removed at every observation period. Leaflets containing the prey were replaced by new ones every three days to maintain adequate physiological conditions. The experiment was considered finished after all mites died.

The software TWOSEX-MSChart by Chi (2020a), available at http://140.120.197.173/Ecology/prod02.htm was used to estimate development and reproduction raw data and to calculate population parameters, using the procedure “two-sex life table” (Chi and Liu, 1985; Chi, 1988). The following parameters were estimated: age-stage–specific survival rate (s_{xj}), age-specific survival rate (l_x), age-specific fecundity (m_x), net reproduction rate (R_0), intrinsic rate of increase (r), finite rate of increase (λ), and average generation time (T).

Predation potential of *P. longipes* was assessed by providing separately TRSM nymphs reared on each genotype. We focused on nymphs (protonymphs and/ or deutonymphs) of TRSM to feed *P. longipes*, as suitable stages to assess the effect of the different trichomes and the compounds ingested by the prey when

feeding on the tested genotypes, as well as predator ability to kill the prey. Based on preliminary tests, 20 TRSM nymphs were supplied daily to predator protonymphs and deutonymphs. No food was given to larvae, as preliminary data indicated that they did not feed. Replenishment of attacked prey was done carefully, to reduce trichome damage. After predator adult emergence, couples were formed and 40 TRSM nymphs were provided daily as food. To distinguish male from female predation rates, the predation rates of 20 males were evaluated under similar conditions. Female daily predation was estimated by subtracting the average male daily predation from couple predation rates (Farhadi et al. 2011). For immatures and adults, the experimental units were examined under a stereomicroscope every 12 h, recording the number of prey attacked until the death of both predators of each unit.

Daily predation of all individuals (males, females, and dead immatures) was used to calculate the age-specific consumption rate (c_{xj}: mean number of prey consumed by an individual predator in age x and stage j), age-specific predation rate (k_x : the mean number of prey consumed by a predator at age x), net age-specific predation rate (q_x), net predatory rate (C_0 : mean number of prey consumed by an average individual predator during its life span), rate of prey transformation into predator offspring (Q_p: mean number of prey that a predator needs to consume to produce an offspring), stable rate of predation (ψ: the total predation capacity of a stable population whose total size is one), finite predation rate (ω: the predation potential of a predator population), intrinsic predation rate (ln ω), and NC_0 (The total number of killed prey by a cohort of size N) (Chi and Yang 2003; Yu et al. 2013).

The software CONSUME-MSChart by Chi (2020b), available at http://140.120.197.173/Ecology/prod02.htm was used to analyze predation rate data. Variations and standard errors of predation parameters were estimated using the bootstrap resampling method. Standard errors of development, fecundity, reproduction period, and population and predation parameters were also estimated using the bootstrap procedure included in the TWOSEX-MS chart software. The bootstrap analysis uses random sampling. With a small number of samples, it will generate variable means and standard errors. Hence, we used 10,000 random resampling to reduce the variability of the estimates of the standard errors (Efron

and Tibshirani 1993). A paired bootstrap (B = 100,000) test based on the 95% confidence interval of differences was used to compare the difference between genotypes (Hesterberg et al. 2005; Smucker et al. 2007; Wei et al. 2020).

Results

Life stage durations, fecundity, sex ratio, and life table parameters

Predator egg, protonymphal and deutonymphal stages lasted longer on TLCV15 and SPJ-05-2018, but no significant differences between genotypes were observed for the larval stage (Table 1). On TLCV15 and SPJ-05-2018, females and males had slightly slower development (respectively 4.6 and 4.5 days), as compared to development on Tounvi (4.2 days) (Table 1).

Predator females had shorter pre-oviposition periods (APOP and TPOP) on TLCV15 and SPJ-05-2018, starting oviposition about 1.5 days earlier on these genotypes (Table 2). Subsequently, the number of days in oviposition was lower on SPJ-05-2018 (5.0), intermediate on TLCV15 (8.8), and higher on Tounvi (12.4 days) (Table 2).

A similar pattern was observed for female longevity, also shorter on SPJ-05-2018 (13.9), intermediate on TLCV15 (18.9), and longer on Tounvi (26.7 days). Male longevity was lower on TLCV15 (13.4) and longer on Tounvi (19.5 days); male longevity on SPJ-05-2018 (16.0) did not differ from longevity on other genotypes. Male and female total longevity, as well as life span, followed the same trend reported for females (Table 2). Difference between Tounvi and TLCV15 was not significant for daily fecundity (2.5 and 2.6 eggs/day, respectively), but daily fecundity was significantly lower on SPJ-05-2018 (1.8). As female longevity was longest on Tounvi, total fecundity was also highest on this genotype (43.6 eggs/ female), followed by TLCV15 (27.2) and SPJ-05-2018 (13.7). No significant difference between genotypes was observed for sex ratio (Table 2).

Table 1 Average duration (± SE), in days, and mortality rate (%) of *Phytoseiulus longipes* immature stages consuming *Tetranychus evansi* nymphal stages on three tomato genotypes (TLCV15, Tounvi and SPJ-05-2018)

Parameter	Plant genotype					
	TLCV15 (susceptible)		Tounvi (highly susceptible)		SPJ-05-2018 (partially-resistant)	
	*n	Average (± SE)	n	Average (± SE)	n	Average (± SE)
Egg	72	1.62 ± 0.03a	66	1.47 ± 0.04b	71	1.67 ± 0.05a
Larva	72	0.58 ± 0.02a	65	0.61 ± 0.03a	71	0.59 ± 0.02a
Protonymph	72	0.98 ± 0.03a	64	0.88 ± 0.04b	64	0.92 ± 0.04a
Deutonymph	70	1.3 ± 0.06a	61	1.12 ± 0.04b	58	1.41 ± 0.07a
Female development time	49	4.49 ± 0.02b	39	4.10 ± 0.07b	37	4.62 ± 0.10a
Male development time	21	4.43 ± 0.04b	22	4.16 ± 0.13b	21	4.55 ± 0.12a
Total development time	70	4.47± 0.02a	61	4.12 ± 0.06b	58	4.59 ± 0.07a
Pre-adult mortality rate	73	4.10 ± 2.31b	66	7.58 ± 3.25b	72	19.44 ± 4.66a

*n: number of individuals for a respective stage. Means within a row followed by the same letter are not significantly different. The SEs were estimated by using 100,000 bootstrap resamplings and means were compared by paired bootstrap test (B = 100,000) based on a 95% confidence interval of the difference between treatments.

Genotypes had different effects on predator population parameters (Table 2). Net reproduction rate (R_0) was significantly lower on SPJ-05-2018 (7.03 eggs/individual), intermediate on TLCV15 (17.90 eggs/individual), and higher on Tounvi (25.78 eggs/individual) (Table 2). The parameters r and λ were significantly lower on SPJ-05-2018 (0.148 and 1.160 day^{-1}) in comparison with those parameters on Tounvi (0.203 and 1.225 day^{-1}) and TLCV15 (0.228 and 1.256 day^{-1}) (Table 2). Average generation time (T) was longer on Tounvi (15.98 days) than on TLCV15 (12.64 days) and SPJ-05-2018 (13.12 days) (Table 2).

Table 2 Duration (mean ± SE), in days, of the pre-oviposition period (APOP), total pre-oviposition (TPOP), longevity, oviposition (Od), sex ratio, total life span, fecundity, and life-table parameters of *Phytoseiulus longipes* consuming *Tetranychus evansi* nymphal stages on three tomato genotypes (TLCV15, Tounvi and SPJ-05-2018)

Parameter	Plant genotype					
	TLCV15 (susceptible)		Tounvi (highly susceptible)		SPJ-05-2018 (partially-resistant)	
	*n	Average (± SE)	n	Average (± SE)	n	Average (± SE)
APOP	49	3.60 ± 0.01c	39	5.19 ± 0.29a	37	3.72 ± 0.26b
TPOP	49	8.09 ± 0.14c	39	9.29 ± 0.29a	37	8.33 ± 0.26b
Oviposition days (Od)	49	8.75 ± 0.47b	39	12.41 ± 0.96a	37	5.01± 0.59c
Female adult longevity	49	18.85 ± 1.03b	39	26.73 ± 1.57a	37	13.86 ± 1.05c
Male adult longevity	21	13.43 ± 1.21b	22	19.52 ± 2.05a	21	15.31 ± 1.57ab
adult longevity (♂+♀)	70	17.22 ± 0.86b	61	24.13 ± 1.32a	58	14.39 ± 0.88c
Total life span	73	20.99± 0.92b	66	26.39 ± 1.46a	72	16.03 ± 1.00c
Sex ratio [(♀/ (♂+♀)]	73	0.70 ±0.05a	66	0.63± 0.06a	72	0.63± 0.06a
Daily fecundity(eggs)	49	2.51 ± 0.11a	39	2.61± 0.14a	37	1.79± 0.14b
Total fecundity(eggs)	49	27.23± 1.67b	39	43.64 ± 3.84a	37	13.68 ± 1.95c
R_0(eggs/individual)	73	17.90 ± 1.86b	66	25.78 ± 3.46a	72	7.03 ± 1.28c
r(day^{-1})	73	0.228 ± 0.008a	66	0.203± 0.009a	72	0.148 ± 0.014b
λ (day^{-1})	73	1.256 ± 0.011a	66	1.225 ± 0.012a	72	1.160 ± 0.016b
T(day)	73	12.64 ± 0.23b	66	15.98 ± 0.51a	72	13.12 ± 0.52b

*n: number of individuals for a respective parameter. Means within a row followed by the same letter are not significantly different. The SEs were estimated by using 100,000 bootstrap resamplings and means were compared by paired bootstrap test (B = 100,000) based on a 95% confidence interval of the difference between treatments.

Age- and stage-specific survival and fecundity rate

Predator age-specific survival rate (s_{xj}) expresses the probability of newly laid eggs surviving until age x at stage j (Fig. 1).

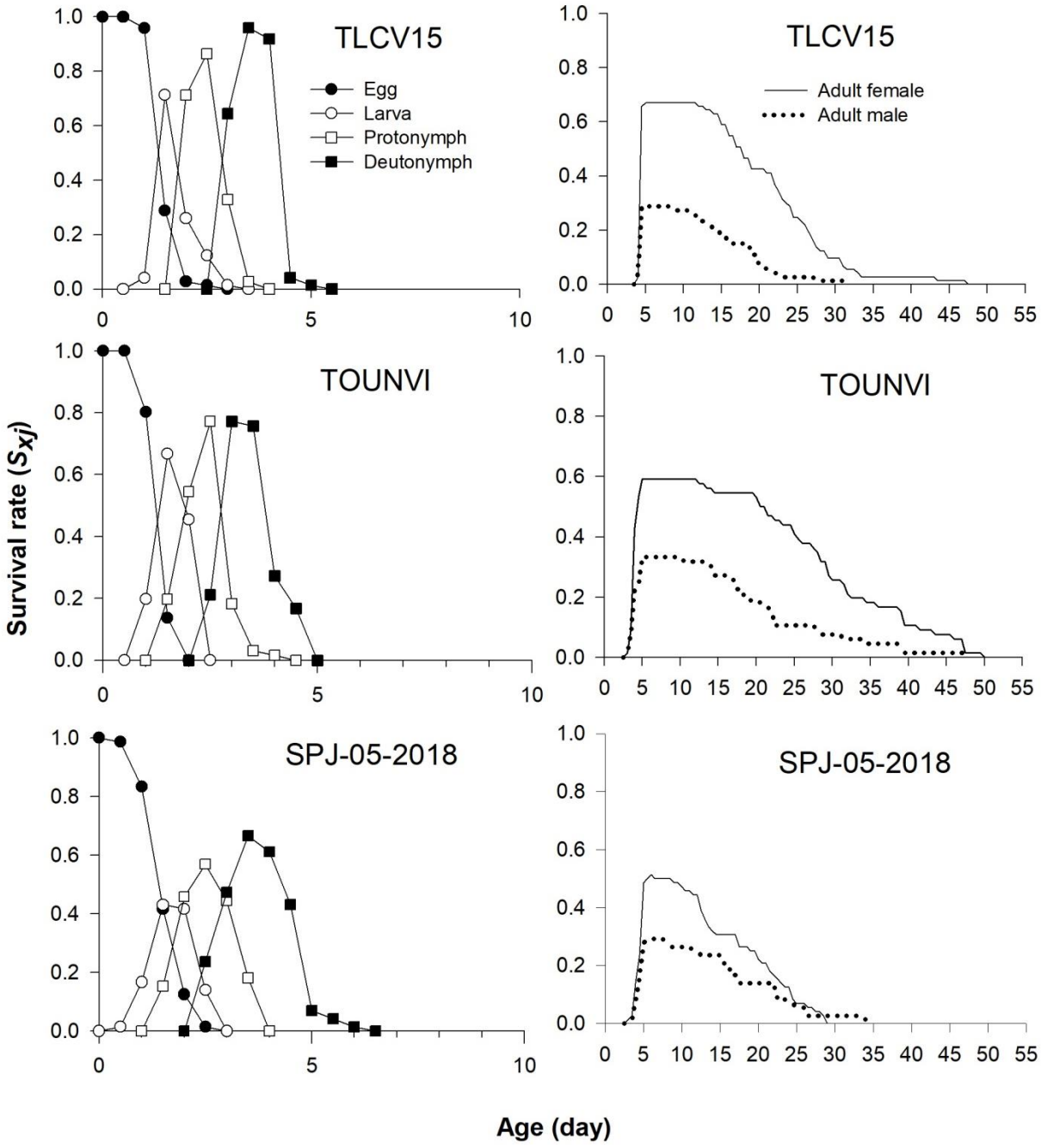

Fig. 1 Age-stage survival rate (s_{xj}) of *Phytoseiulus longipes* consuming *Tetranychus evansi* nymphal stages reared on different tomato genotypes (Tounvi, TLCV15, and SP-05-2018).

On all genotypes, survival rates of all development stages overlapped. Survival curves of immature stages on TLCV5, Tounvi and SPJ-05-2018 were close

to each other. Therefore, after adult emergence, survival patterns varied significantly between genotypes. The female survival curve (s_{xj}) was greater than that of males (Fig. 1). This was observed until the 29th day of females' life on SPJ-05-2018 (Fig. 1). For both sexes on Tounvi and females on TLCV15, survival rates and life span were substantially longer than both sexes on SPJ-05-2018. For all development stages, predator survival probability on the different genotypes until age x was represented by age-specific survival rate (l_x) (Fig. 2). This parameter showed that 51.5% of predators survived to the 25th day of age on Tounvi, while on TLCV15 and SPJ-05-2018 the rates were 27.4% and 12.5%, respectively.

Age-specific fecundity (m_x: average daily fecundity per individuals i.e, this number is divided by all individuals of age x) curves fluctuated throughout the oviposition period (Fig. 2). Oviposition started earlier on Tounvi and TLCV15, which also showed the highest daily f_{xj} and m_x values. The highest peak of specific daily fecundity was 2.0 eggs on the 43rd day for predators on TLCV15 and the 18th day for predators on Tounvi, whereas the lowest was 1.1 eggs on the 18th day for predators on SPJ-05-2018 (Fig. 2).

Predation potential

Average daily rate of predation (k_x) reached the peak earlier on SPJ-05-2018 and TLCV15 (respectively on the 5th and 10th days) than on Tounvi (16th day) (Fig. 3a). The highest k_x peak was observed on TLCV15 (9.0 nymphs), while the lowest were recorded on Tounvi and SPJ-05-2018 (5.0 and 5.2 nymphs, respectively). As predator mortality increased, net age-specific predation rate ($q_x = l_x k_x$) decreased to zero at 50, 46, and 34 days of age on Tounvi, TLCV15, and SPJ-05-2018, respectively (Fig. 3a).

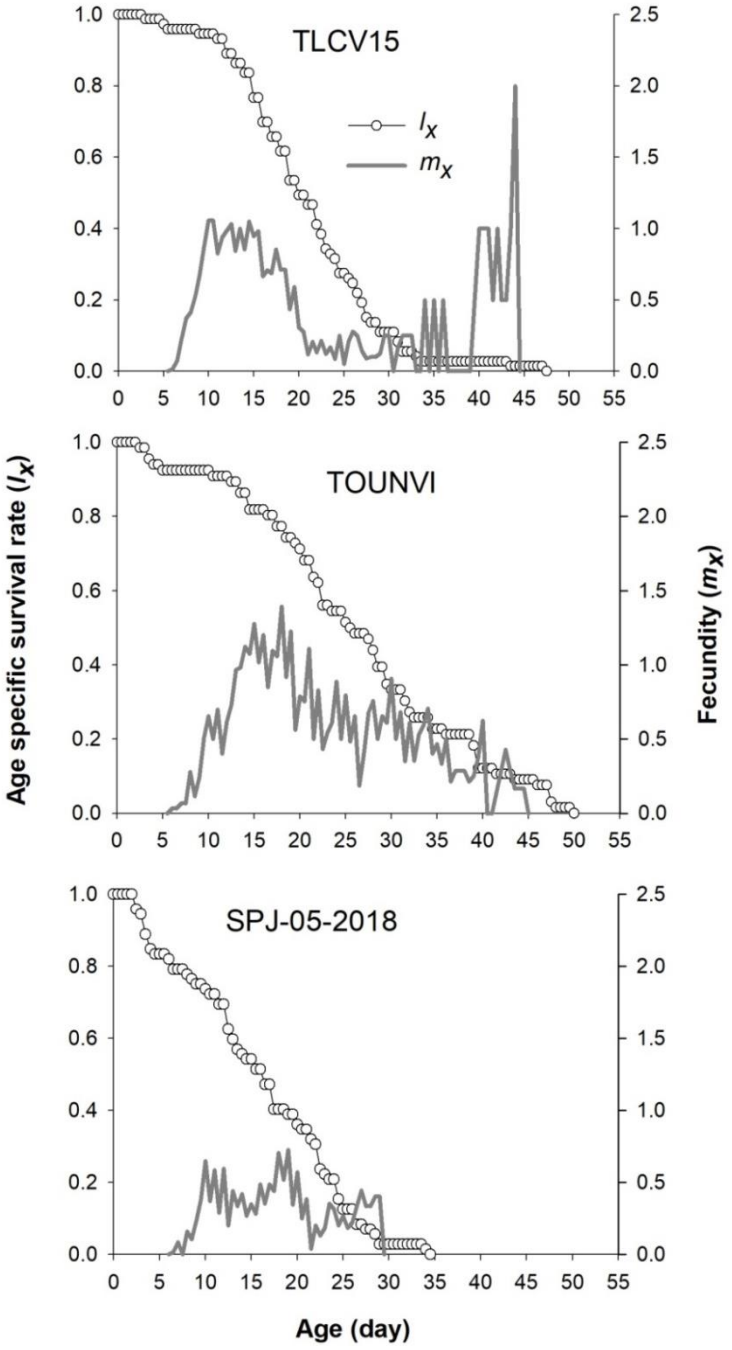

Fig. 2 Age-specific survival rates (l_x) and age-specific fecundity (m_x) of *Phytoseiulus longipes* consuming *Tetranychus evansi* nymphal stages reared on different tomato genotypes (Tounvi, TLCV15, and SP-05-2018).

Predator larvae did not feed during the experiments (Fig. 3b). Protonymphs and deutonymphs preyed on TRSM longer on SPJ-05-2018 and TLCV15 than on Tounvi (Fig. 3b). Both sexes and adult females preyed longer on Tounvi and TLCV15, than on SPJ-05-2018 (Fig. 3b). When compared to the other genotypes, males stopped preying earlier than females on TLCV15, which could be related to male lower survivorship on this genotype (Fig. 3b).

Total predation rate of protonymphs was higher on TLCV15 (5.9) than on Tounvi (4.2) and SPJ-05-2018 (3.9) (Table 3). For deutonymphs, the rate was higher on TLCV15 (10.3) and SPJ-05-2018 (9.8) than on Tounvi (8.6). Therefore, the total number of preyed nymphs during the immature phase was higher on TLCV15 (15.7), intermediate on SPJ-05-2018 (13.6), and lower on Tounvi (12.4). On all genotypes, predator females preyed more than predator males, with the highest total predation rates on Tounvi and TLCV15 (Table 3). For both predator sexes together, the total number of preyed nymphs was substantially higher on TLCV15 and Tounvi than on SPJ-05-2018 (Table 3).

Net predation rate (C_0) was significantly lower on SPJ-05-2018 (77.7) than on Tounvi (166.8) and TLCV15 (180.9) (Table 3). Transformation rate from prey to predator offspring (Q_p), which is the average number of prey a predator needs to attack (and supposedly consume) to produce each offspring, showed that predator females needed fewer TRSM nymphs on Tounvi (6.6) than on TLCV15 (10.1) and SPJ-05-2015 (11.1) (Table 3). Genotypes differed in terms of stable predation rate (ψ), finite predation rate (ω), and TRSM nymph cohort size (NC_0) (Table 3). The first two of those parameters were respectively 2.6 prey/day and 3.3 on TLCV15, 2.3 prey/day and 2.8 on Tounvi, and 2.0 prey/ day and 2.4 on SPJ-05-2018. NC_0 was 13208 (73 x 180.93), 11007 (66 × 166.77), and 5594 (72 x 77.69) preyed nymphs on TLCV15, Tounvi, and SPJ-05-2018, respectively.

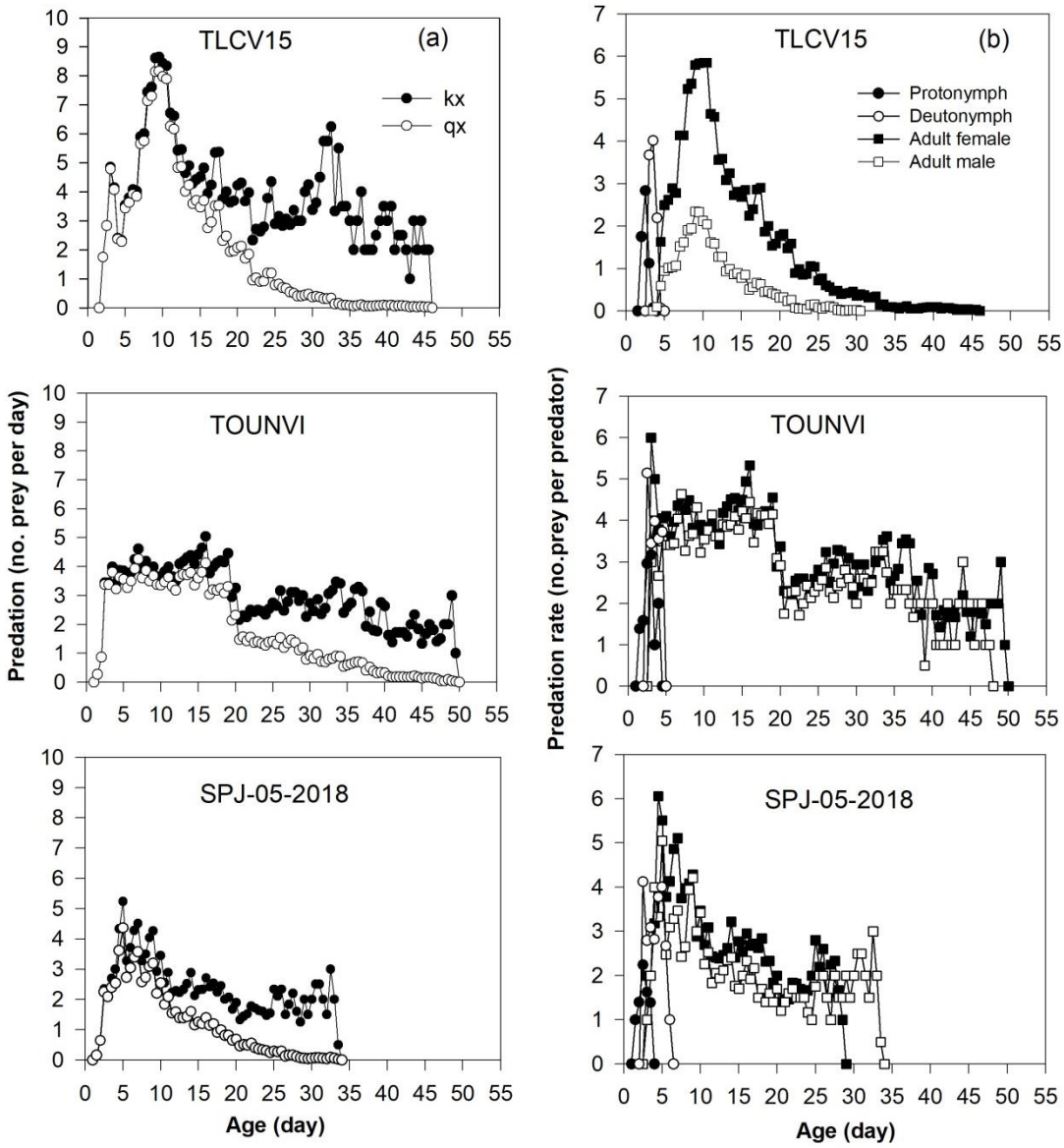

Fig. 3 Predation rate to age x and /or stage j. **a** Age-specific consumption rate (*kx*) and age-specific net consumption rate (q_x) of *Phytoseiulus longipes* fed *Tetranychus evansi* nymphal stages reared on different tomato genotypes (Tounvi, TLCV15, and SP-05-2018). **b** Age-stage predation rate (C_{xj}) derived from an age-stage, two-sex life table for *P. longipes* fed *Tetranychus evansi* nymphal stages reared on three tomato genotypes (Tounvi, TLCV15, and SP-05-2018).

Table 3 Averages (± SE) of total prey consumption for different life stages and consumption parameters of *Phytoseiulus longipes* consuming *Tetranychus evansi* nymphal stages reared on TLCV15, Tounvi, and SPJ-05-2018

Life stage/ parameter	TLCV15 (susceptible)		Tounvi (highly susceptible)		SPJ-05-2018 (partially-resistant)	
	n*	Average (± SE)	n	Average (± SE)	n	Average (± SE)
Protonymph (prey)	72	5.86 ± 0.40a	64	4.16 ± 0.40b	64	3.91± 0.35b
Deutonymph (prey)	70	10.27 ± 0.52a	61	8.61 ± 0.60b	58	9.84 ± 0.81a
Total immature (prey)	73	15.73 ± 1.84a	66	12.42 ± 0.79c	72	13.56 ± 0.93b
Female (prey)	49	189.8 ± 7.0a	39	186.85 ± 9.58a	37	87.35 ± 6.08b
Male (prey)	21	131.48 ± 4.58a	22	131.81 ± 11.62a	21	74.29 ± 6.87b
C_0 (Prey/predator)	73	180.95 ± 8.03a	66	166.77 ± 9.40a	72	77.69 ± 5.63b
Q_p (prey/ offspring)	73	10.12 ± 0.87a	66	6.55 ± 0.70b	72	11.05 ± 1.85a
Stable predation rate (ψ) (prey)	73	2.63 ± 0.06a	66	2.28 ± 0.07b	72	2.02 ± 0.08c
Finite predation rate (ω) (prey/day)	73	3.31 ± 0.09a	66	2.79 ± 0.102b	72	2.35 ± 0.11c
Total cohort predation (NC_0)(prey)	73	13208a	66	11007b	72	5594c

*n: number of individuals for a respective parameter. Means within a row followed by the same letter are not significantly different. The SEs were estimated by using 100,000 bootstrap resamplings and means were compared by paired bootstrap test (B = 100,000) based on 95% confidence interval of the difference between treatments

Discussion

Part of the population growth parameters of *P. longipes* obtained in this study is comparable to those found in previous studies with the same predator. For instance, Furtado et al. (2007) and Ferrero et al. (2013) observed that at 25 or 26 ºC, the immature phase of *P. longipes* fed TRSM reared on *S. lycopersicum* (respectively unspecified and Cheers cultivars) lasted 3.8 – 4.7 days, which is similar to the results of the present study. However, fecundity, sex ratio, and

longevity reported by Furtado et al. (2007) were higher/ longer (59.2 eggs/female, 90% females, and 31.1 days) than in this study, and which in turn were higher/ longer than reported by Ferrero et al. (2013). In addition, the intrinsic rate of increase (*r*) and the finite rate of increase (λ) reported by Furtado et al. (2007) and Ferrero et al. (2013), under similar conditions, were higher (0.284 – 0.363 and 1.306 – 1.44, respectively) than found in this study. These differences may be due to several differences between studies, as genotypes, experimental conditions, and methods used for estimating life table parameters.

Our findings indicated differences between tomato genotypes about the development, survival rate, and reproductive performance of *P. longipes*, as previously reported for other phytoseiids on different plant genotypes (Moghadasi et al., 2014; Khanamani et al. 2015; Fathipour et al. 2019). The longer egg incubation periods on TLCV15 and SPJ-05-2018 have at least two possible explanations. The first refers to the low nutritional quality of the prey reared on these genotypes that served as food to the parent population (Beckerman et al. 2006). Secondly, although eggs are a non-feeding stage, volatile compounds such as terpenes released by the leaflets could interfere with egg development accessing the interior of the egg through the aeropyles (Hilker and Meiners 2011).

Similar explanations could apply to the longer duration of the protonymphal and deutonymphal stages on TLCV15 and SPJ-05-2018, in addition to possible ingestion of other interfering chemical elements. In this regard, several studies have shown that a reduction in carbohydrate to protein proportions in insect diet shortened the developmental duration of predators (Simpson and Raubenheimer 2009; Wang et al. 2018).

The lower mortality of larvae in relation to nymphs of *P. longipes* on the partially resistant genotype SPJ-05-2018 could, at least in part, be due to the smaller sizes, lower activity, and non-feeding habit of the former, which likely reduced their chances of entrapment in glandular trichome sticky substances on this genotype (physical and chemical effects of glandular trichomes) as well as of ingestion of defensive compounds (nutritional or otherwise effect) of other plant tissue (Koller et al. 2007; Sato et al. 2011; Ferrero et al. 2013). As also observed for TRSM (Savi et

al. 2019b; Savi et al. unpublished), the earlier initiation of oviposition on TLCV15 and SPJ-05-2018 may be related to their higher trichome densities, making them less favorable to predators. This seems to be a normal feature for species to perpetuate under conditions that reduce their viability.

Interestingly, the days in oviposition, longevity, and total fecundity of *P. longipes* were longer/higher, intermediate, and shorter/ lower respectively on the genotypes that have few non-glandular trichomes (Tounvi), many non-glandular trichomes (TLCV15), and lower density of glandular trichomes (SPJ-05-2018). These results suggest that there is a relationship between trichomes and the longevity/reproductive performance of *P. longipes*. Therefore, the strong negative effect on longevity and reproductive performance of *P. longipes* on SPJ-05-2018 could be due to the consumption by predators of prey that accumulated a high concentration of toxic compounds (e.g., methyl ketones, 2-tridecanone, and 2-undecanone) found in large amount on diverse plant tissues and type I, IV, and VI trichomes glandular trichomes of this genotype (Williams et al., 1980; Savi et al., 2019a, 2019b). Another possible reason for the strong negative effect of SPJ-05-2018 on *P. longipes* would be the presence of the sticky substance from the glandular trichomes (physical effect) which makes it difficult for the predator to move. This may have forced *P. longipes* to spend more energy freeing themselves from glandular trichome secretions than in reproduction.

Among the life table parameters, the intrinsic rate of increase (*r*) is the one that best reflects the combined effects of biological characteristics such as development, survival, fecundity, and sex ratio (Janssen and Sabelis 1992; Krips et al. 1998). In the present study, the intrinsic rate of increase was higher and statistically similar on TLCV15 and Tounvi compared to that found on SPJ-05-2018. These results suggest that both TLCV15 and Tounvi could allow the highest population growth of *P. longipes*. Higher daily fecundity and immature survival may also have contributed to the rapid population growth of predators on Tounvi and TLCV15.

Our results also indicated that tomato genotypes affected significantly the consumption of prey by *P. longipes* developmental stages. Among genotypes, the

consumption at protonymphal stage on TLCV15 was more than on Tounvi and SPJ-05-2018. However, at the last stage before reaching the adult stage (deutonymph), predators preyed upon more mites on TLCV15 and SPJ-05-2018 than on Tounvi. This can be an adaptive strategy to reduce the negative effects of low nutritional quality on these genotypes, ensuring adult reproductive success (Cogni et al. 2002; Khanamani et al. 2014). In contrast, we found lower consumption of adult males and females on this genotype in relation to TLCV15 and Tounvi. This result could be attributed to the larger size and higher activity of adults when compared to deutonymphs, which likely increased their chances of entrapment in glandular trichome sticky substances found in a larger number on SPJ-05-2018. This might have forced adults on that genotype to spend more energy freeing itself from entrapment instead of consuming prey.

Although the life span on TLCV15 was significantly shorter than on Tounvi, daily consumption rate of adults was significantly higher on TLCV15 so that both sexes had total consumption rates statistically similar on these genotypes. It would therefore explain the similarity in their net predation rates (C_0). The transformation rates of prey into predator offspring (Q_p) showed that *P. longipes* would need to consume fewer individuals on Tounvi (6.6) than on TLCV15 (10.1) or SPJ-05-2018 (11.1). This pattern suggests that either prey developing on Tounvi provided better nutrition (and lower demand for many preys killed by a predator) for maintenance and offspring production, and/ or that predators needed less energy to survive and reproduce on this genotype, given the lower density of trichomes.

Predation potential expressed as finite predation rate (ω) takes finite rate of increase, age-stage structure, and the age-stage predation rate of a predator population into consideration, and is used as a standard parameter to describe and compare the predation potential of natural enemies employed in biological control programs (Chi et al. 2011). Even though the intrinsic rate of increase(r) was similar statistically on TLCV15 and Tounvi and higher than on SPJ-05-2018, finite predation rate was higher on TLCV15. Accordingly, *P. longipes* expressed a greater potential to control TRSM on TLCV15, the genotype with the lowest density of glandular trichomes (0.67/mm^2) and with the highest density of non-glandular trichomes (39.00

mm^2). These relations suggest either the deleterious effect of the glandular trichomes or the beneficial effect of the non-glandular trichomes on the predator. These results refute the hypothesis that the genotype susceptible to TRSM, which has fewer trichomes, is more favorable to *P. longipes* performance and consumption.

There are at least two possible explanations for the higher prey consumption ability of *P. longipes* on TLCV15, a genotype with a large number of non-glandular trichomes. First, this genotype has a predominance of type Va non-glandular trichomes with a characteristic spacing of 350-500 µm from each other on the lower leaflet surface (Savi et al. 2019b), a dimension comparable to the length of the adult female of the predator. This dimension probably facilitates prey capture, even though very often the prey moves within the webbing it produces, connecting trichomes at some distance from the surface of the leaflet. Secondly, there is still the possibility that this genotype provided prey of lower nutritional quality, so that the predator needed to consume more prey items for maintenance and oviposition (Cogni et al. 2002; Khanamani et al. 2015). Other studies have also reported strong positive correlations between density of non-glandular trichomes and performance of phytoseiids, such as *Phytoseiulus macropilis* (Banks), *Phytoseiulus persimilis* Athias-Henriot, and *Typhlodromus pyri* Scheuten (Roda et al. 2001; Loughner et al. 2010; Sato et al. 2011). McMurtry et al. (2013) pointed out the preference of some phytoseiids for leaves with trichomes (without specifying the type of trichomes). However, contrasting results were reported for other predators by different authors, such as *Neoseiulus californicus* McGregor (Koller et al. 2007); *Euseius finlandicus* Oudemans (Seelmann et al. 2007). These correspond to other phytoseiid groups, according to the classification of McMurtry et al. (2013).

Phytoseiulus longipes has been recently introduced to Benin (personal communication with Azandemè-Hounmalon). Given the availability of TLCV15 in that country, it should be included in integrated management programs for TRSM, increasing the chances of success for *P. longipes* as a control agent. As Tounvi is also common in Benin, the biological control of TRSM on that genotype would possibly require a larger scale release of *P. longipes* to obtain similar efficacy as on TLCV15. Although enlightening, these results are certainly preliminary in what

concerns the ability of the predator to control the pest in the field, given that they are related to specific initial densities of prey and predators used in the experiment. Had other densities and other factors such as stage of plant development, and environmental conditions been taken into account, the results could be different.

Acknowledgements

To the Brazilian Federal Agency for theSupport and Evaluation of Graduate Education (CAPES) for granting of a doctoral scholarship to the first author (CAPES Financial Code 001). To Prof. Dr. Tiago Gallina, Universidade Federal do Pampa, campus of Uruguaiana—Rio Grande do Sul (Brazil), for the valuable assistance during P. longipes collections.

References

Abad-Moyano R, Pina T, Ferragut F, Urbaneja A (2009) Comparative life history of three phytoseiid mites associated with Tetranychidae (Acari: Tetranychidae) in Clementine orchards in eastern Spain: implication for biological control. Exp Appl Acarol 47: 121–132.

Azandémè-Hounmalon YG, Affognon HD, Assogba KF, Komlan FA, Tamo M, Fiaboe KKM, Kreiter S, Martin T (2015) Farmers control practices against the invasive red spider mite, *Tetranychus evansi* Baker & Pritchard in Benin. Crop Prot 76: 53 -58.

Beckerman AP, Benton TG, Lapsley CT, Koesters N (2006) How effective are maternal effects at having effect? Proc R Soc Biol Sci 273: 485–493.

Catalogue Béninois des Espèces et Variétés végétales- CaBEV (2016) INRAB/DPVPPAAO/ProCAD/MAEP & CORAF/WAAPP. 339 p. Dépôt légal N° 8982 du 21 octobre 2016, Bibliothèque Nationale (BN) du Bénin, 4ème trimestre. ISBN: 978-99919-2-548-6.

Chi H (1988) Life-table analysis incorporating both sexes and variable development rates among individuals. Environ Entomol 17: 26–34.

Chi H (2020a) TWOSEX-MSChart: a computer program for the age-stage, two-sex life table analysis. (http://140.120.197.173/Ecology/prod02.htm) (accessed May 2020).

Chi H (2020b) CONSUME-MSChart: Computer program for consumption rate analysis based on the age stage, two-sex life table. (http://140.120.197.173/Ecology/prod02.htm) (accessed June 2020).

Chi H, Liu H (1985) Two new methods for the study of insect population ecology. Bull Inst Zool 24: 225–240.

Chi H, Yang TC (2003) Two-sex life table and predation rate of *Propylaea japonica* Thunberg (Coleoptera: Coccinellidae) fed on *Myzus persicae* (Sulzer) (Homoptera: Aphididae). Environ Entomol 32: 327–333.

Chi H, You MS, Atlıhan R, Smith CL, Kavousi A, Özgökçe MS, Güncan G, Tuan SJ, Fu JW, Xu YY, Zheng FQ, Ye BH, Chu D, Yu Y, Gharekhani G, Saska P, Gotoh T, Schneider MI, Bussaman P, Gökçe A, Liu TX (2020) Age-stage, two-sex life table: an introduction to theory, data analysis, and application. Entomol Gen 40: 103-124.

Cogni R, Freitas AVL, Amaral Filho BF (2002) Influence of prey size on predation success by *Zelus longipes* L. (Het, Reduviidae). J Appl Ent 126: 74–78.

de Moraes GJ, Flechtmann CHW (2008) Manual de acarologia: acarologia básica e ácaros de plantas cultivadas no Brasil, 2nd edn. Holos, Ribeirão Preto.

Efron B, Tibshirani RJ (1993) An introduction to the bootstrap. Chapman & Hall, New York.

Fathipour Y, Maleknia B, Bagheri A, Soufbaf M, Zalucki MP (2019) Spider mite host plant resistance traits improve the predatory performance of *Phytoseiulus persimilis* on cucumber, despite negative life history impacts. Biol Control 138: 104064.

Farhadi R, Allahyari H, Chi H (2011) Life table and predation capacity of *Hippodamia variegata* (Coleoptera: Coccinellidae) feeding on *Aphis fabae* (Hemiptera: Aphididae). Biol Control 59: 83–89.

Ferrero M, de Moraes GJ, Kreiter S, Tixier MS, Knapp M (2007) Life tables of the predatory mite *Phytoseiulus longipes* feeding on *Tetranychus evansi* at four temperatures (Acari: Phytoseiidae, Tetranychidae). Exp Appl Acarol 41: 45–53.

Ferrero M, Calvo FJ, Atuahiva T, Tixier MS, Kreiter S (2011) Biological control of *Tetranychus evansi* Baker & Pritchard and *Tetranychus urticae* Koch by *Phytoseiulus longipes* Evans in tomato greenhouses in Spain—Acari: Tetranychidae, Phytoseiidae. Biol Control 58: 30–35.

Ferrero M, Tixier MS, Kreiter S (2013) Different feeding behaviors in a single predatory mite species. Comparative life histories of three populations of *Phytoseiulus longipes* (Acari: Phytoseiidae) depending on prey species and plant substrate. Exp Appl Acarol 62: 313–324.

Furtado IP, de Moraes GJ, Kreiter S, Tixier MS, Knapp M (2007) Potential of a Brazilian population of the predatory mite *Phytoseiulus longipes* as a biological control agent of *Tetranychus evansi* (Acari: Phytoselidae: Tetranychidae). Biol Control 42: 139–147.

Handford CE, Elliott CT, Campbell K (2015). Review of the global pesticide legislation and the scale of challenge in reaching the global harmonization of food safety standards. Integr Environ Asses 11: 525–536.

Hesterberg D, Moore DS, Monaghan S, Clipson A, Epstein R (2005) Bootstrap methods and permutation tests. Chapter 14. In: Moore DS, McCabe GP, Duckworth WM, Sclove SL (eds.), The Practice of Business Statistics, 2nd ed. WH Freeman and Company, New York, NY. pp. 14.1–14.70.

Hilker M, Meiners T (2011) Plants and insect eggs: How do they affect each other? Phytochemistry 72: 1612–1623.

Janssen A, Sabelis MW (1992) Phytoseiid life-histories, local predator-prey dynamics, and strategies for control of tetranychid mites. Exp Appl Acarol 14: 233-250.

Khanamani M, Fathipour Y, Hajiqanbar H, Sedaratian A (2014) Two-spotted spider mite reared on resistant eggplant affects consumption rate and life table parameters of its predator, *Typhlodromus bagdasarjani* (Acari: Phytoseiidae). Exp Appl Acarol 63: 241-252.

Khanamani M, Fathipour Y, Hajiqanbar H (2015) Assessing compatibility of the predatory mite *Typhlodromus bagdasarjani* (Acari: Phytoseiidae) and resistant eggplant cultivar in a tritrophic system. Ann Entomol Soc Am 108: 501-512.

Koller M, Knapp M, Schausberger P (2007) Direct and indirect adverse effects of tomato on the predatory mite *Neoseiulus californicus* feeding on the spider mite *Tetranychus evansi*. Entomol Exp Appl 125: 297–305.

Krips OE, Witul A, Willems PEL, Dicke M (1998) Intrinsic rate of population increase of the spider mite *Tetranychus urticae* on the ornamental crop gerbera: intraspecific variation in host plant and herbivore. Entomol Exp Appl 89: 159–168.

Loughner R, Wentworth K, Loeb G, Nyrop J (2010) Influence of leaf trichomes on predatory mite density and distribution in plant assemblages and implications for biological control. Biol Control 54: 255–262.

Lucini T, Faria MV, Rohde C, de Resende JTV (2015) Acylsugar and the role of trichomes in tomato genotypes resistance to *Tetranychus urticae*. Arthropod Plant Interact 9: 45-53.

McMurtry JA, de Moraes GJ, Sourassou NF (2013) Revision of the lifestyles of phytoseiid mites (Acari: Phytoseiidae) and implications for biological control strategies. Syst Appl Acarol 18: 297–320.

Moghadasi M, Saboori A, Allahyari H, Zahedi Golpayegani A (2014) Life table and predation capacity of *Typhlodromus bagdasarjani* (Acari: Phytoseiidae) feeding on *Tetranychus urticae* (Acari: Tetranychidae) on rose. Int J Acarol 40: 501-508.

Navajas M, de Moraes GJ, Pigeon PAA (2012) Revision of the invasion of *Tetranychus evansi*, biology, colonization pathways, potential expansion and prospective for biological control. Exp Appl Acarol 59: 43–65.

Nyoni BN, Gorman K, Mzilahowa T, Williamson MS, Navajas M, Field LM, Bass C (2011) Pyrethroid resistance in the tomato red spider mite, *Tetranychus evansi*, is associated with mutation of the para-type sodium channel. Pest Manag Sci 67: 891–897.

Roda A, Niro, J, Dicke M, English-Loeb G (2001) Leaf pubescence and two-spotted spider mite webbing influence phytoseiid behavior and population density. Oecologia 129: 551–560.

Sato MM, de Moraes GJ, Haddad ML, Wekesa VW (2011) Effect of trichomes on the predation of *Tetranychus urticae* (Acari: Tetranychidae) by *Phytoseiulus macropilis* (Acari: Phytoseiidae) on tomato, and the interference of webbing. Expl Appl Acarol 54: 21–32.

Savi PJ, de Moraes GJ, Boiça Junior AL, Melville CC, Carvalho RF, Lourenção AL, Andrade DJ (2019a) Impact of leaflet trichomes on settlement and oviposition of *Tetranychus evansi* (Acari: Tetranychidae) in African and South American tomatoes. Syst Appl Acarol 24: 2559–2576.

Savi PJ, de Moraes GJ, Melville CC, Andrade DJ (2019b) Population performance of *Tetranychus evansi* (Acari: Tetranychidae) on African tomato varieties and wild tomato genotypes. Exp Appl Acarol 77: 555–570.

Sarr I, Knapp M, Ogol CKP, Baumgärtner J (2002) Impact of predators on *Tetranychus evansi* Baker and Pritchard populations and damage on tomatoes (*Lycopersicon esculentum* Mill.) in Kenya. Abstract retrieved from Abstract book of the 11th International Congress of Acarology, Merida, Mexico (No 271).

Seelmann L, Auer A, Hoffmann D, Schausberger P (2007) Leaf pubescence mediates intraguild predation between predatory mites. *Oikos* 116: 807– 817.

Silva FR, de Moraes GJ, Gondim Jr MGC, Knapp M, Rouam SL, Paes JLA, de Oliveira GM (2010) Efficiency of *Phytoseiulus longipes* Evans as a control agent of *Tetranychus evansi* Baker & Pritchard (Acari: Phytoseiidae: Tetranychidae) on screenhouse tomatoes. Neotrop. Entomol 39: 991–995.

Simmons AT, Gurr GM (2005) Trichomes of *Lycopersicon* species and their hybrids: effects on pests and natural enemies. Agr Forest Entomol 7: 265–276.
Simpson SJ, Raubenheimer D (2009) Macronutrient balance and lifespan. Aging 1: 875–880.

Smucker MD, Allan J, Carterette B (2007) A comparison of statistical significance tests for information retrieval evaluation. Proceedings of the Sixteenth ACM

Conference on Information and Knowledge Management, CIKM 2007, Lisbon, Portugal, November 6-10, 2007. p. 623-632.

Wang ZL, Wang XP, Li CR, Xia ZZ, Li SX (2018) Effect of dietary protein and carbohydrates on survival and growth in larvae of the *Henosepilachna vigintioctopunctata* (F.) (Coleoptera: Coccinellidae). J Insect Sci 18: 1-7.

Williams WG, Kennedy GG, Yamamoto RT, Thacker JD, Bordner J (1980) 2-Tridecanone: a naturally occurring insecticide from the wild tomato *Lycopersicon hirsutum* f. *glabratum*. *Science* 207: 888–889.

Yu J, Chi H, Chen B (2013) Comparison of the life tables and predation rates of *Harmonia dimidiata* (F.) (Coleoptera: Coccinellidae) fed on *Aphis gossypii* Glover (Hemiptera: Aphididae) at different temperatures. Biol Control 64:1–9.

Wei MF, Chi H, Guo YF, Li XW, Zhao LL, Ma RY (2020) Demography of *Cacopsylla chinensis* (Hemiptera: Psyllidae) reared on four cultivars of *Pyrus bretschneideri* and *P. communis* (Rosales: Rosaceae) pears with estimations of confidence intervals of specific life table statistics. J Econ Entomol 113: 2343-2353.

CHAPTER 4 - Bioactivity of oxymatrine and azadirachtin against *Tetranychus evansi* (Acari: Tetranychidae) and their compatibility with the predator *Phytoseiulus longipes* (Acari: Phytoseiidae) on tomato

Patrice Jacob Savi[1*], Marcio Barbosa Martins[1], Gilberto José de Moraes[2], Fabien Charles Cossi Hountondji[3], Daniel Júnior de Andrade[1]

[1]Faculdade de Ciências Agrárias e Veterinárias, Universidade Estadual Paulista, 14884-900 Jaboticabal- SP, Brazil.

[2]Escola Superior de Agricultura "Luiz de Queiroz", Universidade de São Paulo, 13418-900, Piracicaba-SP, Brazil.

[3]Faculté d'Agronomie, Université de Parakou, Benin

[*]**Corresponding author-** E-mail address: savipatricejacob@yahoo.fr

Abstract

Tetranychus evansi Baker & Pritchard (Acari: Tetranychidae) is one of the main tomato pests in several countries, mainly in Africa, and applications of synthetic acaricides are the main strategy for its control. Efficient biopesticides to suppress pest populations, with low toxicity against natural enemies, are highly desirable for integrated pest management. Here, we evaluated under laboratory conditions the effect of azadirachtin- and oxymatrine-based formulations on each development stage of *T. evansi* and the adult stage of the predatory mite *Phytoseiulus longipes*, the single promising predator of this pest. We also assessed the residual effect of these biopesticides on *T. evansi* under laboratory conditions on leaflets excised from treated tomato plants maintained in a screen-house 1, 5, and 10 days after application. Azadirachtin-based formulations were effective in controlling *T. evansi* immature stages. Oxymatrine-based treatments controlled *T. evansi* immatures and adult females faster than azadirachtin-based treatments. Both biopesticides did not affect *T. evansi* eggs, but oxymatrine-based treatments were highly efficient on newly hatched *T. evansi* larvae. Oxymatrine displayed residual activity that controlled *T. evansi* up to 10 days after application. Azadirachtin formulations caused lower mortality of *P. longipes* adults (8-28%) and slightly reduced fecundity (24.8-56.1%). In contrast, oxymatrine treatments caused higher mortality (60-88%) of the predator and reduced substantiality of its fecundity (73.1-90.7%). Our findings suggest that azadirachtin and oxymatrine provide effective control of *T. evansi*. Azadirachtin may be relatively safer to the predatory mites whereas oxymatrine should be used with caution, to avoid suppression of *P. longipes*.

Keywords: Biopesticides, Tomato red spider mite, Sustainability, Phytoseiid, Predator.

Article published in "Systematic and Applied Acarology" (Impact factor: 1.614)

Introduction

Tomato (*Solanum lycopersicum* L.) has a high economic and social importance in several countries (FAO 2017). This crop is highly affected by pests and diseases, which increase production costs and decrease profitability (Oerke 2006; Savi et al. 2019a). Phytophagous mites have been among the most harmful pests to tomatoes worldwide, and losses due to their damage have been estimated to reach up to 35% of the world's production (Zalom, 2003). The tomato red spider mite, *Tetranychus evansi* Baker & Pritchard (Acari: Tetranychidae), was reported for the first time in 1952 in Brazil and since then it has spread to attack tomato and other solanaceous plants in many countries (Silva 1954; Navajas et al. 2012; Ghazy et al. 2019). It was first recorded in Africa in 1979, attacking tobacco plants (*Nicotiana tabacum* L.) in Zimbabwe (Blair 1983). Nowadays, it has become one of the most destructive pests of solanaceous plants in the African continent, with the potential to reduce tomato yield by up to 90% (Sarr et al. 2002). In several sub-Saharan countries, such as Benin, this pest has been a major challenge for vegetable growers (Azandéme-Hounmalon et al. 2015; Savi et al. 2019 a, b). One of the reasons explaining its rapid spread in different countries is the lack of efficient natural enemies and ineffective chemical control (Navajas et al. 2012; Azandéme-Hounmalon et al. 2015; Ghazy et al. 2019). This mite has been shown to affect mite community composition in infested crops (Ferragut et al. 2013), becoming the dominant species soon after its introduction to a new area (Azandéme-Hounmalon et al. 2015).

Like all other spider mites, *T. evansi* feeds on leaf cell contents, initially causing chlorotic spots that quickly progress to leaf necrosis and death. As a result, it reduces plant size as well as fruit yield and quality (Savi et al. 2019a). In Africa, the application of synthetic products is the main method for controlling red spider tomato mites (Nyoni et al. 2011; Azandémè-Hounmalon et al. 2015). However, this method is not always effective, as this mite develops rapidly and has a high reproductive capacity, which leads producers to intensify chemical sprays to control it (Nyoni et al. 2011; Azandémè-Hounmalon et al. 2015). As consequence, the pest

becomes more prone to evolving pesticide resistance, often leading to the occurrence of environmental pollution and poisoning risks to farmers and consumers as well as outbreaks of secondary pests (Nyoni et al. 2011; Handford et al. 2015; Guedes et al. 2016; Ghazy et al. 2019; Ndolo et al. 2019). For this reason, alternative control measures have been sought worldwide.

Efforts have been devoted in the recent past to the search of prospective *T. evansi* biocontrol agents (Furtado et al. 2006; Navajas et al. 2012). Insects, mites, and entomopathogenic fungi have been evaluated (Wekesa et al. 2005; Furtado et al. 2006; Navajas et al. 2012). However, results have been unsatisfactory, mainly due to their low rates of oviposition and survivorship when *T. evansi* is offered as prey (de Moraes & McMurtry 1985; Navajas et al. 2012). The copious amount of webbing produced and sequestration of secondary plant metabolites by *T. evansi* have been suggested as explanations for poor predator performance (de Moraes & McMurtry, 1985). Among the numerous species studied, only the predatory mite *Phytoseiulus longipes* Evans (Acari: Phytoseiidae), found naturally in association with *T. evansi* in the extreme south of Brazil and northern Argentina, has been shown to be promising for its control (Furtado et al. 2006; Savi et al. 2021).

Compounds isolated from secondary plant metabolism (biopesticides) have been also investigated as alternatives for several arthropod pests control (Reddy et al. 2016; Andrade et al. 2019; Golec et al. 2020). They often have advantages over synthetic pesticides, for their usually low mammal toxicity, low environmental persistence, and risk of resistance development, as well as for their higher selectivity to beneficial and other non-target organisms (Marčić et al. 2014; Bernadi et al. 2012; Golec et al. 2020). Several compounds have been studied and some have shown satisfactory results for phytophagous mites control (Marčić et al. 2014; Zanardi et al. 2015; Andrade et al. 2019; Golec et al. 2020). Among the most used biopesticides, azadirachtin, metabolite extracted from neem, *Azadirachta indica* A. Juss (Meliaceae), has stood out. This compound is a complex tetranortri-terpenoid limonoid with antifeedant effects, reducing fecundity and successful metamorphosis of arthropods antifeedant effects, reducing fecundity, and deleterious effect on the metamorphosis of arthropods (Mordue & Nisbet, 2000). Oxymatrine, extracted from

Sophora flavescens Ait. (Fabaceae), is another metabolite, which has been used for mite control. It is an alkaloid (tetracyclo-quinolizidine) with insecticidal, acaricidal, fungicidal, bactericidal, and nematocidal activities (Wang et al. 2007; Marčić et al. 2014; Zanardi et al. 2015; Andrade et al. 2019).

Commercial azadirachtin- and oxymatrine-based formulations are authorized for agricultural use and available in several countries (Schlesener et al. 2013; Andrade et al. 2019), but their effects on *T. evansi* and *P. longipes* have not been studied. This study aimed to investigate the effects of these biopesticides on these two mite species. The results are expected to help to understand the compatibility of these pesticides for joint use in the control of *T. evansi*.

Materials and Methods

Mite colonies and experimental units were always maintained at 25 ± 1ºC, 70 ± 10% RH, and a daily 12-h photoperiod.

Mite rearing

Specimens of *T. evansi* used in this study were obtained from a colony maintained in a screen-house on tomato plants (commercial var. TLCV15) for three generations. Specimens of *P. longipes* were obtained from a colony initiated about three months before, with specimens collected from *S. lycopersicum*, *S. americamum*, and *Brugmansia suaveolensis* L. plants growing naturally in the urban area of Uruguaiana, Rio Grande do Sul, Brazil (29°49’48.0” S, 57°06’04.0” W and 29°45’12.0” S, 57°04’31.0” W). They were maintained in plastic trays (25 x 17 x 9 cm) containing a 3-cm-thick foam overlaid by a 23.5 x 15.5 cm^2 resin plate (Paviflex®), whose edges were covered with a moistened cotton wool band to prevent mites from escaping (Ferrero et al. 2007). The predators were fed with all stages of *T. evansi* on excised tomato leaflets (var. TLCV15).

Treatments

Three biopesticides were evaluated: two azadirachtin-based (Azamax® and Azact®) and one oxymatrine-based (Matrine®) (Table 1). Treatments consisted of Azamax®, Azact®, and Matrine® at concentrations of 1- and 2-mL of the commercial product per liter of water (cp L^{-1}). Abamectin (Vertimec®) and deionized water were used as positive and negative controls, respectively. Thus, the treatments were named as follows: Azamax® at 1.0 mL cp L^{-1} (Azx 1); Azamax® at 2.0 mL cp L^{-1} (Azx 2); Azact® at 1.0 mL cp L^{-1} (Azt 1); Azact® at 2.0 mL cp L^{-1} (Azt 2); Matrine at 1.0 mL cp L^{-1} (Oxymatrine 1); Matrine at 2.0 mL cp L^{-1} (Oxymatrine 2); Abamectin at 0.2 mL cp L^{-1}, and deionized water (DW).

Table 1. Description of biopesticides and standard control used in bioassays.

Trade Name	**Active Ingredient**	**Concentration of** active ingredient **in commercial product**	**Chemical family**	**Company**
Matrine	Oxymatrine	2.0g i.a.L^{-1}	Quinolizidine alkaloids	Dinagro Agropecuária Ltd., Ribeirão Preto, São Paulo, Brazil
Azamax® EC	Azadirachtin	12 g i.a. L^{-1}	Tetranortriterpenoids	UPL do Brasil – Indústria e Comércio de Insumos Agropecuários S.A., Ituverava, São Paulo Brazil
Azact® EC	Azadirachtin	2.4 g i.a. L^{-1}	Tetranortriterpenoids	LACZA agronegócios,Cravinho, São Paulo, Brazil
Vertimec 18EC	Abamectin	18 g i.a. L^{-1}	Avermectin	Syngenta Proteção de Cultivos Ltda, São Paulo, Brazil

Doses were selected based on previous studies, which assessed these products against *Tetranychus urticae* Koch, *Brevipalpus yothersi* Baker, and *Oligonychus ilicis* (McGregor) mites on strawberry, citrus, and coffee (Bernadi et al. 2012; Andrade et al. 2019, 2020).

Experimental units for Tetranychus evansi

Each unit consisted of a disc of tomato leaflet (var. TLCV15; 3.0 cm diameter) placed with its abaxial surface up into a Petri dish (5.0 cm diameter and 2.0 cm height) lined with a nylon foam mat (1cm thick) covered by a thin cotton-wool layer, both maintained permanently soaked with distilled water. The margin of the disc was covered with a thin cotton-wool band, in contact with the cotton-wool layer, to maintain leaf turgidity and to prevent mites from escaping.

Effect of recently applied treatments on Tetranychus evansi

Separate bioassays were conducted to evaluate treatments on *T. evansi* eggs, larvae, nymphs (protonymphs and deutonymphs), and 6-day-old adult females. Direct contact method was used for the egg bioassay, whereas residual contact method was used for the larva, nymph (mix of protonymphs and deutonymphs), and adult female bioassays. Difference in methods used was based on the generally reduced effect of residual contact on the egg stage and the possible physical effect of the water on the mobile stages. Each bioassay was carried out in a fully randomized design, with eight units (replicates) per treatment.

For the egg bioassay, five adult females were transferred to each unit with a fine brush. Twelve hours later, females and extra eggs were removed leaving only 10 eggs per unit, subjecting the units (with the eggs) to the different treatments under a Potter tower (Burkard Scientific Co., Uxbridge, United Kingdom) calibrated at 27.6 kPa, spraying 2 mL solution, resulting in a deposition of 1.8 ± 0.1 mg fresh residue cm^{-2}. Ovicidal action was determined as the ratio between the numbers of emerged larvae and unhatched eggs in each arena at the end of seven days period after the application.

In bioassays initiated with larvae, nymphs (mix of protonymphs and deutonymphs), and adult females, the units (without mites) were sprayed as previously described and about one hour later, 10 mites of either stage were

transferred to each unit, using a fine brush and under a stereomicroscope. The units were examined under a stereomicroscope 1, 3, 5, and 7 days after spraying (DAIE: days after initial evaluation, considering 0 as the first evaluation, when the experiment was set up and treatments were applied), counting dead and live mites on the leaflets and those trapped in the cotton barrier. Given their continuous development, after the first evaluation mites could be at a stage subsequent to that with which the study was initiated. Mites were considered dead if they did not move for at least the distance equivalent to their body length, after being touched with a fine brush. The eggs laid by females in adult bioassay were also recorded.

Treatment efficiency was calculated in all bioassays using a modified Abbott's formula (Abbott, 1925):

% of corrected mortality = 100 *(Tci - Tti) / Tci

Where: Tci = number of live mites + number of mites found in moistened cotton in the negative control (DW) in the i^{-th} evaluation; Tti = number of live mites + number of mites found in moistened cotton in the treatment in the i^{-th} evaluation after spray.

For each treatment, fecundity was calculated per live female, dividing the total number of eggs laid until the first evaluation and between subsequent evaluations in each unit by the respective mean number of live females within those periods.

Residual effect of biopesticides on Tetranychus evansi

Initially, TLCV15 variety tomato seedlings were grown in Styrofoam trays, filled with Bioplant® substrate (Bioplant Agrícola Ltda., Ponte Nova, Minas Gerais, Brazil) and maintained in a screen-house under automatic irrigation. About 15 days after sowing, fertilizer was applied to the soil (250 mL per plant of a solution of 10 g mono ammonium phosphate in 1 L water). Ten days later, the seedlings had 4–5 leaves and each was transplanted to a 4-L pot filled to about 80% of its capacity with a substrate composed of a mixture of soil, sand, and dry bovine manure at a 1: 1: 1

ratio, previously autoclaved at 120 ºC for 3 h. Ten days after transplanting, each pot received 5 g of urea (44% N) and 5 g of potassium chloride (58% K_2O). Plants were manually irrigated daily to field capacity, avoiding wetting plant shoot. Each treatment was applied at the beginning of the flowering period (about 45 days after sowing) to eight plants, using a hand sprayer (Brudden® Practical; Brudden Equipamento Ltda, Pompeia, São Paulo, Brazil) to runoff stage. After spraying, the plants were arranged in a fully randomized design in a screen-house, at the following conditions (min<average<max: Temperature (º C) - 17.1<25.5<34.7; Relative humidity (%) - 27.4<59.0<91.9; Light: natural environment).

Leaflets were excised from the median third of the canopy of each plant 1, 5, and 10 days after spraying (DAA: days after application), and used for the preparation of experimental units as previously described. Soon after, 10 nymphs (mix of protonymph and deutonymph) were transferred from the stock colony to each unit. Nymphs were used in this bioassay because they were shown to be the most sensitive stage to treatments in the previous phase of the study and because they were easier to handle than larvae. Each treatment had eight replicates, each corresponding to one experimental unit. Nymph mortality was assessed 1, 3, 5, and 7 days after they were transferred. Treatment efficiency was calculated using modified Abbott's formula (Abbott 1925).

Effect of recently applied treatments on Phytoseiulus longipes

Each experimental unit consisted of a rectangle of tomato leaflet (var. TLCV15; 2.0 x 1.5 cm) placed with its abaxial surface up into a Petri dish (12 cm diameter and 2.0 cm height) lined with a nylon foam mat (1.0 cm thick) covered with a thin cotton-wool layer, both maintained permanently soaked with distilled water. The margin of the disc was covered with a thin cotton-wool band in contact with the cotton-wool layer, to maintain leaf turgidity and to prevent mites from escaping. The bioassay was carried out in a fully randomized design, with five replicates per treatment.

Eight adult females and 2 adult males *P. longipes*, all of which 10-day-old, were transferred from the stock colony to each experimental unit. The units and the mites in them were sprayed under a Potter tower, calibrated as previously specified, at a volume of 2 mL of spray solution per unit. The predators were fed *ad libitum* with a mixture of all developmental stages of *T. evansi* offered on pieces of infested tomato leaflets.

Predatory mite survival and fecundity (eggs/ female) was evaluated 3 days after spraying, under a stereomicroscope. Mites were considered dead if they did not move for at least a distance equivalent to their body length after being touched with a fine brush. The eggs recorded on the third day after spraying were, transferred to an untreated experimental unit, which was then evaluated once a day for four consecutive days to determine larval emergence. Mortality in each treatment was calculated by the modified Abbott's formula (Abbott 1925). The percentage of fecundity reduction was also calculated by formula (PFR) = [(AC - AT) / (AC + AT) x 100], where: AC = average of eggs in the control and AT = average of eggs in the treatment.

Based on adult mortality and sublethal effects, i.e. fecundity and fertility of females (egg viability), a reduction coefficient (Ex) was calculated for each treatment using the formula described by Biondi et al. (2012) $E_X = 100 - (100 - M_c) * R_1 * R_2$, where: Mc = Corrected mortality calculated using Abbott's formula (1925); R1 = ratio between the number of eggs laid per female from eggs treated with pesticides and control, and R2 = ratio between the number of hatched larvae of eggs laid per females treated with pesticides and control. These values were compared to the standards of laboratory ecotoxicological tests proposed by the IOBC / WPRS in four categories: I: harmless (Ex <30%); II: slightly harmful (30% <Ex <79%); III: moderately harmful (80% <Ex <99%) and IV: highly harmful (Ex> 99%) (Sterk et al. 1999).

Data analysis

Data were subjected to analysis of repeated measures over time, using generalized linear mixed models (GLMM) of “lme4” package (Bates et al. 2015), with the binomial distribution. The effect of response variables (treated units) was considered fixed, while repeated measures in each unit over time were considered random. The effects of treatment and time were assessed by likelihood-ratio tests (p<0.05) between full and reduced models. The same test was used to verify treatment-time interaction significance, comparing models with and without interaction. Furthermore, for each time, the generalized linear models (GLM) (Nelder & Wedderbun 1972), with binomial and/or Poisson distributions, were used to analyze proportion data (hatching and mortality of eggs, larvae, nymphs, and *T. evansi* adult females) in laboratory tests, and mortality of *T. evansi* sprayed in a greenhouse and assessed in the laboratory. The same models were used to analyze *P. longipes* mortality, fecundity, and egg viability. The fit quality of both parameters was assessed by half-normal plots, with simulation envelopes, using the “hnp” package (Demétrio et al. 2014). When treatments showed significant differences, multiple comparisons were performed using the Tukey's test (p <0.05), using the “glht” function of the “multcomp” package, with fit of p-values (Hothorn et al. 2008). All analyses were performed using the R statistical software, version 3.5.3 (R Development Core Team 2019).

Results

No significant differences were observed between biopesticides (oxymatrine and azadirachtin) and the water control for *T. evansi* egg viability (Table 2); only the positive control (abamectin) significantly reduced hatching rates. On the average, egg hatching occurred about 4.9±0.5 days after treatments were applied to the units. At the two doses, oxymatrine-based products caused high mortality of larvae emerging from the treated eggs (87.1 and 88.0%), but mortality rates caused by the two azadirachtin products at both doses were low (Table 2). High larval mortality rates were observed for both biopesticides and abamectin in the residual method (87.5 - 100%) (Table 3).

Table 2. Ovicidal and larvicidal activities of commercial oxymatrine- and azadirachtin-based formulations (Azact and Azamax) on *Tetranychus evansi* 7 days after spraying. (n= 8 units per treatment)

Treatment	Concentration used (mL c.p. L^{-1})[a]	Hatchability[b](%)	Larval mortality[c] (%)
Deionized water	-	92.5 ± 4.9 a	29.5 ± 1.6 c
Oxymatrine	1	91.3 ± 5.2 a	87.1 ± 8.4 a
	2	96.3 ± 2.6 a	88.0 ± 5.0 a
Azamax	1	85.0 ± 5.3 a	29.5 ± 7.9 b
	2	68.8 ± 9.1 a	25.3 ± 8.7 b
Azact	1	96.3 ± 2.6 a	12.0 ± 4.8 b
	2	73.8 ± 7.3 a	25.8 ± 4.9 b
Abamectin	0.2	46.3 ± 9.8 b	81.3 ± 7.4 a
F		6.490	23.145
Df		7, 56	7, 56
p-value		< 0.001	< 0. 001

[a] Concentration of commercial product in mL per liter of water.
[b] Data (mean ± SE) followed by the same letter in a column do not differ significantly (GLM with quasi-Poisson distribution, followed by post hoc Tukey test; P < 0.05).
[c] Data (mean ± SE) followed by the same letter in a column do not differ significantly (GLM with quasi-Binomial distribution, followed by the post hoc Tukey test; P < 0.05).

Table 3. Mortality (%) of *Tetranychus evansi* larvae after increasing exposure time under different treatments (oxymatrine- and azadirachtin-based formulations Azact and Azamax; n= 8 units per treatment).

Treatment	Concentration (mL c.p. L^{-1}) [a]	Exposure time (days) [b]			
		1	3	5	7
Deionized water	-	2.5 ± 1.6 e	10.0 ± 3.3 e	11.3 ± 6.1 c	12.5 ± 5.9 b
Oxymatrine	1	85.0 ± 5.0 a	92.5 ± 4.9 a	100.0 ± 0.0 a	100.0 ± 0.0 a
	2	67.5 ± 8.0 ab	72.5 ± 8.0 abc	100.0 ± 0.0 a	100.0 ± 0.0 a
Azamax	1	41.3 ± 4.4 bc	63.7 ± 6.5 bc	92.5 ± 4.1 ab	100.0 ± 0.0 a
	2	20.0 ± 3.8 cd	50.0 ± 5.3 cd	88.8 ± 5.8 ab	100.0 ± 0.0 a
Azact	1	15.0 ± 2.7 d	28.8 ± 4.0 d	65.0 ± 5.3 b	96.3 ± 1.8 a
	2	26.3 ± 8.4 cd	47.5 ± 7.5 cd	73.8 ± 6.5 ab	87.5 ± 6.5 a
Abamectin	0.2	86.3 ± 4.6 a	96.3 ± 3.75 a	100.0 ±0.0 a	100.0 ± 0.0 a
F		31.608	26.687	26.484	38.041
df		7, 56	7, 56	7, 56	7, 56
p-value		< 0.001	< 0. 001	< 0.001	< 0.001

[a] Concentration of commercial product in mL per liter of water.
[b]Data (mean ± SE) followed by the same letter in a column do not differ significantly from each other by GLM with quasi-Binomial distribution, followed by the post hoc Tukey's test (p < 0.05).

Besides, a significant interaction was observed between biopesticides and DAIE ($\chi 2 = 30.215$; df = 7; $p < 0.00001$). Both oxymatrine concentrations had quicker action compared to azadirachtin, which showed low larval mortality one day after spraying, increasing progressively over time.

Both biopesticides and abamectin promoted high mortality levels of *T. evansi* nymphs (Table 4). Significant interaction was also observed between treatments and DAIE ($\chi 2 = 47.964$; df = 7; $p < 0.001$), with quicker action in both oxymatrine doses.

Oxymatrine, Azamax, and abamectin caused high mortality of *T. evansi* adults, with significant interaction between treatments and DAIE as well ($\chi 2 = 68.15$; df = 7; $p < 0.001$) (Table 5). In the last evaluation, mortality in Azt treatments was significantly lower than in other treatments. The two oxymatrine concentrations had quicker action (Table 5).

Table 4. Mortality (%) of *Tetranychus evansi* protonymphs and deutonymphs after increasing exposure time under different treatments (Azact and Azamax are commercial azadirachtin-based formulations; n= 8 units per treatment).

Treatment	Concentration (mL c.p. L^{-1})[a]	Exposure time (days) [b]			
		1	3	5	7
Deionized water	-	1.3 ± 1.3 d	6.3 ± 1.8 d	6.8 ± 2.6 c	12.5 ± 3.1 b
Oxymatrine	1	96.3 ±2.6 a	98.8 ± 1.3 a	100.0 ± 0.0 a	100.0 ± 0.0 a
	2	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a
Azamax	1	56.3 ±8.0 b	90.0 ± 2.7 a	95.0 ± 1.9 a	96.3 ± 1.8 a
	2	62.5 ±4.9 b	85.0 ± 4.2 ab	90.0 ± 5.7 a	95.0 ± 5.0 a
Azact	1	22.5 ± 7.7 c	58.8 ± 10.9 b	68.8 ± 8.5 b	88.8 ± 4.4 a
	2	47.5 ±5.9 b	62.5 ± 8.2 bc	83.8 ± 4.2 ab	92.5 ± 3.7 a
Abamectin	0.2	100.0 ±0.0 a	100.0 ±0.0 a	100.0 ±0.0 a	100.0 ± 0.0 a
F		35.774	35.034	70.139	85.22
df		7, 56	7, 56	7, 56	7, 56
p-value		< 0.001	< 0. 001	< 0.001	< 0.001

[a] Concentration of commercial product in mL per liter of water.

[b] Data (mean ± SE) followed by the same letter in a column do not differ significantly from each other by GLM with quasi-Binomial distribution, followed by the post hoc Tukey's test ($p < 0.05$).

Table 5. Mortality (%) of *Tetranychus evansi* adult females after increasing exposure time under different treatments (oxymatrine- and azadirachtin-based formulations Azact and Azamax; n= 8 units per treatment)

Treatment	Concentration (mL c.p. L^{-1})[a]	Exposure time (days) [b]			
		1	3	5	7
Deionized water	-	1.3 ± 1.3 c	2.5 ± 1.6 c	6.3 ± 1.9 d	11.3 ± 2.3 d
Oxymatrine	1	98.8 ± 1.3 a	98.8 ± 1.3 a	98.8 ± 1.3 a	98.8 ± 1.3 a
	2	97.5 ± 1.6 a	98.8 ± 1.3 a	98.8 ± 1.3 a	98.8 ± 1.3 a
Azamax	1	42.5 ± 11.3 b	63.8 ± 11.6 b	72.5 ± 7.7 b	80.0 ± 7.3 ab
	2	31.3 ± 4.0 b	62.5 ± 6.5 b	70.0 ± 6.5 b	86.3 ± 4.2 ab
Azact	1	20.0 ± 3.8 b	40.0 ± 4.6 b	42.5 ± 5.3 c	53.8 ± 4.2 c
	2	20.0 ± 5.7 b	41.3 ± 6.7 b	51.3 ± 4.0 c	68.8 ± 4.0 bc
Abamectin	0.2	100.0 ± 0.0a	100.0 ±0.0 a	100.0 ±0.0a	100.0 ±0.0 a
F		40.852	36.492	56.675	68.525
df		7, 56	7, 56	7, 56	7, 56
P- value		< 0.001	< 0. 001	< 0.001	< 0.001

[a]Concentration of commercial product in mL per liter of water.
[b]Data (mean ± SE) followed by the same letter in a column do not differ significantly from each other by GLM with quasi-Binomial distribution, followed by the post hoc Tukey's test ($p < 0.05$).

Except for the first evaluation (1DAIE), no oviposition was observed with adult females subjected to oxymatrine treatments. Adult females subjected to Azadiractin-based treatments had lower average oviposition rates ($\chi 2 = 189.2.38$; df = 4; p <0.001) (Table 6). In relation to the residual effect (persistence), significant differences were observed between treatments at all DAA (periods between treatment and initiation of evaluations) considered separately (F=24.881–50.85; df = 7, 56; p <0.001) and a significant interaction ($\chi 2 = 36.689$; df = 7; $p < 0.001$) between treatments and residual duration.Oxymatrine treatments resulted in the highest mortality rates at all DAA periods (1, 5, and 10 days) at the last evaluation (7 DAIE) (Fig. A, B, C). A gradual reduction in mortality was observed over time, except for oxymatrine 2 and abamectin, which even after 10 DAA caused high mortality rates. Azadirachtin caused intermediate mortality rates but differed from the negative control in the last evaluations at 1 and 5 DAA. At 10 DAA, all treatments differed from negative control except Azt 1 and Azt 2 (Fig. 1C).

Table 6. Average number of eggs laid (± SE) per female of *Tetranychus evansi* (initial density of ten females per experimental unit) subjected to different treatments after increasing exposure time; numbers in parentheses indicate a number of females in each unit in the first evaluation and between subsequent evaluations (oxymatrine- and azadirachtin-based formulations Azact and Azamax; n= 8 units per treatment).

Treatment	Concentration (mL c.p. L^{-1})[a]	Exposure time (days) [b]			
		1	3	5	7
Deionized water	-	2.0 ± 0.3a (9.8)	5.1 ± 0.5a (9.6)	4.9 ± 0.4a (9.0)	3.4 ± 0.4a (8.3)
Oxymatrine	1	0.2± 0.2(2)	-	-	-
	2	0.2± 0.2(2)	-	-	-
Azamax	1	1.5 ± 0.1b (5.8)	1.1 ± 0.2b (3.4)	0.9 ± 0.1b (2.5)	0.8 ± 0.2b (1.8)
	2	1.1 ± 0.3b (6.6)	1.1 ± 0.2b (3.5)	1.0 ± 0.1b (2.8)	0.6 ± 0.0b (1.1)
Azact	1	0.9 ± 1.6b (8.0)	0.8 ± 0.2b (5.9)	0.7 ± 0.3b (5.6)	0.5 ± 2.0b (4.5)
	2	0.8 ± 0.4b (7.9)	0.9± 0.1b (5.6)	0.3 ±0.1b (3.9)	0.4 ± 0.0b (2.1)
Abamectin	0.2	-	-	-	-
F		24.450	26.815	23.67	26.38
Df		6, 48	4, 32	4, 32	4, 32
P- value		< 0.001	< 0. 001	< 0.001	< 0.001

Numbers in parentheses: average number of surviving females used to estimate average number of eggs laid per surviving female at each evaluation

[a] Concentration of commercial product in mL per liter of water.

[b] Data (mean ± SE) followed by the same letter in a column do not differ significantly from each other by GLM with quasi-Poisson distribution, followed by the post hoc Tukey's test ($p < 0.05$).

Regarding the final effect, the residual efficiency of biopesticide treatments (abamectin remained high) gradually reduced over time after spraying (Fig. 1D).

Azadirachtin had the lowest toxicity to *P. longipes* adults, and its formulations did not differ from each other, showing mortality rates between 8 and 28.0% 3 days after spraying ($F = 11.938$; df = 7, 32; $p < 0.001$) (Table 7). Conversely, both oxymatrine concentrations caused high mortality of *P. longipes*, not differing from abamectin. All treatments negatively affected mite total fecundity (eggs/female). Nevertheless, azadirachtin had less impact on mortality compared to abamectin ($F = 24.221$; df = 7, 32; $p < 0.001$) (Table 7).

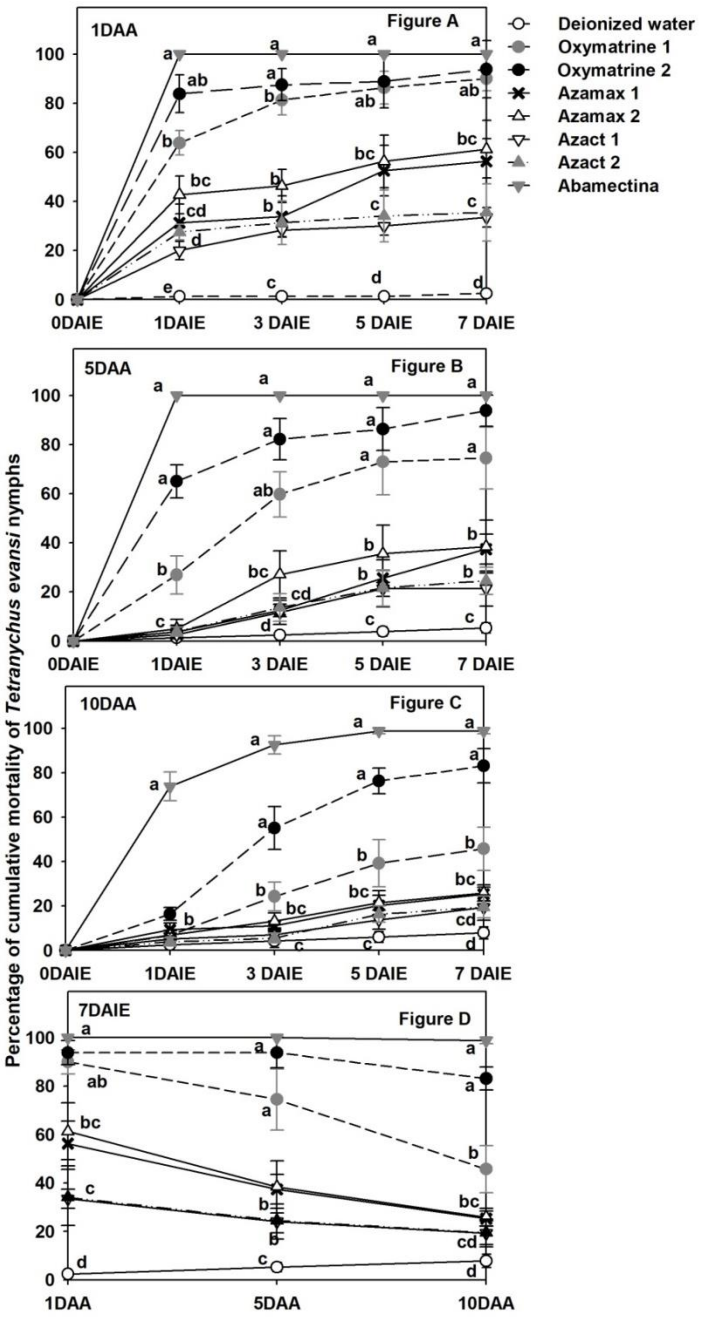

Fig. 1. A- C. Mortality (%) of nymphs of *Tetranychus evansi* at 1, 3, 5, and 7 days after initiation of evaluation (DAIE) on leaflets taken from 1, 5, and 10 days tomato plants after application(DAA) in a screen-house [n= 8]. **D.** Cumulative mortality of nymphs of *Tetranychus evansi* at 7 days of exposure on leaflets taken respectively from 1, 5 and 10 days tomato plants after application(DAA) in a screen-house.[n= 8]. Scatters indicate the level of mortality and standard error of *Tetranychus evansi.* Columns scatter (mean ± SE) followed by the same letter for each assessment do not differ significantly (GLM with quasi-binomial distribution, followed by post-hoc Tukey test; $p < 0.05$).

Table 7. Lethal (adult mortality) and sublethal (fecundity and fertility) effects, reduction coefficient and IOBC/WPRS toxicity categories of *Phytoseiulus longipes* adults after 3days of exposure under different treatments (oxymatrine- as well as azadirachtin-based formulations Azact and Azamax).

Treatments	Concentration used (mL c.p. L^{-1})[a]	Adult Mortality[b] (M_c %)	Effect on reproduction (E_r)			Reduction Coefficient[e] (E_x)	IOBC/WPRS toxicity categories[f]
			Fecundity		Egg viability[c] (% egg hatching)		
			[c]Number of eggs	[d] Reduction (%)			
Control	-	0.0 ± 0.0c	10.3 ±1.1a	-	100.0 ± 0.0a	-	-
Oxymatrine	1	60.0 ± 5.48a	1.6 ±1.1b	73.1	100.0± 0.0a	93.8	III
	2	74.0 ± 18.6a	0.7 ± 0.3c	87.3	88.9 ± 11.11a	98.4	III
Azamax	1	28.0 ± 5.83b	3.8±0.6b	46	96.4 ±2.3a	74.1	II
	2	22.0 ± 3.74b	2.9 ± 0.6b	56.1	100.0 ± 0.0a	77.9	II
Azact	1	8.0 ± 2.0b	4.1 ± 0.5b	43.1	100.0 ± 0.0a	62.7	II
	2	16.0 ± 4.0b	6.2 ± 2.0ab	24.8	100.0 ± 0.0a	49.0	II
Abamectin	0.2	88.0 ± 4.9a	0.5 ± 0.3c	90.7	77.8 ±22.22a	99.5	IV
F		11.938	24.221		2.1202		
d.f.		7, 56	7, 56		7, 56		
P- value		< 0.001	< 0. 001		0.07		

Number of initial adults of *Phytoseiulus longipes* in each treatment = 50. [a] Concentration of commercial product in mL per liter of water.[b] Data (mean ± SE) followed by the same letter in a column followed for each assessment do not differ significantly (GLM with quasi-Binomial distribution, followed by post hoc Tukey test; $P < 0.05$). [c] Number of eggs laid/ female in 3 days; Data (mean ± SE) followed by the same letter in a column do not differ significantly (GLM with quasi-Poisson distribution, followed by post hoc Tukey test; $p < 0.05$). [d] % reduction in relation to a number of eggs laid per female in the control treatment.[e] Reduction coefficient of insecticides calculated by formula $Ex = 100- (100- Mc) \times R_1 \times R_2$ as proposed by Biondi et al. (2012). [f] Toxicity categories proposed by the IOBC/WPRS for pesticide selectivity studies on natural enemies: I: harmless ($Ex < 30\%$); II: slightly harmful ($30\% \leq Ex \leq 79\%$); III: moderately harmful ($80\% \leq Ex \leq 99\%$), and IV: highly harmful ($Ex > 99\%$) as described by Sterk et al. (1999).

Moreover, neither biopesticides affected fertility (% of hatched larvae or egg viability, for eggs produced by treated females) in any treatment ($F = 2.1202$; $df = 7, 32$; $p = 0.07$). According to the reduction coefficient determined after 3 DAIE and the IOBC / WPRS classification, abamectin was highly harmful (class IV) to *P. longipes* adults, while oxymatrine in the different concentrations tested was moderately harmful (class III) (Table 7). Azadirachtin-based formulations in the different concentrations tested were slightly harmful (class II) to *P. longipes* adults.

Discussion

The results of this study can be summarized as follows: a) oxymatrine and azadirachtin-based formulations did not affect egg hatchability, but caused high mortality of the post-embryonic stages of *T. evansi*; b) azadirachtin-based formulations required longer to cause the mortality of the different stages than oxymatrine or abamectin, although, at the longest exposure time of 7 days, all evaluated products and doses had similar effects, except for the lower effect of Azact at the lower concentration; c) oxymatrine and azadirachtin affected oviposition; d) oxymatrine had a relatively prolonged residual activity against *T. evansi*; e) azadirachtin-based formulations caused low mortality and low effect on the oviposition of *P. longipes*. In short, these findings support the use of these biopesticides in the integrated management of *T. evansi*.

The efficacy of oxymatrine was comparable to that of abamectin, the positive control. Oxymatrine was very toxic to larvae, nymphs, and adult females and drastically reduced fecundity. Similar results were reported by Zanardi et al. (2015) for the citrus red mite, *Panonychus citri* (McGregor) (Acari: Tetranychidae), and by Andrade et al. (2019) for the citrus leprosis mite, *B. yothersi*. This compound causes the disturbance of acetylcholinesterase balance affecting acetylcholine receptors and the production of acetylcholinesterase, in addition to acting sodium channels in arthropod nerve cells (Ali et al. 2017; Andrade et al. 2019).

Although oxymatrine did not affect eggs, it was highly toxic to larvae hatching from treated eggs and in contact with the treated substrate. Similar results were obtained by Andrade et al. (2019) for *B. yothersi*. In addition, it produced a quick effect in this study, causing more than 65% mortality of each post-embryonic stage just one day after exposure to the recently treated substrate, indicating its knockdown effect. Azamax showed high efficacy against adult females, causing 80.0–86.3% mortality compared to Azact, which caused only 53.8–68.8% mortality. The drastic reduction caused by both the formulations of Azadiractin and oxymatrine in the eggs laid by *T. evansi* females surviving constituted an important factor for the reduction of the pest population in the future generation. This result suggests that

the biopesticides evaluated have not only a toxic effect, but also an effect on the *T. evansi* oogenesis, which can assist in its control.

Differences in mortality between Azamax and Azact are related to the concentration of active ingredient in the respective formulations, as Azamax has 12 g L^{-1} and Azact only 2.4 g L^{-1} azadirachtin, although their different formulations might also have a bearing, as discussed by Knapp & Kashenge (2003). Inert ingredients and adjuvants can also alter pesticide efficacy against target pests (Mesnage, & Antoniou 2018). Our results corroborate those of Soto et al. (2010) for azadirachtin under other types of formulations, who reported mortality of *T. evansi* females above 95% on tomato after treatments with Natuneem Agrícola® (Natural Rural, Araraquara, São Paulo, Brazil) (31.1 and 20.4 mg ai L^{-1}) and Organic Neem® (Dalquim Indústria e Comércio Ltda, Itajai, Santa Catarina, Brazil) (39.1 and 30.4 mg ai L^{-1}). Conversely, Santos et al. (2017) observed lower efficacy (5-15% mortality) of still other azadirachtin formulations (Organic® and Pironim®, Agroterra Insumos Agrotecnologia, São José do Rio Preto, São Paulo, Brazil) at concentrations of 2, 4, 6, 8, and 10% against *T. evansi* females. Schlesener et al. (2013) reported mortality of 89.7% of females of *T. urticae*, after spraying Azamax at a concentration of 0.5% commercial product in the laboratory. Azadirachtin disrupts arthropod growth by antagonizing ecdysone, a hormone directly involved in ecdysis (Mordue & Nisbet, 2000). Therefore, azadirachtin affects mainly immatures, as observed in the present study (although some effect on adults was also observed). However, studies by Schmutterer (1990), Copping & Men (2000), and Bernardi et al. (2012) indicated that this substance can negatively affect fecundity in females, also observed in our study.

In relation to evaluations done with leaflets taken from sprayed plants after different periods, our results suggested that oxymatrine had a residual effect on *T. evansi* nymphs of up to 10 days (mortality rates of up 85.5% on the last day of evaluation), while the residual effect of azadirachtin was of only one day (mortality rates of at most 30% on the last day of evaluation, on leaflets treated 5 and 10 days before the units were constructed for the test). Biopesticide residues were exposed to environmental conditions in the screen-house, as it was not climatized. In this sense, temperature, air relative humidity, and solar radiation varied in the screen-

house, and these are the main climatic factors responsible for speeding up biopesticide degradation (Yu, 2008; Ahmadi et al. 2018). Xiang et al. (2012) estimated the half-life of oxymatrine to be about seven days, while Caboni et al. (2006) found the half-life of azadirachtin to be lower than 11.3 hours. The longer persistence of oxymatrine is advantageous, for reducing production costs. Andrade et al. (2020) reported good effect of this compound against *Oligonychus ilicis* (McGregor) (Tetranychidae) for up to 10 days. In contrast, Zanardi et al. (2015) reported reduced mortality of *P. citri* by oxymatrine displaying a persistence of approximately one day after application on citrus in a screen-house. However, tomato plants have relatively high densities of leaf trichomes (Savi et al. 2019b), which can increase pesticide persistence (Antonious & Snyder 1993).

Effects on natural enemies should also be considered in acaricide efficacy studies. In the absence of harmful pesticide residues, spider mite populations are often adequately controlled by predatory mites (McMurtry et al. 1970; Schmidt-Jeffris & Beers 2018; Bergeron & Schmidt-Jeffris 2020). Our results showed significantly different toxicity levels and sublethal effects on the fecundity of *P. longipes* adults exposed directly to both biopesticides, without apparent effect on fertility (egg viability). The positive control (abamectin) was highly harmful (Ex > 99%, class IV) to *P. longipes* causing higher mortality of adults and major sublethal effects on reproduction of surviving females. High toxicity and side effects of abamectin have been reported on other phytoseiids, such as *Euseius scutalis* (Athias-Henriot), *Neoseiulus californicus* (McGregor), *Phytoseiulus macropilis* (Banks), and *Phytoseiulus persimilis* Athias- Henriot (Cote et al. 2004, Bernadi et al. 2012; Döker & Kazak 2020). The results of this study suggest that at the tested dosage, abamectin is incompatible with this predator, by the potential to suppress its population, which could limit the potential of *P. longipes* as a biological control agent in tomato crops.

Although oxymatrine was classified as moderately harmful to *P. longipes* (80% ≤ Ex ≤ 99%, IOBC class III), it can cause to its mortality and sublethal effects similar to those of the positive control (abamectin). These results are comparable to previous studies of other phytoseiids, such as *P. persimilis*, *Neoseiulus fallacis*

McGregor, and the predatory beetle *Stethorus punctillum* Weise (Coleoptera: Coccinellidae) (Shah & Appleby 2019). These findings suggest at least partial incompatibility of the use of oxymatrine with *P. longipes*.

In this study, the effect of the biopesticides on the *P. longipes* was assessed only by direct contact exposure. However surviving predators at the egg stage could soon allow the quick recovery of the predator population, as the residual persistence of oxymatrine seems to be low when compared to synthetic acaricides. Fang et al. (2017) found low mortality of *Neoseiulus cucumeris* (Oudemans) females exposed to oxymatrine residual contact. Andrade et al. (2019) also reported that residual contact of this bioacaricide did not affect the population levels of phytoseiids *Amblyseius chiapensis* De Leon, *Amblyseius* sp., and *Iphiseiodes zuluagai* Denmark & Muma on citrus.

Conversely, we found low harmful toxicity (30% ≤ Ex ≤ 79%; IOBC class II) of azadiractin formulations to *P. longipes*, given the low mortality of adult females and low sublethal effects on the offspring produced by the surviving females. These results are similar to those reported for *P. persimilis* and *N. cucumeris* (Spollen & Isman 1996); *N. californicus* and *P. macropilis* (Bernadi et al. 2012), and *Neoseiulus barkeri* (Athias-Henriot) (Lima et al. 2013) exposed to azadirachtin. The low *P. longipes* adult mortality rate caused by azadiractin is likely due to the mode of action of this biopesticide, which acts mainly as molting disruptor (Sieber & Rembold 1983), therefore with expected reduced effect on adult phytoseiids. However, immature stages of P. longipes could likely be more severely affected (Mordue & Nisbet 2000).

Our findings suggest that the use of the biopesticides here evaluated could be more sustainable alternatives in *T. evansi* control than certain synthetic products, for their relatively short persistence in the environment. Furthermore, azadiracthin-based formulations were revealed to be relatively safer to the predatory mite. Although oxymatrine-based formulations have been shown as effective for controlling *T. evansi*, it has the potential to reduce the population levels of predatory mites in tomato crops, hampering their use in IPM programs. Although the combined use of oxymatrine and *P. longipes* may not be recommended on tomatoes, their

alternating use could be feasible, given the relatively short persistence of this biopesticide.

Acknowledgments

The authors thank the Brazilian Coordination for Improvement of Higher Education Personnel (CAPES) - Financial Code 001 - for granting a doctoral scholarship to the first author, and the National Council for Scientific and Technological Development (CNPq) for granting the research productivity scholarship to DJ Andrade (Process No. 301492 / 2018-2). A special thanks to Prof. Dr. Tiago Gallina of the Federal University of Pampa, campus of Uruguaiana, Rio Grande do Sul (Brazil), for his valuable assistance during *P. longipes* collection.

References

Abbott, W.S. (1925) A method of computing the effectiveness of an insecticide. *Journal of Economic Entomology*, 18, 265–267.

Ahmadi, Z., Saber, M., Bagheri, M. & Mahdavinia, G.R. (2018) *Achillea millefolium* essential oil and chitosan nanocapsules with enhanced activity against *Tetranychus urticae*. *Journal of Pest Science*, 91, 837–848.

Ali, S., Zhang, C., Wang, Z., Wang, X.M., Wu, J.H., Cuthbertson, A.G.S., Shao, Z. & Qiu, B.L. (2017) Toxicological and biochemical basis of synergism between the entomopathogenic *fungus Lecanicillium muscarium* and the insecticide matrine against *Bemisia tabaci* (Gennadius). *Scientific Reports*, 7, 46558.

Andrade, D.J., da Rocha, C.M.R., de Matos, S.T.S. & Zanardi, O.Z. (2020) Oxymatrine-based bioacaricide as a management tool against *Oligonychus ilicis* (McGregor) (Acari: Tetranychidae) in coffee, *Crop Protection* https://doi.org/10.1016/j.cropro.2020.105182.

Andrade, D.J., Ribeiro, E.B., Morais, M.R. & Zanardi, O.Z. (2019). Bioactivity of an oxymatrine-based commercial formulation against *Brevipalpus yothersi* Baker and its effects on predatory mites in citrus groves. *Ecotoxicology & Environmental Safety*, 176, 339-345.

Antonious, G.F. & Snyder, J.C. (1993) Trichome density and pesticide retention and half-life. *Journal of Environmental Science & Health*, 28, 205-219.

Azandéme-Hounmalon, G.Y., Affognon, H.D., Komlan, F.A., Tamo, M., Fiaboe, K.K.M., Kreiter, S. & Martin, T. (2015). Farmers control practices against the invasive red spider mite, *Tetranychus evansi* Baker & Pritchard in Benin. Crop Prot. 76, 53–58.

Bates, D., Mächler, M., Bolker, B. & Walker, S. (2015) Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, 67, 1–48.

Bergeron, P.E. & Schmidt-Jeffris, R.A. (2020) Not all predators are equal: miticide non-target effects and differential selectivity. *Pest Management Science*, 2170-2179.

Bernardi, D., Botton, M., Cunha, U.S., Bernardi, O., Malausa, T., Garcia, M.S. & Nava, D.E. (2012) Effects of azadirachtin on *Tetranychus urticae* (Acari: Tetranychidae) and its compatibility with predatory mites (Acari: Phytoseiidae) on strawberry. *Pest Managment Science*, 69, 75-80.

Biondi, A., Desneux, N., Siscaro, G. & Zappalà, L. (2012) Using organic-certified rather than synthetic pesticides may not be safer for biological control agents: Selectivity and side effects of 14 pesticides on the predator *Orius laevigatus*. *Chemosphere*, 87,803–812.

Blair, B.W. (1983) *Tetranychus evansi* Baker & Pritchard (Acari: Tetranychidae); a new pest of tobacco in Zimbabwe. *Coresta Phytopathology & Agronomy Study Groups Bergerac*, pp. 1–6.

Caboni, P., Sarais, G., Angioni, A., Garcia, A.J., Lai, F., Dedola, F. & Cabras, P. (2006) Residues and persistence of neem formulations on strawberry after field treatment. *Journal of Agricultural & Food Chemistry,* 54, 10026-10032.

Copping, L.G. & Menn, J.J. (2000) Biopesticides: a review of their action, application, and efficacy. *Pest Managment Science*, 56, 651–676.

Cote, K.W, Shultz, P.B. & Lewis, E.E. (2004) Using acaricides in combination with *Phytoseiulus persimilis* to suppress *Tetranychus urticae* populations. *Journal of Entomological Science* 39: 267– 274.

Döker, İ. & Kazak, C. (2020). Toxicity and risk assessment of acaricides on the predatory mite, *Euseius scutalis* (Athias-Henriot) (Acari: Phytoseiidae) under laboratory conditions. *Chemosphere*, 127760. https://doi.org/10.1016/j.chemosphere.2020.127760

Demétrio, C.G.B., Hinde, J. & Moral, R.A. (2014) *Models for overdispersed data in entomology*. In: Ferreira, C.P., Godoy, W.A.C. (Eds.), Ecological Modeling Applied to Entomology. Springer, Switzerland, pp. 219–259.

de Moraes G.J. & McMurtry J. A. (1985) Chemically mediated arrestment of the predaceous mite *Phytoseiulus persimilis* by extracts of *Tetranychus evansi* and *Tetranychus urticae*. *Experimental & Applied Acarology*, 1, 127–138.

FAO [Food and Agriculture Organization of the United Nations], 2017. FAOSTAT Database on Agriculture. Food and Agriculture Organization of the United Nations. FAOSTAT, Rome, Italy is available online at: http://faostat3.fao.org/.

Ferragut, F., Garzzon-Luque, E. & Pekas, A. (2013) The invasive spider mite *Tetranychus evansi* (Acari: Tetranychidae) alters community composition and host-plant use of native relatives*. Experimental & Applied Acarology*, 60, 321–341.

Ferrero, M., de Moraes, G.J., Kreiter, S., Tixier, M.S. & Knapp, M. (2007) Life tables of the predatory mite *Phytoseiulus longipes* feeding on *Tetranychus evansi* at four temperatures (Acari: Phytoseiidae, Tetranychidae). *Experimental & Applied Acarology*, 41: 45–53.

Fang, X.D., Ouyang, G.C., Lu, H.L., Guo, M.F. & Wu, W.N. (2017) Ecological control of citrus pests primarily using predatory mites and the bio-rational pesticide matrine. *International Journal of Pest Management*, 64, 262–270.

Furtado, I.P., De Moraes, G.J., Kreiter, S. & Knapp, M. (2006). Search for effective natural enemies of *Tetranychus evansi* in south and southeast Brazil. *Experimental & Applied Acarology*, 40, 157-174.

Ghazy, N. A., Gotoh, T. & Suzuki, T. (2019) Impact of global warming scenarios on life-history traits of *Tetranychus evansi* (Acari: Tetranychidae). *BMC Ecology*, 19:48.

Golec, J.R., Hoge, B. & Walgenbach, J.F. (2020). Effect of biopesticides on different *Tetranychus urticae* Koch (Acari: Tetranychidae) life stages. *Crop Protection*, 128, 105015.

Guedes, R.N.C., Smagghe, G., Stark, J.D. & Desneux, N. (2016) Pesticide-induced stress in arthropod pests for optimized integrated pest management programs. *Annual Review of Entomology*, 61, 43–62.

Hothorn, T., Bretz, F. & Westfall, P. (2008) Simultaneous inference in general parametric models. *Biometrical Journal*, 50, 346–363.

Knapp, M., & Kashenge, S. S. (2003) Effects of different neem formulations on the twospotted spider mite, *Tetranychus urticae* Koch, on tomato (*Lycopersicon esculentum* Mill.). *International Journal of Tropical Insect Science,* 23, 1–7.

Lima, D.B., Melo, J.W.S., Guedes, R.N.C., Siqueira, H.A.A., Pallini, A., Gondim & M.G.C. Jr. (2013) Survival and behavioural response to acaricides of the coconut mite predator *Neoseiulus baraki. Experimental & Applied Acarology*, 60, 381–393.

Marčić, D. & Medo, I. (2014) Acaricidal activity and sublethal effects of an oxymatrine based biopesticide on two-spotted spider mite (Acari: Tetranychidae). *Experimental &Applied Acarology*, 64, 375–391.

McMurtry, J.A., Huffaker, C.B., & van de Vrie, M. (1970) Ecology of tetranychid mites and their natural enemies: A review: I. Tetranychid enemies: Their biological characters and the impact of spray practices Hilgardia 40, p331-390.

Mesnage, R., & Antoniou, M. N. (2018) Ignoring adjuvant toxicity falsifies the safety profile of commercial pesticides. *Frontiers in Public Health, 5.*

Mordue, A.J. & Nisbet, A.J. (2000) Azadirachtin from the neem tree *Azadirachta indica*: its action against insects. *Anais da Sociedade Entomológica do Brasil*, 29, 615-632.

Navajas, M., Moraes, G.J. & Pigeon, P.A.A. (2012) Revision of the invasion of *Tetranychus evansi*, biology, colonization pathways, potential expansion and prospective for biological control. *Experimental and Applied Acarology*, 59, 43–65.

Ndolo, D., Njugunaa, E., Adetunji, C.O., Harborc, C., Rowea, A., Breeyend, A.D., Sangeethae, J., Singhf, G., Szewczykg, B., Anjorinh, T.S., Thangaduraii, D. & Hospeti, R. (2019) Research and development of biopesticides: challenges and prospects. *Outlooks on Pest Management*, 30, 267-276.

Nelder, J.A. & Wedderburn, R.W.M. (1972) Generalized linear models. Journal of the Royal Statistical Society, 135, 370–384.

Nyoni B.N., Gorman, K., Mzilahowa, T., Williamson, M.S., Navajas, M., Field, L.M. & Bass, C. (2011) Pyrethroid resistance in the tomato red spider mite, *Tetranychus evansi*, is associated with mutation of the para-type sodium channel. *Pest Managment Science*, 67, 891–897.

Oerke, E.C. (2006) Crop losses to pests. *Journal of Agricultural Science*, 144, 31-43.

R Development Core Team (2019) R: A Language and Environment for Statistical Computing. Vienna.

Reddy, P.P. (2016) Biorational pest management. In: Sustainable Crop Protection under Protected Cultivations, pp. 99–108.

Santos, M. D. dos, Maciel, A. G. S., Trindade, R. C. P., Silva, E. S. & Duarte, A. G. (2017) Eficiência do óleo de nim e do extrato pironim sobre o ácaro vermelho do tomateiro *Tetranychus evansi* Baker & Pritchard (Acari: Tetranychidae). *Ciência Agrícola*. 15, 53-59.

Savi, P.J., de Moraes, G.J., Melville, C.C. & Andrade, D.J. (2019a) Population performance of *Tetranychus evansi* (Acari: Tetranychidae) on African tomato varieties and wild tomato genotypes. *Experimental & Applied Acarology*, 77, 555–570.

Savi, P.J., de Moraes, G.J., Boiça Junior A.L., Melville C.C., Carvalho, R.F., Lourenção, A.L. & Andrade, D.J. (2019b) Impact of leaflet trichomes on settlement and oviposition of *Tetranychus evansi* (Acari: Tetranychidae) in African and South American tomatoes. *Systematic & Applied Acarology*, 24, 2559–2576.

Savi, P.J., de Moraes, G.J. & Andrade, D.J. (2021) Effect of tomato genotypes with varying levels of susceptibility to *Tetranychus evansi* on performance and predation capacity of *Phytoseiulus longipes*. BioControl. https://doi.org/10.1007/s10526-021-10096-5

Schmutterer, H. (1990) Properties and potential of natural pesticides from the neem tree, *Azadirachta indica*. *Annual Review of Entomology*, 35, 271–297.

Shah, R. & Appleby, M. (2019) Testing the contact and residual toxicity of selected low-risk pesticides to *Tetranychus urticae* koch and its predators. *Sarhad Journal of Agriculture*, 35, 1113-1121.

Sieber, K.P. & Rembold, H. (1983) The effects of Azadirachtin on the endocrine control of molting in *Locusta migratoria Journal of Insect Physiology*, 29, 523-527.

Silva, P. (1954) Um novo ácaro nocivo ao tomateiro na Bahia. *Boletim do Instituto Biológica da Bahia*, *1*, 1–20.

Schlesener, D.C.H., Duarte, A.F., Guerrero, M.F.C., Cunha, U.S. & Nava, D.E. (2013) Effects of neem on *Tetranychus urticae* Koch (Acari: Tetranychidae) and the predators *Phytoseiulus macropilis* (Banks) and *Neoseiulus californicus* (Mcgregor) (Acari: Phytoseiidae). *Revista Brasileira de Fruticultura*, 35, 59-66.

Schmidt-Jeffris, R.A. & Beers, E.H. (2018) Potential impacts of orchard pesticides on *Tetranychus urticae*: A predator-prey perspective. *Crop Protection,* 103, 56–64.

Soto, A., Venzon, M., Oliveira, R.M., Oliveira, H.G. & Pallini, A. (2010) Alternative control of *Tetranychus evansi* Baker & Pritchard (Acari: Tetranychidae) on tomato plants grown in greenhouses. *Neotropical entomology*, 39, 638-644.

Spollen, K.M. & Isman, M.B. (1996) Acute and sublethal effects of a neem insecticide on the commercial biological control agents *Phytoseiulus persimilis* and *Amblyseius cucumeris* (Acari: Phytoseiidae) and *Aphidoletes aphidimyza* (Diptera: Cecidomyiidae). *Journal of Economic Entomology*, 89, 1379–1386.

Wang, Y.L., Guan, Z.G., Jia, X.S., Wu, S.Y. & Wei, H.G. (2007) Study progress of matrine application in farming pest control. *Journal of Shanxi Agricultural University*, 40, 424–428.

Wekesa, V.W., Maniania, N.K., Knapp, M. & Boga, H.I. (2005) Pathogenicity of *Beauveria bassiana* and *Metarhizium anisopliae* to the tobacco spider mite *Tetranychus evansi*. *Experimental & Applied Acarology*, 36, 41–50.

Xiang, Z., Shang, S., Cai, K., Geng, Z. & Chen, X. (2012) Determination and decline study of matrine residue in tobacco by gas chromatography-nitrogen chemiluminescence detector. *Chinese Journal of Pesticide Science*, 14, 115-121.

Yu, S.J. (2008) *The toxicology and biochemistry of insecticides*. Boca Raton, CRC Press, 276pp.

Zalom F. G. (2003) Pests, endangered pesticides and processing tomatoes. *Acta Horticulturae*, 613, 223–233.

Zanardi, O. Z., Ribeiro, L.P., Ansante, T.F., Santos, M.S., Bordini, G.P., Yamamoto, P.T. & Vendramim, J.D. (2015) Bioactivity of a matrine-based biopesticide against four pest species of agricultural importance. *Crop Protection*, 67, 160-167.

CHAPTER 5 - Risk assessment of ten commonly pesticides used in tomato cropping system on the predatory mite *Phytoseiulus longipes* (Acari: Phytoseiidae)

Abstract - The phytoseid mite *Phytoseiulus longipes* is a relatively new bio control agent found in the extreme south of Brazil and northern Argentina for controlling spider mites mostly the invasive pest *Tetranychus evansi*. There is a great interest in its introduction to Africa, where *T. evansi* has caused considerable damage over recent years. Better understanding on risks associated with pesticides used in agro-systems against that phytoseiid mite is important to their successful integration into augmentation and/or conservation programs. Through studies performed under laboratory and screen-house conditions, we assess lethal and sublethal effects of direct exposure as well as the persistence of residual activity of ten pesticides commonly used in western African tomato agro-system on eggs and adults of *P. longipes*. The pesticides include two pyrethroids (cypermethrin and deltamethrin), two organophosphates (dimethoate, chlorpyrifos), a neonicotinoid (imidacloprid), two acaricides (propargite and abamectin) and three biopesticides (oxymatrine, azadirachtin, *H. thompsonii*). The percentage of mortality and the sublethal effects on predator reproductive capacity were summarized in a reduction coefficient (E_x) and the pesticides were classified according to the IOBC (International Organization for Biological Control) toxicity categories. Furthermore, we estimated the life-table parameters of the predator. All pyrethroid and organophosphate insecticides were highly harmful to both stages. Other pesticides resulted in effect moderately harmful to both stages with the exception of azadirachtin and *H. thompsonni* that were slightly harmful to adult. Furthermore, those pesticides reduced life-table parameters *(*R_0, r, λ and T) being r-value of imidacloprid negative. Azadirachtin was classified as harmless for 4-day old residue while the effect of oxymatrine and *H. thompsonii* was reduced from slightly harmful for 4-day residue to harmless for 10-day old residue. The effect of abamectin was reduced from moderately harmful for 4-day old residue to harmeless for 10-day old residue. The effect of propargite and imidacloprid that was moderately harmful for 4- and 10-day old residue reduced to harmless for 20-day whereas that of pyrethroid or organophosphate were highly harmful until 31 days. The results suggest that pyrethroid and organophosphate insecticides are not compatible with both conservation and augmentative releases. Azadirachtin is the safest with augmentative release and conservation (mostly during the period of low presence of eggs). Other pesticides are most suitable with augmentative releases than conservation if appropriate safety deadlines are respected before release.

Key-words: Phytoseiid mites, Augmentative releases, Conservation biological control, IPM *Tetranychus evansi*, selective pesticide, Persistence

Introduction

The tomato red spider mite, *Tetranychus evansi* Baker and Pritchard, is one of the economically major pest problem in tomato and some other solanaceous plants in some countries (Saunyama and Knapp, 2003; Azandémè-Hounmalon et al., 2015; Savi et al., 2019; Migeon and Dorkeld, 2022). Although originally described from Mauritius (Baker and Pritchard, 1960), it is considered to be native to South America, probably northeastern Brazil, from where it has spread to other parts of the American continent and to Africa, Europe and Asia (Silva 1954; Navajas et al., 2012; Migeon and Dorkeld, 2022). It is presently found in 44 countries, but studies have indicated that climate changes can accelerate its spread to new territories (Meynard et al., 2013; Ximénez-Embún et al., 2016; Fan et al., 2021; Migeon and Dorkeld, 2022). In eastern and southern Africa, this spider mite has become one of the most invasive pests, with potential to reduce tomato yield by up to 90% (Saunyama and Knapp, 2003; Navajas et al., 2012).

Control of *T. evansi* populations relies mostly on the use of synthetic acaricides or insecticides with acaricide properties. However, the rapid development and high reproductive capacity (Qureshi et al., 1969; de Moraes and Flechtmann, 2008) allow *T. evansi* to reach high population levels, which often leads producers to repeat several pesticide applications for its control (de Moraes and Flechtmann, 2008; Nyoni et al., 2011; Azandémè-Hounmalon et al., 2015; Savi et al., 2021a,b). Repeated applications of pesticides most often is unsustainable for leading to the development of pesticide resistance, increasing production costs, and causing environmental impacts as well as health risks to the producers and consumers (Handford et al., 2015; Nyoni et al., 2011; Azandémè-Hounmalon et al., 2015).

For this reason, Integrated Pest Management (IPM) has become an essential part of plant protection in several crops worldwide, with a view to reduce to restrain chemical treatments (Furtado et al., 2007; Silva et al., 2010; Ferrero et al., 2007, 2013). Within this context, predatory mites of the family Phytoseiidae have been highlighted on many occasions to be an important component of IPM programs.

Their predatory behavior on phytophagous mites, their short life-cycle and amenability for large-scale production make them good candidates for the control of several pests, especially spider mites (Abad-Moyano et al., 2009). After considerable search efforts for biological control agents of *T. evansi* in South America, the phytoseiid *Phytoseiulus longipes* Evans found in natural association with it in the extreme south of Brazil and northern Argentina; in fact, that has been shown to be the single promising predatory mite of this pest (Furtado et al., 2007; Silva et al., 2010; Savi et al., 2021b,c). Besides, *P*. *longipes* has demonstrated the potential to control also other *Tetranychus* species for belonging to the phytoseiids adapted to the type I-a lifestyle including *Tetranychus urticae* (Ferreiro et al., 2011; McMurtry et al., 2013). Furthermore, this predatory mite is able to survive in a relatively wide range of temperatures, making it promising to be used in different countries and continents where the pest is a source of concern for producers (Ferrero et al., 2011). The potential effectiveness demonstrated by this predatory mite for controlling *T. evansi* and other spider mites has generated great interest in its introduction to Africa, where that invasive pest has caused considerable damage over recent years (Kungu et al., 2020).

However, the incompatibility of *P. longipes* with chemicals commonly used in pest control could hamper its establishment, conservation and augmentation efforts in agroecosystems, as African producers of vegetable crops commonly make use of synthetic pesticides for the control of *T. evansi* and other pests (Nyoni et al., 2011; Azandémè-Hounmalon et al., 2015) . This could negatively affect the incorporation of this predator in that continent. Hence, the assessment of the potential side effects of pesticides commonly used for vegetable pest control in the area where *P. longipes* could be introduced is necessary (Desneux et al., 2006; Stark et al., 2007; Biondi et al., 2012; Put et al., 2015).

Besides short-term mortality (lethal effects), pesticides can also hamper biocontrol agent services by causing sublethal effects on demographic (development time, fecundity, fertility, longevity and sex ratio) and behavioral (predation rate, mobility, orientation and feeding activity) parameters of the agents (Desneux et al., 2007, Biondi et al., 2015; Guedes et al., 2016; Kim et al., 2018; Duso et al., 2020).

Therefore, the determination of the pesticide side effects on biocontrol agents must take into account the assessment of lethal effects as well as sublethal effects that may occur in pesticide-exposed populations (Stark et al., 2011; Biondi et al., 2013; Zanardi et al., 2017; Bozhgani et al., 2018; Duso et al., 2020). As pesticides can act through multiple routes of exposure (direct or residual contact, food ingestion), this should also be considered in assessment of pesticide risks (Rimoldi et al., 2017; Kim et al., 2018; Bergeron and Schmidt-Jeffris, 2020; Duso et al., 2020).

The sequential testing exposition scheme proposed by the International Organization for Biological Control (IOBC) “Pesticides and Beneficial Organisms” working group is a common approach used for the evaluation of pesticide impact on non-target species after pesticide exposure (Hassan 1994; Sterk et al., 1999; van de Veire et al., 2002; Wanumen et al., 2016). Compounds are classified in four toxic classes from harmless to harmful and are tested sequentially, beginning in the laboratory and moving on to further trials under semi-field or field conditions if required. This approach has been widely used in pesticide risk assessment on non-target arthropods among scientists in the safe combination of biological control agents with chemical control (Thomson and Hoffmann 2006, Arnó and Gabarra 2011, Biondi et al., 2012; Wanumen et al., 2016).

Life table parameters are recognized as another important tool to track and understand how pesticides can affect demographic and population parameters of natural enemies at the worst-case scenario (Stark and Banks 2003; Zanardi et al., 2017). Estimation of these parameters helps to elucidate both lethal and sublethal effects resulting from pesticide exposure of biocontrol agents (Alinejad et al. 2014; Zanardi et al., 2017; Shahbaz et al. 2019; Duso et al., 2020).

Given, *P. longipes* is a relatively new bio control agent and there is a lack of data on its susceptibility to pesticides, the objective of this study is to evaluate both the short- and long-term effects of ten pesticides commonly used in western African tomato cropping system on *P. longipes* in accordance with IOBC approach and life table parameters. The results of this study might help to understand whether the pesticides used for tomato pest control in that region can be safely combined with the use of *P. longipes*, and to define the appropriate deadlines for the release of the

predator in the area for eventual augmentative biological control, after pesticide application.

2. Materials and Methods

2.1. Mites

The original colony of *T. evansi*, used in this study to establish laboratory colony was collected from American black nightshade plants (*Solanum americanum* Mill) maintained in a screen house of Acarology laboratory at São Paulo State University, Jaboticabal Campus, Brazil. Some specimens were transferred onto commercial tomato plants (*S.lycopersicum* var. TLCV15), onto which they were reared for several generations (period of about 3 months), in a climate controlled chamber at 25±1ºC, 70± 10% relative humidity and 12-hour photoperiod. Periodically, senescent plants were replaced by new ones, to ensure adequate plant substrate.

The colony of *P. longipes* was established with specimens collected from *S. lycopersicum*, *S. americamum* and *Brugmansia suavolensis* L. growing naturally in the urban area of Uruguaiana, Rio Grande do Sul, Brazil (29°49'48.0”S 57° 06'04.0"W and 29°45'12.0"S 57°04'31.0"W) onto *S.lycopersicum* var. TLCV15. After confirmation of the identity of the species by the examination of specimens mounted in Hoyer´s medium under an optical microscope (Microscope Axio Imager.A2, coded with LED), live specimens were transferred onto an arena consisting of a synthetic plate (Paviflex®; 22 × 15 cm) resting onto a piece (3 cm thick) of foam mat in a plastic tray (25 × 17 × 9 cm). These were periodically fed with all stages of *T. evansi* periodically offered on infested leaflets of TLCV15 tomato genotype. The mat was maintained permanently wet by periodic addition of deionized water. The edge of the plate was covered with a strip of moistened cotton wool, which, in contact with the mat, prevented mites from escaping. To hold longer, the petioles of the leaflets were inserted in the strip of cotton wool. Infested leaflets were periodically replaced, and the trays were maintained in a climate-controlled chamber, under the same conditions mentioned in the previous section, for about three months (about five generations) prior to the initiation of the experiments.

2.2. Chemicals

Ten commercial pesticides belonging to different chemical families and commonly used for controlling caterpillars, aphids, small borer, bed bugs, leaf miner, whitefly or phytophagous mites in tomato crops were tested on *P. longipes* eggs and adults. The pesticides were tested at their field concentrations registered for use with the Brazilian Ministry of Agriculture, Livestock and Food Supply (Agrofit, 2021). The insecticides and the respective producers and recommended field concentrations (milligrams of active ingredient per liter of water) were: imidacloprid 100 (Provado 20% SC, Bayer S.A. SP, Brazil); Propargite 360 (Omite 72% EC, UPL do Brasil – Indústria e Comércio de Insumos Agropecuários S.A.); abamectin 3.6 (Vertimec 1.8% EC Syngenta Proteção de Cultivos Ltda, SP, Brazil); oxymatrine 2.0 (Matrine 0.2% SL, Dinagro Agropecuária Ltd., Ribeirão Preto, SP, Brazil); azadirachtin-based bioinsecticide 24 (Azamax®EC, 1.2% w/v, UPL do Brasil – Indústria e Comércio de Insumos Agropecuários S.A., Ituverava, SP, Brazil); *Hirsutella thompsonii* 8.0 (Skupa-Mite 0.4% SL, Maneogene Agrociências S.A. Jaguariúna – SP, Brazil). chlorpyrifos 450 (Sabre 45% EW, Dow AgroScience Industrial, Barueri, SP, Brazil); dimethoate 400 (Dimetoato Nortox 50% EC, Nortox, Arapongas, PR, Brazil), cypermethrin 62.5 (Cipermetrina Nortox 25% EC, Nortox Sa Arapongas – PR, Brazil); deltamethrin 25.0 (Decis 2.5% EC, Bayer S.A. SP, Brazil). The first two pesticides are respectively from the neonicotinoid group and the alkyl sulfite group whereas the second four products are biopesticides with acaricide activity. The third two and the last two insecticides are respectively from the pyrethroid and oganophosphate groups. Deionized water was used as a control treatment.

2.3. Experimental units

The experimental unit used for each bioassay consisted of a synthetic plate (Paviflex®; 15 × 20 mm) resting onto a 1-cm-thick piece of foam mat inside a rectangular Petri dish (120 × 20 mm) and maintained wet by periodic addition of deionized water. A tomato leaflet, whose petiole was inserted in the wet cotton wool

strip to maintain turgidity, was placed onto the synthetic plate to receive the tested predator. To prevent mites from escaping, the plate edge was covered with a strip of cotton wool, which contacted the mat.

2.4. Topical exposure of *Phytoseiulus longipes* to the pesticides

Separate bioassays were conducted to access lethal and sublethal effects of the topical exposure of *P. longipes* eggs and adults to each insecticide. The bioassays were conducted in a fully randomized design with in a climate controlled chamber, under the same conditions mentioned for the stock colony. The plant material used to perform all bioassays was TLCV15 tomato genotype free from pesticide applications.

Eggs

For the egg bioassay, 50 newly laid eggs (< 6 h old) were transferred onto a tomato leaflet with the abaxial side up in turn placed onto a layer of water-saturated cotton wool in a Petri dish (6 cm in diameter). The Petri dish with eggs was subjected to aqueous solution of every insecticide or deionized water (control) under a Potter tower (Burkard Scientific Co., Uxbridge, United Kingdom) calibrated at 27.6 kPa, spraying 2 mL of the solution, resulting in a deposition of 1.8 ± 0.1 mg fresh residue cm^{-2}. The treated leaflet was allowed to air-dry for 1 h and then the eggs exposed to insecticides were individualized on untreated tomato leaflet of each experimental unit described in item 2.3. A proportional mixture of all stages of *T. evansi* was added to each unit daily *ad libitum* as food when the predatory larvae began to emerge. The units were examined twice a day (6:00 AM and 6:00 PM) under a stereomicroscope to determine the survival rate and the duration of each developmental stage. Mite size, moving ability and presence of exuviae were observed to determine molting. Predatory mites reaching adulthood were sexed and transferred in couples to new experimental units, requiring the use of some males from the stock colony. Observations were performed daily to determine duration of

the pre-oviposition and oviposition periods, longevity of each sex and fecundity (eggs/female). In units in which males died before females, other males were taken from the colony to replace them. Data referring to males taken from stock colony and from mites that died in the cotton barrier while trying to escape were not taken in account in the statistical analyses. Observations were discontinued when all predators died.

To determine the fertility of females originated from eggs exposed to insecticides, a sample of 10 eggs (< 6 h old) was separated daily per treatment from eggs collected in the first five days of fecundity assessments and placed in the new experimental units. The numbers of emerged larvae were counted daily, and eggs that did not hatch after 4 days were considered dead.

Based on immature stage mortality and sublethal effects, i.e. fecundity and fertility of females, a reduction coefficient (E_x) was calculated for each treatment using the formula described by Biondi et al. (2012): $E_X = 100 - (100 - M_c) * R_1 * R_2$, in which M_c= Corrected mortality calculated using the Henderson and Tilton formula (1955); R_1= ratio between the number of eggs laid by females from eggs treated with insecticides and control, and R_2= ratio between the number of hatched larvae from eggs laid by females whose eggs were treated with insecticides and control. The values of E_x obtained were compared to the standards of laboratory ecotoxicological tests proposed by the IOBC / WPRS in four categories: I: harmless (E_x < 30%); II: slightly harmful (30% < E_x < 79%); III: moderately harmful (80% < E_x < 99%) and IV: highly harmful (E_x > 99%) (Sterk et al., 1999).

Adults

For the bioassay with adults, 50 newly emerged mites (25 females and 25males, <24 h old) obtained from the stock colony were used to form five groups of each sex per treatment. Each group consisted of transferring 5 females and 5 males to a tomato leaflet with the abaxial side up placed on a layer of water-saturated cotton wool in a Petri dish (12 cm in diameter). To prevent the predators from escaping, the leaflet edges were covered with a strip of cotton wool, and some *T.*

evansi eggs were added to leaflet. Then, the leaflets with *P. longipes* adults were sprayed with 2 mL of solution of each insecticides or deionized water (control) in a Potter tower as described for egg bioassay. Adult mortality (lethal effect on adults) was recorded 24 h after the spray treatments. Predatory mites were considered dead when they did not respond to touches by a fine brush. Adults surviving the topical spraying were separated by gender, and then couples (1 male and 1 female) were formed and transferred to experimental units described previously in section 2.3. A proportional surplus of all stages of *T. evansi* was added to each unit daily to ensure abundant food.

The units were examined daily under a stereomicroscope to evaluate insecticide effects on fecundity, and adult female and male longevities. Observations were discontinued when all predators died. The effect of insecticides on the egg viability (fertility) laying by females exposed to insecticides was also determined following the same procedure described for the fertility of females originated from eggs exposed to insecticides in egg bioassay. Based on the mortality of adults 24 h after topical exposure of insecticides (lethal effects) and on the sublethal effects (fecundity, fertility, longevity), a reduction coefficient was calculated for each treatment, following the same procedure and criteria described for section of eggs exposure to pesticides.

2.5. Residual effect and duration of the pesticides harmful to *Phytoseiulus longipes*

The residual effect and duration of the harmful activity of pesticides were accessed by exposing *P. longipes* adults to different ages of pesticides residues. For this purpose, TLCV15 variety tomato seedlings were grown in Styrofoam trays, filled with Bioplant® substrate (Bioplant Agrícola Ltda., Ponte Nova, Minas Gerais, Brazil) and maintained in a screen-house under automatic irrigation. About 15 days after sowing, fertilizer was applied to the soil (250 mL per plant of a solution of 10 g monoammonium phosphate in 1 L water). Ten days later, the seedlings had 4–5 leaves and each was transplanted into a 4-L pot filled to about 80% of its capacity

with a substrate composed of a mixture of soil, sand and dry bovine manure at a 1: 1: 1 ratio, previously autoclaved at 120 ºC for 3 h. Ten days after transplanting, each pot received 5 g of urea (44% N) and 5 g of potassium chloride (58% K_2O). Plants were manually irrigated daily to field capacity, avoiding wetting plant parts.

Five plants were sprayed with aqueous solution of each insecticide tested or deionized water (control) to runoff stage using a hand sprayer (Brudden® Practical; Brudden Equipamento Ltda, Pompeia, São Paulo, Brazil). After spraying, the plants were arranged in a fully randomized design in a screen-house, at the following conditions: Temperature (º C) 26.7 ± 0.3 C, RH 59.05 ± 0.60% ; Light: natural environment) to allow aging of pesticides in regular cropping conditions for 4, 10, 20 and 31 days after application. For each treatment, five leaflets (one per plant) were excised from the median third of the plant canopy at 1, 5 and 10 days after spraying, each representing a replicate. The leaflets excised were used for the preparation of five experimental units as previously described in item 2.3. Soon after, ten six-day-old adults (5 males and 5 females) were transferred from the stock colony to each unit. A surplus of all stages in an equitable proportion of *T. evansi* was added to each unit daily to ensure an abundance of food. The experimental units were kept in a climate chamber under the same conditions mentioned for the stock colony. For each treatment, dead adults and number of eggs produced by surviving females after 72-h exposure to different ages of pesticides residues were recorded. Predatory mites were considered dead when they remained immobile after touched with a fine paintbrush.

To determine egg viability (fertility) from females exposed to leaflets pesticide residues, five groups (10 eggs per group, 0-6 h old) were formed daily per treatment from eggs removed during determination of the number of eggs laid. Each group was transferred to a new experimental unit and the numbers of emerged larvae was recorded 3 days later. The trial was stopped when the pesticides were classified as harmless or up to one month after treatment (Hassan 1994). Based on the adult mortality (lethal effects) and the sublethal effects (fertility and fecundity), a total effect of pesticide or reduction coefficient *Ex* was calculated for each treatment for the four different ages of pesticides residues following the same procedure and criteria

described in item 2.3. When the pesticide is classified as harmless, the reduction coefficient is not calculated for other ages of pesticide residues. For the evaluation of the pesticide effects in the persistence test, the four toxicity categories of the IOBC/WPRS for the extended laboratory were used in each aged residue to assess its harmfulness: 1 (harmless, <25 %), 2 (slightly harmful, 25–50 %), 3 (moderately harmful, 51–75 %), and 4 (harmful, >75 %). Pesticide persistence was categorized as follows: A (short-lived: < 5days); B (slightly persistent: 5–15 days); C (moderately persistent: 16–30 days); and D (persistent, 30 days).

2.6. Data analysis

The age-stage two-sex life table model available at http://140.120.197.173/ecology/Download/Twosex-MSChart.rar (Chi, 2021) was used to analyze data referred to development time of different stages, survival rate, fecundity, adult longevity and subsequent populations parameters of *P. longipes* eggs exposed to pesticides (Chi et al., 2020). Age-specific survival rate (l_x), age-specific fecundity (m_x) were calculated for the treatments that at least reached the larval stage whereas net reproduction rate (R_0), intrinsic rate of increase (r), finite rate of increase (λ), and average generation time (T) were estimated for the treatments that have a mortality less than 85%. Standard errors (SE) of development, fecundity, survival and population parameters of treated *P. longipes* eggs were also estimated using the bootstrap procedure included in the TWOSEX-MS chart software. The bootstrap analysis uses random sampling. With a small number of samples, it will generate variable means and SE. Hence, we used 10,000 random resampling in order to reduce the variability of the SE estimates (Efron and Tibshirani 1993). A paired bootstrap (B = 100,000) test based on the 95% confidence interval of differences was used to compare the difference between treatments (Smucker et al., 2007; Wei et al., 2020).

Data referred to treated adult bioassays and persistence of the insecticides toxicity on *P. longipes* were analized with generalized linear models (GLM) (Nelder and Wedderburn, 1972) in statistical program R, version 3.5.3 (R Development Core

Team, 2019). The quasi-binomial distribution, quasi-Poisson and Gaussian distributions were used to analyze the data for proportion (adult mortalities, and female fertility), counts (female fecundity) and duration (female and male longevities), respectively. Data about fertility from treated eggs were also analyzed with GLM model using Gaussian distributions. The fit quality was determined through a half-normal graph with a simulation envelope (Hinde and Demétrio, 1998). In case of significant differences between treatments, multiple comparisons were performed with post-hoc Tukey's test ($p < 0.05$) using the “glht” function of the “multcomp” package, with adjusted *p* values.

3. Results

3.1. Topical exposure toxicity

3.1.1. Lethal and sublethal effects on eggs

Chlorpyrifos, cypermethrin and dimethoate treatments reduced *P. longipes* egg hatching to 0, 6 and 48% respectively (Table1). Deltamethrin, propargite, *H. thompsonii* and oxymatrine applications reduced egg-hatching rate only slightly, to ~72-84%. In contrast, abamectin, imidacloprid and azadirachtin were virtually innocuous to egg, comparable to the control (~88-98% versus 100%). Predator egg stage was longer with abamectin application and shorter with cypermethrin, dethametrin, dimethoate, oxymatrine, propargite and *H. thompsonii* applications, whereas azadirachtin and imidacloprid did not interfere with egg stage duration, in comparison with the control. No larva originating from eggs treated with cypermethrin application survived to the next development stage. Eggs exposed to other insecticides, except abamectin and azadiracthin, had lower larval survival in comparison with the control; for those two insecticides, larval survival was not significantly different from the control. Larval development time was significantly prolonged following treatment of eggs with dimethoate or dimethoate, intermediate when eggs were treated with other insecticides and faster in control (Table 1). All persticides significantly reduced nymph survival in comparison with the control.

Table 1. Means (±SE) duration (days) and survival (%) of immature stages of *Phytoseiulus longipes* when eggs were treated with pesticides.

Treatment	Concentration used (mg i.a. L^{-1})	Eggs		Larvae		Nymphs		Preadult survival (%)	Immature duration (days)
		Hatching (%)	Duration (days)	Survival (%)	Duration (days)	Survival (%)	Duration (days)		
Control	-	100.0± 0.0a	1.64±0.04b	100.0±0.0a	0.58± 0.03c	94.0±3.4a	2.27±0.1cd	94.0±3.4a	4.5± 0.1d
Abamectin	3.6	98.0±0.0 a	2.03 ±0.07a	90.0 ± .2ab	0.83± 0.04b	60.0± 6.9b	2.43±0.3bc	60.0± 6.9b	5.2± 0.2ab
Azadirachtin	24	87.2 ± 4.8ab	1.62±0.07b	92.7±4.1ab	0.74± 0.1b	48.9±7.3bc	2.02± 0.1e	48.9±7.3bc	4.6±0.1cd
Chlorpyrifos	450	-	-	-	-	-	-	-	-
Cypermethrin	62.5	6.1± 3.4d	1.42 ±0.08c	-	-	-	-	-	-
Deltamethrin	25	72.0±6.3b	1.39± 0.08c	47.2±8.2d	1.0± 0.0a	8.0±3.6d	3.14± 0.3a	8.0±3.6d	5.3± 0.1a
Dimethoate	400	48.0±7.0c	1.42± 0.1 c	83.3±7.6bc	1.0± 0.0a	14.0±4.8d	2.5± 0.3b	14.0±4.8d	4.75 ±0.2bc
Imidacloprid	100	95.6± 2.9a	1.75 ±0.08b	88.6±4.8b	0.76±0.04b	45.7±7.3bc	2.79± 0.2ab	45.7± 7.3bc	5.5± 0.2a
Oxymatrine	2	84.4±5.4b	1.20±0.07d	86.8±5.5b	0.74± 0.04b	57.8±7.2bc	2.37± 0.1c	57.8±7.3bc	4.5± 0.1d
Propargite	360	78.0±5.8b	1.23 ±0.07d	51.3± 7.9d	1.0± 0.0a	16.0±5.1d	2.9± 0.12a	16.0±5.0d	5.1 ± 0.1b
Hirsutella thompsonii	8	79.6± 5.7b	1.32±0.08cd	74.4± 7.0c	0.9±0.1a	38.8±6.9c	2.16±0.1d	38.8±7.0c	4.6± 0.2cd

In each column, means followed by a same letter do not differ from each other. Standard errors (SE) were estimated using 100,000 bootstraps and means were compared using the paired bootstrap test at 5% significance

Azadiracthin application reduced nymphal stage, whereas other treatments followed the same pattern reported for the larval stage, except for abamectin and oxymatrine, which did not differ from the control. The mean survival values (%) until adult emergence for eggs exposed to abamectin, oxymatrine, azadirachtin and imidacloprid was reduced to ~ 50-60%, compared to 94% in the control. Exposure of eggs to other insecticides resulted in immature survival of less than 40%. No significant difference in immature development time was observed between control and exposure of eggs to azadirachtin, oxymatrine or *H. thompsonii*. However, eggs exposed to abamectin, deltamethrin, dimethoate, imidacloprid or propargite increased immature development time of *P. longipes* (Table 1).

Age-specific survival rate (l_x) expresses the probability of survival of a *P. longipes* individual until age x (Figure 1). Exposure of *P. longipes* eggs to all insecticides produced clear negative effects on the predator suvival curves compared to that of the control. The age range for 50% survivorship in the control (19.0 ± 0.91 days) was substantiality longer than the range for eggs treated with insecticides, as follows: abamectin (11.0±2.1), oxymatrine (7.5±2.4), azadirachtin (6.5±1.9), imidacloprid (5.0±2.3), *H. thompsonii* (3.0±0.9), propargite (1.6±0.2), deltamethrin (1.5±0.2) and dimethoate (1.2±0.4). Life span of *P. longipes* individuals of control and propargite treatment were similar up to 46 days-old, but substantially longer than observed with other treatments. The shortest life span was observed for mites from eggs exposed to dimethoate and deltamethrin applications. Age-specific fecundity (m_x; average daily fecundity per individuals at age x) fluctuated throughout the oviposition period (Figure 1). Females obtained from eggs exposed to imidacloprid and propargite applications started oviposition a little latter than the control and other treatments. In comparison with the control, *P. longipes* females from all other treatments stopped oviposition much earlier before dying, except for propargite application, in which predators virtually oviposited until the end of life span. The highest maximum peak of specific daily fecundity was observed for females originating from eggs exposed to propargite. The highest values of specific daily fecundity was 1.3 egg (on the 15th day) for predators of the control, 1.2 egg (on the 33rd day) for predators exposed to propargite, and 1.0 egg (on the 12th and 10th

days) for predators exposed to azadirachtin and dimethoate treatments, respectively. In the other treatments, the maximum m_x value was less than 1.0 egg.

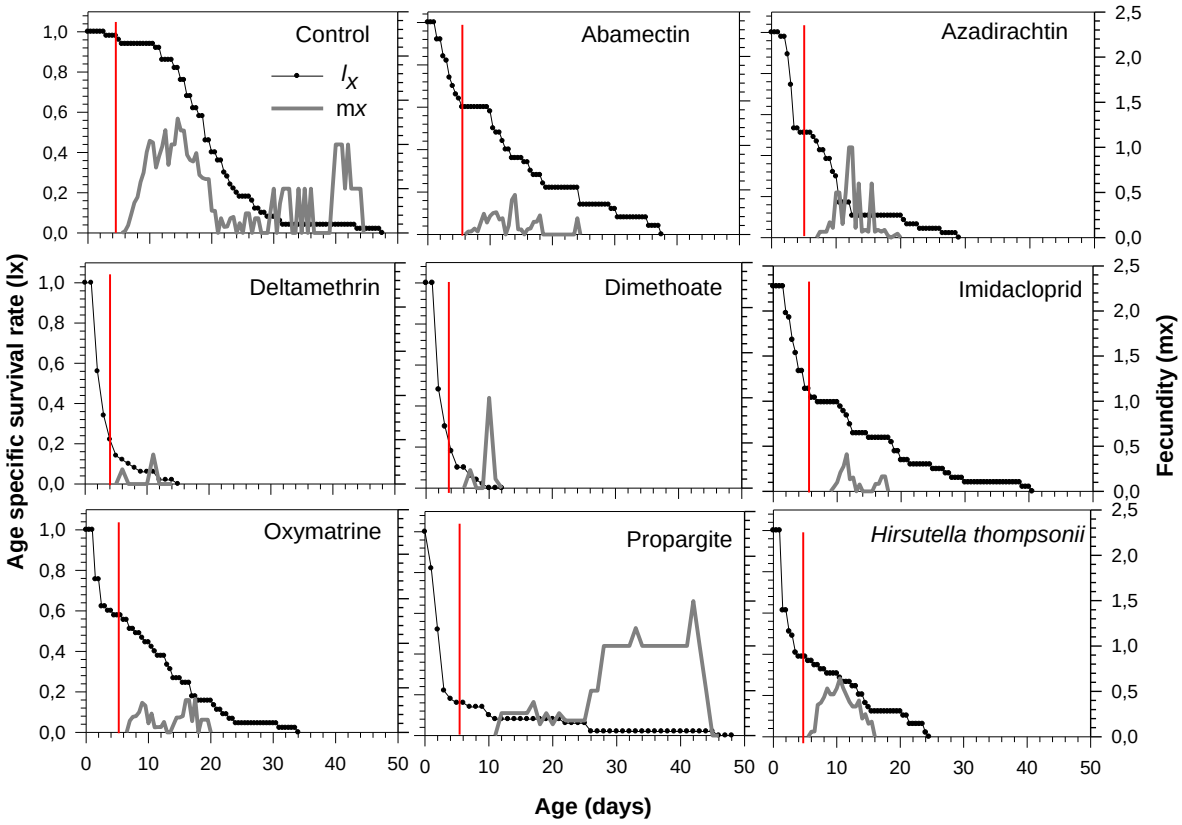

Figure1. Age-specific survival rates (l_x), age-specific fecundity (m_x) of *Phytoseiulus longipes* when eggs were treated with pesticides. The lines in red in different treatments indicate day of *P. longipes* adult emergence.

Total fecundity was significantly higher in the control than in any other treatment (Table 2), despite the relatively high variation observed mainly for propargite. Eggs exposed to deltamethrin, dimethoate, and propargite had significantly lower fertility than eggs of the control or exposed to other insecticides (GLM with binomial distribution: F=3.62; df =8, 49; p=0.002). All treatments, except abamectin, significantly reduced female longevity. However, male longevity was statistically the same for the control, abamectin, imidacloprid, oxymatrine and *H. thompsonii* treatments, but significantly lower for other treatments. Based on the reduction coefficient (E_x values) and according to the IOBC/WPRS classification,

Table 2. Lethal (corrected mortality of immature stage) and sublethal (total fecundity, fertility, and female and male longevities) effects, reduction coefficient and IOBC/WPRS toxicity categories of pesticides applied on eggs of *Phytoseiulus longipes*.

Treatment	Concentration used (mg i.a. L^{-1})	Corrected mortality of immature stage (M_c%)	Effect on reproduction (*Er*)		Longevity		Ex [b]	IOBC class [c]
			Total fecundity (eggs $female^{-1}$)	[a] Fertility (%) (number of hatched larvae	Female	Male		
Control	-	6.0	28.1± 2.1a	96.8± 1.5a	18.3± 1.4a	12.5 ± 0.9a	-	-
Abamectin	3.6	36.17	6.46± 2.1b	87.0 ± 8.3ab	18.04± 2.88a	12.9 ± 1.5a	86.82	III
Azadirachtin	24	47.94	9.02± 3.6b	93.8 ± 6.3a	12.63±2.27b	7.1± 0.3c	83.82	III
Chlorpyrifos	450	100.0	-	-	-	-	100	IV
Cypermethrin	62.5	100.0	-	-	-	-	100	IV
Deltamethrin	25	85.11	2.0±0.1c	53.2± 2.3c	4.5± 1.13c	4.4± 0.70c	99.42	IV
Dimethoate	400	91.49	2.0±0.1c	68.9± 5.6c	3.0± 0.38c	3.25± 1.22c	99.57	IV
Imidacloprid	100	51.43	3.33± 2.1c	85.0±9.57ab	13.55± 2.13b	14.50±3.19a	94.95	III
Oxymatrine	2	38.53	7.88± 3.4b	88.3± 3.33ab	14.25±3.31b	9.83±1.24ab	87.85	III
Propargite	360	82.98	17.6±6.8b	78.13± 7.7b	9.4± 5.6b	8.00±2.81b	91.4	III
Hirsutella thompsonii	8	58.75	12.0±4.1b	90.0± 10.0a	10.75±2.44b	9.22± 1.51ab	83.6	III

Means followed by the same letters in a column do not differ from each other. Standard errors (SE) were estimated using 100,000 bootstraps and means were compared using the paired bootstrap test at 5% significance. [a]Data (mean ± SE) followed by the same letter in a column do not differ significantly (GLM with quasi-binomial distribution, followed by post hoc Tukey test; $p < 0.05$). [b]Reduction coefficient of insecticides calculated by formula $Ex= 100-(100-Mc)x\ R_1xR_2\ x\ R_3\ x\ R_4x\ R_5$. [c] IOBC class toxicity in laboratory: I: harmless (Ex < 30%); II: slightly harmful (30% < Ex < 79%); III: moderately harmful (80% < Ex < 99%), and IV: highly harmful (Ex > 99%).

chlorpyrifos, dimethoate, cypermethrin, deltamethrin were highly harmful (class IV), while abamectin, azadirachtin, imidacloprid, oxymatrine, propargite and *H thompsonii* were moderately harmful (class III) to *P. longipes* eggs. Exposure of *P. longipes* eggs to abamectin, azadirachtin, imidacloprid, oxymatrine, propargite and *H. thompsonii* treatments reduced the net reproduction rate (R_0), intrinsic rate of increase (r) and the finite rate of increase (λ) of the predator in comparison with the control (Table 3). Average generation time (T) of eggs exposed to propargite was higher than that for the control, which in turn was higher than that for other treatments. The nearly 100% mortality of *P. longipes* immature stages prevented life table parameters estimation for predators exposed to chlorpyrifos, dimethoate, cypermethrin and deltamethrin treatments.

Table 3. Mean (± SE) life-table parameters of *Phytoseiulus longipes* when eggs were treated with pesticides.

Treatment	Concentration used (mg i.a. L^{-1})	R_0 (Offspring/ individual)	r(day^{-1})	λ (day^{-1})	T (day)
Control	-	18.0 ± 2.32a	0.228 ± 0.011a	1.256 ± 0.013a	12.648 ± 0.294b
Abamectin	3.6	1.68 ± 0.65b	0.036±0.03b	1.036 ± 0.031b	14.379± 3.059b
Azadirachtin	24	1.531±0.75b	0.039±0.069b	1.040±0.065b	10.831±0.729b
Imidacloprid	100	0.652±0.43b	-0.033± 0.05c	0.967±0.04c	12.966±0.876b
Oxymatrine	2	1.4± 0.72b	0.028±0.05b	1.029±0.05b	11.762±1.83b
Propargite	360	1.76± 1.54b	0.031±0.10b	1.031±0.09b	18.01± 4.20a
Hirsutella thompsonii	8	1.95±0.88b	0.068±0.05b	1.070± 0.05b	10.53± 0.84b

Means within a column followed by the same letter are not significantly different. The SEs were estimated by using 100,000 bootstraps and means were compared by using paired bootstrap test at 5% significance level. . R_0 = net reproductive rate; r = intrinsic rate of increase; λ = finite rate of increase; T = mean offspring time.

3.1.2. Lethal and sublethal effects on adults

All treatments caused a significantly higher adult mortality than the control ($F=24.403$; df =10, 44; $p<0.01$) (Table 4). Oxymatrine, chlorpyrifos, cypermethrin, deltamethrin and dimethoate treatments resulted in 88 to 100% mortality of adults within 24h of exposure. Abamectin, imidacloprid and propargite caused moderate levels of mortality (52-82%), whereas azadirachtin and *H. thompsonii* applications caused just 36-37.5% mortality within 24 h of exposure. Females surviving exposure to azadirachtin, imidacloprid and *H. thompsoni* treatments had fecundity 2–3 times lower than control, whereas exposure to abamectin, oxymatrine and propargite had fecundity 5–11 times lower than the control ($F=19.003$; df=6,69; $p<0.001$). No significant differences for egg viability were observed between the control and treatments in which the surviving females had oviposited ($F=19,003$; df = 6, 50; $p=0.3817$). For all insecticide treatments, longevity was reduced by half in comparison with the control for surviving adult females ($F= 24.039$; df = 6, 88; $p<0.001$) and males ($F= 16.149$; df = 6, 72; $p<0.001$) (Table 4). Based on the determined reduction coefficient and the IOBC/WPRS classification, chlorpyrifos, cypermethrin, deltamethrin, and dimethoate were highly harmful (class IV) whereas abamectin, imidacloprid, oxymatrine, and propargite were moderately harmful (class III) to *P. longipes* adults (Table 4). Only azadirachtin and *H. thompsonii* were slightly harmful (class II) to *P. longipes* adults.

3.2. Residual effects and duration of harmful activity of pesticides

The residue-ages of pesticides sprayed under screen-house conditions produced significant effects on adult mortality and offspring production of surviving individuals (Table 5). The interaction between residue-ages and treatments was also significant for adult mortality ($\chi2 = 41.03$, df =10; $p <0.001$) and egg production of surviving females ($\chi2 = 116.91$; df =10; $p <0.001$), but not for egg viability ($\chi2 = 96.5$; df = 10; $p = 0.643$).

Table 4. Lethal (corrected adult mortality) and sublethal effects (total fecundity, fertility, and female and male longevities), reduction coefficient and IOBC class toxicity of pesticides applied on adult *Phytoseiulus longipes*.

Treatment	Concentration used (mg i.a. L^{-1})	Adult mortality[a] (M_c %)	Effect on reproduction (*Er*)		Longevity [c]		Ex [d] (%)	IOBC/Class[e]
			Total fecundity[b] (eggs $female^{-1}$)	Egg viability (%)	Female	Male		
Control	-	2.4±1.1e	27.6±1.91a	98.5±1.47a	18.4±1.2a	12.6±0.93a	-	-
Abamectin	3.6	70.0±5.48b	3.5±0.83cd	84.4±8.10a	4.5±0.34b	4.2±0.31b	96.74	III
Azadirachtin	24	37.5±5.48d	10.8±5.2b	97.0±5.0a	8.0±2.16b	4.9±0.91b	75.91	II
Chlorpyrifos	450	100.0±2.00a	-	-	-	-	100	IV
Cypermethrin	62.5	100.0±0.0a	-	-	-	-	100	IV
Deltamethrin	25	100.0±0.0a	-	-	-	-	100	IV
Dimethoate	400	100.0±0.0a	-	-	-	-	100	IV
Imidacloprid	100	68.0±13.56b	8.3±3.9b	93.8±6.25a	5.6±1.44b	6.1±0.81b	90.84	III
Oxymatrine	2	82.0±8.60a	2.5± 0.5d	87.5±12.5a	4.0± 0.14b	3.7±0.33b	98.6	III
Propargite	360	52.0±3.74c	5.9±2.3c	95.0±3.62a	6.4±1.26b	4.0±0.97b	90.1	III
Hirsutella thompsonii	8	36.0±13.26d	9.4±2.4b	96.0±6.12a	6.0±0.99b	5.3±0.8b	78.75	II
F		24.403	19.003	1.089	24.039	16.149		
d.f.		10,44	6,69	6,50	6,88	6,72		
P		<0.001	<0.001	0.3817	<0.001	<0.001		

[a] Data (mean ± SE) followed by the same letter in a column do not differ significantly (GLM with quasi-binomial distribution, followed by post hoc Tukey test; $p < 0.05$). [b] Data (mean ± SE) followed by the same letter in a column do not differ significantly (GLM with quasi-Poisson distribution, followed by post hoc Tukey test; $p < 0.05$).[c] Data (mean ± SE) followed by the same letter in a column do not differ significantly (GLM with Gaussian distribution, followed by post hoc Tukey test; $p < 0.05$). [d] Reduction coefficient of insecticides calculated by formula $Ex= 100-(100-Mc)x\ R_1xR_2\ x\ R_3\ xR_4$ as proposed by Biondi et al. (2012). [e] IOBC toxicity class in laboratory I: harmless (Ex < 30%); II: slightly harmful (30% < Ex < 79%); III: moderately harmful (80% < Ex < 99%), and IV: highly harmful (Ex > 99%)

For 4-day old residues, exposures to dimethoate, deltamethrin, cypermethrin and chlorpyrifos caused 100% adult mortality whereas exposure to abamectin, propargite, oxymatrine and imidacloprid reduced mortality to 20–54% ($F=78.801$, $df = 10, 44$; $p<0.001$) (Table 5). Mortality rates with exposure to azadirachtin and *H. thompsonii* treatments did not differ from the control, all ranging between 8 and 10%.

Number of eggs produced by surviving females from azadirachtin treatment did not differ from the control, whereas other treatments resulted in fecundity 1.4–3.8 times lower than in the control ($F= 57.072$; $df = 10, 44$; $p<0.001$). Viability of eggs produced by females surviving abamectin, propargite, and imidacloprid treatments was reduced to 70–80% , while exposure to other treatments did not significantly affected viability, in comparison with the control ($F = 13.129$; $df = 6,28$; $p<0.001$).

For 10-day old residues, exposure to *H. thompsonii,* abamectin, oxymatrine and imidacloprid had statistically the same effect as the control, whereas exposure to propargite reduced mortality to 24%. For other treatments, adult mortality remained high (~94-98%) ($F = 98.1$, $df = 9, 40$; $p<0.001$). Number of eggs produced by surviving females after 72-h exposure to 10-day old residues of *H. thompsonii*, oxymatrine and abamectin treatments did not differ from the control, but fecundity of females surviving propargite and imidacloprid treatments was 1.5–2.5 times lower than the control ($F=63.1$; $df = 9,40$; $p <0.001$). For other treatments, fecundity was very close to zero. Egg viability of females exposed to chlorpyrifos, cypermethrin, deltamethrin and dimethoate treatments was 6–24%, whereas other treatments followed similar trend as reported for 4-day old residues except imidacloprid, which also did not differ from the control ($F=78.8$; $df= 9,40$; $p<0.001$).

For 20-day old residues, dimethoate, deltamethrin, cypermethrin or chlorpyrifos caused 70–86% adult mortality ($F=29.6$, $df = 6,28$; $p<0.001$) whereas imidacloprid, abamectin and propargite treatments did not affect adult mortality in comparison to the control, all ranging between 4 and 8%. Number of eggs produced by surviving females exposed to imidacloprid and propargite treatments was statistically the same as the control, but other treatments caused substantial reduction in fecundity ($F=76.7$; $d.f = 6, 28$; $p<0.001$).

Table 5. Mean (±SE) of corrective mortality and reproductive parameters of adult *Phytoseiulus longipes*, reduction coefficient and IOBC class toxicity of pesticides applied after 72-h exposure to 4, 10, 20 and 31 DAA aged residues of pesticides under screen-house (26.7 ± 0.3º C, RH 59.05 ± 0.60%, light environment)

Treatment	Concentration used (mg i.a. L^{-1})	Adult mortality[a] (M_c %)	Effect on reproduction (*Er*)		Ex (%)	IOBC Class [e]	Adult mortality[a] (M_c %)	Effect on reproduction (*Er*)		Ex (%)	IOBC Class [e]
			Number of eggs/ live females[b]	% of egg viability[a]				Number of eggs/ live females[b]	% of egg viability[a]		
		4DAA					**10 DAA**				
Control	-	2.7±1.4c	38.4±1.5a	100.0±0.0a	-	-	3.1±1.1c	39.2±2.2a	100.0±0.0a	-	-
Abamectin	3.6	38.0±6.6b	23.4±1.9b	70.1±8.1b	72.45	III	4.0±2.4c	31.6±2.6ab	98.0±6.8b	24.2	I
Azadirachtin	24	8.1 ± 2.0c	35.0±3.5a	94.0±2.5a	17.89	I					
Chlorpyrifos	450	100.0±0.0a	0.0 ± 0.0d	-	100	IV	98.0±1.3a	2.2 ±1.2d	6.0±2.4c	100	IV
Cypermethrin	62.5	100.0±0.0a	0.0±0.0d	-	100	IV	94.0±2.5a	2.4±0.6d	24.0±6.8c	100	IV
Deltamethrin	25	100.0±0.0a	0.0±0.0d	-	100	IV	98.0±0.0a	1.1±1.1d	22.0±4.9c	100	IV
Dimethoate	400	98.0.0±0.0a	0.0±0.0d	-	100	IV	97.0±0.0a	1.5±1.2d	6.0±2.4c	100	IV
Imidacloprid	100	36.2±2.3b	23.2±3.5b	72.0±3.7b	70.99	III	14.0±8.7bc	23.6±3.5bc	78.0±4.9b	57.9	III
Oxymatrine	2	20.1±4.5b	27.8±2.6b	92.0±3.8a	44.5	II	8.0±3.7c	36.4±3.3a	94.0±2.5ab	16.4	I
Propargite	360	54.0±9.3b	10.0±1.9c	80.0±6.3b	90.1	III	30.0±7.7b	16.0±2.7c	86.0±4.1b	74.4	III
Hirsutella thompsonii	8	10.0±3.2c	26.6±2.2b	94.0±2.5a	39.0	II	4.3±2.2c	37.8±1.1a	96.0±2.5a	7.4	I
F (d.f)		78.8(10,44)	57.1(10,44)	13.1(6,28)			98.7(9,40)	63.1(9,40)	78.8(9, 40)		
P		<0.001	<0.001	<0.001			<0.001	<0.001	<0.001		
		20 DAA					**31 DAA**				
Control	-	2.1±1.1b	37.3± 2.2a	98.1± 1.2a	-	-	2.3±1.4b	35.2±2.2a	98.6± 0.9a	-	-
Chlorpyrifos	450	86.0±8.7a	3.6±0.2b	16.0± 4.0b	99.8	IV	57.0±3.7a	7.6±1.4b	49.8±6.0b	95.3	IV
Cypermethrin	62.5	82.0±9.7a	4.6±1.5b	36.0±5.1b	99.2	IV	67.0.0±2.8a	8.4±0.6b	64.6±6.2b	94.8	IV
Deltamethrin	25	78.0±10.2a	5.4±0.6b	39.0±5.6b	98.7	IV	56.4±4.9a	7.6±0.2b	58.4±3.1b	94.4	IV
Dimethoate	400	70.0± 9.4a	6.6±1.2b	28.0±11.1b	98.5	IV	54.8± 4.8a	10.5±1.3b	54.0±7.2b	92.6	IV
Imidacloprid	100	6.0±4.9b	33.5±3.4a	97.0±4.0a	16.5	I					
Propargite	360	8.0±3.5b	34.8± 2.3a	98.0±1.2a	14.3	I					
F (d.f)		29.6(6,28)	76.7(6,28)	47.1(6,28)			53.8(4,20)	60.1(4,20)	56.3(4,20)		
P		<0.001	<0.001	<0.001			<0.001	<0.001	<0.001		

[a] Data followed by the same letter and for the same age residue in a column do not differ significantly (GLM with quasi-binomial distribution, followed by post hoc Tukey test; $p < 0.05$). [b]Data followed by the same letter in a column do not differ significantly (GLM with quasi-Poisson distribution, followed by post hoc Tukey test; $p < 0.05$).[c] Data followed by the same letter in a column do not differ significantly (GLM with Gaussian distribution, followed by post hoc Tukey test; $p < 0.05$). [d]Ex= 100-(100-Mc)x R1xR2. [e] IOBC toxicity class used in extended laboratory test: I: harmless (Ex < 25%); II: slightly harmful (25% < Ex < 50%); III: moderately harmful (51% < Ex < 75%), and IV: highly harmful (Ex > 75%).

Viability of the eggs laid by surviving females from different pesticides followed the inverse trend reported for adult mortality ($F = 47.1$; $df = 6, 28$; $p<0.001$).

For 31-day old residues, mortality due to the dimethoate, deltamethrin, cypermethrin or chlorpyrifos treatments was 54-67% ($F = 53.8$; $df = 4, 20$; $p<0.001$) higher than the control. The number of eggs produced by surviving females ($F = 60.1$; $df = 4, 20$; $p <0.001$) and egg viability ($F = 56.3$; $df = 4, 20$; $p<0.001$) followed the inverse trend reported for adult mortality.

Based on the reduction coefficient (E_x) and the IOBC/ classification, azadirachtin was classified as harmless for 4-day old residue (Table 5). The effect of oxymatrine and *H. thompsonii* was reduced from slightly harmful for 4-day residue to harmless for 10-day old residue while that of abamectin was reduced from moderately harmful for 4-day old residue to harmless for 10-day old residue. The effect of propargite and imidacloprid that was moderately harmful for 4- and 10-day old residue, reducing to harmless for 20-day residue. Other insecticides (chlorpyrifos, cypermethrin, deltamethrin and dimethoate) were classified as highly harmful at all evaluated residue ages. Based on the persistence toxicity class of IOBC (Table 6), azadirachtin was classified as short-lived (< 5days),

Table 6. Duration of the pesticides harmful to *Phytoseiulus longipes*

Treatment	Concentration used (mg i.a. L^{-1})	Persistence	
		Days	IOBC Persistence classes
Abamectin	3.6	5-15 days	B
Azadirachtin	24	< 5days	A
Chlorpyrifos	450	> 31days	D
Cypermethrin	62.5	> 31days	D
Deltamethrin	25	> 31days	D
Dimethoate	400	> 31days	D
Imidacloprid	100	16-30 days	C
Oxymatrine	2	5-15 days	B
Propargite	360	16-30 days	C
Hirsutella thompsonii	8	5-15 days	B

IOBC Persistence classes: A = short lived (<5 days); B= slightly persistent (5–15 days); C = moderately persistent (16–30 days); D = persistent (>30 days)

whereas abamectin, oxymatrine and *H. thompsonii* were classified as slightly persistent (5–15 days). Propargite and imidacloprid were moderately persistent (16–30 days) while other insecticides (chlorpyrifos, cypermethrin, deltamethrin and dimethoate) were persistent (> 31 days).

4. Discussion

The ten insecticides assessed at the field recommended rate in this study showed contrasting non-target effects on the predatory mite *P. longipes*, depending on studied life stage, the chemical group of the insecticide or exposure route. The non-target effects evaluated through topical exposure showed that chlorpyrifos and cypermethrin were acutely toxic to both egg and adult stages, but the inverse was observed for azadirachtin. On the other hand, abamectin, deltamethrin, dimethoate, oxymatrine and imidacloprid were more acutely toxic to adults than to eggs, whereas propargite and *H. thompsonii* caused moderate toxicity to both stages. These results are comparable to what has been reported in previous studies for other phytoseiid mites (Franco et al., 2017; Zanardi et al., 2017; Bergeron and Schmidt-Jeffris, 2020; Döker and Kazak, 2020). Besides the acute toxicity, significant sublethal effects, on development time, fecundity, fertility or longevity of surviving *P. longipes* life stages were verified with apparent effect of insecticides belonging to the chemical group of pyrethroids (cypermethrin and deltamethrin) and organophosphates (dimethoate). On the other hand, the insecticides that proved to be highly harmful to *P. longipes* through topical exposure under laboratory conditions were not necessarily highly harmful or persistent to it under screen-house conditions. This high variability of insecticide response to *P. longipes* highlights the importance to access the non-target effects of every pesticide on each predator life stage and through of different exposure routes.

Pyrethroid and organophosphate insecticides are still intensively used in horticultural crops in western African countries due to their broad-spectrum activity, affecting several pests (Azandémè-Hounmalon et al., 2015). However, the topical

exposure of two organophosphates, chlorpyrifos and dimethoate or the two pyrethroids, cypermethrin and deltamethrin, evaluated in this study proved to be highly harmful (class IV) to both egg and adult stages of *P. longipes*, as they caused 100% mortality of adults within 24 hours of exposure. In addition, 90-100% of the eggs exposed to chlorpyrifos and cypermethrin did not hatch. Conversely, acute toxicity on the exposed mite stage does not seem to be the only effect of dimethoate and deltamethrin. Despite the fact that 48 and 72 % of eggs exposed to those respective products had hatched, the emerging mites suffered severe sublethal effects, especially the high reduction of the survivorship of the post-embryonic stages (8–14%), and decreasing in fecundity (2.0 vs 28 eggs/ female on control) and adult longevity (3-4 vs 12-18 days on control). High acute toxicity, reduction in reproductive parameters or inability to reproduce were also reported for several other phytoseiid mites treated with pyrethroid or organophosphate insecticides (Croft, 19910; Castagnoli et al., 2005; Prischmann et al., 2005 ; Broufas et al., 2008; Silva et al., 2009; Hamby et al., 2013; Uddin et al., 2015; Beers and Schmidt, 2014; Franco et al., 2017 ; Schmidt-Jeffriset al., 2021).

Apart from high harmful effects of topical exposure, the residual activity of these insecticides have been shown also highly harmful (IOBC, Class IV) and persistent (IOBC, Class D) to *P. longipes* adults, as they did not lose the residual effects even 31 days after application, exhibiting a higher toxicity and significant reductions in reproductive parameters of *P. longipes* adult. Such high harmful effects and persistence of pyrethroid and organophosphate residues to phytoseiids, are likewise well documented (Croft, 1990, Croft and Whalon, 1982; Abou-Awad and El-Banhawy, 1985; Villanueva and Walgenbach 2005; Bostanian et al., 1985, Broufas et al., 2008; Hamby et al., 2013; Beers and Schmidt 2014, Férnandez et al., 2017; Franco et al., 2017). Therefore, the insecticides belonging to pyrethroid and organophosphates groups should not be used in tomato IPM programs that aim for the conservation of *P. longipes* populations or augmentative biological control, after pesticide application in area.

Imidacloprid proved to be moderately harmful (80% < Ex < 99%, Class III) when applied directly to eggs and adults. Furthermore, the value of intrinsic rate of

increase (*r*, the life table parameter that best reflects the combined effects of biological attributes such as survival, sex ratio, developmental duration and fecundity) of egg treated proved to be negative. In agreement with our findings, with findings of Argolo et al. (2013) also reported negative effect of imidacloprid on the demographic parameters of *Neoseiulus californicus.* Our results suggest therefore that topical exposure of imidacloprid could lead to the suppression of *P.longipes* population overtime. In general, the moderate harmfulness of imidacloprid to *P. longipes* adults is due to the high acute toxicity (68% mortality) recorded within 24 h after exposure and the significant reduction in the oviposition of surviving females. Conversely, the moderate harmfulness of imidacloprid against egg stage or negative value of intrinsic rate of increase (*r*) seems to be due to the dramatic sublethal effects observed on immature duration, survival of post-embryonic stages, decrease in egg production or reduction of longevity because the insecticide had limit impact on egg mortality. High acute toxicity to adult or pronounced sublethal effects of imidacloprid to *P. longipes* stages in the present study are in line with what has been reported for other phytoseiid predators, such as *N. californicus* (Villanueva and Walgenbach, 2005), *Phytoseiulus persimilis* (Duso et al., 2008), *Euseius gallicus* Kreiter & Tixier (Put et al., 2015), *Galendromus occidentalis*, *Neoseiulus fallacis* and *Amblyseius andersoni* (James 2003). In contrast to our result, James (1997) reported a beneficial effect of imidacloprid, by causing an increased egg production of *Amblyseius victoriensis* by up to 54% after exposure.

Neonicotinoids do not affect non-target arthropods through topical exposure alone, but may also display significant and prolonged side effects by residual contact and consumption of contaminated prey arthropods; they are labelled to be key insecticides in vegetable crop protection against sucking pests as aphids, whiteflies and thrips (Tomizawa and Casida, 2003). This seems to be the case of residual activity of imidacloprid on *P. longipes* adults, as its harmful activity lasted longer than 10 days in the present study. Residual effects of imidacloprid have been also reported on *Neoseiulus collegae*, *P. persimilis*, *G. occidentalis N. fallacis*, *N. californicus Phytoseiulus macropilis*, *Proprioseiopsis mexicanus* (Mizell and Sconyers 1992; James, 2003; Duso et al., 2008; Bostanian et al., 2010; Argolo et

al., 2013), *Iphiseius degenerans* (Döker et al., 2015) and *Iphiseiodes zuluagai* (Zanardi et al., 2017). However, in contrast to the moderate persistence (16–30 days) found for imidacloprid on *P. longipes* in this study, Franco et al. (2017) and Zanardi et al. (2017) observed in citrus that its harmful effects on *Euseius concordis* and *I. zuluagai* lasted 3 days. The harmful effects of the same product on *Macrolophus basicornis* (Stal) (Heteroptera: Miridae) reported by Wanumen et al. (2016) remained for 34 days, which is somewhat different from the finding in our study. Contrasting reported durations of harmful effects of imidacloprid between non-target arthropods could be due to differences in sensitivity of the species studied, plant substrate, light intensity and UV radiation during the aging of the residue (Wanumen et al., 2016; Fernandez et al., 2017; Savi et al., 2021b). Our results suggest that imidacloprid can be apparently safe for the mass releasing of *P. longipes* 20 days after pesticide application.

The bacterial fermentation product abamectin and alkyl sulfite product propargite are designed to be modern acaricides or insecticides used to control mites, leafminers, suckers, beetles and fire ants (Copping and Duke 2007) in tree, herb and vegetable crops including tomato (Bai and Ogbourne, 2016). The direct exposure of these two pesticides (80% < Ex < 99%, Class III) have been shown moderately harmful to either egg or adult stages. These two pesticides may not be therefore suitable for combined applications with *P. longipes* in IPM programs, but the positive value of the intrinsic rate of increase (*r*), though low, observed in present study is indicative that the *P. longipes* population could still increase over time. In general, these two acaricides did not have lethal effect when applied on egg but they caused delays in the development time of post-embryonic stages, reduced immature survival (case of propargite) and decreased significantly the female reproductive capacity (fecundity and viability)and adult longevities . Conversely, the moderate harmfulness of two acaricides to adult proved to be due to a high acute toxicity suffered as well as sublethal effects caused as well as reproductive parameters (fecundity and viability). In agreement with our findings, other studies reported high acute toxic or dramatic sublethal effects after direct exposure of abamectin or propargite against the phytoseiids *Typhlodromus pyri*, *Euseius*

scutalis, *N. californicus*, *N. fallacis*, *P. macropilis, E. gallicus* and *P. persimilis* (Hardman et al., 2003; Cote et al., 2004; Bostanian and Akalach 2006; Put et al., 2015; Döker and Kazak, 2020).

Apart from direct exposure effects, residue of these two acaricides also proved to harm *P. longipes*, abamectin becoming harmless only after 10 days of the application (slightly persistence) and propargite, only 20 days after application (moderate persistence). These results suggest that after abamectin and propargite applications in an area, *P. longipes* should not be released in the area within those periods. Residual toxicity of abamectin or propargite has been reported for other phytoseids, such as *N. californicus P. persimilis*, *Neoseiulus cucumeris*, *N. fallacis* and *I. degenerans*, *Amblyseius swirskii* (Kim et al., 2005; Bostanian and Akalach 2006; Ruiz and Moraes, 2008, Gentz et al., 2010; Uddin et al., 2015; Férnandez et al., 2017). On the other hand, Morse et al. (1987) and Udin et al. (2015) reported a moderate persistence (16–30 days) of harmful activity of propargite residues respectively for *Euseius stipulatus* and *N. carliforniicis,* which was consistent with our findings. In agreement with our findings, a slight persistence (5–15 days) of abamectin residues was reported for *N. carlifornicus*, *P. persimilis* and *A. swirskii* (van de Veire et al., 2001; Sáenz-de-Cabezón Irigaray et al., 2007; Nadimi et al., 2011; Ruiz and de Moraes, 2008; Udin et al., 2015, Férnandez et al., 2017).

Azadirachtin, oxymatrine, and the pathogenic fungus *H. thompsonii*, are products generally perceived as environmentally-safe with prevailing use in organic agriculture (Mordue and Nisbet 2000; Chandler et al., 2005; Biondi et al., 2013; Andrade et al., 2019). However, difference in their selectivity to the studied stages of *P. longipes* were obseved. Oxymatrine was determined as moderately harmful to both egg and adult stages, whereas azadirachtin and *H. thompsonni* were determined as moderately harmful to directly exposed eggs (80% < Ex < 99%, Class III) but as slightly harmful to adult (30% < Ex < 79%, Class III). These results suggest oxymatrine as unsuitable for conservation of the *P. longipes* population in the field; however, the difference in the susceptibility of both stages to azadirachtin and *H. tompsonni* could be an indicative that these products may partially be compatible with biological conservation control during the low egg population period in the field.

However, data of persistence duration after application of azadirachtin and *H. tompsonni* in crops are still necessary to validate such findings.

Moderately harmful effect of azadirachtin, oxymatrine, and *H. thompsonii* to eggs is due to the pronounced sublethal effects on the survivorship of post-embryonic stages, fecundity and longevity because they had limited lethal impact (low acute mortality). Such significant sublethal effects of these biopesticide resulted also in important delays of parameters of population growth. In the case of adults, the moderate harmfulness of oxymatrine is due to high acute toxicity and drastic sublethal effects on fecundity and longevity. Conversely, azadirachtin and *H. tompsonni* have a limit lethal impact on adult but they caused significant long term effects, reducing fecundity and adult longevity.

In agreement with our findings, Shah and Appleby (2019) reported high acute toxicity and dramatic sublethal effects on fecundity of *P. persimilis*, *N. fallacis* and *Stethorus punctillum* Weise (Coleoptera: Coccinellidae) after direct exposure to oxymatrine. The high acaricidal activity of oxymatrine may be associated with its mode of action. This biopesticide acts not only on the nicotinic acetylcholine receptors but also on the sodium channels of arthropod nerve cells (Ali et al., 2017; Andrade et al., 2019), matching high acute toxicity of adult or significant adverse effects on fecundity or longevity caused by oxymatrine.

Similar to our findings, relatively low acute mortality but harmful effects on reproductive parameters caused by azadirachtin were reported for other phytoseiid mites (Spollen and Isman, 1996, Sterk et al., 2003; Duso et al., 2008; Lima et al., 2015). Mordue and Blackwell (1998) and Biondi et al.(2012, 2013) reported that high susceptibility of immature stages and reduction of reproductive capacity are some consistent properties of neem-based formulations on non-target species, which are consistent with the low immature survival, decreased fecundity, and differential susceptibility of *P.longipes* by life stage (egg versus adult) to azadirachtin in the present study.

In agreement with the harmfulness of *H. thompsonii*, direct exposure to the studied stages of *P. longipes,* Fernando et al. (2007) also reported significant reduction of the population of *Neoseiulus baraki* in the coconut area treated with the

same pathogen fungus although, signs of dead mites were rarely seen. Phytoseiid mites exposed directly to other pathogen fungi, such as *Beauveria bassiana* (Balsamo) (Cordycipitaceae) or *Isaria fumosorosea*) (Wize) Brown & Smith, were also reported to be adversely affected in terms of survival, longevity and fecundity (Pozzebon and Duso, 2009; Seiedy et al., 2015, Ullah and Lim, 2017). Early stages are often less susceptible to fungal infection due to germinating conidia being shed during ecdysis (Midthassel et al., 2016), contrasting with the minimal harmfulness of adult in comparison eggs found in present study. Therefore, additional studies should be carried out to assess the physiological processes involved in difference of harmfulness between *P. longipes* stages. In contrast to our results, Wekesa et al. (2007) reported that the fecundity of *P. longipes* was not affected by feeding on the mites *Tetranychus evansi* Baker or *Tetranychus urticae* Koch infected with *Neozygites floridana* (Weiser & Muma). In the present study, the prey offered to *P. longipes* was not infected by the fungus *H. thompsonii*, but the prey could have been affected chemically by the insecticides used in the different treatments.

With respect to the results of residual effects and duration of harmful activity, azadirachtin proved to be harmless to *P. longipes* (Class I) because its residual activity lasted less than four days (short-live persistence, IOBC A), and no mortality or sublethal effects to the phytoseiid mite were observed. This is consistent with findings of other studies, in which this product was also reported as harmlessness and short-lived to phytoseiid mites such as *E. stipulatus* and *E. gallicus* (Viggiani and Bernado, 2001, Put et al., 2015). The harmlessness and the short-live persistence of azadirachtin suggest that this biopesticide would be the safest toward augmentative releases. Furthermore, the short-live persistence combined with the minimal harmful effect on adults in comparison with eggs support that azadirachtin could actually be suitable with conservation of *P. longipes* population, mostly during the periods of low presence of *P. longipes* eggs allowing therefore a rapid recolonization of predator.

In contrast to azadirachtin, oxymatrine and *H. thompsonii* proved to be slightly harmful to *P. longipes* adults exposed to 4-day old residue because at this age both biopesticides caused slight reduction of oviposition, in addition to a slight

lethal impact caused by oxymatrine. However, lethal impact or sublethal effects of these two biopesticides on *P. longipes* did not persist 10 days after the application (slight persistence, IOBC class B) suggesting that oxymatrine and *H. thompsonii* would be compatible with augmentative biological control if this 10-day period were respected before the release.

5. Conclusion

Through studies performed under laboratory and screen-house conditions, we have shown that the pesticides commonly used in tomato crop in West Africa greatly differed in their lethal and sub lethal toxicity to *P. longipes*, a new biocontrol agent of *T. evansi.* The results suggest insecticides belonging to pyrethroid (cypermethrin and deltamethrin) and organophosphate (dimethoate, chlorpyrifos) groups are not compatible with both conservation and increase of *P. longipes* population in IPM programs. Azadirachtin is the safest for augmentative biological control, as well as for conservation mostly during periods of low presence of *P. longipes* eggs. Other pesticides (abamectin, propargite, imidacloprid, oxymatrine, *H. thompsonii*) are most suitable for augmentative biological control than conservation if appropriate safety deadlines are respected before release.

6. References

Abad-Moyano, R., Pina, T., Ferragut, F., Urbaneja, A., 2009. Comparative life-history traits of three phytoseiid mites associated with *Tetranychus urticae* (Acari: Tetranychidae) colonies in clementine orchards in eastern Spain: implications for biological control. Exp Appl Acarol 47, 121–132.

Abou-Awad, B.A., El-Banhawy, E.M., 1985. Comparison between the toxicity of synthetic pyrethroids and other compounds to the predacious mite *Amblyseius gossipi* (Mesostigmata: Phytoseiidae). Exp Appl Acarol 1, 185–191.

Agrofit (2020) Sistema de Agrotóxicos Fitossanitários - Ministério da Agricultura, Pecuária e Abastecimento, Brasil. http://extranet.agricultura.gov.br/agrofit_cons/principal_agrofit_cons. Accessed 14 Jan 2020

Ali, S., Zhang, C., Wang, Z., Wang, X.-M., Wu, J.-H., Cuthbertson, A.G.S., Shao, Z., Qiu, B.-L., 2017. Toxicological and biochemical basis of synergism between the entomopathogenic fungus *Lecanicillium muscarium* and the insecticide matrine against *Bemisia tabaci* (Gennadius). Sci Rep 7, 46558.

Alinejad, M., Kheradmand, K., Fathipour, Y., 2014. Sublethal effects of fenazaquin on life table parameters of the predatory mite *Amblyseius swirskii* (Acari: Phytoseiidae). Exp Appl Acarol 64, 361–373.

Andrade, D.J. de, Ribeiro, E.B., de Morais, M.R., Zanardi, O.Z., 2019. Bioactivity of an oxymatrine-based commercial formulation against *Brevipalpus yothersi* Baker and its effects on predatory mites in citrus groves. Ecotoxicol. Environ. Saf. 176, 339–345.

Argolo, P.S., Banyuls, N., Santiago, S., Mollá, Ó., Jacas, J.A., Urbaneja, A., 2013. Compatibility of *Phytoseiulus persimilis* and *Neoseiulus californicus* (Acari: Phytoseiidae) with imidacloprid to manage clementine nursery pests. Crop Prot. 43, 175–182.

Arnó, J., Gabarra, R., 2011. Side effects of selected insecticides on the *Tuta absoluta* (Lepidoptera: Gelechiidae) predators *Macrolophus pygmaeus* and *Nesidiocoris tenuis* (Hemiptera: Miridae). J Pest Sci 84, 513–520.

Azandémè-Hounmalon, G.Y., Affognon, H.D., Komlan, F.A., Tamò, M., Fiaboe, K.K.M., Kreiter, S., Martin, T., 2015. Farmers' control practices against the invasive red spider mite, *Tetranychus evansi* Baker & Pritchard in Benin. Crop Prot. 76, 53–58.

Bai, S.H., Ogbourne, S., 2016. Eco-toxicological effects of the avermectin family with a focus on abamectin and ivermectin. Chemosphere 154, 204–214.

Baker, E.W., Pritchard, A.E., 1960. The tetranychoid mites of Africa. Hilg 29, 455–574.

Beers, E.H., Schmidt, R.A., 2014. Impacts of orchard pesticides on *Galendromus occidentalis*: Lethal and sublethal effects. Crop Prot. 56, 16–24.

Bergeron, P.E., Schmidt-Jeffris, R.A., 2020. Not all predators are equal: miticide non-target effects and differential selectivity. Pest Manag Sci 76, 2170–2179.

Biondi, A., Campolo, O., Desneux, N., Siscaro, G., Palmeri, V., Zappalà, L., 2015. Life stage-dependent susceptibility of *Aphytis melinus* DeBach (Hymenoptera: Aphelinidae) to two pesticides commonly used in citrus orchards. Chemosphere 128, 142–147.

Biondi, A., Desneux, N., Siscaro, G., Zappalà, L., 2012. Using organic-certified rather

than synthetic pesticides may not be safer for biological control agents: Selectivity and side effects of 14 pesticides on the predator *Orius laevigatus*. Chemosphere 87, 803–812.

Biondi, A., Zappalà, L., Stark, J.D., Desneux, N., 2013. Do biopesticides affect the demographic traits of a parasitoid wasp and its biocontrol services through sublethal effects? PLoS ONE 8, e76548.

Bostanian, N.J., Akalach, M., 2006. The effect of indoxacarb and five other insecticides on *Phytoseiulus persimilis* (Acari: Phytoseiidae),*Amblyseius fallacis* (Acari: Phytoseiidae) and nymphs of *Orius insidiosus* (Hemiptera: Anthocoridae). Pest. Manag. Sci. 62, 334–339.

Bostanian, N.J., Hardman, J.M., Thistlewood, H.A., Racette, G., 2010. Effects of six selected orchard insecticides on *Neoseiulus fallacis* (Acari: Phytoseiidae) in the laboratory: Effects of six insecticides on *Neoseiulus fallacis*. Pest. Manag. Sci. 66, 1263–1267.

Bozhgani, N.S.S., Kheradmand, K., Talebi, A. asghar, 2018. The effects of spirotetramat on the demographic parameters of *Neoseiulus californicus* (Phytoseiidae). saa 23, 1952.

Broufas, G.D., Pappas, M., Vassiliou, G., Koveos, D.S., 2008. Toxicity of certain pesticides to the predatory mite *Euseius finlandicus* (Acari: Phytoseiidae). IOBC/WPRS Bull 35, 85–91.

Castagnoli, M., Liguori, M., Simoni, S., Duso, C., 2005. Toxicity of some insecticides to *Tetranychus urticae*, *Neoseiulus californicus* and *Tydeus californicus*. Biocontrol 50, 611–622.

Chandler, D., Davidson, G., Jacobson, R.J., 2005. Laboratory and glasshouse evaluation of entomopathogenic fungi against the two-spotted spider mite, *Tetranychus urticae* (Acari: Tetranychidae), on tomato, *Lycopersicon esculentum*. Biocontrol Sci Technol 15, 37–54.

Chi H (2021) TWOSEX-MSChart: a computer program for the age-stage, two-sex life table analysis. (http://140.120.197.173/Ecology/prod02.htm) (Accessed June 2021).

Chi, H., You, M., Atlıhan, R., Smith, C.L., Kavousi, A., Özgökçe, M.S., Güncan, A., Tuan, S.-J., Fu, J.-W., Xu, Y.-Y., Zheng, F.-Q., Ye, B.-H., Chu, D., Yu, Y., Gharekhani, G., Saska, P., Gotoh, T., Schneider, M.I., Bussaman, P., Gökçe, A., Liu, T.-X., 2020. Age-Stage, two-sex life table: an introduction to theory, data analysis, and application. Entomol. Gen. 103–124.

Copping, L.G., Duke, S.O., 2007. Natural products that have been used

commercially as crop protection agents. Pest. Manag. Sci. 63, 524–554.

Cote, K.W., Schultz, P.B., Lewis, E.E., 2004. Using acaricides in combination with *phytoseiulus persimilis* athias-henroit to suppress *Tetranychus urticae* Koch populations. J. Entomol Sci. 39, 267–274.

Croft, B.A., 1990. Arthropod biological control agents and pesticides, environmental science and technology. Wiley, New York.

Croft, B.A., Whalon, M.E., 1982. Selective toxicity of pyrethroid insecticides to arthropod natural enemies and pests of agricultural crops. Entomophaga 27, 3–21.

de Moraes, G.J., Flechtmann, C.H.W., 2008. Manual de acarologia: acarologia básica e ácaros de plantas cultivadas no Brasil,2nd edn. Holos, Ribeirão Preto.

Desneux, N., Decourtye, A., Delpuech, J.-M., 2007. The sublethal effects of pesticides on beneficial arthropods. Annu. Rev. Entomol. 52, 81–106.

Döker, İ., Kazak, C., 2020. Toxicity and risk assessment of acaricides on the predatory mite, Euseius scutalis (Athias-Henriot) (Acari: Phytoseiidae) under laboratory conditions. Chemosphere 261, 127760.

Döker, İ., Pappas, M.L., Samaras, K., Triantafyllou, A., Kazak, C., Broufas, G.D., 2015. Compatibility of reduced-risk insecticides with the non-target predatory mite *Iphiseius degenerans* (Acari: Phytoseiidae): Risk assessment of reduced-risk insecticides to *I. degenerans*. Pest. Manag. Sci. 71, 1267–1273.

Duso, C., Malagnini, V., Pozzebon, A., Castagnoli, M., Liguori, M., Simoni, S., 2008. Comparative toxicity of botanical and reduced-risk insecticides to Mediterranean populations of *Tetranychus urticae* and *Phytoseiulus persimilis* (Acari Tetranychidae, Phytoseiidae). Biol. Control 47, 16–21.

Duso, C., Van Leeuwen, T., Pozzebon, A., 2020. Improving the compatibility of pesticides and predatory mites: recent findings on physiological and ecological selectivity. Curr. Opin. Insect Sci. 39, 63–68.

Efron, B., Tibshirani, R., 1993. An introduction to the bootstrap, Monographs on statistics and applied probability. Chapman & Hall, New York.

Fan, Q.-H., Li, D., Bennett, S., Balan, R.K., 2021. Diagnosis of a new to New Zealand spider mite, *Tetranychus evansi* Baker and Pritchard, 1960 (Acari: Tetranychidae). N Z Entomol 44, 59–70.

Fernández, M.M., Medina, P., Wanumen, A., Del Estal, P., Smagghe, G., Viñuela, E., 2017. Compatibility of sulfoxaflor and other modern pesticides with adults

of the predatory mite *Amblyseius swirskii*. Residual contact and persistence studies. BioControl 62, 197–208.

Ferrero, M., Calvo, F.J., Atuahiva, T., Tixier, M.-S., Kreiter, S., 2011. Biological control of *Tetranychus evansi* Baker & Pritchard and Tetranychus urticae Koch by *Phytoseiulus longipes* Evans in tomato greenhouses in Spain [Acari: Tetranychidae, Phytoseiidae]. Biol. Control 58, 30–35.

Ferrero, M., de Moraes, G.J., Kreiter, S., Tixier, M.-S., Knapp, M., 2007. Life tables of the predatory mite *Phytoseiulus longipes* feeding on *Tetranychus evansi* at four temperatures (Acari: Phytoseiidae, Tetranychidae). Exp Appl Acarol 41, 45–53.

Ferrero, M., Tixier, M.S., Kreiter, S., 2014. Different feeding behaviors in a single predatory mite species. 1. Comparative life histories of three populations of *Phytoseiulus longipes* (Acari: Phytoseiidae) depending on prey species and plant substrate. Exp Appl Acarol 62, 313–324.

Franco, A.A., Zanardi, O.Z., Jacob, C.R. de O., de Oliveira, M.B.R., Yamamoto, P.T., 2017. Susceptibility of *Euseius concordis* (Mesostigmata: Phytoseiidae) to pesticides used in citrus production systems. Exp Appl Acarol 73, 61–77.

Furtado, I.P., Moraes, G.J. de, Kreiter, S., Tixier, M.-S., Knapp, M., 2007. Potential of a Brazilian population of the predatory mite *Phytoseiulus longipes* as a biological control agent of *Tetranychus evansi* (Acari: Phytoseiidae, Tetranychidae). Biol.Control 42, 139–147.

Gentz, M.C., Murdoch, G., King, G.F., 2010. Tandem use of selective insecticides and natural enemies for effective, reduced-risk pest management. Biol. Control 52, 208–215.

Guedes, R.N.C., Smagghe, G., Stark, J.D., Desneux, N., 2016. Pesticide-Induced Stress in Arthropod Pests for Optimized Integrated Pest Management Programs. Annu. Rev. Entomol. 61, 43–62.

Hamby, K.A., Alifano, J.A., Zalom, F.G., 2013. Total effects of contact and residual exposure of bifenthrin and λ-cyhalothrin on the predatory mite *Galendromus occidentalis* (Acari: Phytoseiidae). Exp Appl Acarol 61, 183–193.

Handford, C.E., Elliott, C.T., Campbell, K., 2015. A review of the global pesticide legislation and the scale of challenge in reaching the global harmonization of food safety standards: Global Harmonization of Pesticide Legislation. Integr Environ Assess Manag 11, 525–536.

Hardman, J.M., Franklin, J.L., Moreau, D.L., Bostanian, N.J., 2003. An index for selective toxicity of miticides to phytophagous mites and their predators based

on orchard trials. Pest. Manag. Sci. 59, 1321–1332.

Hassan, S.A., Bigler, F., Bogenschütz, H., Boller, E., Brun, J., Calis, J.N.M., Coremans-Pelseneer, J., Duso, C., Grove, A., Heimbach, U., Helyer, N., Hokkanen, H., Lewis, G.B., Mansour, F., Moreth, L., Polgar, L., Samsøe-Petersen, L., Sauphanor, B., Stäubli, A., Sterk, G., Vainio, A., Veire, M., Viggiani, G., Vogt, H., 1994. Results of the sixth joint pesticide testing programme of the IOBC/WPRS-working group «pesticides and beneficial organisms». Entomophaga 39, 107–119.

Henderson, Chas.F., Tilton, Elvin.W., 1955. Tests with acaricides against the brown wheat mite. J. Econ. Entomol. 48, 157–161.

Hinde, J., Demétrio, C.G.B., 1998. Overdispersion: Models and estimation. Comput. Stat. Data Anal. 27, 151–170.

James, D.G., 2003. Toxicity of imidacloprid to *Galendromus occidentalis*, *Neoseiulus fallacis* and *Amblyseius andersoni* (Acari: Phytoseiidae) from hops in Washington State, USA. Exp Appl Acarol 31, 275–281.

James, D.G., 1997. Imidacloprid increases egg production in *Amblyseius victoriensis* (Acari: Phytoseiidae). Exp Appl Acarol 21, 75–82.

Kim, S.S., Seo, S.G., Park, J.D., Kim, S.G., Kim, D.I., 2005. Effects of selected pesticides on the predatory mite, *Amblyseius cucumeris* (Acari: Phytoseiidae). J Entomol Sci. 40, 107–114.

Kim, S.Y., Ahn, H.G., Ha, P.J., Lim, U.T., Lee, J.-H., 2018. Toxicities of 26 pesticides against 10 biological control species. J. Asia-Pac. Entomol. 21, 1–8.

Kungu, M., Subramanian, S., Salifu, D., Fiaboe, K.K.M., Azandémè-Hounmalon, G.Y., Gitonga, L., Onyambu, G.K., Deletre, E., Martin, T., 2020. Influence of predatory mites, *Phytoseiulus longipes* Evans. on the within-plant diurnal migration and distribution of the red spider mite, *Tetranychus evansi*, Baker and Pritchard on african nightshade, *Solanum scabrum*, in: Niassy, S., Ekesi, S., Migiro, L., Otieno, W. (Eds.), Sustainable management of invasive pests in Africa. Springer International Publishing, Cham, pp. 267–282.

Lima, D.B., Melo, J.W.S., Guedes, N.M.P., Gontijo, L.M., Guedes, R.N.C., Gondim, M.G.C., 2015. Bioinsecticide-predator interactions: azadirachtin behavioral and reproductive impairment of the coconut mite predator *Neoseiulus baraki*. PLoS ONE 10, e0118343.

Mcmurtry, J.A., Moraes, G.J.D., Sourassou, N.F., 2013. Revision of the lifestyles of phytoseiid mites (Acari: Phytoseiidae) and implications for biological control strategies. Syst. Appl. Acarol. 18, 297.

Meynard, C.N., Migeon, A., Navajas, M., 2013. Uncertainties in predicting species distributions under climate change: a case study using *Tetranychus evansi* (Acari: Tetranychidae), a widespread agricultural pest. PLoS ONE 8, e66445.

Midthassel, A., Leather, S.R., Wright, D.J., Baxter, I.H., 2016. Compatibility of *Amblyseius swirskii* with *Beauveria bassiana*: two potentially complimentary biocontrol agents. BioControl 61, 437–447.

Migeon A, Dorkeld F (2006-2021) Spider Mites Web: a comprehensive database for the Tetranychidae. INRA, http://www.montpellier.inra.fr/CBGP/spmweb/

Mizell, R.F., Sconyers, M.C., 1992. Toxicity of imidacloprid to selected arthropod predators in the laboratory. Fla. Entomol. 75, 277.

Mordue (Luntz), A.J., Blackwell, A., 1993. Azadirachtin: an update. J. Insect Physiol. 39, 903–924.

Mordue, A.J.L., Nisbet, A.J., 2000. Azadirachtin from the neem tree Azadirachta indica: its action against insects. An. Soc. Entomol. Bras. 29, 615–632.

Nadimi, A., Kamali, K., Arbabi, M., Abdoli, F., 2011. Study on persistence tests of miticides abamectin and fenproximate to predatory mite *Phytoseiulus persimilis* (Acarina: Phytoseiidae) Afr. J. Agric. Res. 6, 338-342.

Navajas, M., de Moraes, G.J., Auger, P., Migeon, A., 2013. Review of the invasion of Tetranychus evansi: biology, colonization pathways, potential expansion and prospects for biological control. Exp Appl Acarol 59, 43–65.

Nelder, J.A., Wedderburn, R.W.M., 1972. Generalized Linear Models. Journal of the Royal Statistical Society. Series A (General) 135, 370.

Nyoni, B.N., Gorman, K., Mzilahowa, T., Williamson, M.S., Navajas, M., Field, L.M., Bass, C., 2011. Pyrethroid resistance in the tomato red spider mite, Tetranychus evansi, is associated with mutation of the para-type sodium channel. Pest. Manag. Sci. 67, 891–897.

Pozzebon, A., Loeb, G.M., Duso, C., 2009. Grape powdery mildew as a food source for generalist predatory mites occurring in vineyards: effects on life-history traits. Annals of Applied Biology 155, 81–89.

Put, K., Bollens, T., Wäckers, F., Pekas, A., 2016. Non-target effects of commonly used plant protection products in roses on the predatory mite *Euseius gallicus* Kreiter & Tixier (Acari: Phytoseidae): Pesticide side effects on *Euseius gallicus*. Pest. Manag. Sci. 72, 1373–1380.

R Development Core Team, 2019. R: a language and environment for statistical

computing. R foundation for statistical computing, Vienna

Rimoldi, F., Fogel, M.N., Ronco, A.E., Schneider, M.I., 2017. Comparative susceptibility of two Neotropical predators, *Eriopis connexa* and *Chrysoperla externa*, to acetamiprid and pyriproxyfen: Short and long-term effects after egg exposure. Environ. Pollut. 231, 1042–1050.

Ruiz, M.G., Moraes, G.J. de, 2008. Mortalidade do ácaro predador *Neoseiulus californicus* (Acari: Phytoseiidae) em testes de toxicidade residual de inseticidas e acaricidas usuais em pomáceas. Rev. Bras. Frutic. 30, 919–924.

Sáenz-de-Cabezón Irigaray, F.J., Zalom, F.G., Thompson, P.B., 2007. Residual toxicity of acaricides to *Galendromus occidentalis* and *Phytoseiulus persimilis* reproductive potential. Biological Control 40, 153–159.

Saunyama, I., Knapp, M., 2004. Effect of pruning and trellising of tomatoes on red spider mite incidence and crop yield in Zimbabwe. African Crop Science Journal 11, 269–277.

Savi, P.J., de Moraes, G.J., Carvalho, R.F., de Andrade, D.J., 2021a. Bottom-up effects of breeding tomato genotypes on behavioural responses and performance of *Tetranychus evansi* population. J Pest Sci. https://doi.org/10.1007/s10340-021-01437-5

Savi, P.J., de Moraes, G.J., de Andrade, D.J., 2021b. Effect of tomato genotypes with varying levels of susceptibility to *Tetranychus evansi* on performance and predation capacity of *Phytoseiulus longipes*. BioControl 66, 687–700.

Savi, P.J., Martins, M.B., De Moraes, G.J., Hountondji, F.C.C., Andrade, D.J., 2021. Bioactivity of oxymatrine and azadirachtin against *Tetranychus evansi* (Acari: Tetranychidae) and their compatibility with the predator *Phytoseiulus longipes* (Acari: Phytoseiidae) on tomato. Syst. Appl. Acarol. 26, 1264-1279.

Savi, P.J., de Moraes, G.J., Melville, C.C., Andrade, D.J., 2019. Population performance of *Tetranychus evansi* (Acari: Tetranychidae) on African tomato varieties and wild tomato genotypes. Exp Appl Acarol 77, 555–570.

Schmidt-Jeffris, R.A., Beers, E.H., Sater, C., 2021. Meta-analysis and review of pesticide non-target effects on phytoseiids, key biological control agents. Pest Manag Sci 77, 4848–4862.

Seiedy, M., Tork, M., Deyhim, F., 2015. Effect of the entomopathogenic fungus Beauveria bassiana on the predatory mite *Amblyseius swirskii* (Acari: Phytoseiidae) as a non-target organism. Syst. Appl. Acarol. 20, 241-250.

Shah, R., Appleby, M., 2019. Testing the contact and residual toxicity of selected

low-risk pesticides to *Tetranychus Urticae* Koch and its predators. Sarhad J. Agric. 35, 1113–1121.

Shahbaz, M., Khoobdel, M., Khanjani, M., Hosseininia, A., Javadi Khederi, S., 2019. Sublethal effects of acetamiprid on biological aspects and life table of *Amblyseius swirskii* (Acari: Phytoseiidae) fed on *Aleuroclava jasmini* (Hemiptera: Aleyrodidae). Syst. Appl. Acarol. 24, 814-824.

Silva, F.R. da, Moraes, G.J. de, Gondim Jr, M.G.C., Knapp, M., Rouam, S.L., Paes, J.L.A., Oliveira, G.M. de, 2010. Efficiency of *Phytoseiulus longipes* Evans as a control agent of *Tetranychus evansi* Baker & Pritchard (Acari: Phytoseiidae: Tetranychidae) on screenhouse tomatoes. Neotrop. entomol. 39, 991–995. 2

Smucker, M.D., Allan, J., Carterette, B., 2007. A comparison of statistical significance tests for information retrieval evaluation, in: Proceedings of the Sixteenth ACM Conference on Conference on Information and Knowledge Management - CIKM '07. Presented at the the sixteenth ACM conference, ACM Press, Lisbon, Portugal, p. 623.

Spollen, K.M., Isman, M.B., 1996. Acute and sublethal effects of a neem insecticide on the commercial biological control agents *Phytoseiulus persimilis* and *Amblyseius cucumeris* (Acari: Phytoseiidae) and *Aphidoletes aphidimyza* (Diptera: Cecidomyiidae). J. Econ. Entomol. 89, 1379–1386.

Stark, J.D., Banks, J.E., 2003. Population-level effects of pesticides and other toxicants on arthropods. Annu. Rev. Entomol. 48, 505–519.

Stark, J.D., Vargas, R., Banks, J.E., 2007. Incorporating ecologically relevant measures of pesticide effect for estimating the compatibility of pesticides and biocontrol agents. J. Econ. Entomol 100, 1027–1032.

Sterk, G., Hassan, S.A., Baillod, M., Bakker, F., Bigler, F., Blümel, S., Bogenschütz, H., Boller, E., Bromand, B., Brun, J., Calis, J.N.M., Coremans-Pelseneer, J., Duso, C., Garrido, A., Grove, A., Heimbach, U., Hokkanen, H., Jacas, J., Lewis, G., Moreth, L., Polgar, L., Rovesti, L., Samsoe-Peterson, L., Sauphanor, B., Schaub, L., Stäubli, A., Tuset, J.J., Vainio, A., Van de Veire, M., Viggiani, G., Viñuela, E., Vogt, H., 1999. Results of the seventh joint pesticide testing programme carried out by the IOBC/WPRS-Working Group 'Pesticides and Beneficial Organisms'. BioControl 44, 99–117.

Sterk, G., Jans, K., Put, K., Wulandari, O.V., Uyttebroek, M., 2003. Toxicity of chemical and biological plant protection products to beneficial arthropods. In: Colloque international tomate sous abri, protection integree agriculture biologique, Avignon, France, 17–19 September 2003, pp. 113–118.

van de Veire, M., Tirry, L., 2003. Side effects of pesticides on four species of

beneficials used in IPM in glasshouse vegetable crops: “worst case” laboratory tests. IOBC/wprs Bull 26, 41–50.

Viggiani, G., Bernardo, U., 2001. Side effects of some pesticides on predatory mites (Phytoseiidae) in citrus orchards. IOBC Bulletin 24:97–101.

Thomson, L.J., Hoffmann, A.A., 2006. Field validation of laboratory-derived IOBC toxicity ratings for natural enemies in commercial vineyards. Biol.Control 39, 507–515.

Tomizawa, M., Casida, J.E., 2003. Selective toxicity of neonicotinoids attributable to specificity of insect and mammalian nicotinic receptors. Annu. Rev. Entomol. 48, 339–364.

Uddin, N., Alam, Z., Miah, R.U., Mian, I.H., Mustarin, K.-E.-, 2015. Toxicity of pesticides to *Tetranychus urticae* Koch (Acari:Tetranychidae) and their side effects on *Neoseiulus californicus* (Acari:Phytoseiidae). Int. J. Acarol. 41, 688–693.

Ullah, M.S., Lim, U.T., 2017. Synergism of *Beauveria bassiana* and *Phytoseiulus persimilis* in control of *Tetranychus urticae* on bean plants. Syst. Appl. Acarol. 22, 1924.

Van de Veire, M., Sterk, G., van der Staaij, M., Ramakers, P.M.J., Tirry, L., 2002. Sequential testing scheme for the assessment of the side-effects of plant protection products on the predatory bug *Orius laevigatus*. BioControl 47, 101–113.

Villanueva, R.T., Walgenbach, J.F., 2005. Development, Oviposition, and Mortality of *Neoseiulus fallacis* (Acari: Phytoseiidae) in Response to Reduced-Risk Insecticides. . J. Econ. Entomol. 98, 2114–2120.

Wanumen, A.C., Carvalho, G.A., Medina, P., Viñuela, E., Adán, Á., 2016. Residual acute toxicity of some modern insecticides toward two mirid predators of tomato pests. J Econ Entomol 109, 1079–1085.

Wei, M., Chi, H., Guo, Y., Li, X., Zhao, L., Ma, R., 2020. Demography of Cacopsylla chinensis (Hemiptera: Psyllidae) reared on four cultivars of *Pyrus bretschneideri* (Rosales: Rosaceae) and *P. communis* pears with estimations of confidence intervals of specific life table statistics. J. Econ. Entomol.113, 2343–2353.

Wekesa, V.W., Moraes, G.J., Knapp, M., Delalibera, I., 2007. Interactions of two natural enemies of *Tetranychus evansi*, the fungal pathogen *Neozygites floridana* (Zygomycetes: Entomophthorales) and the predatory mite, *Phytoseiulus longipes* (Acari: Phytoseiidae). Biol. Control 41, 408–414.

Ximénez-Embún, M.G., Ortego, F., Castañera, P., 2016. Drought-stressed tomato plants trigger bottom–up effects on the invasive *Tetranychus evansi*. PLoS ONE 11, e0145275.

Zanardi, O.Z, Pavan Bordini, G., Aparecida Franco, A., Jacob, C.R.O., Takao Yamamoto, P., 2017. Sublethal effects of pyrethroid and neonicotinoid insecticides on *Iphiseiodes zuluagai* Denmark and Muma (Mesostigmata: Phytoseiidae). Ecotoxicology 26, 1188–1198.

CHAPTER 6 – Final Regards

In recent decades, agriculture on the African continent has faced various threats from invasive insects and mites, whose one of them is the red spider mite *Tetranychus evansi* Baker e Pritchard (Acari: Tetranychidae). This situation led the farmers to the extensive use of broad-spectrum synthetic pesticides for dealing with these pests menace due to the lack of effective local natural enemies. Unfortunately, this measure is unsustainable in the long run as it leads inevitably to the development of resistance, compromise environmental health, negatively affect beneficial ecosystem service providers as well as human and livestock health, besides increasing crop production cost. This concern has led to identify alternatives that could sustainably prevent and manage this invasive pest, while mitigating their adverse effects on the environment. In other words, the chemical control practice should be subordinate or integrate with other control methods, such as biological control, development of resistant genotypes, etc.

Within this context, source of resistance (glandular trichomes) has been found expressively in wild genotypes from South America and could be explored to increase resistance level of varieties of interest to this pest. Furthermore, endeavors involving the collaboration of African and South American researchers in region of *T. evansi* origin (South America) have also led to the identification of *Phytoseiulus longipes* Evans (Phytoseiidae), a promising predatory mite. However, the incorporation of this predatory mite into IPM programs where *T. evansi* is a serious problem requires detailed knowledge and understanding of the interactions of these mites with other crop management practices. Thus, we decided for the case of Benin to establish in the present study an integrated management system for *T. evansi* with the acquisition of tomato genotypes resistant to *T. evansi*, a suitable genotype that could optimize the performance of predatory mite *Phytoseiulus longipes* Evans (Phytoseiidae) and with the definition of selective pesticides to this predator.

In view of this research, we found that the progenies F1, SPJ-05–2018 and SPJ-10–2017 obtained by crossing the wild-resistant genotype [Solanum habrochaites, access PI 134417] with *Solanum lycopersicum* cv TLCV15 [an important cultivated genotype widely grown in Benin] have inherited significant

glandular trichomes types I, IV and VI from their resistant parent(PI 134417). We observed that those densities of glandular trichomes inherited by progeny genotypes were able to mitigate the damage caused by *T. evansi*. These results open many research opportunities on genetic improvement programs for promising tomato genotypes and adapted to the sustainable production in Republic of Benin.

We found however that the progeny genotype SPJ-05–2018 (partially resistant with many glandular trichomes) caused important delays population growth and reduced significantly a survival, and the predation potential of *P. longipes*. In contrast to SPJ-05-2018, we found the survival and population growth of the predatory mite on Tounvi (susceptible, with few trichomes), TLCV15 (susceptible, and with many trichomes), two cultivated genotypes widely in Benin, were similar, however, the predation potential was found to be higher on TLCV15. Based on these results we rejected the presumption that a susceptible genotype (with fewer trichomes) would be more favorable to *P. longipes* than susceptible (with a higher numbers) or partially resistant genotypes (with higher numbers glandular trichomes). This aspect must be taken into account when choosing the variety to be included in the envisioned IPM program to optimize *P. longipes* as a biocontrol agent. This aspect is also important in defining the number of predators that must be released in a given variety to expect a given effectiveness. In practice, our result suggest that TLCV15 is more suitable genotype to optimize *P. longipes* as a biocontrol agent in IPM program of *T. evansi*. However, the control of *T. evansi* on Tounvi would possibly require a larger scale release of *P. longipes* to achieve similar efficacy as on TLCV15.

We found that the use of azadirachtin- and oxymatrine based biopesticides had high activity against *T. evansi* and may be an important alternative in the management of the mite in replacement or rotation with synthetic acaricides. Furthermore, azadirachtin proved also to be safer to the predatory mites for both augmentative biological control and conservation whereas oxymatrine should be recomendaded for augmentative biological control 10 days before release. These products coud be important to sustainably manage this invasive pest while mitigating their adverse effects on the environment. Other pesticides commonly used in tomato

cropping system as abamectin, propargite, imidacloprid and the enthomopathogenic fungus *Hirsutella thompsonii* (Fischer)(Deuteromycetes) proved to be more suitable toward augmentative biological control than conservation if appropriate safety deadlines are respected before release. In contrast, the insecticides belonging to pyrethroid (cypermethrin and deltamethrin) and organophosphate (dimethoate, chlorpyrifos) groups are not compatible with both conservation and increase of *P. longipes* population in IPM programs. These results are important in implementation of IPM program that aim to preserve or increase the use of *P. longipes* as predator. Further work in this area should focus on filling any gaps in our current knowledge of non-target- effects from other pesticides, especially fungicides and herbicides and crop protection products new to the market in the most informative way possible (field experiments) for producers to whom it will most benefit.