EXPLORING THE POTENTIAL OF USING ARTHROPOD-RESISTANT TOMATO PLANTS IN WHITEFLY MANAGEMENT

by

SWIKRITI PANDEY

(Under the direction of Jason M. Schmidt)

ABSTRACT

Plant traits impact the dynamics of plant-arthropod interactions. Defensive plant traits have negative/positive impacts on interacting arthropods (herbivores and natural enemies). Pest management strategies focus on manipulating the expression of plant defense traits to repel herbivores and/or attract suitable biological control agents, which are considered alternative (i.e. non-insecticide) tactics to reduce pests in vegetable systems. Success in exploiting biological control agents depends on compatibility with host plants expressing defense traits. Whitefly, *Bemisa tabaci* (Gennadius) (Hemiptera: Aleyrodidae) control is challenging in tomato (*Solanum lycopersicum* L.) and is currently reliant on synthetic insecticides. To overcome this challenge, arthropod-resistant tomato plants bred with enhanced defense traits (high trichome density and acylsugar content) from wildtypes, have the potential to repel whiteflies and reduce the need for insecticides. Here I use experimental tomato lines bred to express elevated levels of acylsugar produced from glandular trichomes and predaceous mites and study their efficacy in reducing whitefly abundance in both open field and enclosed conditions. Our results suggest acylsugar-producing tomato lines provide resistance to whiteflies, but mite releases were not effective.

INDEX WORDS: Arthropod resistance, defense traits, *Solanum lycopersicum*, trichomes, acylsugar, *Bemisia tabaci*

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SWIKRITI PANDEY

B.S., Agriculture and Forestry University, Nepal, 2017

A Thesis Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment of Requirements of the Degree

MASTER OF SCIENCE

ATHENS, GEORGIA

2022

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SWIKRITI PANDEY

Major Professor:	Jason M. Schmidt
Committee:	David G. Riley
	Bhabesh Dutta

Electronic Version Approved:

Ron Walcott
Vice Provost for Graduate Education and Dean of the Graduate School
The University of Georgia
May 2022

DEDICATION

I would like to dedicate this work to my parents and my siblings. Your unfaltering trust, love and support is what keeps me moving forward.

ACKNOWLEDGEMENTS

I would like to extend my foremost gratitude to my advisor Dr. Jason M. Schmidt for his continuous support, encouragement and patience throughout the process. Thank you for the opportunity to learn, grow and widen my perspective on what research can and should be. I am thankful to all the members of Schmidt Biocontrol Lab for helping with all aspects of my project. I am forever grateful to all the members for providing me a comfortable, fun and exciting workspace. I would also like to thank the members of my advisory committee, Dr. David G. Riley and Dr. Bhabesh Dutta for all their invaluable assistance and insights throughout the program. Thanks to the Tomato Breeding Program, Cornell University specifically Dr. Martha A. Mutschler for providing us with tomato seeds. Lastly, I am thankful to all my friends for their endless support, confidence boost and laughter.

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Plants harbor many arthropods including herbivores and natural enemies. Herbivores in plant-insect interactions consume plant resources and also act as resources themselves (to the natural enemies). The interaction between plant-herbivore-natural enemies results in a tri-trophic interaction, which is governed by various factors including diet breadths, host plant quality, availability of resources and plant defense traits. The understanding of tri-trophic interactions is also incomplete without taking into consideration that each one of the trophic levels can modify and alter other trophic levels (Abdala-Roberts et al., 2019; Inbar & Gerling, 2008; Kessler et al., 2011; Price et al., 1980; Turlings et al., 2018; Vet et al., 1992). Defensive traits conferred by plants can influence herbivores and natural enemies directly or indirectly (Abdala-Roberts et al., 2019). A classic paper by Price et al. (1980) argues that plants protect against herbivores with mostly sub-lethal effects on herbivores causing impaired growth, development and oviposition. The defense traits may directly hinder herbivore feeding and eventually diminish resources for natural enemies. The secondary metabolites (e.g. volatile compounds) produced by plants, and the morphological features such as the plant architecture, and leaf domatia can indirectly aid natural enemies or may ultimately prove to be detrimental by creating unfavorable environments for natural enemies. This may lead to reduced movement, foraging efficiency and reduced herbivory overall. The influence of plant traits on the herbivores and natural enemies can therefore be positive or negative (Abdala-Roberts et al., 2019; Koller et al., 2007; O'Dowd & Willson, 1991; Orre et al., 2010; Price et al., 1980; Verheggen et al., 2009).

Plant defense traits and arthropod resistance

Defensive traits are expressed as plant structure and chemistry exerting effects on herbivores (e.g. survival and reproduction rates), and effects on upper trophic levels (Ode, 2006). Plant defense traits may be expressed in the form of indirect defense such as herbivore-induced plant volatiles (HIPV; volatiles emitted from plants in response to herbivore attack) that may attract natural enemies (Orre et al., 2010). HIPVs are utilized by natural enemies like predatory mites to detect their prey (Nomikou et al., 2005). However, these volatiles also attract some pests (see Table 1.1) (Zhang et al., 2019). Defense traits can also be expressed in the form of physical traits such as leaf domatia and leaf pubescence/trichomes and plant secondary metabolites that create unfavorable, anti-digestive, anti-nutritive environments for herbivores (Agrawal, 2000; Orre et al., 2010; Stipanovic, 1983; Peterson et al., 2016). Structural plant traits also alter multiple trophic levels. For example, in a study by Grevstad & Klepetka (1992), ladybird beetles, *Hippodamia* spp. and *Coccinella* spp. (Coleoptera: Coccinellidae) were found to constantly fall off the waxy leaves of crucifers which hindered their predation on aphids, *Brevicoryne brassicae* (Linnaeus) (Hemiptera: Aphididae). Likewise, various studies on tomato (*Solanum lycopersicum*, Solanaceae) plants revealed that physical defense traits, such as trichomes, are responsible in deterring tomato fruitworm *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae) and Colorado Potato Beetle *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae) (Tian et al., 2012) as well as reducing the efficiency of natural enemies such as *Telenomus sphingis* (Hymenoptera: Scelionidae) *(*an egg parasitoid of tobacco hornworm) (Farrar & Kennedy, 1991) and *Orius insidiousus* (Say) (Hemiptera: Anthocoridae) (a predator of western flower thrips) (Coll & Ridgway, 1995). Appropriate use of plant defense traits, therefore, is crucial for improving pest management (Cortesero et al., 2000).

Plant traits are of interest for efficient crop production and protection, but defense traits may also present trade-offs when detrimental to herbivores as well as their natural enemies (Agrawal, 2000; Peter et al., 1995). For instance, glandular and non-glandular trichomes, hair-like projections developing from the epidermis of leaves, stems and other organs of plant species (Peter et al., 1995), are found to negatively impact at least 19 arthropod pests including whiteflies, aphids, spider mites and leaf miners (Kennedy, 2003) by affecting their mobility, development, oviposition and predation abilities (Riddick & Simmons, 2014). However, trichomes also reduce the pest control efficiency of predatory mites and hoverflies (Krips et al., 1999; Peter et al., 1995; Verheggen et al., 2009). Glandular trichomes produce “cocktails” of plant secondary compounds including terpenoids, phenolics, sucrose esters, methyl ketones and organic acids which are found to confer antibiosis in plants. (Simmons & Gurr, 2005; Tian et al., 2012). Antibiosis refers to the process in which feeding on plants by insects results in reduced growth, development, disruption in physiological processes and/or mortality in insects (Rector et al., 2000). Various blends of chemical compounds are produced as an indirect defense by plants in response to herbivore damage and attract natural enemies. These chemicals induced in response to herbivore attacks are known as HIPVs (Cortesero et al., 2000). The role of HIPV was observed in a study where the synthetic application of Methylsalicylate (MeSA), HIPV produced by Brassicacae, in turnip plants *Brassica rapa* L. attracted a diamondback moth parasitoid *Diadegma semiclausum* Hellén (Hymenoptera: Ichneumonidae), and lacewing parasitoid *Anacharis zealandica* Ashmead (Hymenoptera: Figitidae). Unfortunately, along with natural enemies, leaf-mining dipteran pest *Scaptomyza flava* Fallén (Diptera: Drosophilidae) was also attracted to HIPV-treated plants (Orre et al., 2010). Other defense traits in plants are linked with altering both herbivore and natural enemy populations (see Table 1.1), including the waxy surface of leaves (Grevstad & Klepetka, 1992) and axillary leaf domatia which may be present in form of pits, pockets or hair tufts present underside of the leaf (O'Dowd & Willson, 1991). Therefore, the expression of plant defense traits presents trade-offs in herbivore control and the efficacy of biological control (Inbar & Gerling, 2008).

Tri-trophic interactions in tomato systems

The cultivated tomato (*Solanum lycopersicum* L.: Solanaceae) is an economically important crop (Tian et al., 2012) and ranks fourth among the most produced vegetables in the United States (USDA NASS, 2017). It is susceptible to the attack by an estimated 100-200 species of insects (Leckie et al., 2016), which includes devastating insect pests like tomato fruitworm *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae), common armyworm *Spodoptera litura* (Lepidoptera: Noctuidae), spider mites *Tetranychus urticae* Koch (Acari: Tetranychidae), whiteflies *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) and thrips *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae) (Srinivasan, 2010). Among these, whiteflies are a major challenge to tomato production in the Southeastern US (Riley & Srinivasan, 2019).

Cultivated tomato is susceptible to a plethora of insects and diseases both in greenhouse and field conditions (Shipp & Wang, 2006). Various cultural control strategies include disease-resistant cultivars, sanitation in the nursery and field, weed control, trap crops, fertilizer and nutrient management, and use of reflective mulches (Castañé et al., 2020; Srinivasan, 2010). In the tropics and sub-tropics, even with the use of Integrated Pest Management (IPM) strategies, the cultivation of tomatoes is largely dependent on the use of pesticides (Firdaus, 2012; Mutschler, 2021). Tomato growers often rely on chemical control methods (Riley & Srinivasan, 2019), but insecticide resistance is recognized for many modes of action (Rattan, 2010).

Resistance and non-target effects of pesticides necessitate the development of alternative, cost-effective and environment-friendly methods of pest management (Leckie et al., 2012). Biological control of the pests and the development of arthropod-resistant plant varieties are considered pillars of integrated pest management to reduce pesticide use (Oriani & Vendramim, 2010)

Biological control of tomato pests

The aim of applying biological control strategies is to control pest populations at below economically damaging pest levels, which in turn can reduce pesticide use. Biological control offers an ecologically sound tool for pest mitigation in an Integrated Pest Management (IPM) system. The success or failure of biological control often depends on the interaction between the plant, herbivores and biological control agents. Therefore, the selection of biocontrol agents and timing of the release is crucial for pest management (Gigon et al., 2016; Messelink et al., 2008; Baker et al., 2020). The augmentative release of natural enemies provides promising results for controlling major tomato pests (see Table 1.2) (Silva et al., 2018), but efforts to identify and enhance the indigenous natural enemies should also be a priority when using biological control as a preferred pest management strategy (Urbaneja et al., 2012). The use of biological control agents targeting tomato pests is suited for controlled environments such as greenhouse and a plethora of examples and studies for the same are available. It is more effective to apply biological control strategies in controlled environments due to the limited dispersal ability of the biological control agents and the feasibility of controlling conditions that favor natural enemies (Walgenbach, 2018).

The use of *Encarsia formosa* (Hymenoptera: Aphelinidae) as a predator of greenhouse whiteflies *Trialeurodes vaporariorum* (Hemiptera: Aleyrodidae) dates back to 1926, however, increased pesticide use halted its production and distribution. Insecticide resistance eventually causes the resurgence of *E. formosa* uses in greenhouses. So, there is no definitive strategy for how biological control agents can be identified and selected, most of it occurs through trial and error over time (van Lenteren & Woets, 1987). Application of biological control programs in tomato is not simple due to its morphological characteristics, foliage pigments and volatiles, presence of defense structures, which is often linked to incompatibility with the arthropods (Lange & Bronson, 1981). The predatory mite *Ambylseuis swirskii* Athias-Henriot (Acari: Phytoseiidae) has been considered an efficient biocontrol agent of tomato pests such as whiteflies *B. tabaci*, thrips *Frankliniella occidentalis* and two-spotted spider mites *Tetranychus urticae* in the vegetable system (Bolckmans et al., 2005; Calvo et al., 2010; Soleymani et al., 2016), but studies including its use in tomato plants are scarce and provide mixed results. For example, some studies with detached tomato leaflets show success of the establishment and suppression of pests such as *Aculops lucopersici* (Massee) (Acari: Eriopyidae) and *Tuta absoluta* (Momen and Abdel-Khalek., 2008; Park et al., 2010), while other studies indicate their failure of establishment in tomato plants and subsequent pest suppression (Paspati et al., 2021; van Houten et al., 2013). Nevertheless, many biological control agents for the control of tomato pests have been identified, and tested and are also available commercially through mass production. The predatory mirid bugs *Dicyphus hesperus* Knight (Hemiptera: Miridae), *Nesidiocoris tenuis* Reuter (Hemiptera: Miridae) and *Macrolophus pygmaeus* (Hemiptera: Miridae) have been used in tomato crops for the control of whiteflies *B. tabaci* and tomato moth *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). The adults of these predators are capable of consuming about 100 eggs/day if predators such as leafminers, aphids and spider mites (Calvo et al., 2009; McGregor et al., 1999; Sanchez et al., 2018; Urbaneja et al., 2012). Studies show efficacy of the hymenopteran parasitoid *Eretmocerus mundus* Mercet (Hymenoptera: Aphelinidae) in controlling whiteflies (Stansly et al., 2005b; Urbaneja et al., 2006). Besides arthropods as biological control agents, entomopathogens such as the bacterium *Bacillus thringiensis* Berliner (Firmicutes: Bacillaceae) (Ladurner et al., 2011), fungi *Beauveria bassiana* (Balsamo) Vuillemin (Ascomycota: Clavipitaceae) (Gregorio et al., 2009), nematodes *Steinernema carpocapsae* (Weiser) (Nematoda: Steinernematidae), *Steinernema feltiae* (Filipjev) (Nematoda: Steinernematidae) and *Heterorhabditis bacteriophora* Poinar (Nematoda: Heterorhabditidae) (Batalla-Carrera et al., 2010) have also been used in the tomato pest management systems (Urbaneja et al., 2012).

Arthropod resistance in tomato plants

The most important resistant factor in tomato against insect herbivores are trichomes (Chatzivasileiadis & Sabelis, 1997). Trichomes are hair-like projections developing from the epidermis of leaves, stems and other organs of plant species (Peter et al., 1995) and are used by the plants as morphological defenses against herbivores (Kennedy, 2003). Foliar trichomes of *Lycopersicon spp.* are categorized as types I–VII, with types I, IV, VI and VII being glandular and types II, III and V being non-glandular. The glandular trichomes possess sticky and/or exudates that may either poison or repel the herbivores while the non-glandular trichomes have heads that physically hinder the movement or feeding behavior of herbivores (Tian et al., 2012). The allelochemicals such as 2-tridecanone, 2-undecanone present in type VI glandular trichomes of wild tomato *Lycopersicum hirsutum* f. *glabratum* is reported to be toxic and repellent to two-spotted spider mites (Chatzivasileiadis & Sabelis, 1997). Similarly, the wild relatives of tomatoes such as *Solanum pennellii, and Solanum pimpinellifolium* are found in several studies to be resistant to various herbivores. LA716, a wild accession of *S. pennellii* is associated to a high density of Type IV glandular trichome. Acylsugars exuded from the glandular trichomes result in non-preference for oviposition by *B. tabaci* (Leckie et al., 2012; Rodriguez-Lopez et al., 2011; Silva et al., 2014).

The defense traits associated with trichomes are observed in high amounts in wild tomatoes compared to common cultivars and can be transferred from wild varieties to cultivated tomato varieties (Lawson et al., 1997; Leckie et al., 2012; Oriani & Vendramim, 2010; Smeda et al., 2018). The transfer of quantitative trait loci (QTL) from wild accession to cultivated varieties that pertains to increased acylsugar production has been the main focus of the tomato breeding program (Ben-Mahmoud et al., 2019). In tomatoes, the resistance to insects conferred by acylsugar is found to be directly correlated with the density of type IV glandular trichomes. The resistance to herbivores is caused either by the direct toxicity or its sticky nature that helps immobilize or suffocate the arthropods (Glas et al., 2012). The impact of the host plant traits on trophic interactions is evident in wild relatives of tomatoes in which glandular trichomes are found to produce acylsugars that are associated with insect resistance repelling a wide array of arthropods. Although the repellence of pests like whiteflies, thrips, and mites may improve crop health (Ben-Mahmoud et al., 2018), trichomes also negatively affect biological control agents by decreasing their walking speed, and predation rates (Chatzivasileiadis & Sabelis, 1997). Therefore, the expression level of plant resistance traits likely impacts natural enemies of pests, and the possibility of incompatibility with the resistance conferred by the host plants' morphological and physiological defense traits (Bergman & Tingey, 1979).

The mode of action of the acylsugar is not yet determined, but many members of the nightshade family Solanaceae are found to confer resistance to various pests through both antixenosis and antibiosis (Leckie et al., 2012; Oriani & Vendramim, 2010, Le Roux et al., 2008). Antibiosis occurs when feeding on plants results in reduced growth, development, disruption in physiological processes and/or mortality in insects. Antixenosis also called non-preference is a process in which the insects/arthropods are repelled by the host plants they generally prefer. Although both these processes can be assessed separately, more often they overlap (Rector et al., 2000; Le Roux et al., 2008). High acylsugar-producing tomato lines appear successful in reducing whitefly oviposition and development (Leckie et al. 2012, Silva et al. 2014). Furthermore, the rate of oviposition of western flower thrips (*Frankliniella occidentalis*) in the sepals of the flowers of acylsugar lines of tomato was also found lower compared to current commercial cultivars. The pests preferred petals or stamens that had a lower density of type IV trichomes and acylsugar content (Ben-Mahmoud et al., 2018). Similarly, the western flower thrips oviposition and Tomato Spotted Wilt Virus inoculation were found increasingly suppressed with an increased amount of acylsugar in tomato leaves (Ben-Mahmoud et al., 2019). The survival of the tomato psyllids (Li et al., 2019), oviposition and survival of two-spotted spider mite were lower on high acylsugar-producing tomato lines (Alba et al., 2009; Lucini et al., 2015).

Whiteflies: a major pest of tomato system

A current major challenge to the sustainable production of vegetable and cotton systems worldwide is the sweet potato whitefly (Stansly et al., 1997) through direct or indirect damage to the plants. Direct damage is caused by phloem-feeding which causes reproductive and vegetative disorders and indirect damage is caused through excretion of honeydew that promotes sooty mold development (Brown et al., 1995). Whiteflies have a wide host range feeding on upwards of 600 host plants in agricultural landscapes, which further increases the difficulty of control (Rodriguez-Lopez et al., 2011; Silva et al., 2014; Wang et al., 2017). Whiteflies also vector viruses that causes destructive diseases to the plants (Leckie et al., 2012; Silva et al., 2014). One of the widespread whitefly transmitted viruses in Southeastern US in tomato plants is Tomato Yellow Leaf Curl Virus (TYLCV) (family Geminiviridae, genus *Begomovirus*) that can cause up to 100% incidence and yield loss (Marchant et al., 2020; Srinivasan et al., 2012).

Whitefly management primarily relies on the use of insecticides, but whiteflies are highly efficient in detoxifying insecticides and are resistant to numerous commonly used insecticides (Horowitz et al., 2005). Therefore, whiteflies challenge Integrated Pest Management (IPM), and necessitate the urgent need for alternative pest management strategies (Ozores-Hampton et al., 2010). Various tomato cultivars have been developed that are resistant to whitefly transmitted virus but are not immune to whitefly feeding and infestation acting as a reservoir for vectors and viruses (Srinivasan et al., 2012)**.** Cultural practices like the use of reflective mulches deter whiteflies at the early stages of infestation by disorienting the pest (Nyoike et al., 2008; Polston & Lapidot, 2007; Simmons et al., 2010), but higher costs appear to discourage grower implementation (Riley & Srnivasan, 2019).

Plant breeding for resistance against pests like whitefly to improve pest management and disease has been widely studied in the tomato system since several wild-type genes show potential in pest management (Lawson et al., 1997). Tomato lines developed by transferring genetic materials responsible for insect repellence from wild relatives to cultivated tomato have shown potential for whitefly control in many studies (Leckie et al., 2016, 2012; Marchant et al., 2020; Rodríguez-López et al., 2020; Silva et al., 2014). These lines significantly reduce the numbers of whiteflies which provides evidence of the efficacy of breeding plants as a useful defense strategy in whitefly management. The use of biological control of whiteflies shows a high degree of success in greenhouse-controlled environments, and some success in the field. Some examples include hymenopteran parasitoids (*Eretmocerus* sp.), and predatory taxa such as coleopteran beetle (*Delphastus* sp.) and Acari (*Neoseiulus bakeri* and *Ambylseius swirskii)* (Greenberg et al., 2002; Heinz & Zalom, 1996; Nomikou et al., 2001; Stansly et al., 2005a; Tellez et al., 2017) (see Table 1.2). However, incompatibility of the biological control agents with host plant traits may pose a problem in pest management, as some plant resistance traits can interfere with predation or the biology of natural enemies (e.g. trichomes, Riddick & Simmons, 2014).

Trade-offs of tomato pest management strategies

Non-target effect of pesticides on natural enemies is one of the major trade-offs of using insecticides as a sole pest management tactic (Wanumen et al., 2016). The continuous use of pesticides, associated with the pesticide treadmill, eventually causes pests to develop resistance which is followed by the use of pesticides in higher volumes that causes severe harm to natural enemies. A side effect of the pesticide treadmill is a lack of natural enemies to control the pests, often generating secondary pest outbreaks (Castañé et al., 2020). Resistance to insecticides developed by pests such as aphids and whiteflies is also a looming issue (Verheggen et al., 2009; Horowitz et al., 2005).

The other challenge that exists is the compatibility of biological control agents with tomato plants. This has been an impending issue in breeding tomatoes for insect resistance (Chatzivasileiadis & Sabelis, 1997). Highly successful resistance traits in tomatoes are glandular and non-glandular trichomes that render insects immobile (Peter et al., 1995). Although glandular trichomes provide defense against the pests they are often non-selective and can therefore affect biocontrol agents by decreasing their walking speed, and predation rates and their efficacy to targeted pests ultimately (Krips et al., 1999; Madadi et al., 2007; Riddick & Simmons, 2014).

Ambylseius swirskii (Acari: Phytoseiidae) is a successful generalist predator of common vegetable pests (Calvo et al., 2015; Xiao et al., 2012). Originally *A. swirskii* was investigated as a potential biocontrol agent against whiteflies as it was found to feed on its eggs and nymphal stage (Nomikou et al., 2001, Nomikou et al., 2002). The commercialization of this predatory mite in 2005 sparked its widespread use as a biological control agent of various pests of vegetables including whiteflies *B. tabaci*, thrips species *F. occidentalis* in cucumber (Calvo et al., 2010), zucchini (Tellez et al., 2017), melon thrips *Thrips palmi* Kamy (Thysanoptera: Thripidae) in cucumbers and eggplants (Razzak et al., 2019), broad mites *Polyphagotarsonemus* spp (Acari: Tarsonemidae) in sweet pepper and eggplants (Stansly et al., 2009) and spider mites *T. urticae* (Razzak et al., 2019). In some vegetables, *A. swirskii* is found to perform better against common tomato pests better than other commercially available biological control agents (Razzak et al., 2019; Messelink et al., 2008; Tellez et al., 2017). The use of *A. swirskii* for biological control in tomato production may be negatively impacted by the presence of trichomes on the stem and leaves that impair movement (van Houten et al., 2013). However, studies of *A. swirskii* compatibility with tomatoes provide inconsistent results for pest management. In some cases, the presence of trichomes does not impact *A. swirskii* establishment and effectiveness against the tomato russet mite (Park et al., 2010), while in others *A. swirskii* performed poorly in the presence of glandular trichomes on the stems and leaves of tomato plants (Paspati et al., 2021; van Houten et al., 2013). Hence, it is becoming increasingly clear that the expression of plant defense traits produces trade-offs and expression levels must be balanced with natural enemy compatibility.

Research objectives and approach

<u>Objective 1</u>: Determine the compatibility of acylsugar-producing tomato lines with the biocontrol agent *Ambylseius swirskii* and its efficacy in whitefly control

a. Evaluate the success of whitefly control in acylsugar tomato lines as compared to commercial cultivars in open field and greenhouse condition
b. Assess the whitefly abundance in acylsugar tomato lines in response to three *A. swirskii* mite treatments (no mite, dusting on top of plants, basal application and slow-release mite sachet) application

<u>Hypothesis</u>

- Tomato plants with enhanced defense traits (high acylsugar content and trichome density) will have significantly lower whitefly numbers as compared to commercial cultivars.
- The presence of defense traits in tomato plants will hinder the establishment of the biocontrol agent *A. swirskii*.

<u>Objective 2</u>: Exploring the efficacy of whitefly control in experimental acylsugar tomato lines in comparison to commercial tomato cultivars

a. Assess the whitefly abundance in the field and enclosed conditions when using experimental acylsugar tomato lines.
b. Comparison of whitefly abundance in experimental tomato lines and commercial cultivars both in the open field and enclosed conditions.

<u>Hypothesis</u>

- Experimental acylsugar lines will have a significant effect in reducing whitefly abundance as compared to commercial cultivars

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Table 1.1. Summary of studies focused on the type of plant defense traits and their positive or negative impact on herbivores and predators. “Negative” and “positive” recorded under the type of effects for the predators/parasitoids indicates that the defense traits negatively and positively impacted the predator’s ability of pest suppression and establishment on the host plants respectively. “Negative” recorded under the type of effects for the herbivores indicate that the defense traits aided in pest suppression thereby conferring a negative impact on pests and “positive” indicates that the defense traits aided in their establishment and attack on the host plants.

Plant defense trait	Nature of defense tested (Physical/ Chemical)	Herbivores/Predators	Nature of insect	Type of Effect	References
Trichomes	Physical	*Phytoseiulus persimilis*	Predator	Negative	(Krips et al., 1999a)
		Neoseiulus californicus	Predator	Negative	(Cédola et al., 2001; Koller et al., 2007)
		Trichogramma exigum	Parasitoid	Negative	(Keller, 1987)
		Aculops lycopersici	Herbivore	Positive	(van Houten et al., 2013)
		Ambylseius swirskii	Predator	Negative	(Buitenhuis et al., 2014; Paspati et al., 2021)
Methyl ketone from glandular trichomes	Chemical	*Telenomus sphingis*	Parasitoid	Negative	(Farrar et al., 1991)
		Tetranychus urticae	Herbivore	Positive	(Chatzivasileiadis & Sabelis, 1997)
Acylsugar from glandular trichomes	Chemical	*Bemisia tabaci*	Herbivore	Negative	(Leckie et al., 2016; Leckie et al., 2012; Marchant et al., 2020; Silva et al., 2014)
		Trialeurodes vaporarium	Herbivore	Negative	(Rodríguez-López et al., 2020)
		Frankliniella sp.	Herbivore	Negative	(Ben-Mahmoud et al., 2019; Ben-Mahmoud et al., 2018; Leckie et

					al., 2016; Smeda et al., 2018)
		Bactericera cokerelli	Herbivore	Negative	(Li et al., 2019)
		Tetranychus urticae	Herbivore	Negative	(Lucini et al., 2015)
Leaf doamtia	Physical	*Frankliniella occidentalis*	Herbivore	Positive	(Agrawal & Karban, 1997)
		Orius tristicolor	Predator	Positive	(Agrawal & Karban, 1997)
		Geocoris sp.	Predator	Positive	(Agrawal & Karban, 1997)
		Metaseiulus occidentalis	Predator	Positive	(Grostal & O'Dowd, 1994)
		Ambylseius andersoni	Predator	Positive	(Norton et al., 2001)
Waxy surface	Physical	*Hippodamia convergens*	Predator	Positive	(Eigenbrode et al., 1995, 1996)
		Orius insidiosus	Predator	Positive	(Eigenbrode et al., 1995, 1996)
		Chrysoperla carnea	Predator	Positive	(Eigenbrode et al., 1995, 1996)
Herbivore-induced plant volatiles	Chemical	*Diadegma semiclausum*	Parasitoid	Positive	(Orre et al., 2010)
		Scaptomyza flava	Herbivore	Positive	(Orre et al., 2010)
		Anacharis zealandica	Parasitoid	Positive	(Orre et al., 2010)
		Bemisia tabaci	Herbivore	Positive	(Zhang et al., 2019)
		Rhopalosiphum padi	Herbivore	Negative	(Glinwood et al., 2009)
		Coccinella spetempunctata	Predator	Positive	(Glinwood et al., 2009)
		Aphidus colemani	Parasitoid	Positive	(Glinwood et al., 2009)

Table 1.2. Summary of studies focused on pest and biological control agents (BCA) used in the tomato production system in greenhouse and laboratory conditions. Major pests in the tomato production system and the biological control agents used to control the pests were reviewed. I also recorded if the studies were conducted in greenhouse or laboratory conditions and the release rate of BCA required to achieve whitefly control in tomato plants.

Pests	BCA	Context	BCA release rates	References
Sweetpotato whitefly (*Bemisia tabaci* Gennadius)	*Nesidiocoris tenuis*	Greenhouse	One application of 1-4 *N. tenuis*/plant	(Calvo et al., 2009)
	Eretmocerus mundus	Greenhouse	7.5 wasps/plant/week	(Stansly et al., 2005b)
Greenhouse whitefly (*Trialeurodes vaporariorum* Westwood)	*Eretmocerus eremicus*	Laboratory	1 parasitoid female/25 whitefly nymphs	(Greenberg et al., 2002)
Sweetpotato whitefly	*Macrolophus caliginosus*	Greenhouse	1 *M. caliginosus* adult/plant	(Gabarra et al., 2006)
	Dicyphus hesperus	Greenhouse	1.25 *D. hesperus* adults/tomato plants/week	(Smith & Krey, 2019)
Two-spotted spider mites (*Tetranychus urticae* Koch)	*Phytoseiulus macropilis*	Greenhouse	1 *P. macropilis*/5 spider mites	(Gigon et al., 2016)
Western flower thrips (*Franklienella occidentalis* Pergande)	*Dicyphus hesperus*	Greenhouse	0.5-10 D. Hesperus/60-150 thrips/plant	(Shipp et al., 2006)

CHAPTER 2

EFFECTS OF ACYLSUGAR TOMATO LINES ON THE PREDATORY MITE, *AMBYLSEIUS SWIRSKII*, *AND* WHITEFLY SUPPRESSION [1]

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[1] Swikriti Pandey, Andre Luiz Biscaia Ribeiro da Silva, Bhabesh Dutta, Juang-Horng Chong, Martha Ann Mutschler-Chu and Jason M. Schmidt. (submitted to Entomologia experimentalis et applicata)

Abstract

Arthropod- and disease-resistant crop varieties and biological control tools provide alternatives to insecticide use. Successful integration of host plant resistance and biological control has the potential to substantially reduce pest damage and pesticide application. However, plant defense traits can hinder the establishment of biological control agents, resulting in biocontrol-host incompatibility. In a series of greenhouse and field experiments, we explored whether tomato plants with augmented defense traits (i.e., production of acylsugars) could reduce sweetpotato whitefly (*Bemisia tabaci*) populations and enhance the effectiveness of the predatory mite, *Ambylseius swirskii.* In the field experiment, commercial tomato cultivars and acylsugar-producing tomato lines received no predatory mite or mites released via three different methods (dusting on top or bottom, and slow-release sachets). In the first greenhouse experiment, predatory mites were released onto the commercial and acylsugar-producing tomato plants via sachets. The second greenhouse experiment using a similar design, we augmented the mite diet with an alternative non-prey resource (i.e. cattail pollen). Acylsugar-producing tomato plants supported significantly fewer whiteflies than the commercial lines in all experiments. However, in the field, Tomato Yellow Leaf Curl Virus was detected at higher frequencies in acylsugar-producing lines compared to commercial lines. Few mites were recovered from all commercial and acylsugar-producing lines in the field or greenhouse experiments. Our results suggest *A. swirskii* does not establish well on tomatoes, and acylsugar lines successfully decreased whitefly populations but not a viral disease incidence even at low whitefly abundance.

Keywords:

Arthropod resistance, acylsugar, tomato plants, trichomes, *Bemisia tabaci*, biological control

Introduction

Tomatoes (*Solanum lycopersicum* L.) are highly susceptible to various pests and diseases, therefore, often requiring tomato growers to depend on pesticides (Riley & Srinivasan, 2019). A major pest of tomato, sweetpotato whitefly, *Bemisia tabaci* (Gennadius), is also a global pest in many crop systems (Horowitz et al., 2011). Whiteflies cause direct damage to the plants by feeding on phloem sap and indirect damage is caused by honeydew secretion and the associated sooty mold growth (Brown et al., 1995; Firdaus et al., 2012). Whiteflies also vector devastating plant viruses, such as Tomato Yellow Leaf Curl Virus (TYLCV) (family Geminiviridae, genus *Begomovirus*), a role that has led to zero tolerance by many growers (Leckie et al., 2012; Silva et al., 2014; Friedmann et al., 1998; Marchant et al., 2020; Srinivasan &Riley, 2012). Whitefly control is highly reliant on insecticides but they rapidly evolve resistance to insecticides (Horowitz et al., 2020) which has necessitated the need for alternative pest management strategies (Ozores-Hampton et al., 2010; Pickett et al., 2004), including the development of whitefly- or disease-resistant tomato varieties. Currently, multiple commercial tomato cultivars are resistant to whitefly-transmitted viruses; however, these varieties are not immune to whitefly infestation (Srinivasan et al., 2012).

The presence of glandular and non-glandular trichomes on tomato plants is associated with the deterrence of arthropod pests such as tomato fruitworm (*Helicoverpa zea*), Colorado potato beetles (*Leptinotarsa decemlineata*), whiteflies (Oriani & Vendramim, 2010; Tian et al., 2012). Non-glandular trichomes are capable of trapping arthropods, such as leafminers and aphids (Johnson, 1953; Xing et al., 2017). Glandular trichomes, on the other hand, exude secondary compounds, such as terpenoids, methylketones, sucrose esters and organic acids that confers antibiosis (Simmons & Gurr, 2005; Tian et al., 2012). Acylsugars are found in various solanaceous species and may confer arthropod resistance through antixenosis and antibiosis (Smeda et al., 2017). Enhanced acylsugar production from tomato glandular trichomes helps repel whiteflies (Leckie et al., 2016; Leckie et al., 2012; Marchant et al., 2020; Rodríguez-López et al., 2020).

Plant defense traits, such as acylsugars, are non-selective and may reduce pest populations in addition to reducing the efficacy of natural enemies (Coll et al., 1997; Farrar & Kennedy, 1991; Schmidt, 2014). Therefore, breeding for traits that deter pests and (coincidentally) natural enemies may present a trade-off between host plant resistance and biological control (van Houten et al., 2013). Multiple biological control agents are documented to provide whitefly suppression in greenhouse and field conditions (Greenberg et al., 2002; Heinz & Zalom, 1996; Nomikou et al., 2001; Stansly et al., 2005; Tellez et al., 2017). The phytoseiid mite, *Ambylseius swirskii*, is an effective generalist predator and is considered an excellent biological control agent of whiteflies in numerous vegetable systems (Tellez et al., 2017; Calvo et al., 2010; Calvo et al., 2012, Calvo et al., 2015). However, the predatory mites may have difficulty establishing on tomato plants (Sakamoto et al., 2012; van Houten et al., 2013; Paspati et al., 2021), especially in varieties with heightened expression of defense traits (Paspati et al., 2021). In this study, we evaluated the ability of acylsugar-producing tomato lines to suppress whitefly abundance and the associated viral disease prevalence. We hypothesized that the enhanced defense traits in acylsugar-producing tomato plants will have significantly lower whitefly abundance compared to commercial cultivars. We also tested the hypothesis that the establishment of *A. swriskii* might be hindered by this defense trait. To test our hypotheses, we evaluated the efficacy of combining *A. swirskii* with tomato plants that had either Tomato Yellow Leaf Curl Virus (TYLCV) resistance, or experimental lines with enhanced acylsugar production in suppressing the whitefly population in a series of greenhouse and field experiments.

Materials and Methods

Plant lines, whiteflies and predatory mites

Seeds of the experimental acylsugar-producing tomato lines (henceforth called acylsugar lines) were provided by the Tomato Breeding Program at Cornell University, Ithaca, New York. Acylsugar lines, CU0701026 and FA7/AS, and the commercial cultivars, Skyway 687 and SV7631TD, were evaluated in the field experiments. For the greenhouse experiments, we used the acylsugar lines, FA7/AS, CU071026 and QTL6/AS, and the commercial cultivars, Florida 47 (purchased from Tomato Growers Supply Company, Fort Myers, Florida) and Amelia F1 Hybrid (purchased from Harris Seeds, Rochester NY). The commercial cultivars for the field experiments Skyway 687 (purchased from Johnny’s Selected Seeds, Winslow Maine) and SV7631TD (Seedway, LLC, Hall, NY) represented resistance and susceptibility to the Tomato Yellow Leaf Curl Virus (TYLCV) respectively. The acylsugar lines range from moderate to high acylsugar content and trichome density. CU071026 is a benchmark acylsugar line obtained by transferring increased levels of acylsugars from *S. pennellii* accession LA716 into the cultivated tomato. It is reported to produce 15% of acylsugar levels produced by *S. pennellii* LA716 lines. FA7/AS was created by the introgression of quantitative trait loci FA7 which contains acylsugar fatty acid profiles into CU071026. The Introgression of QTL6 within CU071026 is associated with increased acylsugar level and trichome IV density (Leckie et al., 2014; Leckie et al.,2012). We produced tomato seedlings in a greenhouse using nursery trays filled with organic in-ground soil blended with organic compost.

We obtained the adult whiteflies for greenhouse experiments from a colony maintained in at the University of Georgia, Tifton GA. The colony was maintained in 4-5 leaf stage cotton seedlings in a rearing room at 27±2°C and 14:10 light: dark cycle.

Ambylseius swirskii was purchased from Koppert Biological System (Michigan, United States). Two products were used: Swirski-Mite (50000 predatory mite nymphs and adults per liter in loose bran) and Swirski-Mite Plus (250 mites in each bran-filled sachet). Following manufacturer recommendations and standard protocols, prior to each experiment, We assessed the viability of the predatory mites immediately upon receipt. Three randomly selected sachet was placed in a plastic cup over a container filled with water. After two days, the water was observed under the microscope for the presence of *A. swirskii*. For the Swirski-Mite product, approximately 0.5 gm of loose bran was sprinkled into a Petri dish and observed under microscope for *A. swirskii*. *A. swirskii* was distinguished from the bran mites and we only took counts for *A. swirskii*. The process was repeated 5 times and the average number of predatory mites (*A. swirskii*) in 0.5 gm of product was 155.4±13.5 (Mean ± 1SE).

Field experiment

Field experiments were conducted in a certified organic field located at the University of Georgia Tifton Campus, Tifton, GA. Tomato seedlings (4-5 leaf stage) were transplanted into raised beds at the between-plant spacing of 0.46 m. Each bed was 4.6 m long, 0.91 m wide and 0.2 m high, 0.31 m away from the adjacent beds, and covered with black plastic mulch. Ten plants of each commercial line (Skyway 687 and SV7631TD) were transplanted in each bed, whereas only five plants of each acylsugar line (CU071026 and FA7/AS) were transplanted in each bed because of lower availability of seedlings due to poor germination. Dead seedlings were replaced for all the tomato lines after 10 days of transplantation to ensure equivalent numbers of plants per plot. A total of four blocks, each containing 16 beds, were prepared.

A fully factorial treatment combination of mite release methods (no mite, basal application, top application and sachet application) and tomato lines (CU071026, FA7/AS, Skyway 687 and SV7631TD) were assigned to beds following a Randomized Complete Block design (RCBD). We introduced predatory mites one day after replacing the dead transplants. The three different mite treatments were achieved by not applying any mite (no mite), sprinkling a dosage of Swirski-Mite at the base (on top of soil) of the tomato plants (basal application), sprinkling a dosage of Swirski-Mite to the canopy of the plants (top application), or attaching two Swirski-Mite Plus sachets to each bed of tomato plants (sachet). The dosage for all three types of treatments was applied as per Koppert Biological System's recommendation for the usage of the mites. A "mite dosage" per Koppert Biological System's recommendation was approximately 0.5 gm of Swirski-Mite product (mites mixed with bran) sprinkled on the top or bottom of the plants or approximately 120 mites based on our pre-release quality assessment. Initially, we placed the sachets under the plastic mulch in between the plants with a small opening for the mites to emerge from the sachet. When the plants reached 8-10 leaf stage, the mite sachets were moved to be hung in the tomato canopies. We allowed whiteflies to infest the tomato plants naturally. Plants were irrigated with drip irrigation as needed and weeds between beds were suppressed three times during the sampling period by mowing. In addition to mowing, an organic herbicide, Biosafe Weed and Grass Killer® at the rate of 0.1 liter/1litre of water was sprayed between beds two times during the sampling period.

We sampled for predatory mites and whitefly abundance every two weeks for a period of four sampling weeks after the mite release. We randomly selected a leaflet from the middle section of each of the six tomato plants in each bed. Adult whiteflies on the abaxial surface of the leaflet were counted and recorded in the field (field scouting) following Diehl et al. (1995) before taking leaflet samples. The leaflets were collected, placed in individual plastic bags, transported to the laboratory, and stored overnight at -20ºC. Whitefly eggs and nymphs, *A. swirskii* eggs and adults, and any other herbivores on each leaflet were counted. Tomato yellow leaf curl virus (TYLCV) presence was assessed based on visual symptoms exhibited as slight to pronounced yellowing of leaflet margins, curling and cupping of leaflets with reduction in size and stunted plants (Friedmann et al., 1998). The number of plants in a bed exhibiting TYLCV symptoms was recorded.

Greenhouse experiments

We conducted two greenhouse experiments. For both experiments, seedlings in nursery trays and plants in pots (grown from the seedlings) were maintained in Bugdorms (60 W x 60 D x 60 H cm with mesh size 150x150 or 160 µm aperture; MegaView Science Co., Taiwan) to exclude all insects prior to the experiments. The seedlings were potted in 12.7 cm diameter plastic pots with in ground soil mix (Miracle-Gro ®, Lowe's, Tifton, GA). N: P: K= 20:20:20 general purpose fertilizer (Peter's Professional Brand) was applied to the tomato plants immediately after potting and a week after the first fertilizer application.

Greenhouse experiment 1: Role of tomato lines and *A. swirskii* application in whitefly population suppression

A setup with a bugdorm consisting of five whitefly-infested tomato plants of each of the five lines (Florida 47, Amelia, CU071026, FA7/AS and QTL6/AS) was prepared for the experiment. The setup was replicated six times. To prepare a bugdorm setup, we firstly infested the tomato seedlings with whiteflies at the 5-leaf stage. Cotton seedlings at (4-5 leaf stage) with approximately 15-20 whitefly adults each were placed inside the bugdorms with uninfested tomato seedlings. We allowed the whiteflies to establish on the uninfested tomato plants for 3 weeks, which is the amount of time whiteflies take to complete their lifecycle under favorable conditions (White, 2014). Once, we observed whitefly establishment on the tomato seedlings, we divided the tomato plants into 6 bugdorm setups. The whitefly-infested tomato plants inside the bugdorms were undisturbed for three weeks allowing times for the new generation of whiteflies to develop. During these three weeks, we took leaflet samples from each plant line in the bugdorms weekly. A commercial mite sachet (Swirski-Mite plus, Koppert Biological System) was placed on each plant 3 weeks after the infested tomato plants were divided into bugdorm setups. We continued to take leaflet samples weekly for additional four weeks after mite sachets were applied to the tomato plants. So, we took leaflet samples for a total of seven weeks which included leaflet samples taken for three weeks before mite application and four weeks after mite application. Samples were collected by taking a leaflet from the middle portion of each tomato plant weekly and placing them in a plastic bag. The leaflet samples were then transported to the lab for the count of the number of whiteflies and predatory mite *A. swirskii* at all life stages. To reduce possible feeding on tomato pollen, we removed all inflorescence from the tomato plants for the duration of the experiment (Calvo et al., 2015).

Greenhouse experiment 2: Non-pest food effects on *A. swirskii* establishment and whitefly suppression

The second greenhouse experiment included three treatments: *B. tabaci* only, *B. tabaci* + *A. swirskii,* and *B. tabaci* + *A. swirskii* + cattail pollen replicated over 12 tomato plants each. We used the commercial tomato cultivar Skyway 687 in this experiment at the 4-5 leaf stages. *A.*

swirskii (Swirskii-System) and cattail pollen (Nutrimite^{TM}) for this experiment were purchased from Biobest Sustainable Crop Management, Belgium. Similar to other experiments, I followed manufacturer protocols to assess the quality of the mites. I counted the number of mites in 0.5 grams of the product. The mixture contained an average of 120 mites/0.5 grams of treatment, which was calculated by averaging the number of mites in 0.5 grams of products 5 times. Aside from herbivores, *A. swirskii* can also feed on pollen as a supplementary food source (Calvo et al., 2015) and cattail pollen is observed as an alternative food source for predatory mites (Delisle et al., 2015a)

Each treatment received twelve tomato plants. Twelve tomato plants assigned to each treatment were divided into three bugdorms with four plants in each bugdorm. Twenty-five whitefly adults were released in each bugdorm and were allowed to establish on the tomato plants. We observed whitefly establishment on the tomato plants two weeks after introduction. Following whitefly establishment, we applied mites and cattail pollen to the tomato plants by sprinkling 0.5 grams of products on the top of the tomato plants. Both mites and cattail pollen were applied to the plants two times at an interval of two weeks during the four-week sampling period. We collected samples a week after the mites and cattail pollen were applied to the plants. One plant from each of the three bugdorms were sampled. Five randomly chosen leaflets per plant per bugdorm were selected and carefully observed under stereo-microscope for the count of whitefly and *A. swirskii* at all life stages.

Statistical analyses

In field-collected data, the whitefly abundance data was not normally distributed. Both whitefly incidence and TYLCV incidence was analyzed by Kurskall-Wallis test. We conducted Bonferroni adjusted Dunn test as our post-hoc test for mean comparisons of all the whitefly stages (eggs, nymphs and adults) in relation to tomato lines. We excluded the mite application treatments from data analyses due to the poor recovery of predatory mites in our field experiment. In the greenhouse experiment 1 and 2, whitefly counts and mite counts data for all life stages were log-transformed to fit normality and variance assumptions of ANOVA models, which was confirmed by the Shapiro-Wilk test. We then conducted ANOVA to test for the main effect of acylsugar lines followed by Tukey HSD (honestly significant difference) test for comparisons of whitefly and mite counts between tomato lines. Whitefly egg and nymph counts before mite treatment and after the mite treatment in greenhouse experiment 1 was compared using t-test. JMP® Pro 15.0.0 (2019 SAS Institute Inc.) was used for all statistical analyses. All differences are determined to be significant at α=0.05.

Results

Field experiment

Whitefly population, mite population and virus prevalence in the field

We released approximately 8800 mites in the entire field including mite application by sprinkling the product on the top and bottom of the plants and mite sachet application to the plants. We took leaflet samples from the field at an interval of two weeks for four weeks. On each sampling date, we sampled approximately 352 leaflets from the entire field. However, we recovered only 9 mites from all of our sampling efforts in tomato plants subjected to all three mite aoolication types, which provides evidence that mites did not successfully establish on the tomato plants.

Whiteflies at all life stages were high during the first four weeks of the field experiment (Figure 2.1). The number of whiteflies declined by the end of the sampling period (Figure 2.1). There was significant effect of tomato lines on whitefly eggs (ChiSq= 443.835, df= 3, P <

0.001), nymphs (ChiSq= 606.697, df=3, $P < 0.001$) and adults (ChiSq= 627.941, df=3, $P < 0.001$) abundance (Figure 2.2A). Both commercial lines (Skyway and SV7631TD) demonstrated a higher abundance of all stages of whiteflies compared to acylsugar lines (CU071026 and FA7/AS). Despite the lower abundance, whitefly abundance was not significantly different in both acylsugar tomato lines (Figure 2.1). In contrast, mean comparisons for TYLCV disease incidence indicated significantly lower virus incidence on the commercial lines compared to the acylsugar lines (ChiSq= 47.143, df=3, $P < 0.001$) (Figure2.2B).

Greenhouse experiment 1: The role of plant lines and *A. swirskii* in whitefly suppression in the greenhouse experiment

Tomato lines had a significant effect on whitefly nymph counts ($F_{4,38}$=4.873, P=0.0028) (Figure 2.3A). However, no significant effect of tomato lines was observed for whitefly egg counts ($F_{4,38}$=2.267, P=0.082) (Figure 2.3A). Furthermore, whitefly egg and nymph counts before and after the application of mites was not significantly different indicating that mites did not affect whitefly eggs (t-test: $p>0.2651$) or nymphs (t-test: $p>0.5898$; Figure 2.3B).

Greenhouse experiment 2: Non-pest food effects on *A. swirskii* establishment and whitefly suppression

The application of alternative food did not significantly enhance *A. swirskii* abundance on tomato plants (*A. swirskii* eggs, $F_{2,7}$=0.208, P>0.661, adults, $F_{2,9}$=1.459, P>0.257) (Table 2.1). Therefore, it was surprising to observe lower whitefly egg abundance on plants where cattail pollen was provided as an alternative food for the mites ($F_{2,14}$=8.205, P=0.0044; Table 2.1), but no effect on either whitefly nymphs ($F_{2,21}$=2.196, P=0.136) or adults ($F_{2,6}$=1.427, P=0.3112) was observed (Figure Table 2.1).

Discussion

My study shows that the experimental acylsugar lines with enhanced defense traits had lower whitefly numbers when compared to commercial cultivars. We observed similar results in both field and greenhouse experiments. The tomato lines used in our study (CU071026, FA7/AS and QTL6/AS) are bred from a wild relative *Solanum pennellii* LA716 for high acylsugar production. Although the level of acylsugar production in the experimental lines is not as high as the wild relative (Leckie et al., 2012, Smeda et al., 2016), my results are showing a consistent trend of significantly reduced whitefly oviposition, and development (Smeda et al., 2016,2018, Leckie et al., 2012), which has also been shown for piercing/sucking pests (Smeda et al, 2018, Li et al., 2019).

The results suggest that the effects of acylsugar lines may have context dependencies and perform better in some environmental conditions than others. The abundance of whitefly eggs, nymphs and adults was not significantly different among both acylsugar lines (CU071026 and FA7/AS) in our field experiment. In our greeenhouse experiment, however, we observed the lowest whitefly abundance on the tomato line FA7/AS followed by CU071026 and QTL6/AS. The presence of acylsugars on tomato leaves is associated with the deterrence of piercing and sucking insects (Goffreda et al., 1989; Li et al., 2019). The experimental acylsugar lines express enhanced densities of type IV glandular trichomes which exudes the secondary metabolite acylsugars compared to the trace amount of acylsugar found in *S. lycopersicum* (Lawson et al., 1997; Smeda et al., 2016; Leckie et al., 2012). Differences in acylsugar production potentially help explain differences in the abundance between some of the acylsugar lines and also the responses of whiteflies to the acylsugar lines appear context dependent (i.e. field or greenhouse led to different outcomes). Although we did not measure the acylsugar levels in our study, it is known that the accumulation of secondary metabolites such as acylsugar is highly dependent on environmental factors (Shapiro et al., 1994). Leckie et al. (2012) reports higher acylsugar levels in greenhouse-grown CU071026 compared to field-grown CU071026. Similar results are reported for wild tomato *S. pennelii* where higher acylsugar content was observed from greenhouse-grown versus field-grown wild tomatoes. (Shapiro et al., 1994). Smeda et al. (2016) reports a higher accumulation of acylsugars in line FA7/AS than CU071026, and combined results suggest further work is needed to optimize and clarify the differences in the production of acylsugars in relation to variable environmental conditions.

In our field experiments, although whitefly abundance was lower on acylsuagr lines, we observed high TYLCV incidence in acylsugar lines when compared to the commercial cultivars. The lowest incidence of the virus was observed in TYLCV resistant and susceptible cultivars. *B. tabaci* feeding is detrimental to tomato plants as they are capable of transmitting viruses such as TYLCV that cause serious damage to the crop (Marchant et al., 2020). The experimental tomato lines used in this study have moderate to high acylsugar content and high trichome density, however, currently, none of the lines possess virus-resistant genes (Ben-Mahmoud et al., 2018, Smeda et al., 2016,2018). Given the whitefly pressure in the field conditions (Figure 2.1), a leaf is considered infested if it has >5 immature whiteflies, (Barman et al., 2020), acylsugar lines are not completely immune to whitefly feeding and resultant virus acquisition and transmission. This is also true for the TYLCV-resistant cultivars available commercially (Srinivasan et al., 2012). Lower whitefly populations on acylsugar lines can help lower TYLCV infections overall, but once the plants are infected with the virus both acylsugar and non-acylsugar lines acquire similar viral loads capable of transmission (Marchant et al., 2020). However, Rodriguez et al., (2011) conducted a study to understand the level of viral loads and transmission in acylsugar lines compared to the commercial cultivar Moneymaker. The study demonstrated that, although virus symptoms and load were similar in acylsugar and non-acylsugar lines, the virus spread from acylsugar lines was lower, which indicates that acylsugar effects go beyond influencing within plant populations of whiteflies.

Acylsugar production has the potential to alter the compatibility of biological control agents, which could alter biological control efficacy. We tested the effectiveness of pairing *A. swirskii* with the acylsugar lines of tomatoes for the management of whitefly in my field and greenhouse experiments. In the field and greenhouse, even though we repeatedly released mites or used time-release sachets, we recovered few *A. swirskii* on leaf samples. We attribute this to the possibility that glandular trichomes are non-selective plant defenses and hinder the growth and foraging of *A.swirskii*, a result that is consistent with the recent review of natural enemies (Simmons & Gurr, 2005; Tian et al., 2012, Peter et al., 1995; Farrar & Kennedy, 1991). Currently, plant breeding efforts to accommodate natural enemies and predatory insects are minimal (Bergman & Tingey, 1979; Cortesero et al., 2000; Agrawal, 2000). For example, trichomes negatively impact the oviposition rate (Koller et al., 2007) and searching efficiency of predatory mite *Neoseiulus californicus* for the control of two-spotted spider mites (Cédola et al., 2001). Studies also demonstrate reduced movement, oviposition and predation by phytoseiid mite such as *Phytoseiulus macropilis, P. longipes, Amblydromalus limomicus* on tomato leaves (Sato & Mochizuki, 2011; Davidson et al., 2016). Two other studies show specifically that *A. swirskii* survival was significantly impacted by trichomes and secondary metabolite (acylsugar) accumulating on the bodies (Buitenhius et al., (2014), Paspati et al. (2021). Hence, the presence of trichomes and their exudates appears to have negative effects on *Ambylseius swirskii*, a topperforming predator of whiteflies (Calvo et al., 2015, Nomikou et al., 2001, Sakamoto et al., 2012).

Ambylseius swirskii appears to avoid or have difficulty establishing on all the tomato lines we tested. In an attempt to stimulate *A. swirskii* biological control, we provided a non-prey food source, cattail pollen. Our hope was that the application of cattail pollen would provide an additional food source to help establish the predatory mite because *A. swirskii* can feed on pollen as an alternative food source (Calvo et al., 2015, Ragusa & Swirski, 1975). For example, Nomikou et al. (2002) observed aggregation of mites in leaves where the pollen was applied, which improved the survival of *A. swirskii* in the absence of whiteflies and only pollen. Cattail pollen, apple pollen and flour moth pollen were observed as good food sources of *A. swirskii* (Delisle et al., 2015a, Vangansbeke et al., 2016). Delisle et al. (2015b) also reported improved control of thrips when *A. swirskii* was supplemented with apple pollen. In our greenhouse experiment, we observed slightly higher numbers of *A. swirskii* adults on tomato plants sprinkled with cattail pollen. We observed that the whitefly egg abundance was lower on the plants treated with cattail pollen. However, we observed no significant effect on whitefly nymphs and adult numbers. My results indicate that although the addition of non-prey resources can help establish *A. swirskii* on tomato plants, mite establishment was still poor and, in this instance, non-prey food did not improve whitefly control.

Conclusions:

The current study highlights the success of higher acylsugar-producing tomato lines for whitefly resistance. We also conclude that the predatory mite *Ambylseius swirskii* is not compatible with tomato as a host plant. While we only used mites as the biological control agents for whiteflies, there are various biological control agents available commercially. Future studies should test other arthropod compatibility with acylsugar lines to potentially synergize the pest control benefits of plant defenses and biological control.

Acknowledgments

We thank our experimental farm managers, Andy Carter, for help setting up plots in addition to Michael Foster and Melissa Thompson for field and laboratory technical support. This research work was supported by the Sustainable Agriculture Research and Education (SARE) program (project LS19-305).

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Table 2.1. Mean (± 1SE) *A. swirskii* eggs and adults count and whitefly eggs, nymphs and adults counts per 5 leaflets per treatment. Different letters represent significant difference amongst lines (Tukey HSD test: p<0.001). B represents *B. tabaci* only, B+A represents *B. tabaci*+*A. swirskii* treatment and B+A+C represents *B. tabaci*+*A. swirskii* +cattail pollen treatment.

Treatments	*A. swirskii* eggs	*A. swirskii* adults	Whitefly eggs	Whitefly nymphs	Whitefly adults
B	0.00(0.00)	0.00(0.00)	82.67(25.99)a	69(22.10)a	5.33(3.17)a
B+A	1.25(0.71)a	0.58(0.29)a	81.42(41.61)a	123.33(66.38)a	2.42(1.55)a
B+A+C	1.83(0.88)a	10(6.74)a	5.5 (2.91)b	15.08(7.55)a	0.58(0.43)a

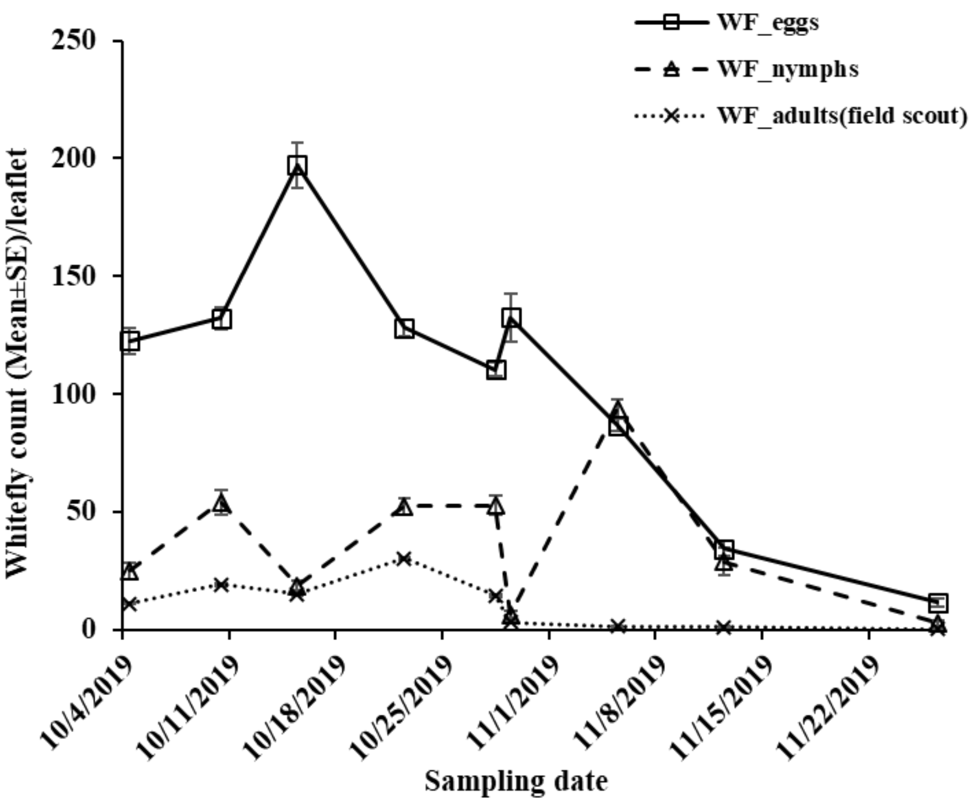

Figure 2.1. Whitefly eggs, nymphs and adults observed per leaflet on all lines on open field grown tomato plants (Mean ± 1SE). The whitefly adult counts represent field scouting counts across the sampling dates for all tomato lines. Field scouting was conducted by counting the number of whiteflies in the abaxial surface of tomato leaflets in the field. Adult whitefly counts were taken by selecting a leaflet from the middle section of each of the six tomato plants in each bed.

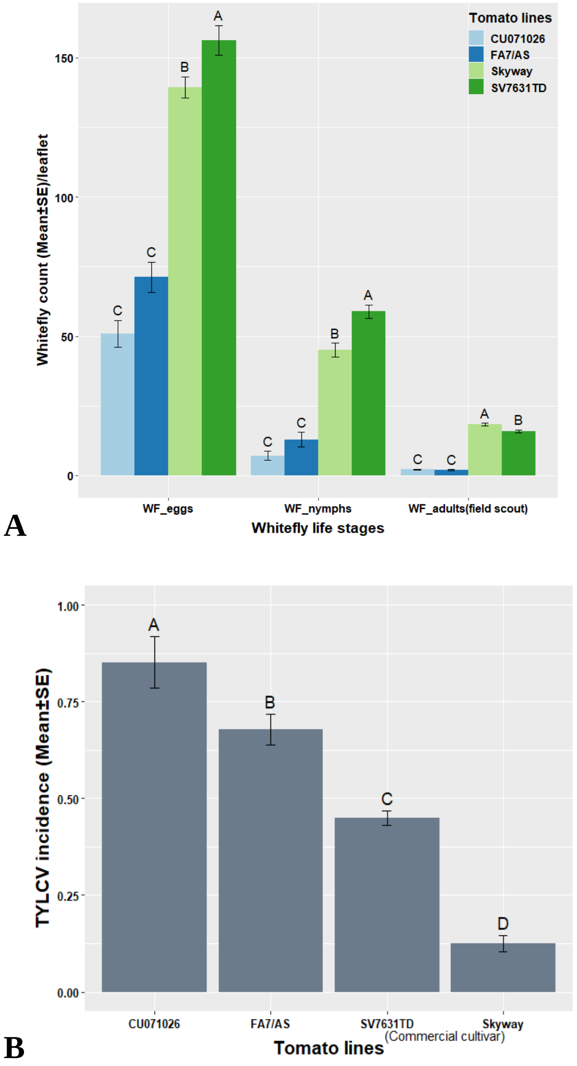

Figure 2.2. Mean ± 1SE whitefly abundance (A) and Mean ±1SE proportion of TYLCV infected plants (B). (A) Whitefly egg, nymph and adult counts per leaflet per line (Mean±1SE). Whitefly adults were counted from leaflets in the field (field scouting). A leaflet from the middle section of each of the six tomato plants in each bed was selected and the abaxial surface of the leaflet was observed for the presence of adult whiteflies. Egg and nymph counts were conducted under a microscope. Different letters represent significant difference amongst tomato lines (B) Proportion of plants (Mean ±1SE) infected by TYLCV compared to total number of plants in the plot. Different letters represent significant difference amongst tomato lines (Bonferroni adjusted post hoc Dunn test: $p<0.001$.

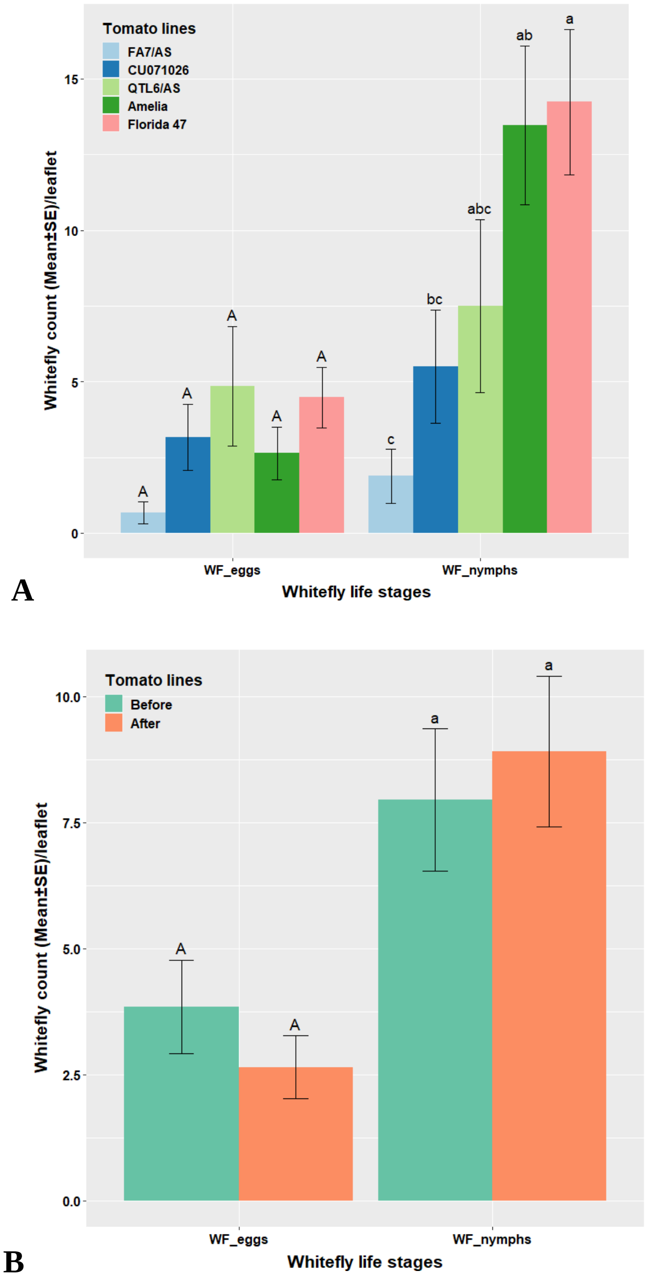

Figure 2.3. Mean ± 1SE whitefly abundance per leaflet per line (A) and Mean ± 1SE whitefly abundance before and after mite treatment(B). (A)Whitefly eggs and nymphs count per leaflet per line (Mean ± 1SE). Different letters represent significant difference amongst lines (Tukey HSD test: $p<0.001$). (B) Whitefly eggs and nymphs count per leaflet before and after mite treatment (Mean ± 1SE). Different letters represent significant difference amongst lines (t-test at 95% CI).

CHAPTER 3

EVALUATING THE PREFERENCE OF WHITEFLIES TO ARTHROPOD-RESISTANT TOMATO LINES

Abstract

Glandular and non-glandular trichomes in tomato (*Solanum lycopersicum* L.) plants are a primary insect defense structure. Exudates from glandular trichomes of wild tomato species produce acylsugar which confers resistance against various arthropods. Repeated crossing and back crossing of wild relatives with high acylsugar content to cultivated tomato has produced tomato lines with high trichome density and acylsugar content. Various studies demonstrate the potential of these lines with enhanced acylsugar production to confer resistance against arthropods such as whiteflies, *Bemisia tabaci* (Gennadius). In this study, I explore the preference of whiteflies for tomato lines with known differences in acylsugar production. I hypothesized that a lower abundance of whiteflies will be observed in tomato lines with high trichome density and acylsugar content and vice versa. To test this hypothesis, an open field experiment was paired with both a choice and no-choice test of five experimental acylsugar tomato lines and one commercial tomato cultivar. For the field experiment, I allowed the plants to infest naturally following the transplant to the field. For both choice and no-choice tests, a standardized number of whiteflies were released into the cages to infest the leaflets for six days. For all three experiments, leaflet samples were collected and observed under a stereomicroscope to count whitefly stages. Results from both field and choice tests showed significantly lower whitefly counts in experimental tomato lines when compared to commercial cultivars. In a no-choice test,

however, the results for whitefly counts were not significantly different. Hence, we demonstrate that whiteflies prefer tomato cultivars with reduced defense traits. This indicates that using experimental acylsugar tomato lines suppresses whiteflies.

Introduction

Plant characteristics, morphological or biochemical, direct or indirect, can impact the behavior, development, physiology and preference of herbivores to the host plants. Plant structures like leaf domatia (Norton et al., 2001), glossy vs waxy leaf surfaces (Eigenbrode et al., 1996), and glandular trichomes (Farrar & Kennedy 1991) play an important role in influencing predator-prey interactions by acting as direct defense structures and/or conferring resistance to herbivores (Stout et al., 2002; Cortesero et al., 2000; Price et al., 1980). The role of plant traits that confer resistance to arthropods is understood as an important aspect of an integrated pest management system (Smith & Clement., 2011). Hence, plant breeding efforts are focusing on selecting plant traits that provide defense against herbivores and enhance plant defenses to improve pest management (Cortesero et al., 2000). One such plant defense structure of interest in plant breeding for arthropod resistance is the trichome. Trichomes are hair-like projections present in the foliar region of the plant and can be glandular and non-glandular. Non-glandular trichomes confer resistance by trapping arthropods and limiting their movement while glandular trichomes exude phytochemicals such as terpenoids, methylketones, and sucrose esters that hinder their oviposition and development (Riddick & Simmons, 2014; Simmons & Gurr 2005; Tian et al., 2012; Cardoso, 2008). Tomato (*Solanum lycopersicum*) plants utilize these glandular and non-glandular trichomes as a primary defense structure against herbivores (Chatzivasileiadis & Sabelis 1997).

Trichomes in tomatoes are categorized as types I–VII, with types I, IV, VI and VII being glandular and types II, III and V being non-glandular (Tian et al. 2012). Type VI glandular trichomes in tomatoes constitute phytochemicals (plant-produced chemicals) such as 2-tridecanone, methylketone and acylsugar which is toxic to various herbivores and display arthropod-resistant properties (Burke et al., 1987; Smeda et al., 2017). Herbivores are often known to choose plants lacking defense traits or lower expression levels of defense traits. For example, tobacco hornworm, *Manduca sexta* (L.), Colorado potato beetle, *Leptinotarsa decemlineata* (Say), leafminer, *Liriomyza trifolii* preferred a commercial tomato cultivar with low glandular trichome density compared to a wild tomato with higher type VI glandular trichome density (Kauffman & Kennedy., 1989; Fernandes et al., 2012). Therefore, one method for improving tomato resistance involves the transfer of genetic material from wild tomato relatives with high trichome density and acylsugar production to cultivated tomatoes (Lawson et al., 1997; Alba et al., 2009; Rodriguez-Lopez et al., 2012). My studies and recent studies show the success of acylsugar-enhanced lines in repellence of pests such as two-spotted spider mites, tomato psyllids and thrips (Alba et al., 2009; Li et al., 2019; Smeda et al., 2018), including globally important pest of tomato whiteflies (Rodriguez-Lopez et al., 2011,2012; Leckie et al., 2012).

Plant traits affect insect performance and help determine insect resistance or tolerance to the plants (Inbar & Gerling, 2008). Plant traits such as trichomes (glandular and non-glandular) and leaf domatia can affect herbivore settling and survival on host plants (Cortesero, 2000). For example, piercing and sucking insects such as aphids, *Myzus persicae* (Hemiptera; Aphididae) and whitefly nymphs prefer to settle on the abaxial surface of the leaf rather than the adaxial surface (Calabrese et al., 1976; Simmons, 2002). Leaf domatia harbor predatory arthropods and enhance their performance in reducing herbivore pests (Agrawal & Karban, 1997). Studies that provide arthropods with a choice of host plants differing in the expression of defense traits helps understand arthropod's preference (Withers & Mansfield, 2005; Rodriguez et al., 2011; Silva et al., 2014; Simmons, 2002). Results measuring whether an arthropod favors the plant or not is often determined through the arthropod's responsiveness to factors such as time taken to establish, oviposit and move within the host plants (Withers & Mansfield, 2005). Choice and no-choice tests to determine arthropod performance are mostly limited to a controlled-environments such as greenhouse or laboratories and provide an opportunity for predicting arthropod performance in field conditions (Withers & Mansfield, 2005; Murray et al., 2010). These tests are widely used in experiments targeted at understanding the preference of whiteflies to a different range of host plants or host plants with varying levels of defense characteristics. Results from these experiments have been instrumental in understanding the role of plant traits and their impacts on whitefly performance (Rodriguez et al., 2011,2020; Silva et al., 2014, Simmons, 2002).

Whiteflies, *Bemisia tabaci* (Gennadius), (Hemiptera: Aleyrodidae) are a major pests of concern to tomato growers worldwide (Brown et al., 1995; Simmons, 2002). Direct damage to the plants is caused by feeding on phloem sap and honeydew production which encourages sooty mold in tomato plants (White, 2014). Whiteflies also carry viruses that are transmitted to the plants causing various plant diseases such as Tomato Yellow Leaf Curl Virus (TYLCV) (family Geminiviridae, genus *Begomovirus*). Damage symptoms include leaf wilting, irregular ripening of the fruits and reduced yield (Brown et al., 1995; Firdaus et. al., 2012; Srinivasan et al., 2012). Although tomato cultivars with virus resistance are available, a majority of whitefly management is insecticide dependent which is also not reliable due to the ability of whiteflies to develop rapid resistance to insecticides (Horowitz et al., 2005, Riley & Srinivasan, 2019, Srinivasan et al., 2012). Hence, enhancing phytochemicals that can repel pests can provide an alternative to insecticide use for whitefly control (Mutschler et al., 2021).

In this study, we evaluate the preferences of whiteflies to tomato plants differing in the levels of defense trait expression. Our hypothesis was that the enhanced trichome structures and higher acylsugar production in experimental tomato lines would hinder whitefly feeding and oviposition thereby reducing whitefly abundance. For this purpose, we combined open field with controlled environment choice and no-choice tests. Our goal was to compare the preferences of whiteflies to tomato lines differing in the presence or absence of enhanced defense traits.

Materials and Methods

Tomato plant lines

We used experimental acylsugar lines of tomato provided by the Tomato Breeding Program at Cornell University, Ithaca New York and we purchased a commonly grown commercial cultivar. For all experiments (field, choice and no-choice tests) we use five acylsugar lines with varying levels of acylsugar content and trichome density namely CU071026, CU17NBL, QTL6/sw5/AS, QTL6/CU17 and QTL6/sw5/CU17NBL (Table 3.1). We used Grand Marshall as our commercial cultivar for the field experiment and Amelia F1 Hybrid for choice and no-choice tests. We planted the seeds of each tomato line in the greenhouse in nursery trays with Black Gold® Natural and Organic Seedling Mix. 20-20-20 general purpose fertilizer (Peter's Professional Brand) was applied to the seedlings as needed.

Field experiment

We conducted field experiments at the University of Georgia-Tifton Campus, Tifton GA. We transplanted the seedlings at 5-6 leaf stage to the raised bed plots covered with white plastic mulch. Each plot measured 1.8m by 6m (6ft by 20ft) with 0.32m (1ft) alleyway on all sides. Each plot represented a replicate of the tomato line and received 10 plants each. We had 4 replicates of each line and the plots were randomly assigned for each line. We used CU071026, CU17NBL, QTL6/sw5/AS, QTL6/CU17 and QTL6/sw5/CU17NBL experimental acylsugar lines of tomato and Grand Marshall, a commercial cultivar as control (Table 3.1).

After transplanting the tomato seedlings in the field, we allowed the whiteflies to infest the tomato plants naturally. We irrigated the plots regularly through drip irrigation. Weeds were suppressed by hand weeding as needed. One-time Radiant® @ 0.7L/ha insecticide application as well as hand-picking was conducted two times to manage the caterpillar pests, tobacco hornworm, *Manduca sexta* (L.) and fall armyworm caterpillars *Spodoptera frugiperda*, (Smith) in the tomato plants. Scouting data for tobacco hornworm was taken for two different scouting dates (Table 3.4).

Estimating whitefly abundance, bacteria/virus prevalence and arthropod populations in the field

Two weeks after transplanting the tomato seedlings to the field, we started weekly leaflet sampling from the field plots for ten weeks. A leaflet sample was taken by selecting three plants randomly out of 10 plants in a plot and taking a leaflet from the middle portion of the selected plants. We placed the collected leaflet in separate plastic bags, transported it to the lab and stored them in a freezer at -20ºC overnight. The leaflets were then observed under a stereomicroscope to count whitefly eggs, nymphs and adults. Prior to taking leaflet samples, we scouted for adult whiteflies following Diehl et al. (1995). We randomly selected three plants from a plot and the number of adult whiteflies settling on the abaxial surface of the leaflet of selected plants was counted and recorded.

We conducted visual inspections to assess the presence of Tomato Yellow Leaf Curl Virus (TYLCV) weekly following the methods developed by Friedmann et al. (1998). However, only two plants out of ten plants in a plot with the experimental acylsugar line QTL6/sw5/CU17NBL displayed symptoms of TYLCV. We also conducted disease ratings from bacterial leaf spots caused by *Xanthomonas* spp. at the end of the sampling period. We assessed the disease severity by visually inspecting all plants in the plots for the symptoms of bacterial leaf spots. I converted the disease severity (percentage of symptomatic foliage observed in the plants within a plot) into modified Horsfall-Barrett scale by assigning corresponding numbers to the scale where 0= 0%, 1 = 0-3%, 2=3-6%, 3 = 6-12%, 4 = 12-25%, 5 = 25-50%, 6 = 50-75%, 7 = 75-88%, 8 = 88-94%, 9 = 94-97%, 10 = 97-100% (Fayette et al., 2012; Horsfall & Barratt, 1945).

Scouting and hand-picking of tobacco hornworm and fall armyworm caterpillars were conducted on two dates following caterpillar incidence (Table 3.4). I counted the number of tobacco hornworm caterpillars observed on all the tomato plants. We were interested in understanding the diversity of arthropods (pest and non-pest) in the tomato field as well as their effect on non-pest arthropods that are potentially beneficial to the tomato system. Since, I didn't observe arthropod diversity on tomato leaflets collected for whitefly counts we conducted a 30-second suction sample from the canopy of the middle three tomato plants in each plot using a modified reverse-flow leaf blower into a fine mesh bag (Bowers et al., 2020) at the end of our sampling period. I then transferred all the contents suctioned in the mesh bag into a plastic bag (i.e. one sample per plot) and transported them to the laboratory. The samples were stored and preserved in a -20ºC freezer until further identification. To process the suction samples, each was sieved, individuals sorted, and categorized to taxa (order level or higher; see Supplemental Table 3.1), to provide estimates of arthropod counts in each tomato line.

Choice and no-choice tests

We conducted no-choice tests in greenhouse and choice experiment in a temperature-controlled environmental chamber located at the University of Georgia, Tifton GA. We transplanted the tomato seedlings at 4-5 leaf stage in 3-inch plastic pots with in ground soil mix (Miracle-Gro ®). The tomato lines used in the choice and no-choice tests were five acylsugar lines namely CU071026, CU17NBL, QTL6/sw5/AS, QTL6/CU17, QTL6/sw5/CU17NBL and a commercial tomato cultivar Amelia F1 Hybrid used as control (Table 3.1). Peter’s Professional Brand 20-20-20 general purpose fertilizer was applied to the plants as needed. We collected the whiteflies for the experiment by aspirating using a pipette tip attached to the aspirator tubine. The whiteflies for the experiments were obtained from the greenhouse colony maintained in Red Snapper tomato plants at the University of Georgia-Tifton Campus, Tifton GA. The tomato plants with whitefly colonies were isolated in bugdorms (© MegaView Science Co., Ltd., Taiwan) with dimensions 60*60*60 cm (W*D*H) and mesh size 150*150|160µm aperture.

Leaf-cage experiment (no-choice test)

The no-choice test was conducted in a greenhouse by placing a tomato plant from each line in a bugdorm (© MegaView Science Co., Ltd., Taiwan) with dimensions 60*60*60 cm (W*D*H) with the mesh size 150*150|160µm aperture. We selected two leaflets within the plants in each of the six tomato lines and enclosed them with a leaf cage on both abaxial and adaxial sides. We introduced 10 whitefly adults into each leaf cage. After 6 days of introducing whiteflies into the leaf cage, we carefully removed the leaflets and transported the leaflets into a plastic bag. We then observed the leaflets under a stereo microscope and counted and recorded the number of whitefly eggs and nymphs in each leaflet. We repeated the experiment five times, hence we obtained ten leaflet samples per tomato line.

Detached leaflet experiment (choice-test)

We conducted the choice test with detached leaflets in a temperature-controlled environmental chamber (30°C, 100 µml photoperiod 16L:8D hours). Petioles of a fully expanded leaflet of all six lines were obtained from tomato plants maintained in the greenhouse. The leaflets were checked for the presence of arthropods or infestation. Only clean leaflets were used in the experiment.

We created an experimental setup with a transparent cylindrical cage made of a transparent plastic sheet. The cylindrical cages were made by gluing two opposite edges of a transparent plastic sheet in a circular fashion to form a hollow tube-like structure. The top opening of the cylindrical cage was covered with a fine mesh cloth to prevent the escape of whiteflies. We also made two openings on either side of the cage and covered them with a fine mesh cloth. The cylindrical cage was 20cm high and had a diameter of 13.7 cm. We filled a scintillation vial (4ml) with water and inserted a petiole of a fully expanded leaflet of a tomato line in. This was repeated for all six tomato lines (five acylsugar CU071026, CU17NBL, QTL6/sw5/AS, QTL6/CU17, QTL6/sw5/CU17NBL and a commercial cultivar Amelia F1 hybrid, Table 3.1). The scintillation vials were sealed with transparent plastic tape and a hole was punctured to insert petioles through the tape. We made sure that the petioles were immersed in water inside the scintillation vial. All six scintillation vials were placed in a circular plastic container with a diameter of 14.2 cm. The plastic container with the scintillation vials was then covered with a cylindrical cage. We prepared five of these experimental setups. Twenty whitefly adults were introduced per setup. We collected 20 whitefly adults in an aspirator prepared as mentioned above and allowed the whiteflies six days to interact with the leaflets. The experiment was repeated three times. Hence, we obtained 15 replicates per line. At the end of six days, we removed the experimental setup from the environmental chamber and collected the adult whiteflies and leaflets. Each leaflet was observed under a stereo microscope. We counted and recorded the number of eggs and nymphs present on a leaflet.

Statistical Analyses

In our field experiment data, to assessed the seasonal pattern of whitefly abundance observed on tomato leaves. We used log transformed whitefly counts in relation to the fixed effect, sampling date, fit using a Linear Mixed-Effects Model (LME). We accounted for repeated measures of plots by partitioning a portion of the error as a random effect of plot (i.e. =~1|plot). To assess changes in whitefly abundance in relation to different tomato lines, we used a similar structured LME with log transformed total whitefly counts in relation to lines as the fixed effect and sampling date as our random effect. For significant main effects in both models, we conducted linear contrasts with adjusted estimates using {emmeans}.

For the greenhouse and environmental chamber experiments, the counts for whiteflies for both the choice and no-choice tests was square root transformed. Since we completed the greenhouse experiments in groups of replicated plants for each line, we used experimental time blocks as our random effect. Comparisons between different tomato lines was conducted by pairwise adjusted Tukey HSD (Honestly Significant Difference). Analyses were conducted in R version 4.1.2 “Bird Hippie” (RCoreTeam 2021). We natural log transformed the data obtained from field scouting of tobacco hornworms to fit normality according to the Shapiro-Wilk test. Significant main effect of tomato line was assessed using Tukey HSD test (α=0.05). Bacterial leaf spot data were converted to Horsfall-Barratt scale as described above. Data were analyzed using the non-parametric Kruskal-Wallis test and means were separated by Student-Newman-Keuls (SNK) test. Tobacco hornworm field scouting data and bacterial leaf spot data were analyzed using JMP® Pro 15.0.0 (2019 SAS Institute Inc.)

Results

Field experiment

Estimating whitefly population responses

Whiteflies were generally more abundant later in the season as compared to early August, and appeared to peak in September (LME: $F_{9,207}$=6.42, P<0.0001; Fig. 3.1A, Table 3.2). Tomato lines had significant effects on the number of whiteflies observed on leaves (LME: $F_{5,225}$=22.86, P<0.0001; Fig.3.1B). The commercial cultivar had significantly higher whitefly pressure as compared to any of the experimental acylsugar lines (Fig. 3.1B). Of the acylsugar lines, the CU17NBL had the highest whitefly numbers, and the QTL6/CU17 had the lowest. However, the three with the lowest were not significantly different (Fig. 3.1B). Furthermore, to view the results at the overall leaf collection level of 702 leaves sampled (Fig.3.1C), all of the acylsugar lines commonly had below 10 whiteflies, and only the CU17NBL or the QTL6/sw5/AS lines had whitefly counts on leaves greater than 20. Whereas the commercial line had upwards of 60 and commonly greater than 10 (Fig.3.1C).

Estimating bacterial prevalence, tobacco hornworm abundance and arthropod diversity

Tomato lines had a significant effect on bacterial leaf spot severity. We observed significantly high disease incidence in the commercial cultivar Grand Marshall. Of the five experimental acylsugar lines, QTL6/sw5/AS and CU17NBL were observed to have the lowest bacterial leaf spot severity (Table 3.3). There was a significant effect of tomato lines on tobacco hornworm incidence ($F_{4,23}$=10.89, P<0.001, Table 3.4). We didn’t observe tobacco hornworms on the commercial cultivar Grand Marshall and hence we discarded the cultivar from analyses. The highest caterpillar abundance was observed in lines CU071026 and CU17NBL and with no significant difference between the two lines. The lowest caterpillar abundance was observed in the lines QTL6/sw5/AS. We observed a high abundance of arthropods of the order Hemiptera including families including high numbers (3827) of Miridae. Predatory bug families such as Geocoridae (76) and Anthocoridae (4) were also observed. Diptera was the second most abundant with a count of 722. A total of 121 parasitic wasps (order: Hymenoptera) was also observed. See supplemental table 3.1 for more details.

Greenhouse and environmental chamber experiments

Detached leaf experiment (choice test) and leaf cage experiment (no-choice test)

We observed no significant difference in whitefly oviposition and nymphs across different lines in the no-choice test (LME: $F_{5,50}$=0.5941, P=0.7045; Fig. 3.2A). Conversely, in the choice-test (detached-leaf experiment), tomato lines had significantly higher numbers of whiteflies eggs and nymphs on the leaflets of the commercial cultivar, as compared to the acylsugar lines (LME: $F_{5,82}$=6.3742, P<0.0001; Fig. 3.2B). The number of whiteflies eggs and nymphs was not significantly different between the different acylsugar lines (Figure 3.2B; see Supplemental Table 3.2)

Discussion

Our study demonstrates that whiteflies do not prefer tomato plants with enhanced defensive traits such as high density of glandular trichomes and acylsugar levels. We report significantly high whitefly abundance on commercial/non-acylsugar lines. Acylsugars, a secondary metabolite secreted from type IV glandular trichomes in tomato plants is associated with decreased survival and fitness of whiteflies (Liedl et al., 1995; Leckie et al., 2012,2016; Rodriguez-Lopez et al., 2011). These defense traits are, however, found non-functional in cultivated/commercial tomato lines (Lawson et al., 1997). Hence, the results indicate the preference of whiteflies to non-acylsugar compared to acylsugar tomato lines.

In both the open field and choice test, whiteflies had a choice of five acylsugar tomato lines and a commercial line. The acylsugar lines included in this study consisted of two benchmark lines CU071026 and CU17NBL and three other sister lines (QTL6/sw5/AS, QTL6/sw5/CU17NBL, QTL6/CU17) derived from the benchmark lines (Mutschler, 2021). The benchmark lines accumulate lower levels of acylsugar compared to the sister lines derived from CU071026 and CU17NBL (Leckie et al., 2012; Marchant et al., 2020). In the field experiment, among the acylsugar lines, we observed the highest whitefly abundance in benchmark line CU17NBL and the lowest on the line QTL6/CU17. Although we didn’t measure the level of acylsugars, high counts of whiteflies in benchmark acylsugar line and commercial lines suggest whiteflies prefer plants with low acylsugar content. In our choice test with detached leaflets conducted in an environmental chamber, whitefly counts among acylsugar lines didn’t significantly differ. Marchant et al. (2020) highlights that total acylsugar content in benchmark line and lines with QTL6 were not different as a result greenhouse whitefly (*Trialeurodes vaporariorum*) had no settling preference when a choice between two acylsugar lines was provided. It is possible that the accumulation of acylsugars in different lines was impacted by the environmental conditions which are known to be a determining factor for acylsugar level fluctuations (Shapiro et al., 1994). When no-choice of lines were available, whitefly numbers were not different among all tomato lines.

Whiteflies also host viruses that are transmitted to plants causing various diseases (Moreno-Ripoll et al., 2014). None of the acylsugar lines used in these experiments possessed Tomato Yellow Leaf Curl virus-resistant genes yet the TYLCV incidence was observed on only two plants out of all the plants in the field experiment. This could be attributed to lower whitefly abundance in acylsugar tomato lines resulting in hindered TYLCV acquisition and transmission (Marchant et al., 2020; Smeda et al., 2018). The sister lines derived from the benchmark lines CU071026 and CU17NBL differ in the levels and compositions of acylsugars due to the addition of one or more quantitative trait locus (QTL) (Mutschler, 2021). Along with higher levels of acylsugars than the benchmark lines, some of these sister lines used in our study (QTL6/sw5/AS, QTL6/sw5/CU17NBL) include sw-5 gene introgressions responsible for thrips transmitted Tomato Spotted Wilt Virus (TSWV) resistance (Stevens et al., 1995; Mutschler, 2021). Since, our experiment was conducted in the fall when thrips typically overwinter in the southern US (Reitz, 2002), we have no data that support the efficacy of these lines against TSWV. Some studies show decreased thrips oviposition and associated TSWV suppression in CU071026 and their sister lines (Ben-Mahmoud et al., 2018,2019), however, in our study we are unable to provide accurate information on how acylsugar tomato lines behave in the presence of viral pathogens.

Aside from observing both whitefly abundance differences and disease, our field tomato plants became infested with tobacco hornworm caterpillars hence we decided to record the caterpillar numbers. Surprisingly, no caterpillars were observed on the commercial cultivar. However, we observed the highest numbers of tobacco hornworms on the benchmark acylsugar tomato lines CU071026 and CU17NBL. This trend was similar to what we observed regarding the whitefly numbers. Tobacco hornworms numbers were higher on benchmark acylsugar lines compared to other acylsugar lines. However, Weinhold & Baldwin (2011) highlights that unlike piercing and sucking insects, acylsugars may act as a sugary first meal to the Lepidopteran herbivores. This provides one explanation for why the caterpillars were potentially attracted to acylsugar tomato lines. Studies also suggest that applying acylsucroses produced by wild tomato *S. pennellii* LA716 provides resistance to caterpillar pests such as tomato fruitworm (*Helicoverpa zea* Boddie) (Lepidoptera: Noctuidae) (Tian et al., 2012), tomato pinworm (*Tuta absoluta* Meyrick) (Lepidoptera: Gelechiidae) (Dias et al., 2019; de Resende et al., 2022). Therefore, variable results are present within the literature regarding the presence of secondary metabolite such as acylsugars and chewing pests such as tobacco hornworms. Similarly, various arthropods are present in the tomato system and the defensive traits such as acylsugars and trichomes can exert both positive and negative effects on interacting arthropods.

Suction sampling of tomato plants provided us with an idea of arthropods interacting in the tomato field within different tomato lines (See Supplemental table 3.1). We observed high numbers of insects belonging to the family Miridae in our suction samples. Mirid bugs are considered pests in tomato systems. Some mirid bugs are also known to be attracted by herbivore-induced volatiles produced by *T. absoluta* and whitefly-infested tomato plants (Ayelo et al., 2021). Although we are not sure of the specific reasons for their high numbers, it could also simply be spill over from adjacent cotton fields. We were also interested in understanding the diversity of non-pest arthropods that are beneficial to the tomato systems. Albeit in low numbers, we observed beneficial insects such as *Geocoris* spp. (Family: Geocoridae), *Orius* spp. (Family: Anthocoridae) and parasitic wasps of the order Hymenoptera. The low numbers of beneficials observed could be due to the high density of trichomes in tomato plants used in the study which is considered unfavorable exerting negative effects such as reduced oviposition, survival and herbivory (Riddick & Simmons, 2014).

Finally, our study demonstrates that commercial hybrids are preferred by whiteflies when compared to acylsugar-producing tomato lines. This opens up the possibility of utilizing acylsugar tomato lines in commercial settings to control challenging tomato pests such as whiteflies. Currently, the studies on experimental acylsugar lines lack encouraging results on major attributes such as plant quality, fruit quality and yield which are required for utilizing tomato lines on a commercial scale. An integrated pest management strategy could be developed by utilizing arthropod-resistant lines in a large scale. This could potentially help reduce the grower’s reliance on insecticides. However, limitations of the experimental lines regarding fruit production and yields must be addressed before the lines can be integrated into the pest management system.

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Table 3.1: Associated characteristics of the acylsugar lines and commercial cultivar of tomato used in the study. The acylsugar sugar lines are bred from the wild relative of tomato *Solanum pennellii* (Correll) d'Arcy accession LA716 (Lawson et al., 1997; Leckie et al., 2012).

Tomato lines	Acylsugar/Commercial lines	Characteristics of the lines	References
CU071026	Acylsugar	- Benchmark acylsugar line - Moderate levels of acylsugar (15% of S. pennellii LA716) - No virus resistance	(Leckie et al., 2012)
CU17NBL	Acylsugar	- Comparable to CU071026 - No virus resistance - Good fruit, seed set and germination	(Mutschler., 2021)
QTL6/CU17	Acylsugar	- Increased acylsugar content and trichome density (chromosome 6 introgression of QTL6/AS in the background of CU17NBL - Better fruit, seed set and germination - No virus resistance	(Mutschler., 2021)
QTL6/sw5/AS	Acylsugar	-Consists of sw-5 resistant gene to TSWV combined with high acylsugar content and trichome density as in QTL6/AS	((Mutschler., 2021); Leckie et al., 2012; Spassova et al., 2001)
QTL6/sw5/CU17NBL	Acylsugar	-80-100% increase in acylsugar than in CU17NBL - Increased acylsugar content and trichome density (chromosome 6 introgression of QTL6/AS in the background of CU17NBL) -Consists of sw-5 resistant gene to TSWV combined with high acylsugar content and trichome density as in QTL6/AS	(Mutschler., 2021)
Amelia F1 Hybrid	Commercial	Resistant to TYLCV	
Grand Marshall	Commercial	Resistant to Bacterial Leaf Spot	

Table 3.2 Total Mean (±1SE) whitefly count per sampling date per tomato line. Whitefly count data represents counts of whitefly eggs, nymphs and adults from field scout pooled together per sampling date per tomato line

	Tomato lines					
Sampling dates	CU071026	CU17NBL	QTL6/CU 17	QTL6/sw 5/AS	QTL6/sw5/ CU17NBL	Grand Marshall
07/29/2021	0.50(0.34)	1(0.51)	1.25(0.51)	4.83(3.15)	1.58(0.85)	1.83(0.78)
08/07/2021	0.17(0.17)	0.42(0.19)	0.58(0.42)	0.5(0.26)	1.42(0.83)	0.92(0.34)
08/13/2021	0.58(0.19)	0.50(0.23)	0.25(0.13)	0.33(0.22)	0(0)	0.67(0.51)
08/20/2021	1.08(0.49)	4.42(2.22)	0.33(0.22)	0.33(0.14)	0.33(0.33)	3.00(0.78)
08/27/2021	1.16(0.61)	2.50(1.43)	0.58(0.34)	2.33(1.08)	0.58(0.34)	5.25(1.43)
09/03/2021	2.67(1.53)	4.08(2.28)	0.58(0.31)	1.42(1.02)	2.67(1.29)	12.75(3.47)
09/10/2021	1.08(0.62)	3.17(0.99)	0.83(0.51)	1.42(0.63)	0.58(0.25)	22.08(7.06)
09/17/2021	0.58(0.36)	4.75(1.75)	0.83(0.39)	2.67(1.26)	0.67(0.47)	17.00(4.59)
09/24/2021	1.50(0.59)	1.42(0.61)	0.92(0.47)	2.17(1.06)	1.33(0.48)	10.67(2.80)
10/01/2021	0.92(0.53)	2.67(0.82)	1.83(0.84)	2.67(1.37)	0.58(0.56)	7.83(2.13)

Table 3.3. Severity of bacterial leaf spot in experimental and commercial tomato lines. Different or non-overlapping letters indicate significant differences between different lines according to SNK test ($p<0.05$). The disease severity data is based on Horsfall-Barrett scale for rating bacterial leaf spot severity.

Tomato line	Bacterial leaf spot severity
Grand Marshall	8a
QTL6/CU17	6.75b
CU071026	6.75b
CU17NBL	6.5b
QTl6/sw5/CU17NBL	6.5b
QTL6/sw5/AS	6.25b

Table 3.4. Mean (± 1SE**)** tobacco hornworm caterpillar counts over two scouting dates per tomato line per plot. Tobacco hornworm scouting data were taken in two different dates 09/08/2021 and 09/14/2021 respectively. Different letters represent significant difference amongst varieties (Tukey HSD test: p<0.001).

Tomato lines	Caterpillar counts
CU17NBL	5.38(1.03)a
CU071026	2.75(0.90)a
QTL6/sw5/CU17NBL	2.00(0.59)ab
QTL6/sw5/AS	1.00(0.27)b
Grand Marshall	0

Supplemental Table 3.1. Total counts of arthropod taxa (order level or higher) in each tomato line obtained from suction sampling. The arthropods were collected through a 30-second suction sampling of three middle plants out of ten plants in a plot by using a modified leaf blower. Each tomato line was assigned four plots with ten plants each in our field experiment so we suction sampled twelve plants per tomato line and the samples are representative of one sampling date. Listed in order of highest to lowest count

	Tomato lines						
Arthropod taxa	CU071026	CU17NBL	QTL6/ CU17	QTL6/sw5/AS	QTL6/sw5/ CU17NBL	Grand Marshall	Total
Miridae	831	834	476	667	430	589	3827
Diptera	52	38	59	46	15	512	722
Coleoptera	68	28	41	16	37	26	216
Collembola	24	31	7	21	3	62	148
Psocoptera	39	18	17	15	18	14	121
Hymenoptera (Parasitic wasps)	20	15	11	16	6	21	89
Coreidae	0	7	0	0	7	62	76
Geocoridae (*Geocoris* spp.)	24	17	5	10	3	5	64
Aleyrodidae	2	9	2	12	13	1	39
Araneae	6	4	1	2	1	13	27
Reduviidae	3	7	1	8	3	4	26
Berytidae	3	11	3	1	2	4	24
Lepidoptera	2	12	2	3	2	0	21
Pentatomidae	1	4	0	1	2	3	11
Cicadellidae	3	0	1	2	0	1	7
Formicidae	1	2	0	0	0	1	4
Anthocoridae (*Orius* spp.)	1	1	0	1	0	0	3
Blattidae	0	2	0	0	0	1	3
Thysanoptera	0	1	0	0	2	0	3
Nabidae	0	1	0	0	1	0	2
Membracidae	0	0	1	0	1	0	2
Total	1080	1042	627	821	546	1319	5435

Supplemental Table 3.2. Data shows Mean (±1SE) whitefly eggs and nymphs counts per leaflet per line for choice and no-choice tests.

Tomato line	Eggs	Nymphs
No-choice test		
Amelia	44.3(6.27)	2.7(3.43)
CU071026	43.3(10.89)	1.8(2.80)
CU17NBL	53.6(9.41)	0.2(0.2)
QTL6/CU17	35.5(8.75)	2.8(2.16)
QTL6/sw5/AS	45.5(14.67)	1.2(0.76)
QTL6/sw5/CU17NBL	35.3(9.93)	0.2(0.2)
Choice- test		
Amelia	6.00(4.18)	16.80(3.43)
CU071026	0.87(0.46)	5.33(2.80)
CU17NBL	4.20(2.16)	6.60(1.57)
QTL6/CU17	2.33(1.17)	2.87(1.06)
QTL6/sw5/AS	1.33(0.75)	1.00(0.44)
QTL6/sw5/CU17NBL	1.00(0.61)	4.47(1.72)

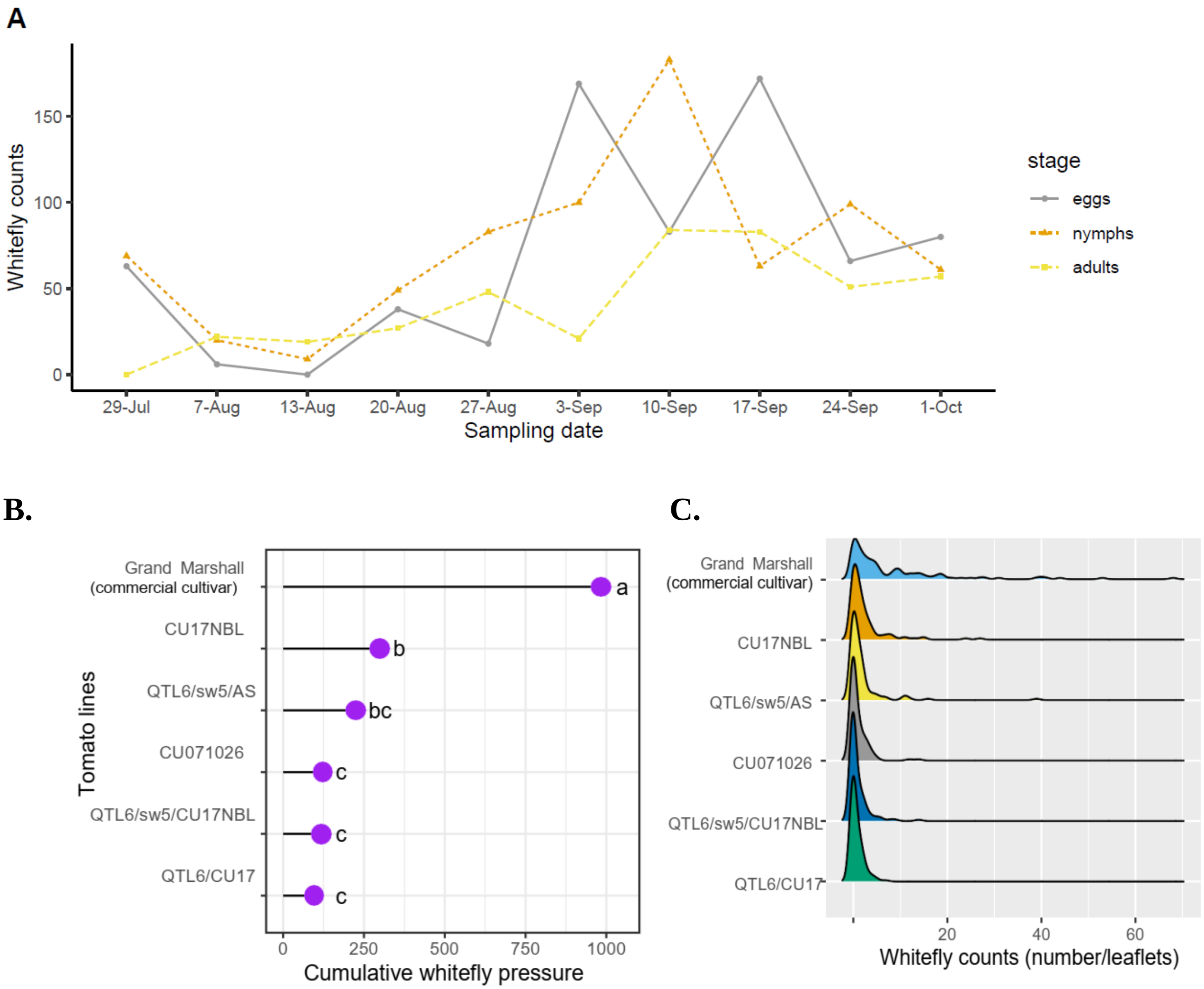

Figure 3.1. Seasonal pattern of whitefly counts on tomato leaves (A). Counts were combined across replicates of plots and tomato lines and cultivars for each life stage. Comparison of whiteflies observed on tomato lines and cultivars (B). We combined seasonal counts from leaf samples taken across the season to form an estimate of whitefly pressure. Letters indicated adjusted linear contrasts where different letters or nonoverlapping letters indicate a significant difference between lines (p<0.05). Frequency distribution of counts of whiteflies on leaves of different lines (C).

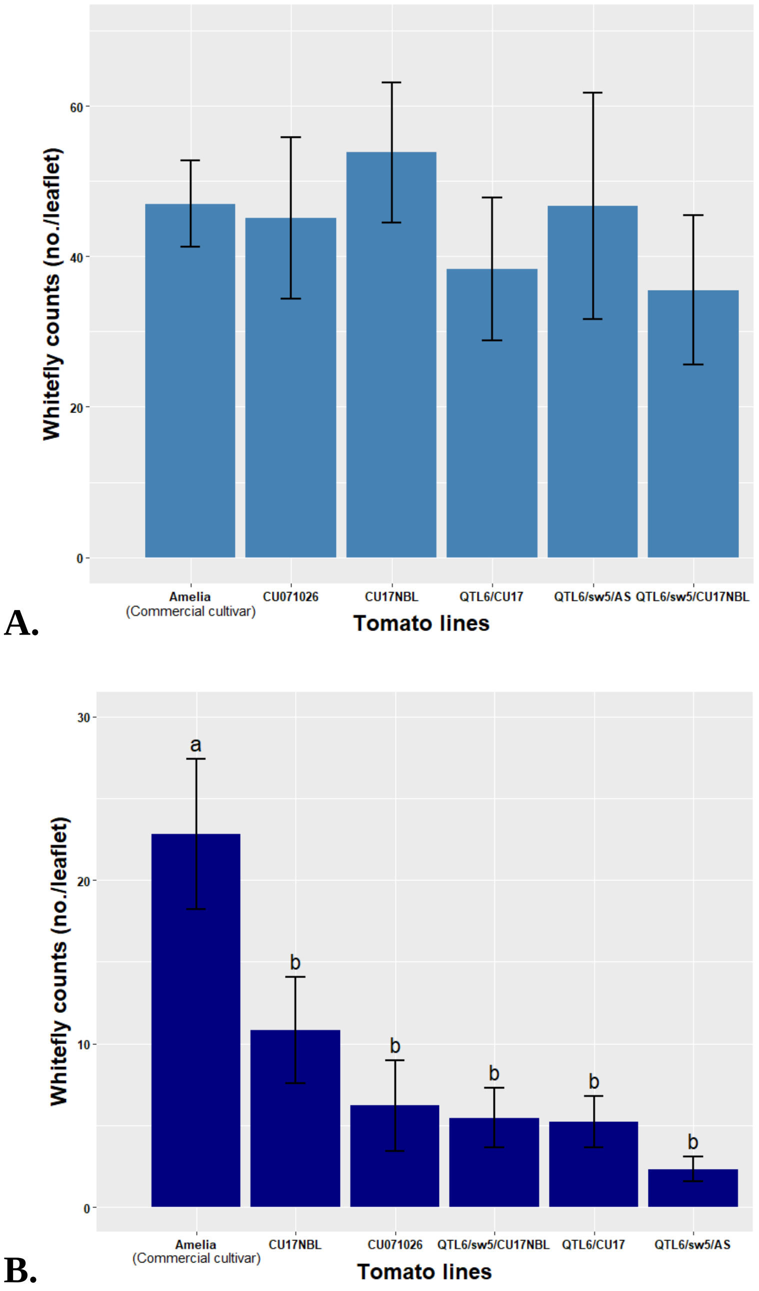

Figure 3.2. Whitefly counts per leaflet in a leaf-cage experiment (no-choice test) (A) and detached leaf experiment (choice test) (B). Counts were combined across replicates for whitefly eggs and nymphs. The estimates represent the back-transformed whitefly count Mean ± 1SE. Different or non-overlapping letters indicate a significant difference between different tomato lines ($p<0.05$)

CHAPTER 4

SUMMARY AND CONCLUSION

The interaction between plants and arthropods in a multitrophic system is dynamic even when they occur in simplified habitats. The manipulation of the elements (plants and arthropods) of the interaction is a key to successful pest management practices (Stout, 2013) and sustainable alternatives to the insecticide-dependent insect-pest control tactics (Baker et al., 2020). Studies on pest management strategies have focused on understanding the evolution of plant-arthropod interactions and implementing the knowledge towards utilization and modification of plant traits that confer arthropod resistance (Stout, 2013). Various plant defense traits and more than 100,000 plant secondary metabolites are reported to directly or indirectly display defensive properties through the process of antixenosis (adverse effect on pest behavior) and antibiosis (adverse effect on pest's life history) (Peterson et al., 2016; Stout et al., 2013). In tomato plants, trichomes (glandular or non-glandular) act as defense structures against numerous arthropods (Simmons & Gurr, 2005). Acylsugars, secondary metabolites exuded by glandular trichomes, are known to play a role in arthropod resistance (Mutschler, 2021). In the present study, we investigated the effect of arthropod-resistant tomato plants for improving management of whiteflies in combination with mite natural enemies. Our results demonstrate the success of acylsugar-producing tomato lines in reducing whitefly populations when compared to current commercial tomato cultivars. However, mites did not establish on any tomato line.

Tomato production is rife with pest attacks which has caused tomato growers to be dependent on insecticides for control. Whiteflies are one of the key pests in the tomato causing damage through feeding and transmission of viruses that cause disease (Riley & Srinivasan, 2019). Building new management systems containing plants bred with heightened plant defensive traits is identified as one approach to improving pest control. For example, wild tomato varieties are found to be more resistant to herbivore attack than the cultivated varieties, which was linked to the high density of glandular trichomes and associated acylsugar production (Liedl et al., 1995). Cultivated tomato plants often produce insignificant amounts of secondary metabolites and have low trichome densities. Trichomes in wild tomato plants act as a physical hindrance and additionally produced secondary metabolite acylsugars that were not favored by arthropod pests. Thus, breeding programs focused on the introgression of arthropod-resistant traits from wild relative to cultivated tomato cultivars to produce tomato plants with augmented defensive properties against various tomato pests including whiteflies (Liedl et al., 1995; Lawson et al., 1997; Mutschler, 2021). However, the tomato plants bred to resist arthropods are not devoid of trade-offs. Experimental tomato lines have not quite tapped into the aspects of horticultural attributes. The experimental lines have poor seed germination rates, heightened vegetative growth (small-bush type), poor fruit set, fruit size, off-flavor fruit and poor overall yield (Mutschler, 2021) which is a requirement for the marketability of the product at a commercial scale. Studies conducted with experimental tomato lines do not highlight this aspect of the tradeoff which is a huge barrier to commercializing these experimental tomato lines. Hence, considerable progress in the development of acylsugar lines is still required before they can reach the grower’s field.

Our study detailed in Chapter 2 focuses on understanding how the tomato lines bred with enhanced trichome density and acylsugar content interact with the pest whitefly in impacting its abundance. We compared the performance of the experimental tomato lines with enhanced acylsugar content to the commonly cultivated tomato cultivars. In all our experiments, whiteflies population was higher on commercial cultivars indicating that whiteflies are repelled by the enhanced traits in the experimental tomato plants. The study detailed in Chapter 3 focuses on understanding the preference of whiteflies to acylsugar tomato lines differing in levels of defensive traits they exhibit. We also used commercial tomato cultivar to compare the preference to the tomato lines displayed by whiteflies. We conducted an open field experiment, laboratory choice test and greenhouse no-choice test. Our results show that when given a choice, whiteflies prefer non-acylsugar tomato lines indicated by high whitefly abundance on non-acylsugar lines compared to acylsugar lines.

The use of natural enemies is another important facet to utilizing the elements of the multi-trophic system for efficient pest control. Various natural enemies and predators are reported to successfully reduce whitefly populations. Commercially, various biological control agents of whiteflies are available in the market and reported to successfully reduce pests (Greenberg et al., 2002; Heinz & Zalom, 1996; Téllez et al., 2020). However, biological control agents will interact with the host plants and defense traits may interfere with or negatively affect the fitness and survival of biological control agents decreasing, which would decrease their efficiency (Ode, 2006; Orre et al., 2010). Trichomes in tomato plants are non-selective, and often associated with incompatibility with several predators/natural enemies (Riddick & Simmons, 2014; Simmons & Gurr, 2005; Kennedy, 2003). We encountered the biocontrol-host plant incompatibility issue in our study as well. In one of the experiments (Chapter 1), we incorporated the predatory mite, *Ambylseius swirskii*, as a biological control agent for whiteflies. *A. swirskii* is a successful whitefly biological control agent and commercially available (Calvo et al., 2015). However, the *A. swirskii* established poorly on the tomato plants as evident by the few numbers of mites recovered from intensive and repeated sampling in the field. Our attempt to augment their population by providing alternative food sources also did not help with *A. swirskii* establishment. Hence, we conclude that tomato plants do not provide a favorable environment for the predatory mite's establishment.

Plant fitness is a crucial component of plant-arthropod interaction (Price et al., 1980). Integrating pest management strategies with major consideration to plant traits and their interaction with the arthropod pests and predators is an area of research that needs more concentrated efforts. Most studies considering trophic interaction components are conducted in a controlled environment setting which is not always translated in an open field setting on a large scale. Here we conducted an open field study and sought to support the results with greenhouse and lab experiments. Considering the tomato plants, we used in the experiments are experimental, their performance on a commercial scale is still uncertain. However, our study added tounderstanding the performance of the experimental tomato lines with enhanced defense traits in relation to a global pest. Experimental acylsugar lines definitely provides a basis of restructuring our integrated pest management system considering current commercial lines with disease resistance are frequently competing with the evolving pathogens. Research efforts in breeding tomato plants is continuously progressing. Along with breeding for arthropod-resistance future studies should also focus on troubleshooting tradeoffs of the acylsugar tomato lines relating to the horticultural attributes so that growers too can benefit from using the lines.

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