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Breeding for Tomato Resistance to Spider Mite *Tetranychus urticae* Koch
(Acari: Tetranychidae)

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DISSERTATION

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A dissertation submitted in partial fulfillment of the
requirements for the degree of Doctor of Philosophy in the
College of Agriculture, Food and Environment
at the University of Kentucky

By

Ammar Sami AL-Bayati

Lexington, Kentucky

Co- Directors: Dr. John C. Snyder, Associate Professor of Horticulture

and   Dr. Arthur G. Hunt, Professor of Plant Physiology

Lexington, Kentucky

2019

ABSTRACT OF DISSERTATION

Breeding for Tomato Resistance to Spider Mite *Tetranychus urticae* Koch
(Acari: Tetranychidae)

Cultivated tomato plants are extremely susceptible to the two-spotted spider mite *Tetranychus urticae* Koch. Selection for pest resistance is usually a crucial step required to achieve successful genetic resistance transfer from wild into cultivated tomato genotypes. *S. habrochaites* LA2329, a wild relative of tomato, is highly resistant to arthropods. Its resistance has been attributed to the presence of a high density of type IV and type VI trichomes and abundant production of 7-epi-zingiberene, a sesquiterpene hydrocarbon. The interspecific backcross hybrids used in this research were derived from the cross between the wild relative tomato, *S. habrochaites* LA2329, and the cultivated tomato, *S. lycopersicum* 'Zaofen 2' (ZH2). This population has been directly selected for type IV trichome density and zingiberene. The arthropod resistance status of the backcross hybrids was unknown when this research was initiated. Thus, the main objective of the research was to verify the transfer of arthropod resistance from *S. habrochaites* to cultivated tomato. The effects of glandular trichome densities and leaf zingiberene contents on spider mite behavior and biology were also explored. Also, the chemical composition of the trichome secretions in the wild tomato donor is segregating for presence and abundance of sesquiterpenoids related to zingiberene. The bioactivity of these sesquiterpenoids was explored in this research.

To evaluate the relative bioactivities of zingiberene alcohol and 7-epizingiberene, extracted from glandular trichomes of *Solanum habrochaites* accession LA2329, as well as alpha-zingiberene obtained from ginger oil, these were purified by silica gel chromatography and bioassayed with two-spotted spider mites, *Tetranychus urticae* Koch (Acari: Tetranychidae) using a bean leaf disc bioassay. Zingiberene alcohol was most efficacious and alpha-zingiberene, was least efficacious, while the efficacy of 7-epizingiberene was intermediate. Thus, tomato breeders should consider introgression of the genes responsible for the oxidation of 7-epizingiberene into zingiberene alcohol to potentially improve the spider mite resistance of cultivated tomato. Also, it is possible that this compound may be exploited as eco-biopesticide approach for integrated pest management against a broad spectrum of herbivorous pests.

To verify transfer of arthropod resistance, a bioassay utilizing whole leaves was employed. Nine hybrids (BC_3F_3 and BC_3F_4) were chosen for this bioassay, based on variation of type IV trichome density and zingiberene concentration among the hybrids. The experiment also included three susceptible and three resistant control plants. Mite responses on some of the hybrids were similar to those on the resistant wild donor parent, *S. habrochaites,* as indicated by number of leaflet surfaces infested by mites, degree of mite webbing and feeding damage. Egg density on four backcross hybrids was

similar to that on the *S. habrochaites* resistant controls. Based these results, we concluded that resistance had been successfully transferred from the wild accessions to the hybrids by deployment of backcrossing and indirect selection. There was a significant negative correlation of almost all mite behavioral and biological responses with Type IV trichome density and zingiberene content. This bioassay illuminated behavioral variations of mites associated with presence or absence of leaf compounds and glandular trichome densities. Also, the results support the idea that introgression of type IV trichomes and zingiberene has led to effective spider mite resistance.

In another bioassay-based experiment to verify transfer of resistance, seven interspecific backcross hybrids (BC$_3$F$_2$), the resistant parent LA2329, and two susceptible cultivated tomato lines, the recurrent parent ZH2 and 'Small Roma', were used in thumbtack bioassays. Mite movement was measured by imaging bioassayed leaves at 15, 20, 30, 45, and 60 min intervals. In addition to confirming transfer of spider mite resistance, other objectives included determination of the relative contributions of type IV and VI trichome densities and leaf compounds to mite behavior over time intervals. Our findings confirmed the transfer of mite repellency from the wild resistant parent to advanced backcross hybrids. Several backcross hybrids performed similarly to the wild donor parent, displaying shorter distances traveled on the leaves after 15 and 30 min. The type IV and type VI trichome densities as well as zingiberene contents had a significant positive correlation with the number of spider mites remaining on tack. There was a significant negative correlation of type IV density and zingiberene concentration with the total distance travelled by mites for both the abaxial and adaxial surfaces across most time intervals. Stepwise multiple regression analysis showed that the type IV trichome density was the most critical factor, and zingiberene content was a secondary factor across over most time intervals. *T. urticae* remained longer on the thumbtack heads and traveled shorter distances on the leaf surface of the wild donor parent LA2329 and the interspecific hybrids compared to *S. lycopersicum* leaves. These results indicated that introgression of genetic resistance, especially repellence, against spider mite from the wild relative into cultivated tomato varieties has been successfully achieved.

In conclusion, trichome type IV and/or zingiberene content has been successfully transferred from the wild relative into interspecific tomato hybrids, and the hybrids show significant adverse impact on spider mite behavior and/or biology in whole leaf and thumbtack bioassays. Type IV trichome density is the most crucial factor in mite deterrence while zingiberene seemed to be a second key factor across most of time durations for both surfaces. Collectively, several backcross hybrids had similar leaf characteristics to the *S. habrochaites* LA2329, also may be a potential source of resistance to other insect pests.

KEYWORDS: *Solanum habrochaites*, Tomato, Trichomes, 7-epi-Zingiberene,

Alpha-zingiberene, Arthropod resistance

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April 15, 2019

Date

Breeding for Tomato Resistance to Spider Mite *Tetranychus urticae* Koch
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April/15/2019
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DEDICATION

I dedicate my dissertation work to the souls of my parents, to the lifelong companion, my wife Hind AL-Delfi, my daughters Mawadah, Rahma, and my son Aliarridah. A special feeling of gratitude to my friends ...... whose words of encouragement supported me throughout the entire PhD program.

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CHAPTER 1. Tomato Breeding for Arthropod and Insect Resistance: General Background

1.1   Diversity of Wild Tomato Species

Tomato relatives are comprised of thirteen species, including the cultivated one *Solanum lycopersicum*. These wild relatives tend to have more or less compatibility when crossed with the cultivated lines (Peralta et al. 2008). The habitats of wild species are primarily in Peru and northern Chile, the Andes area in Ecuador, as well as the Galapagos Islands. They grow in a wide array of elevations and climates ranging from dry to rainy regions (Bergougnoux 2014). From a breeding perspective, wild relatives of tomato are precious sources for resistance genes against pest and pathogens, ecological stresses, as well as for genes that can confer higher nutritional value to the fruit (Gonçalves et al. 2007; Lucini et al. 2016). Eco-diversity has greatly contributed to the other formation of divergent tomato phenotypes (Nakazato and Housworth 2011).

Resistance to arthropod pests has been investigated in wild species including *S. habrochaites* f. *hirsutum* (Carter and Snyder 1985; Bleeker et al. 2012), *S. habrochaites* f. *glabratum* (Antonious and Snyder 2015), *S. pimpinellifolium* (Rodríguez-López et al. 2011; Rakha et al. 2016), *S. pennellii* (Liedl et al. 1995; Maciel et al. 2018). *S. cheesmaniae* (Rakha et al. 2017), *S. galapagense* (Lucatti et al. 2013), and *S. peruvianum* (Channarayappa et al. 1992). Collectively, there is a necessity to delineate the genetic basis of resistance along with compatibility between wild and cultivated tomato genotypes to allow the transmission of genes responsible for resistance. Examples of valuable genes would be those controlling presence of glandular trichomes and/or leaf

chemical secretions, also called allelochemicals (Aragão et al. 2000; Rodríguez-López et al. 2011; Lucini et al. 2016; de Oliveira et al. 2018).

1.2   Breeding Tomato for Herbivore Resistance

The tomato plant, *S. lycopersicum* (Solanaceae), is cultivated worldwide and is utilized as fresh or as processed products, with global gross production more than 182 metric tons. 75.26% of world's total production occurs in a Asia and America (FAO 2017). Tomato growers face economic challenges due to pests, e.g. herbivorous arthropods, beginning from germination until harvest. One important pest of tomato is the two-spotted spider mite (*T. urticae* Koch) (Acari: Tetranychidae). This mite is polyphagous, meaning that it attacks a wide plant-host range feeding on more than 140 different plant families (Grbic et al. 2011). In fact, cultivated tomato lines are susceptible to spider mite. Mite feeding on tomato plants causes severe damage on leaves and fruits resulting in yield loss under severe infestation levels (Meck et al. 2013).

Screening genetic resources of tomato for resistance to arthropods such as two-spotted spider mites is needed. Also needed is the ability to evaluate novel hybrids by bioassay, subjecting plants to mite infestation and then studying mite behavior to select resistant plants. Breeding for durable genetic resistance in crops is urgently required as an alternative for pesticide-based pest control. Moreover, considering the need for promising substitutes for chemical insecticide application for mite control, tomato breeding research oriented toward investigating and developing resistant varieties should be considered as a critical role for integrated management of this pest (de Oliveira et al.

2018). A number of wild tomato accessions are remarkably resistant to a wide array of herbivorous pests (Rick 1982; Guo et al. 1993; de Azevedo et al. 2003; Vosman et al. 2018), but cultivated tomatoes experience lower commercial value due to lack of plant defense mechanisms. Domestication of many crops with increasing selection for desirable characters has narrowed genetic variability (Liedl et al. 1996). It has been stated that there is more genetic variability in a single accession of wild tomato than there is all of cultivated tomato (Lindhout 2005).

Although resistance to insects and arthropods has been documented in wild tomato relatives, the presence of Dobzhansky-Muller interactions may preclude or reduce the breeder's ability to utilize these genetic resources (Dobzhansky 1937; Rieseberg and Willis 2007; Lowry et al. 2008). Other crossing barriers also exist. For example, (Liedl et al. 1996) characterized unilateral incongruity (UI) in the interspecific hybrids derived from crossing the wild tomato species *S. pennellii* X cultivated variety *S. lycopersicum* used as a model for non seed set, suggesting that (UI) is responsible for the interspecific barrier distinct from self incompatibility (SI) which is expressed as intraspecific barrier.

Because of the existence of these barriers, successful introgression of resistance genes into cultivars requires an efficient breeding method. The breeding scheme that underpins this dissertation research, a modified backcross scheme, is provided in Figure 1-1. The modified backcross scheme permits direct selection for factors known to be associated with resistance such as for high foliar zingiberene content and for presence and abundance of certain trichome types with additional emphasis on reproductive traits

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like fruit and seed set. The presumption is that direct selection for factors associated with arthropod resistance will lead to lines that are actually resistant. Studies reporting resistance to arthropods have often cited the importance of the presence and density of different trichome types, especially glandular trichomes (Carter and Snyder 1985; Weston et al. 1989; Gonçalves et al. 2006; Schilmiller et al. 2010; de Oliveira et al. 2018), suggesting that trichome characteristics may be valuable breeding tools. Also, Snyder et al. (2005) provided evidence that the spider mite-repellency present in the resistant parent, due to high levels of 2,3-dihydrofarnesoic acid, was successfully transferred to the interspecific F_2 backcross hybrids, as demonstrated by thumbtack bioassays. In other breeding work, similar bioassays on an interspecific cross, BPX-368, obtained from crossing *S. lycopersicum* × *S. habrochaites* showed that zingiberene and type IV trichome densities were negatively correlated with mite *T. evansi* deterrence measured as distance travelled by mites on the leaf surface (Maluf et al. 2001).

1.3   Diversity of Tomato Leaf Trichomes

Trichomes are hair-like appendages growing on the aerial plant epidermis. In tomato species, trichomes have been reported to play a role in arthropod resistance. Glandular trichomes on wild tomato leaves can play key anti-herbivory roles via production of chemical secretions and mechanical impairment (Kang et al. 2010). Trichomes can serve as repellent barriers to small herbivores due to allelochemical secretions (Guo et al. 1993; Snyder et al. 2005; Bergau et al. 2015). They can also physically hamper insect movement on the leaf surface , due to trichome length and

density (Baur et al. 1991; Aragão et al. 2000; Simmons and Gurr 2005). Basically, there are two major forms of trichomes: glandular and non-glandular. Trichomes in wild tomato relatives were first documented by Luckwill (1943) who described seven types of trichomes. Subsequently eight distinct types were described by Channarayappa et al. (1992), based on trichome shape and size. Leaves of *S. lycopersicum* genotypes tend to have copious type III and V trichomes whilst *S. habrochaites* accessions tend to have abundant type IV trichomes, few type III, and a lack of type V trichomes. The type VI trichomes are ubiquitous glandular trichomes in tomato and have been studied extensively in the genus *Solanum* (Bergau et al. 2015). Therefore, the existence of specific types of trichomes can differentiate wild species *S. habrochaites* from the cultivated one, *S. lycopersicum* (Snyder and Carter 1985).

QTLs associated with tomato trichomes have been studied by Andrade et al. (2018) who identified two QTLs, located on chromosome 2 (gal.IV-2) and on chromosome 3 (gal.IV-3), associated with presence and abundance of type IV trichome in an F2 population derived from the interspecific cross of *S. lycopersicum* TOM-684 X S. *galapagense* LA1401. These two QTLs are responsible for the formation of the glandular head and density of type IV trichomes. High density of type IV trichomes in this interspecific tomato population was associated with whitefly (*Bemisia tabaci* biotype B) resistance. Recovered resistance was similar to that present in the wild *S. galapagense* LA1401 (Andrade et al. 2017). Also, resistance to *T. urticae* was correlated with the density of type IV trichomes in *S. pimpinellifolium* accession 'TO-937' and in the BC_1 hybrids generated by crossing between 'TO-937' and *S. lycopersicum* 'Moneymaker'

(Fernández-Muñoz et al. 2003). Therefore, introgression of type IV trichomes into cultivated tomato may improve tomato resistance to arthropod pests.

1.4   Tomato Foliar Allelochemical Secretions and Pest Resistance

Studies of tomato–arthropod interactions have emphasized the role of allelochemical content associated with glandular trichomes. Glandular trichomes are often secretive for secondary metabolites and these can often act as substantial weapons that impact herbivore nutrition and growth progress (Baldwin et al. 2001; Vandenborre et al. 2010; Bleeker et al. 2011; Huang et al. 2018). These secondary metabolites (e.g. terpenoids, phenylpropanoids, flavonoids, and methyl ketones) are critical components of repellency or toxicity to herbivores (Simmons and Gurr 2005; Kortbeek et al. 2016). Tomato species that exude different main chemical compositions are called chemotypes (Lundgren et al. 1985). Hence, different accessions belonging to the wild species *S. pennellii* have different acylsugar chemotypes. One class of chemotypes produce primarily acylglucoses and the other class produces a mixture of acylglucoses and acylsucroses (Leckie et al. 2014). Similarly, different chemotypes of *S. habrochaites* exude different volatiles via glandular trichomes, e.g. LA2329 exuding mainly sesquiterpene hydrocarbons, but LA407 secreting methyl ketone volatiles (Guo et al. 1993).

Terpenoids such as sesquiterpenes and derivatives are one of the major components of secretions in tomato glandular trichomes, and are a highly diverse class of plant secondary metabolites that can perform numerous biological functions (Bleeker et al. 2012). Zingiberene, a monocyclic sesquiterpene hydrocarbon consisting of three

isoprene units, is mostly stored and released by type IV and/or VI glandular trichomes in accessions of *S. habrochaites* f. *hirsutum*. Zingiberene confers potent resistance to spider mites (Freitas et al. 2002; Gonçalves et al. 2006; Bleeker et al. 2012). Also, zingiberene has been reported as having bio-pesticidal activity on Colorado Potato Beetle (Carter et al. 1989; Gianfagna et al. 1992), spider mites (Weston et al. 1989; Gonçalves et al. 2006), white flies (Neiva et al. 2013), and tomato pinworm (de Azevedo et al. 2003; Lima et al. 2015). The wild tomato accession LA2329, *S. habrochaites,* used in this research is rich in 7-epizingiberene (Snyder, personal communication). It is noteworthy that a novel sesquiterpenoid, zingiberene alcohol, has been discovered in *S. habrochaites* (EP Patent No. 3178313A1 2017). However, its effects have not been studied before on the two-spotted spider mites or other arthropods. Characterizing and discovering additional phytochemicals that may confer insect resistance in some wild crop relatives could allow for breeding cultivars with pest resistance. Leaves of *S. habrochaites* that produce the anti-arthropod sesquiterpenoid compound known as zingiberene alcohol, may provide a source for natural acaricidal/insecticidal agents instead of synthetic pesticides. This foliar extract may be a component of integrated pest management and have activity against a broad spectrum of herbivorous pests.

1.5   Spider Mite as a Pest and a Model

The two-spotted spider mite *Tetranychus urticae* Koch belongs to the phylum Arthropoda and family Tetranychidae. It is known as a poly-phytophagous, devastating pest worldwide responsible for causing considerable damage on tomato leaves and fruits.

Damage includes slow growth, leaf wilting, yield loss and low fruit quality particularly under favorable conditions for infestation (Pokle and Shukla 2015). This mesophyll-feeding mite (Egas et al. 2003), invades a wide range of host plant species, feeding on 1200 plant species belonging to more than 140 different plant families (Grbic et al. 2011). Feeding damage can be observed after several days of heavy infestation as necrotic spots on leaf surfaces, due to chlorophyll sucking (Meck et al. 2009) and gold flecking on tomato fruits (Meck et al. 2012). Oku et al. (2009) reported that *T. urticae* webbing (Figure 1-2) previously formed by conspecifics on leaf surfaces can promote oviposition rate of other females that visit later the same leaf patch. In addition, this arthropod pest is known to rapidly evolve pesticide resistance at a high rate, one that is amongst the highest for arthropods (Whalon et al. 2016). Mites can prevail under field and greenhouse conditions by virtue of several biological mechanisms, including rapid development during its life cycle, high fecundity of up to 25 generations per year, haplodiploid sex determination (Van Leeuwen et al. 2010) and high adaptability of mate competition (Macke et al. 2011). Female spider mites have short developmental times (10 to 14 days) from egg to adult stage depending on environmental conditions and host plant (Hance and Van Impe 1999). Cultivated tomato varieties, *S. lycopersicum,* experience a broad array of arthropods pests, including the two-spotted spider mite, *T. urticae.*

The frequent development of resistance by *T. urticae* to synthetic pesticides, which are also associated with environmental and human health consequences, has motivated us to identify novel, appropriate replacements for plant protection with fewer adverse impacts. Additionally, understanding trichome secretive chemicals having

potentially growth-inhibiting, antifeedant, anti-ovipositional and lethal effects against

mites or insects is crucial for motivating introgression of these characters into cultivated

varieties to improve resistance.

S. lycopersicum × S. habrochaites
↓
F1
↓⊗
F2 → (selection for mite resistance)
↓
S. lycopersicum × F2 (selected)
↓
BC1F1 → (selection for mite resistance)
↓
S. lycopersicum × BC1F1 (selected)
↓
BC2F1 → (selection for self fruiting, zingiberene, type
IV trichome density)
↓
BC2F1 (selected)
↓⊗
BC2F2 → (selection for self fruiting, zingiberene, type IV
trichome density)
↓
BC2F2 (selected) × S. lycopersicum
↓
BC3F1
↓⊗
BC3F2

Figure 1–1: Modified backcross scheme used for transferring high type IV trichome density, and high concentration of 7-epizingiberene from the wild *S. habrochaites* to the cultivated tomato (Courtesy of my advisor, Dr. John Snyder).

Figure 1–2: Extreme webbing and feeding damage caused by two-spotted spider mite
T. urticae Koch.

CHAPTER 2. Mortality Fecundity of Two-spotted Spider Mites in a Bean Leaf Disk Bioassay Treated with 7-EpiZingiberene, Zingiberene Alcohol and Alpha-Zingiberene

2.1 Abstract

The isolation and characterization of novel allelochemical extracted from leaves of wild tomato relatives is important for their introgression into cultivated varieties to improve mite resistance. Zingiberene alcohol and 7-epizingiberene, present in trichome secretions of *Solanum habrochaites* LA2329, as well as alpha-zingiberene from ginger oil, were purified by silica gel chromatography and bio-assayed with two-spotted spider mites, *Tetranychus urticae* Koch (Acari: Tetranychidae). The objective was to compare the relative efficacies of these sesquiterpenoids for their ability to cause mite mortality and to reduce fecundity (number of eggs per female mite). Based on results using a bean leaf disc bioassay, zingiberene alcohol was most efficacious, alpha-zingiberen isolated from ginger oil was least efficacious, and the efficacy of 7-epizingiberene was intermediate. The highest concentration tested of zingiberene alcohol caused complete mortality of spider mites; 7-epizingiberene and alpha-zingiberene tested at similar concentrations did not cause complete mortality. The results support the idea that tomato breeders should consider introgression of the genes responsible for the oxidation of 7-epizingiberene from wild to cultivated tomato. Furthermore, evaluation of other unidentified volatiles present in trichome secretions of *S. habrochaites* LA2329 could lead to the identification of compounds with higher efficacy to control insects.

2.2   Introduction

Tomato, *Solanum lycopersicum* L., is grown worldwide in gardens as well as an agricultural commodity providing an important source of vitamins and nutrients (Labate et al 2007). Cultivated tomato plants are susceptible to a wide range of arthropod pests (Kennedy 2003) which requires the use of chemical sprays to protect the crop. One of these arthropod pests is the two-spotted spider mite (*T. urticae* Koch) (Acari: Tetranychidae) which attacks 1200 plant species belonging to more than 140 different plant families (Grbic et al. 2011). Whalon et al. (2016) found that *T. urticae* has one of the highest rates of pesticide resistance amongst arthropods and also rapidly evolves pesticide resistance under field and greenhouse conditions (Van Leeuwen et al. 2010). This frequent development of *T. urticae* resistance to active components of synthetic pesticides, and the cumulative environmental and health-related consequences associated with the utilization of synthetic pesticides provide the rationales behind current research for novel, appropriate approaches to plant protection having fewer adverse impacts.

Resistance to arthropods has been explored in some wild tomato relatives including *S. habrochaites* (Carter and Snyder 1985; Antonious and Snyder 2008; Bleeker et al. 2012), *S. pennellii* (Liedl et al. 1995; Maciel et al. 2018), and *S. pimpinellifolium* (Alba et al. 2009). The resistance in these wild species has been associated with the presence of certain leaf glandular trichomes (Carter and Snyder 1985; Bleeker et al. 2012), which domesticated tomato lacks. Glandular trichomes secrete secondary metabolites that often play roles as natural defensive systems against pests (Antonious and Snyder 2006;

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Bleeker et al. 2011; Huang et al. 2018). Secondary metabolites have been shown to impact herbivore nutrition and growth progress (Baldwin et al. 2001; Vandenborre et al. 2010). For example, alkaloids, glucosinolates, terpenes, phenolics, and polyphenol oxidases are secreted from glandular trichomes from various plant species such as wild relatives of tomato (Antonious and Snyder 2006; Schilmiller et al. 2010), *Artemisia annua* (Tan et al. 2015), sweet basil (Maria et al. 2016), Arabidopsis (Barczak-Brzyzek et al. 2017), and tobacco (Huang et al. 2018).

Terpenes are particularly diverse in tomato trichomes. The bioactivity of tomato terpene synthases (TPS) were identified in vitro as enzymatic functions (Falara et al. 2011). They reported additional clades found in tomato of functional TPS genes belong mainly to TPS-a clade but partially to TPS-b clade genes, which both are responsible for encoding only sesquiterpene synthases. They suggested if the TPS genes clustering of 2 to 5 genes located in close proximity with genes encoding enzymes such as genes encoding putative cytochrome P450 proteins, then these could possibly modify terpenes. Thus, characterizing and quantifying modified or oxidized sesquiterpene hydrocarbons, e.g. 7-epizingiberene alcohol, in *Solanum* species is of interest to test the biochemical activities against arthropods would be useful towards breeding resistant into cultivated tomato.

Understanding the bioactivity of chemicals secreted from trichomes such as their growth inhibition, antifeedant, anti-ovipositional and toxic or lethal effects against insects is a fundamental aspect of understanding host resistance. Terpenoids such as sesquiterpenes and their derivatives are one of the major components secreted by glandular trichomes of tomato and are a highly diverse class of plant secondary

metabolites with numerous biological activities (Bleeker et al. 2012). The sesquiterpene hydrocarbon, zingiberene, that is present in extracts of wild tomato *S. habrochaites* has efficacy as a pesticide against Colorado Potato Beetle (Carter et al. 1989), spider mites (Weston et al. 1989; Gonçalves et al. 2006), and white flies (Freitas et al. 2002; Bleeker et al. 2012).

Breeden and Coates (1994) extracted 7-epizingiberene from the leaves of two wild tomato accessions *S. habrochaites* f. *hirsutum* PI 365906 and *S. habrochaites* f. *glabratum* PI 199381 then purified it by chromatography. They reported that 7-epizingiberene is a diastereoisomer of alpha-zingiberene present in ginger oil (*Zingiber officinalis)*. They also proposed that the presence of this sesquiterpene could be a useful taxonomic character of certain *Solanum* accessions. The stereoisomers alpha-zingiberene and 7-epizingiberene differ in their configurations of the hydrogen and methyl group at carbon 7 (Figure 2-1). Bleeker et al. (2011) isolated and purified 7-epizingiberene from *S. habrochaites* PI127826 and also isolated and purified alpha-zingiberene from ginger oil. They reported that the tomato zingiberene, 7-epizingiberene, acted as a repellent to whiteflies, *Bemisia tabaci*, while the stereoisomer, alpha-zingiberene from ginger oil, had no effect. The *S. habrochaites* accession, LA2329, used in this research is rich in 7-epizingiberene (John Snyder, personal communication). This *S. habrochaites* accession has been used as a donor parent to transfer spider mite resistance into cultivated tomato. Several years ago, our lab identified two distinct segregating chemotypes of LA2329 based on glandular trichome extracts of leaves detected by gas chromatography. These chemotypes have been maintained using selfing or sib-mating within chemotype for our

breeding projects. One chemotype exudes one major terpene, 7-epizingiberene, while the other chemotype produces two 7-epizingiberene derivatives. Recently, we also found similar terpenoid components in another *S. habrochaites* accession PI127826.

This research investigated the effect of isolated and purified sesquiterpenes on the two spotted spider mites and addresses this question: can alpha zingiberene, 7-epizingiberene, and zingiberene alcohol in a leaf disc bioassay exhibit distinct effects on spider mite

2.3   Materials and Methods

2.3.1   Plant Materials:

Seeds of *Solanum habrochaites* S Knapp and DM Spooner, formerly named *Lycopersicon hirsutum* f. *typicum* LA2329 were obtained from the Tomato Genetics Resource Center (TGRC) at Davis, California, USA and seeds of the other accession, PI127826 were obtained from the USDA, ARS Plant Genetic Resources Unit at Geneva, NY. Seeds were germinated on moistened filter paper in a 9 cm glass petri dish kept in an incubator in the dark at 28 C. After germination, seedlings were transplanted into 72-cell plastic trays filled with growing medium (Pro Mix BX, Premier Horticulture Inc., Quakertown, PA, USA) and were maintained in the laboratory at the Horticulture Department, University of Kentucky, Lexington, KY with continuous fluorescent lighting. Once seedlings had six leaves, they were transplanted into 20 cm diameter plastic pots filled with Pro Mix BX and set on greenhouse benches, spaced in 30 cm apart. Greenhouse conditions for plant growth included natural light, day temperatures of 25C and night temperatures of 20C. Plants were irrigated as necessary using a fertigation system

consisting of two 1:100 injectors. One injector was supplied with a stock solution of 97 g/L of Ultrasol® Hydroponic Plus 3-15-28 fertilizer (SQM North America, Atlanta, GA). The other injector was supplied with a stock solution of 118 g/L of greenhouse grade CaNO3 (Yara North America, Tampa, FL).

Bean plants (*Phaseolus vulgaris* 'Dwarf Horticultural') were grown and maintained in the lab of the Horticulture Department at University of Kentucky. Growth conditions were constant light from cool white florescent lights and a temperature of 23 ± 2C. These plants were used for producing mites and leaf discs for bioassays described later.

2.3.2   Maintenance of Mite Colony:

Two-spotted spider mite, *Tetranychus urticae* Koch, were reared on the bean plants as described by Weston and Snyder (1990). Weekly, non-infested plants having 3-4 trifoliate leaves were inoculated by transferring several infested bean leaves to non-infested plants. Mite reproduction on the newly infested plants was checked after two days and after 5 – 6 days, adult female mites were used for the leaf disk bioassay described later.

2.3.3   Gas Chromatographic Analysis:

A gas chromatograph equipped with a flame ionization detector (GC-FID) (Hewlett Packard 5890 Series II) was used for quantitation of sesquiterpenoids. The column was an RTX-5 (5% diphenyl/95% dimethyl polysiloxane, 15 m, 0.53-mmID, 0.5 µm d_f (Restek Corporation, Bellefonte, PA, USA). Helium gas was used as a carrier was flowing at 16 ml/min. Temperatures were as follows: injector 250°C, detector 300°C, oven initial temperature 50°C for 1 min, then increasing at 20°C/min to 260°C.

2.3.4 Validation of Ethanol as a Solvent for Zingiberene:

Ten and 100 µL of ginger oil (Berje, Bloomfield, NJ, USA) were dissolved individually in 1.0 ml hexane or in 1.0 ml 100% pure ethanol in triplicate and then analyzed by GC-FID. Mean concentrations of zingiberene in each solution were calculated and then the concentrations in ethanol were calculated as a percentage of the concentration in hexane. A preliminary experiment used the two accessions of wild tomato, *S. habrochaites* LA2329 and PI127826, to verify the solubility of sesquiterpenoids in ethanol versus hexane. Three leaflets of each accession were collected from the third leaf from the apex to make leaf hexane extract (2mL) which was then sub divided into two samples. One of these was used as a control. For the other sample, hexane was evaporated by use of a nitrogen gas stream and the residue was re-dissolved in 100% pure ethanol. All leaflet samples were prepared in triplicate and were analyzed by GC-FID to quantify the major sesquiterpenoid components, 7-epi-zingiberene, zingiberene alcohol, and zingiberene epoxide.

2.3.5 Silica Gel Column Preparation for Column Chromatography:

A ball of glass wool was inserted into the bottom of the glass column to serve as a plug and then about one gram of sand was poured with the aid of a scoopula into the column to provide a flat surface. A slurry packing method was carried out using silica gel (230 – 400 mesh SiO_2, FW 60.08, Natland International Corporation) (Khrimian et al. 2014) by mixing it with hexane and pouring into the glass column (20 cm long x 0.8 cm diameter). Afterwards, the upper interior column wall was rinsed and then the silica gel was left for 5 minutes to allow the silica gel layer to settle. Bed length was then measured.

About a half gram of sand was added to the top of the column to avoid the disturbance while running the sample.

2.3.6 Isolation and Purification of Alpha-zingiberene from Ginger Oil (*Zingiber officinale* L.):

A sufficient number of tubes (12 x 75mm) were labeled for collecting fractions. Preliminary trials helped optimize conditions for separation and yield. To allow isolation of sufficient quantities of purified alpha-zingiberene, 200 µL (~172 mg) of ginger oil (Berje, Bloomfield, NJ, USA) was applied to the top of the column. The column was then eluted with hexane:methyl tert-butyl ether (MTBE). The initial ratio of hexane:MTBE was 100:0. Fractions (0.5 mL) were collected and evaluated for the presence or absence of uv-light absorbance. This was done by placing a drop from each fraction onto a thin layer chromatography plate (TLC) (Silica gel 60 A with fluorescent indicator, Whatman Int Ltd, England) which was then illuminated with uv-light (254-nm UVGL 25Mineralight Lamp, UVP Inc, CA USA). Once a uv positive fraction was detected on a TLC plate, the eluant concentration was changed, first to 99:1, and subsequently to 98:2, and 97:3 with elution volume of ~10mL for each ratio. Subsequently 1 µL of each fraction that contained *uv*-absorbing material was manually injected into the gas chromatograph (GC-FID) to confirm the presence of and to quantify alpha-zingiberene as well as other compounds. The fraction chosen for use in the bioassays were stored in the freezer (-20°C) until used for bioassay.

2.3.7 Isolation and Purification of Zingiberene and Zingiberene Alcohol from

S. habrochaites LA2329:

To obtain this extract, leaflets were collected in March 2018 from S. habrochaites LA2329 maintained in the greenhouse. After collection, leaflets were then steeped in hexane. The next day, the leaflets were removed, and the extract was left under hood until most of the hexane evaporated. The concentrated extract was transferred to a 20-ml vial, and the remaining hexane was removed with the aid of a gentle stream of N2. This very concentrated extract was then stored in a -20C freezer.

After warming the concentrated extract, 200 μL (~176 mg) was added to top of the silica gel column. The column was then eluted initially with 99:1 hexane:MTBE. Elution was monitored in a similar fashion to that described for the separation of ginger oil. Fraction volume was about 1.0 mL collected in small tube. The ratio of hexane:MTBE in the eluent was sequentially changed to 95:5, 93:7, 91:9, 89:11, 87:13, and 85:15 based on the pattern of uv-positive spots observed on the TLC plate. Then 1 μL of each uv-positive fraction was manually injected into the GC to quantify and identify 7-epi-zingiberene, zingiberene alcohol, and other compounds as well. The fractions were stored in the -20C freezer until used for bioassay. In addition, the separation process for 7-epi-zingiberene and zingiberene alcohol collected from wild tomato accession LA2329 extract was validated by repetition, three times.

2.3.8 Preparation of Ethanol Solutions for Bioassays:

To prepare ethanol solutions of sesquiterpenoids fractionated by silica gel chromatography for use in the bioassay, fractions containing alpha-zingiberene, 7-epi-zingiberene, or zingiberene alcohol were taken from the -20°C freezer. The residue of each fraction was dissolved in 1.0 ml of 100% ethanol (Decon Lab Company, King of Prussia, PA) to prepare a stock solution for each fraction. Serial dilutions were then prepared to create 0.1X and 0.01X dilutions to cover concentrations in the range of $10^5 - 10^7$ GC area units of the compound of interest per μl. One μl of each dilution was then manually injected into the GC-FID to quantify alpha-zingiberene, 7-epi-zingiberene, and zingiberene alcohol as appropriate. Periodically, a known concentration of tetradecane in hexane was injected into the GC-FID to verify chromatographic performance.

2.3.9 Bean Leaf Disk Bioassay:

The bioassay consisted of spraying bean leaf discs with isolated fractions and then bioassaying the disks with spider mites. Intact bean trifoliate leaves were removed from the plant and disks were prepared by punching discs using a 3.17 cm diameter punch. Three disks were placed on a paper towel with the abaxial surface oriented up, which were then sprayed using a small sprayer (spray bottle-fine plastic mist, 2.7 oz, Juvo Plus Inc Irwindale, China) with either an ethanol solution of sesquiterpenoids isolated by silica gel chromatography or ethanol only as control. The measured average amount of ethanol solution applied on each disk was approximately 22.3 mg. The solution on the leaf disk surface was allowed evaporate under the fume hood. The treated discs were placed on a moistened filter paper (9 cm- Shanghai Haoen Chemical, China) in a 9 cm glass petri

dish. Another filter paper was attached by transparent tape onto the petri dish lid to absorb condensation that could occur during incubation. Ten lab-reared female spider mites (2-4 days in age) were gently positioned on each leaf disk using a fine-tipped brush (Antonious et al. 2014). All dishes were closed with a tight-fitting lid, sealed with parafilm, labeled and transferred into a 30C incubator in the dark. Spider mite number and viability were checked by recounting and assessing their movement just before incubation. Moistness of the lower filter paper in each petri dish was monitored with water added as needed. Additional bean leaf discs were treated with water and bioassayed as described to determine if ethanol was a confounding factor in comparison with the control samples (ethanol only).

2.3.10 Data Recorded:

Replications of each concentration were assessed on the same day to reduce replication variability. Data were recorded after 3 days of incubation. Mortality was evaluated by poking the spider mites gently with a fine-tipped-brush and observing their response with the aid of a stereo microscope. When no appendage moved, mites were considered dead. For fecundity assessment, eggs were counted with the aid of the stereo microscope on both adaxial and abaxial surfaces of the bean leaf disk and then the total number of eggs was divided by 10, the number of female mites placed on each leaf disk. An ocular microgrid installed in the stereo microscope allowed accurate counting of eggs. Data were statistically analyzed according to a one-way ANOVA using PROC GLM via SAS software version 9.4 (SAS Institute Inc. 2012). Duncan's multiple range test was

implemented for mean comparisons. Graphs were created by Microsoft® Office Excel 365 ProPlus.

2.4 Results

2.4.1 Isolation and Purification of Alpha-zingiberene of Ginger Oil and Zingiberene as well as Zingiberene Alcohol of *S. habrochaites* LA2329:

The purity of the alpha-zingiberene separated from other components in the ginger oil by silica gel chromatography was calculated from the GC-FID data. The area units/µL of alpha-zingiberene, provided in GC-FID data file, was divided by the total area units/µL of that data disregarding the injection peak. Based on purity, and concentration of alpha-zingiberene, fraction 28 (83% purity) was chosen for use in the bioassay (Figure 2-2, Table 2-1).

The purities of 7-epi-zingiberene and zingiberene alcohol were computed from the GC-FID data for each fraction as mentioned above. Fractions 6 and 45 contained 7-epi-zingiberene (92% purity) and zingiberene alcohol (73% purity), respectively, and were selected for use in the leaf disk bioassays (Table 2-3, Figure 2-3 A, B).

2.4.2 Validation of Ethanol as a Solvent:

Results from the two solutions (10 and 100 µL of ginger oil per ml of solvent) indicated that the amount of zingiberene detected in the ethanol solution compared to that in the hexane solution was 103% with standard error ± 3.04 for the 10 µL sample of ginger oil, and 95% with standard error ± 1.76 for the 100 µL sample. Therefore, it appeared that solubility of zingiberene was nearly identical between ethanol and hexane at the concentrations tested (Table 2-4). Similar results were obtained for the three

terpenoids present in extracts of wild tomatoes; relative recoveries in ethanol vs. hexane ranged from 79 to 90%, depending on the particular compound evaluated and plant source of the extract (Table 2-5). Recovery in all cases was sufficient to permit use of ethanol as the solvent for the planned bioassays.

2.4.3   Ethanol as a Non-toxic Solvent to Spider Mites:

The average number of eggs per mite in the control samples sprayed with water was 18.96 ± 1.79 and for the samples sprayed with ethanol, 17.7 ± 4.44. In addition, average percent mortality of spider mites in the control samples was 43.33 ± 8.81 and for the samples treated with ethanol, 50.00 ± 5.77 (Table 2-6). Statistically, a t-test verified that there was no significant difference between control and ethanol-treated samples for both eggs per mite ($P = 0.81$) and mortality percent ($P = 0.56$). Consequently, we concluded that ethanol can be used as a solvent for the bean leaf disk bioassay without introducing a confounding factor.

2.4.4   Mortality:

Of the three compounds tested in the leaf disk bioassay against spider mites, zingiberene alcohol was the most toxic to female spider mites when tested at the highest concentration, with 100% dead mites on the treated bean leaf disks three days after infestation (Figure 2-4). Conversely, bioassays with alpha-zingiberene from ginger oil resulted in the least mortality, 60% when tested at the highest concentration. Mortality associated with exposure to 7-epi-zingiberene was intermediate. Even at the lowest concentration of zingiberene alcohol tested, 53% of the mites died compared with only 33% mortality for alpha zingiberene at a similar concentration. These results suggested

that zingiberene alcohol and 7-epi-zingiberene had higher efficacy against spider mites compared to that for alpha-zingiberene obtained from ginger oil (Figure 2-4).

2.4.5   Fecundity:

Of the three compounds tested in the leaf disk bioassay against the spider mite, zingiberene alcohol had the greatest adverse effect on eggs per mite at all concentrations tested compared to 7-epizingiberene from wild tomato or alpha-zingiberene from ginger oil (Figure 2-5). In contrast, alpha-zingiberene isolated from ginger oil was least effective in reducing the number of eggs laid per female. 7-epi-zingiberene was intermediate in effectiveness across all concentrations (Figure 2-5). Furthermore, the number of eggs per female mite were reduced to nearly zero at the highest rate of zingiberene alcohol application (10^7). Although the greatest reduction of fecundity was realized by highest concentration of zingiberene alcohol, it may be confounded with mite mortality.

2.5   Discussion

We identified zingiberene alcohol in trichome exudates from wild tomato LA2329 and compared its toxicity against spider mites with 7-epizingiberene and alpha-zingiberene. Zingiberene alcohol was the most toxic of the three compounds tested whilst alpha zingiberene was the least effective.

Bleeker et al. (2012) reported that leaf bioassays of transgenic tomato (line 2) that produced 7-epizingiberene at 1.5% of the concentration of the wild parent had 40% higher mite mortality than that for the control genotype S. lycopersicum cv. Moneymaker after 4 days incubation. Other studies have reported that 7-epizingiberene was not only

toxic to spider mites, but also had pesticidal potential for white flies, tomato pinworm, beet armyworm and Colorado potato beetle; Freitas et al. (2002), de Azevedo et al. (2003), Eigenbrode et al. (1994), and Carter et al. (1989) respectively. Spider mite mortality caused by zingiberene alcohol could have resulted from ingestion by the mites, or by vapor toxicity. These potential effects should be tested at lower concentrations to assess the threshold impact on mite survival and to determine LC50 values, the concentration that kills 50% of the population. The other compound tested from wild tomato, 7-epi-zingiberene, had a higher percent mite mortality and anti-fecundity than the alpha-zingiberene isolated from ginger oil. To the best of our knowledge, based on the literature, this is the first report on the acaricidal properties of zingiberene alcohol.

Because zingiberene alcohol appears to have greater toxic effects on spider mites, plant breeders should make an effort to introgress this compound into tomato. This could lead to tomato plants that produce zingiberene alcohol and these would, perhaps, be more resistant to arthropods, compared to plants producing zingiberene alone. It is likely that a cytochrome P450 terpene oxidase is responsible for the conversion of 7-epi-zingiberene into zingiberene alcohol (EP Patent No. 3178313A1 2017). Therefore, tomato hybrids that possess leaf glandular trichomes secreting zingiberene, such as those reported on in other chapters of this dissertation, could produce and secrete zingiberene derivatives after introgression of the cytochrome P450, terpene oxidase from wild parent to the hybrid. A GMO approach to such a plant has been patented (EP Patent No. 3178313A1 2017). Because introgression of the genes responsible for high levels of zingiberene production have been successfully introgressed from wild to

cultivated tomato (Snyder, personal communication), the concept that introgression of the oxidase might also be successful, permitting a non-GMO path toward greater arthropod resistance in tomato could also be successful.

Reducing insect and spider mite fecundity and preventing attendant leaf and fruit damage caused by herbivores is crucial in crop protection. Bleeker et al. (2012) reported that egg production of T. urticae and T. evansi was reduced after 4 d by 81% and 54% respectively, compared to control plants, on the transgenic line 2 that was expressing low levels of 7-epizingiberen. Foliar 7-epizingiberene in tomato plants is known to be toxic as well as to have an adverse impact on whitefly oviposition and feeding (Muigai et al. 2002; Freitas et al. 2002), while foliar application of ginger oil containing mostly alpha-zingiberene had weak repellent effect to the whitefly Bemisia tabaci biotype B adults at distance < 1 mm from the odor source (Zhang et al. 2004). In this study, the reduction of number of eggs per mite was an indirect effect with mortality as the primary mite response.

2.6 Conclusion

This study was designed to understand the relative efficacy of zingiberene alcohol and 7-epizingiberene extracted from wild tomato leaves 'LA2329OH', and alpha zingiberene extracted from ginger oil for mite mortality and fecundity. We performed a leaf disk bioassay of these compounds isolated by silica gel chromatography which allowed measurement of mite mortality and fecundity. Testing was limited to a few concentrations, and so this should be kept in mind when considering future research or

deployment of these results. Surprisingly, alpha-zingiberene was less effective in our bioassays than was 7-epizingiberene, indicating that stereochemistry of natural products may be an extremely important aspect of their pesticidal characteristics. Also, we found that zingiberene alcohol had greater activity for mite mortality compared to 7-epizingiberene and, zingiberene alcohol had greater anti-fecundity effects than those of 7-epizingiberene at the lowest concentrations tested. These observations support the idea that plant breeders should consider introgression of the presence of zingiberene alcohol from wild to cultivated tomato. Doing so may lead to tomato lines having greater arthropod resistance, compared to those producing only 7-epizingiberene.

Table 2–1: GC-FID area units/μL and purity (%) of alpha-zingiberene in selected fractions collected from silica gel chromatography of ginger oil.

Fraction	Alpha-zingiberene concentration (GC-FID area units/ μL)	Purity (%)
26	1.4×10^7	74
27	8.5×10^6	79
28	1.1×10^7	83
29	5.3×10^6	84

Table 2–2: Eluant concentrations of hexane:MTBE and their elution volume (mL) for serial fractions collected from wild tomato accession LA2329 extract separated by silica gel chromatography.

Eluant concentration (hexane:MTBE)	Fraction Number	Total elution volume (mL)
99:1	1-4	~ 6
95:5	5-15	~ 13
93:7	16-25	~ 12
91:9	26-35	~ 12
89:11	36-46	~ 13
87:13	47-54	~ 10
85:15	55-62	~ 10

Table 2–3: GC-FID area units/µL and purity (%) of 7-epizingiberene and zingiberene alcohol in selected fractions collected from silica gel chromatography of wild tomato accession LA2329 extract.

Fraction	Chemical ID	Concentration (GC/FID Area unit/ µL)	Purity (%)
5	7-epi-zingiberene	2.2×10^7	84
6	7-epi-zingiberene	6.7×10^7	92
7	7-epi-zingiberene	4.9×10^7	79
32	Zingiberene alcohol	1.9×10^7	62
33	Zingiberene alcohol	1.5×10^7	64
35	Zingiberene alcohol	1.4×10^7	62
36	Zingiberene alcohol	1.4×10^7	64
37	Zingiberene alcohol	1.2×10^7	62
38	Zingiberene alcohol	1.1×10^7	62
40	Zingiberene alcohol	8.2×10^6	66
41	Zingiberene alcohol	8.1×10^6	66
43	Zingiberene alcohol	5.3×10^6	64
45	Zingiberene alcohol	1.4×10^7	73

Table 2–4: Concentrations (GC area units/μL) of alpha-zingiberene detected in ethanol and hexane solutions of ginger oil prepared at 10 or 100 μl per mL and relative recovery of in ethanol compared to hexane determined by GC-FID. SE refers to standard error.

	Solvent				
	Ethanol	Hexane	Relative Recovery		
Ginger Oil Concentration	**GC Area Units/μL**		**%**	**±**	**SE**
10 μL/ml	5.7×10^6	5.6×10^6	103	±	3.04
100 μL/ml	3.9×10^7	4.1×10^7	95	±	1.76

Table 2–5: Concentrations of three sesquiterpenoids, zingiberene, zingiberene alcohol, and zingiberene epoxide in ethanol and hexane solutions of oleoresins obtained from two accessions of *S. habrochaites*, LA2329 and PI127826 as determined by GC-FID. Relative recovery is the amount of the indicated compound present in the ethanol extract compared to that in the hexane extract, expressed as a percentage. SE refers to standard error.

| | | Solvent | | Relative Recovery | | |
| | | Ethanol | Hexane | | | |
Plant source	Sesquiterpenoid	GC Area Units/μL		%	±	SE
LA2329	Zingiberene	2.2×10^5	2.5×10^5	85	±	0.91
	Zingiberene alcohol	1.7×10^5	1.8×10^5	90	±	1.13
PI127826	Zingiberene	1.1×10^5	1.5×10^5	79	±	5.29
	Zingiberene alcohol	9.6×10^4	1.1×10^5	84	±	5.44
	Zingiberene epoxide	8.9×10^4	1.1×10^5	79	±	3.99

Table 2–6: Fecundity and mortality percent of spider mites using the bean leaf disk bioassay with water and ethanol samples. SE refers to standard error.

Control	Eggs Per Mite	±	SE	Mortality (%)	±	SE
Water	18.96	±	1.79	43.33	±	8.81
100% Ethanol	17.73	±	4.44	50.00	±	5.77
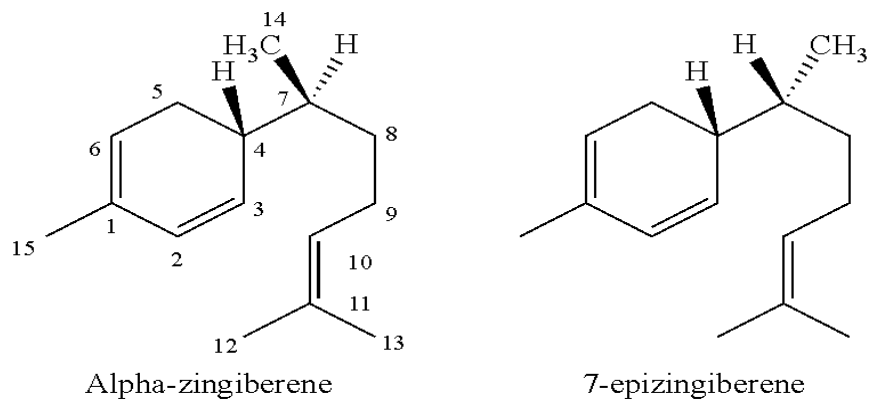
Figure 2–1*:* Stereochemical configuration of alpha-zingiberene isolated from ginger oil and 7-epizingiberene isolated from two wild tomato accessions *S. habrochaites* f. *hirsutum* PI 365906 and *S. habrochaites* f. *glabratum* PI 199381 (Breeden and Coates 1994).
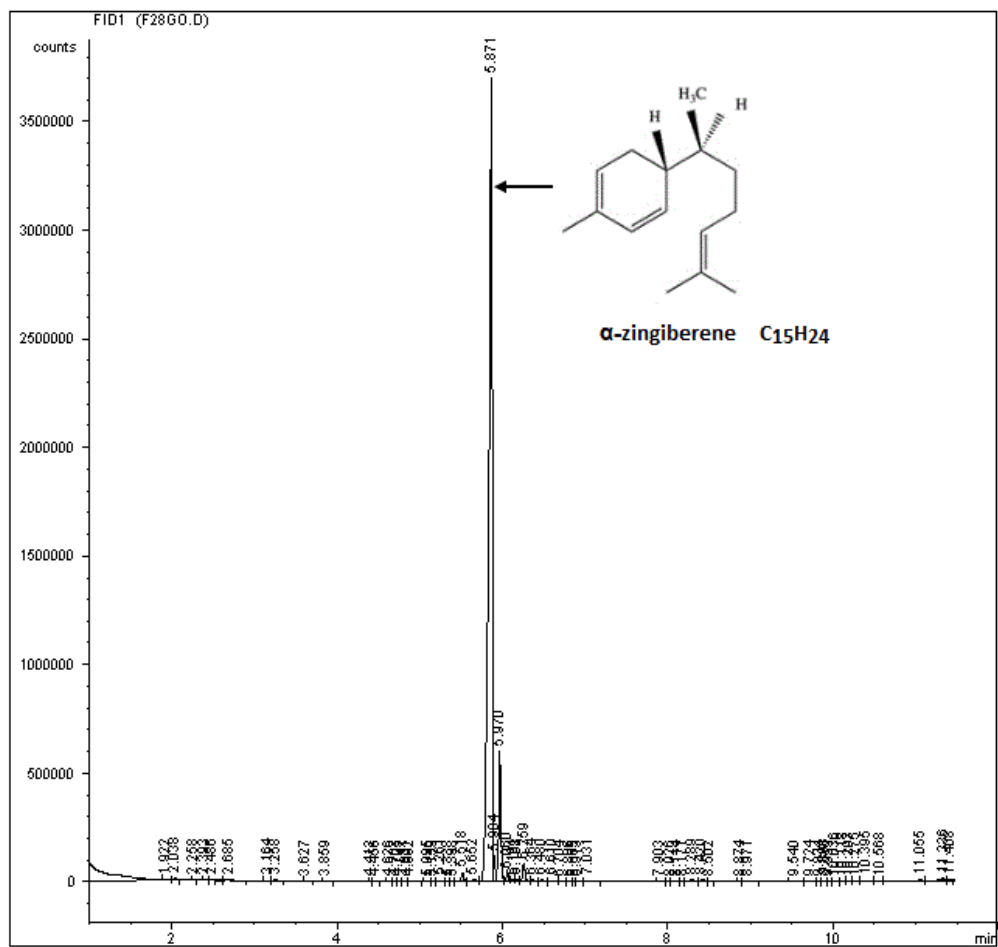
Figure 2–2: GC-FID chromatogram of fraction 28 of ginger oil separated on silica gel demonstrating purity and quantity of alpha-zingiberene. Chemical structure of alpha-zingiberene obtained from Bleeker et al. (2011).
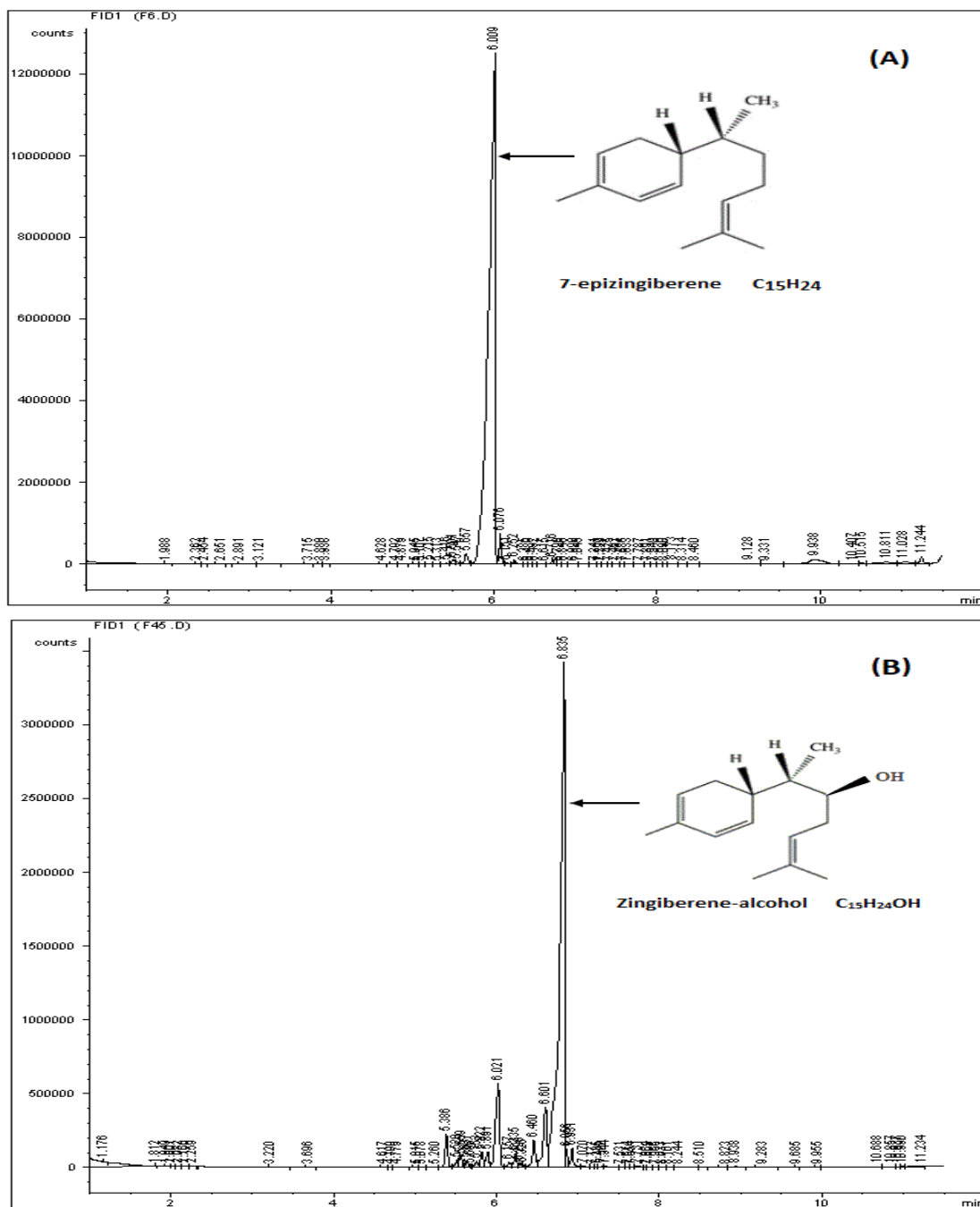
Figure 2–3: GC-FID Chromatogram of fraction 6, containing 7-epi-zingiberene (A) and fraction 45 containing zingiberene alcohol (Breeden and Coates) obtained by silica gel chromatography of LA2329 extract. Chemical structures obtained from (Bleeker et al. 2011; EP Patent No. 3178313A1 2017).
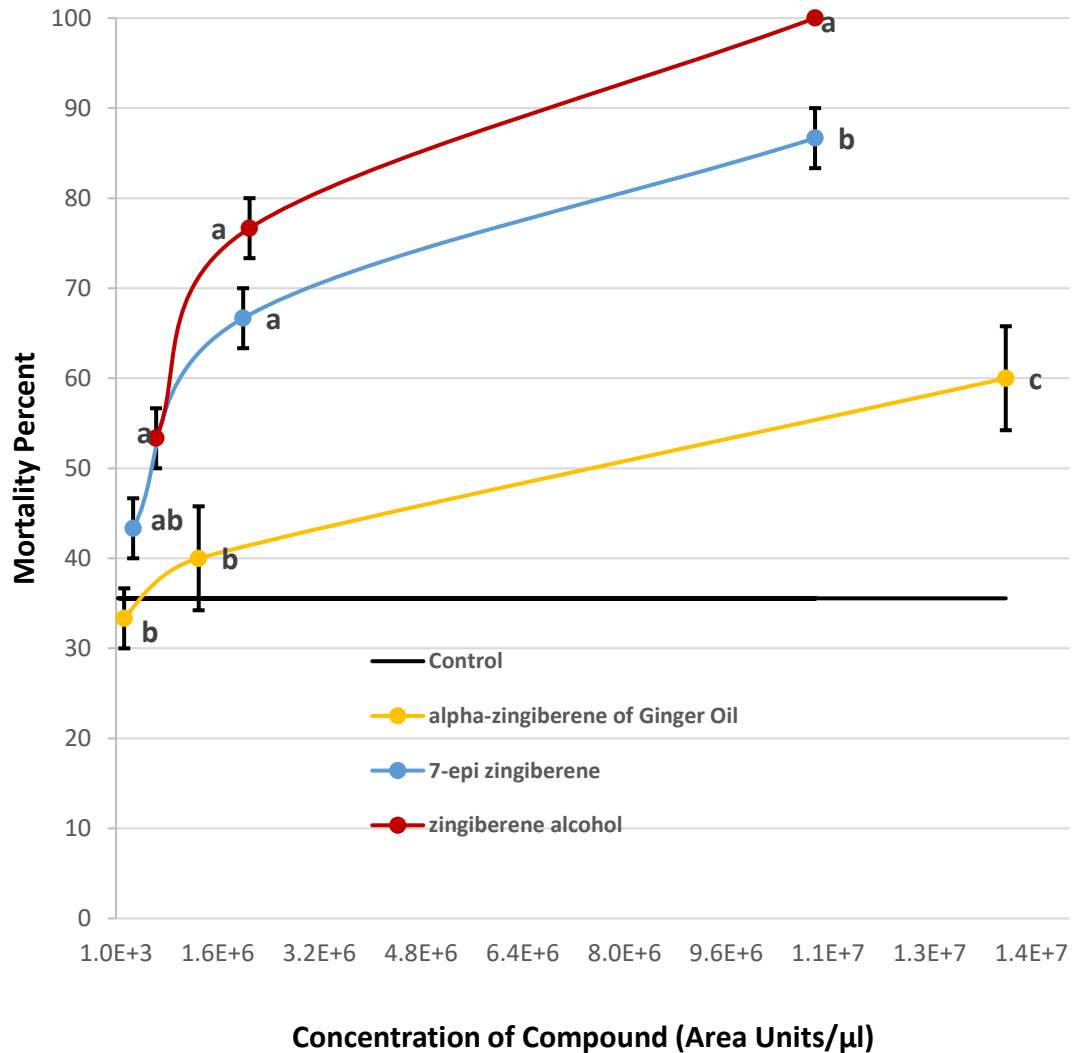
Figure 2–4: Mean mortality (%) of female spider mites in bean leaf disk bioassays of 7-epizingiberene and zingiberene alcohol isolated from trichome secretions of the wild tomato *S. habrochaites* 'LA2329' and alpha-zingiberene isolated from ginger oil. Each leaf disc was triplicated per concentration sprayed. Control sample contained ethanol only. Actual extract concentrations were: alpha-zingiberene 1.4×10^7, 1.3×10^6, and 1.3×10^5; 7-epizingiberene 1.1×10^7, 2.0×10^6, and 2.7×10^5; and zingiberene alcohol 1.1×10^7, 2.1×10^6, and 6.3×10^5. Letters indicate significant differences among treatments at each concentration. Vertical lines designate to standard errors.
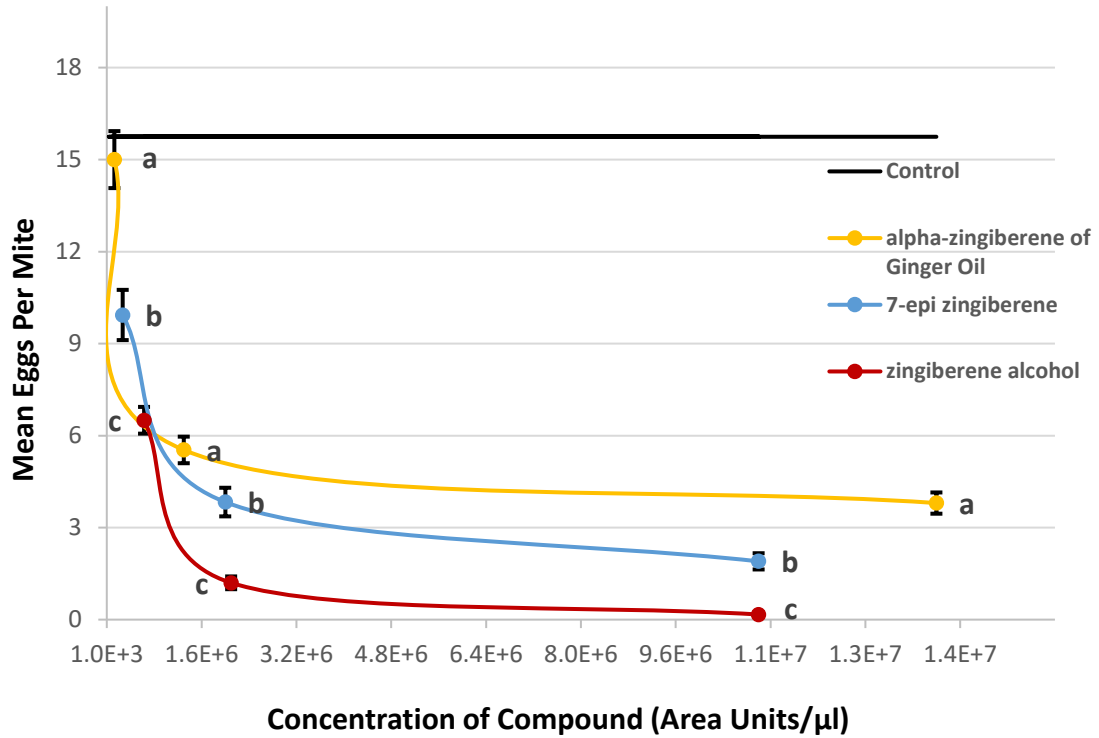
Figure 2–5: Mean eggs per female mite in bean leaf disk bioassay of 7-epizingiberene and zingiberene alcohol isolated from trichome secretions of the wild tomato S. habrochaites 'LA2329' and alpha-zingiberene isolated from ginger oil. Each leaf disc was triplicated per concentration sprayed. Control sample contained ethanol only. Actual extract concentrations were: alpha-zingiberene 1.4×10^7, 1.3×10^6, and 1.3×10^5; 7-epizingiberene 1.1×10^7, 2.0×10^6, and 2.7×10^5; and zingiberene alcohol 1.1×10^7, 2.1×10^6, and 6.3×10^5. Letters indicate significant differences among treatments at each concentration. Vertical lines designate to standard errors.

CHAPTER 3. **Two-spotted Spider Mite Resistance in Tomato Hybrids by Trichome Secretions and Densities of _Solanum habrochaites_ Accession LA2329**

3.1 Abstract

Selection for pest resistance is essential to the genetic transfer of resistance between a wild species and cultivated tomato. _Solanum habrochaites_ LA2329, a wild relative of tomato, is known to be highly resistant to arthropods due to high density of type IV and type VI trichomes with high levels of foliar zingiberene. The primary objective of this work was to confirm the transfer of resistance from _S. habrochaites_ accession LA2329 to cultivated tomato. Also investigated was the interaction of type IV trichome densities and leaf zingiberene contents with spider mite behavior.

In 2017, nine tomato genotypes consisting of BC$_3$F$_3$ and BC$_3$F$_4$ hybrids, three susceptible genotypes and three resistant wild species controls were used. These genotypes were selected based on densities of trichome type IV and zingiberene concentrations. Whole tomato leaves, consisting of five leaflets, were bioassayed with spider mites, _Tetranychus urticae_ Koch under laboratory conditions. Mite responses on some hybrids were almost the same as on the resistant wild donor parent, _S. habrochaites_ as indicated by leaflet surface infested by mites, mite webbing, and feeding damage by mites. At the end of the bioassay, egg density on four backcross hybrids was similar to that on the resistant S_. habrochaites_ accessions. Based on reduced mite success on some of the backcross hybrids, we infer the resistance has been successfully transferred from the wild accession to the selected genotypes by deployment of indirect selection. Trichome-type IV density and zingiberene content had a significant negative correlation

with most of the mite behavioral and biological responses. This bioassay identified behavioral differences of mites based on the presence or absence of leaf compounds and glandular trichome densities and supported the hypothesis that introgression of type IV trichome and zingiberene will lead to greater spider mite resistance.

3.2 Introduction

Breeding for durable genetic resistance in crops is an alternative to pesticide-based pest control. The development of crops and cultivars with desirable traits (large seed size, high yield, pest and disease resistance, etc.) has been realized by utilization of plant genetic diversity as germplasm resources (Govindaraj et al. 2015). For instance, the secondary and tertiary tomato germplasm pool is highly diverse providing a wide range of phenotypes that may have economic potential. Recent trends have moved to improve resistance of tomato plants to herbivores such as pinworm (Antônio et al. 2011), and whitefly (Freitas et al. 2002; Neiva et al. 2013). Hence, there is a significant necessity for assay platforms to assess and identify levels of resistance to insect performance, also to understand fundamentals of tomato–pest interactions and for the development of resistant varieties.

Two-spotted spider mite (*T. urticae* Koch) is known as a poly-phytophagous pest responsible for causing damage on tomato leaves and fruits leading to a reduction in fruit yield (Pokle and Shukla 2015). Aznar-Fernández and Rubiales (2018) identified two major mechanisms of plant resistance to pea aphid *Acyrthosiphon pisum*; antixenosis and antibiosis. Both have been characterized in tomato host-pest interaction papers (Carter

and Snyder 1985; Vijaykumar et al. 2009; Kim et al. 2013; Kamphuis et al. 2013). Out of 99 interspecific backcrosses made by crossing *S. habrochaites* LA1363 x *S. lycopersicum,* 16 hybrids resistant to spider mites were identified by Snyder et al. (2005), using a whole leaf bioassay. Repellent effects were attributed to foliar secretions of 2,3-dihydrofarnesoic acid.

Although resistance to insects and arthropods has been proven in wild tomato relatives, the presence of Dobzhansky-Muller interactions may preclude or reduce gene flow, preventing introgression or hybridization between species (Dobzhansky 1937; Rieseberg and Willis 2007; Lowry et al. 2008). Therefore, the key for successful introgression of genetic resistance into cultivars requires using an efficient breeding method. For instance, direct selection, as used in the breeding program at the University of Kentucky for high foliar zingiberene and specific trichome types as well as emphasizing additional traits like fruit size and color, is an efficient procedure aimed at indirect selection for spider mite resistance.

Extensive characterizations of foliar trichome types, hair-like appendages growing on the aerial plant epidermis, have been reported for tomato. Many of these studies focused on the role of trichomes in arthropod resistance, often related to their chemical secretions (Guo et al. 1993; Snyder et al. 2005; Bergau et al. 2015) and/or their ability physically entrap arthropods (Baur et al. 1991; Simmons and Gurr 2005). Basically, there are two major forms of trichomes: glandular and non-glandular. Seven type of trichomes on tomato and its wild relatives were first documented by Luckwill (1943). Subsequently, eight distinct types based on shape and size were described by Channarayappa et al.

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(1992). The *S. lycopersicum* genotypes tend to have copious type III and V trichomes whilst *S. habrochaites* accessions tend to have abundant type IV trichomes, few type III, and a lack of type V trichomes. The type VI trichomes are ubiquitous glandular trichomes and have been studied extensively in the genus *Solanum* (Bergau et al. 2015). Therefore, the existence of specific types of trichomes can differentiate the wild species *S. habrochaites* from the cultivated one, *S. lycopersicum* (Snyder and Carter 1985). Fernández-Muñoz et al. (2003) reported that resistance to *T. urticae* was correlated with the density of type IV trichomes in *S. pimpinellifolium* accession 'TO-937' and in the BC_1 hybrids generated by crossing between 'TO-937' and *S. lycopersicum* 'Moneymaker'. They indicated that genetic resistance was controlled by a major dominant gene.

Multiple studies of tomato–arthropod interactions have emphasized the role of allelochemical content associated with glandular trichomes. For instance, secondary metabolite synthesis in tomato trichomes (e.g. terpenoids, phenylpropanoids, flavonoids, and methyl ketones) is the most important in stems and leaves, often leading to production of compounds that provide resistance against herbivores (Simmons and Gurr 2005; Kortbeek et al. 2016). Exploitation of these foliar compounds by plant breeders could be an effective approach to integrated pest management.

The sesquiterpene hydrocarbon zingiberene, mostly stored and released by type IV and/or VI glandular trichomes of some accessions of *S. habrochaites*, is responsible for high levels of resistance against spider mites even at low levels, reflected in a severe reduction (~ 81%) of *T. urticae* eggs (Bleeker et al. 2012). The pesticidal activity of zingiberene has been studied in Colorado potato beetle (Carter et al. 1989; Gianfagna et

al. 1992), beet armyworm (Eigenbrode et al. 1994), whiteflies (Freitas et al. 2002; Bleeker et al. 2011; Neiva et al. 2013), tomato pinworm (de Azevedo et al. 2003; Lima et al. 2015), red and two spotted spider mites and whiteflies (Bleeker et al. 2012). Direct selection for genotypes with specific types of glandular trichome densities and zingiberene levels seemed to be an efficient technique for indirect selection for arthropod resistance/repellence (Azevedo et al. 1999; Maluf et al. 2001; Júnior et al. 2018). Consequently, glandular leaf-trichomes and their chemical exudates found in wild genotypes can be recovered in cultivated tomatoes providing a defensive system against pests.

The objectives of this study were to use the whole leaf bioassay to determine if we are successfully introgressing spider mite resistance from wild tomato into cultivated tomato by direct selection for type IV trichome density and foliar zingiberene concentration. Secondly, we wanted to illustrate the relationships of type IV trichome density and zingiberene content with mite resistance criteria.

3.3 Materials and Methods

3.3.1 Plant Materials:

An interspecific population between *Solanum lycopersicum* ZH2 'Zaofen 2' (lacking type IV trichomes and zingiberene; susceptible to spider mites) and a wild tomato species, *Solanum habrochaites* LA2329 (rich in type IV trichomes and zingiberene; highly resistant to spider mites), provided the plant material used in this research. The population has undergone selection for high concentrations of zingiberene and high type IV trichome densities on leaflets as well as selection for fruit set and seeds per fruit. The entire

population was prescreened based on rating type IV trichomes using a stereomicroscope and collecting leaf samples for zingiberene quantification. From this screening, nine tomato backcross hybrids of two generations (BC_3F_3 & BC_3F_4) were chosen based on the presence and absence of type IV trichomes, and zingiberene concentrations. An additional six genotypes were chosen, three considered as positive controls (highly resistant to spider mite) and three others as negative controls (highly susceptible to spider mite). All genotypes studied were pre-characterized from preliminary observations (Table 3-1). Details on obtaining the plant material, transplanting, and fertigation management in greenhouse were mentioned in Chapter 2. Plants were grown at the Horticulture Research Farm, University of Kentucky-Lexington, KY and managed as recommended in ID-36 (http://www2.ca.uky.edu/agcomm/pubs/id/id36/id36.pdf).

3.3.2   Maintenance of Mite Colony: as described in Chapter 2.

3.3.3   Quantification of Chemicals in Tomato Leaflets and Trichome

Assessment:

Three leaflets from a leaf adjacent to the bioassayed leaf (see item 4 below) were excised from each genotype. Leaflet tips and bases were removed by scissors and the center leaflet segments were placed in 2.0 ml hexane. After agitation, the extracts were analyzed for zingiberene and the primary monoterpene, β-phellandrene, by GC-FID as described previously in Chapter 2. All leaf segments were scanned to calculate leaf area (cm^2) by image analysis using ImageJ software (https://imagej.nih.gov/ij/). Then leaf chemical concentration was divided by its leaf area to obtain the final value, in units of GC area units/cm^2.

The remaining leaflets on the leaf were used for counting number of type IV

trichomes on abaxial and adaxial surfaces by use of a Meiji stereo microscope equipped

with 10X10 microscopic grid (4.3 mm^2) at 50X for the entire grid.

3.3.4   Whole Leaf Bioassay:

The whole leaf bioassay (Snyder et al. 2005) was used for this research. An excised

whole tomato leaf, fully expanded and positioned at the third node from the apex,

consisting of five leaflets, was transferred from the field to the laboratory. Three leaves

from each genotype were bioassayed with spider mites to provide replication. Each whole

leaf was inserted into a 250-ml Erlenmeyer flask filled with tap water (Figure 3-1-A). Next,

a leaf from a bean plant, *Phaseolus vulgaris* L., infested with about thirty female two-

spotted-spider mites, *T. urticae* Koch, was placed at the base of the detached tomato leaf.

(Figure 3-1-B). Conditions during the bioassay were temperature range of 20-22 ^{O}C and

light in the range of 60-100 μE m^{-2} s^{-1} provided by fluorescent lamps. Flasks were

monitored daily and refilled with water until the seventh day of the bioassay setup.

Several parameters of mite performance were evaluated during the seven-day

course of the bioassay. In most cases, visual evaluations were aided by use of a 3X

magnifying glass. Most of the evaluations were based on counting the leaflets and

surfaces involved for a particular mite parameter. For example, data for the number of

leaflet surfaces infested by mites were obtained by examining the abaxial surfaces of all

five leaflets of a leaf, and recording the total number of abaxial leaflet surfaces where at

least one mite was present. A similar operation was used to evaluate the adaxial surfaces

of the same five leaflets. Data recorded for each surface ranged from 1 to 5. Leaflets with

mite webbing, feeding damage, and presence of eggs were similarly evaluated. Number of leaflets infested by mites, regardless surfaces, and number of each leaflet surface infested by mites were determined on days 1 and 2 of the bioassay. Number of each leaflet surface, abaxial and adaxial, with webbing and feeding damage were determined on day 3 and 7 of the bioassay. Rating scales of 0-3 were also used to score webbing and feeding damage on the whole leaf surface. For these scales 0 represented no webbing or no feeding damage, and 1 represented light webbing or feeding damage based on the intensity and number of spots involved in the whole leaf. Also, a score of 3 represented extensive webbing or feeding damage while a score of 2 was intermediate between score 1 and 3. Leaf surfaces involved with webbing or feeding damage is basically an objective method, however, a rating scale of 0-3 is a subjective measurement.

The total number of each abaxial and adaxial leaflet surfaces having any eggs was determined on day 7 of the bioassay. Egg counting was aided by use of a Meiji stereo zoom microscope and a sum of total number of eggs per surface of each leaf was calculated after the seventh day of the bioassay. Each whole leaf sample, containing five leaflets without petioles, was scanned then processed by ImageJ software to calculate leaf area. Subsequently, egg density (number/cm^2) for each abaxial and adaxial surface of each sample was determined by dividing the total number of eggs on a leaf by leaf area.

3.3.5 Statistical Analysis:

Data for the plant characteristics, β-phellandrene and, zingiberene concentrations (GC area units/cm² of leaflet), and trichome type IV density (No./mm²), and data obtained from the bioassay were analyzed by SAS v 9.4 (SAS Institute Inc. 2012). All data were

analyzed according to a completely randomized design (CRD) using the general linear model (GLM) procedure. The effects of genotype on β-phellandrene and zingiberene were evaluated using a one-way ANOVA. The effects of genotype, surface, and their interaction (genotype X surface) were evaluated through a two-way ANOVA for type IV trichome density and for mite data obtained from the bioassays. Post-hoc Tukey HSD tests were conducted for mean comparisons at P< 0.05 if a significant effect (P < 0.05) of treatments was present. LSmeans was used to compare genotype X surface interaction means when a significant interaction (P < 0.05) was observed. To calculate Pearson's correlation coefficients among variables, the parameters measured for adaxial and abaxial surfaces were summed prior to calculation of coefficients. Doing so avoided false replication with β-phellandrene and zingiberene contents, which were not determined for each surface of the tested leaves. Additional analyses were performed by use of hierarchical cluster analysis based on Ward's method performed by JMP statistical software (JMP® version 12.1.0 SAS Institute Inc, Cary, NC, USA). This analysis included trichome type IV density and zingiberene concentrations as well as all spider mite data obtained from whole leaf bioassay.

3.4 Results

3.4.1 Tomato Leaflet Chemistry and Type IV Trichome Density:

The ANOVA results of the leaflet chemical composition (Table 3-2) indicated that there were significant differences among tomato genotypes in terms of zingiberene and β-phellandrene concentrations per cm^2 of leaf area (P <.0001). Only two hybrids Z116 and X155 had high concentrations of β-phellandrene (Table 3-3). β-phellandrene was

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detected in three tomato cultivars, W126, W129, and W160, and the two additional hybrids, W126 and X71. For the remaining hybrids, β-phellandrene concentrations were below the detection limits (Table 3-3).

The backcross hybrid X71 had the highest concentration of zingiberene/cm^2 leaf area with 5.7 X 10^7 AU/cm^2 followed by the wild accessions LA2329 and LA2329OH, 2.8 X 10^7 and 2.3 X 10^7 respectively (Table 3-4). Six genotypes, three tomato cultivars and three interspecific hybrids W126, W129, and W160, X155, Z116, and Z120 had no detectable zingiberene. The remaining six plants, W75, Z70, PI127826, X166, Z58 and Z161 produced intermediate concentrations of zingiberene, with the latter two, Z58 and Z161, producing at the lowest non-zero concentrations. With the exception of PI127826, these plants were hybrids.

Type IV trichome densities varied among the genotypes and surfaces (Table 3-5). PI127826 had the most abundant density of type IV trichomes followed by the backcross hybrids X155 and X71. In contrast, the cultivated tomato varieties, W126, W129, and W160, as well as three of the hybrids had no type IV trichomes (Table 3-6). Significant differences were found on adaxial (5.49/mm²) and abaxial (21.51/mm²) leaflet surfaces (Table 3-7). However, these means are influenced by presence of six plants, three hybrids and three cultivated tomatoes that did not possess any type IV trichomes. The presence of these six genotypes in the ANOVA likely also explains the significant genotype X surface interaction in the analysis; genotypes lacking type IV trichomes cannot differ in density between surfaces. Conversely, for genotypes having type IV trichomes, density can and

did differ between surfaces with the abaxial surface density for type IV trichomes always significantly greater than the density on the adaxial surface of a genotype (Table 3-8).

Considering the phenotypic variability of the interspecific tomato hybrids chosen for this study. They represent a wide range of type IV trichome density as well as zingiberene production. Thus, understanding mite behavior on these hybrids may provide important information for their relationship to spider mite resistance.

3.4.2 Mite Responses:

3.4.2.1 Number of Leaflets and Surfaces Infested by Spider Mites:

Results of the ANOVA analysis indicated significant differences among tomato genotypes in number of leaflets infested by spider mites on the first and second day of the whole leaf bioassay (Table 3-9) as well as how many leaflet surfaces were infested by spider mites on the same days (Table 3-10). The negative controls W126, W129, and W160 and two of the backcross hybrids, Z161 and Z116, had the highest number of leaflets with mite infestation, 4.33, 3.67, 3.67, 3.67, and 3.33 respectively on the first day while PI127826 and X166 showed the lowest mean, only one leaflet, infested by spider mites (Table 3-11). On the second day, the hybrid Z161 had the highest mean number of leaflets with for mite infestation, 4.67, followed by the negative controls, W126, W129, W160 at 4.33, while the wild accessions used as positive controls, PI127826 and LA2329OH had two or fewer leaflets infested by spider mites (Table 3-12).

For number of leaflet surfaces infested by spider mites on the first and second days of the whole leaf bioassay, the number of leaflets infested by mites were the highest and similar for the negative control genotypes Z58 and W129 (Table 3-13 and 3-14). In

contrast, the positive controls LA2329OH and PI127826 plus the hybrid X166 had similarly low numbers of leaflet surfaces with mites on the first day, 1.33, 0.83, and 1.17 leaflets with mites, respectively, and on the second day with 2.00, 1.33, and 1.67 leaflets with mites, respectively (Table 3-13 and 3-14). On both rating days, adaxial surfaces were more extensively infested than were abaxial surfaces (Table 3-15).

There was a significant genotype X surface interaction (P=0.013 and P=0.028) for number of leaflet surfaces with mites on day 1 and day 2 of the whole leaf bioassay (Table 3-10). For number of leaflet surfaces with mite infestation on day 1, there were no differences between surfaces for PI127826, X166, LA2329OH, LA2329, Z116, X155, Z70, or W126 (Table 3-16). However, for the remaining genotypes, W75, Z120, X71, Z161, W160, W129 and Z58 there was a difference between leaflet surfaces for number of surfaces with mites (Table 3-16). On day 2 of the whole leaf bioassay (Table 3-17) the abaxial and adaxial surfaces on all of the hybrids except one, Z70, had different numbers of surfaces with mite infestation; there was no difference between surfaces in number of surfaces infested by mites for the positive or negative controls.

3.4.2.2 Mite Webbing:

There were significant differences among tomato genotypes for mite webbing scores on day 3 and day 7 but surfaces only differed on day 3 (Table 3-18). The interaction of genotype and surface was only significant for webbing score on Day 7 (Table 3-18). For number of surfaces having webbing on day 3 and day 7, there were significant differences among genotypes and between surfaces on day 3 and day 7; the genotype X surface interaction was not significant on either rating day (Table 3-19).

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The negative controls, W129, W126, and W160 had high mite webbing scores, 1.50, 1.33, 0.83, respectively on the third day while the hybrids X166, X71, and Z116 showed the lowest mean webbing score (no webbing), similar to the positive controls, LA2329, LA2329OH, and PI127826 (Table 3-20). Correspondingly on the seventh day, the negative controls, W126, W129, and W160 had the highest mean of mite webbing scores of 2.67, 2.50, and 2.00 respectively while the wild accession, PI127826 followed by the backcross hybrid X155 had webbing scores of 0.33 and 0.50 mite as the lowest means, respectively (Table 3-21). The adaxial leaflet surface on the third day had a higher mite webbing score than that on the abaxial surface (Table 3-22); average mite webbing scores did not differ between surfaces on the seventh day (data not shown).

The genotype X surface interaction for webbing score was only significant on day 7 of the bioassay (Table 3-23). For the 3 negative controls, W160, W129 and W126, for one of the three positive controls, PI127826, and for five of the nine hybrids, X166, Z161, Z120, Z70, X71 there were no differences in webbing score between surfaces. For the remaining entries in the bioassay, the webbing scores for abaxial and adaxial surfaces did significantly differ for four of the nine hybrids X155, Z116, W75, and Z58, and for the positive controls LA2329 and LA2329OH (Table 3-23).

For number of leaflet surfaces with spider mite webbing on day 3, the *S. lycopersicum* controls W129 and W126, had the maximum number of leaflet surfaces with mite webbing, 2.67 and 1.67 respectively (Table 3-24). Contrarily, the *S. habrochaites* controls, LA2329, LA2329OH, and PI127826, as well as the hybrids, Z116, X71, and X166

had no leaflet surfaces with mite webbing (Table 3-24). On the seventh day the negative

controls, W129, W160, and W126, had 4.16, 4.00, and 3.83 leaflet surfaces with   mite

webbing as the highest means respectively, whereas the positive controls, PI127826 and

LA2329OH, had the lowest mean number of leaflets with webbing of 0.33 and 0.83,

respectively (Table 3-25). Webbing scores on hybrids X155, X166, W75, Z116, Z70 and

that for the positive control, LA2329 were not significantly different from those of the

resistant controls, PI127826 and LA2329OH (Table 3-25).

For number of surfaces with webbing, the adaxial leaflet surfaces for both third

and seventh day of the bioassay had higher means than those on the abaxial surface

(Table 3-26).

3.4.2.3   Mite Feeding Damage:

There were significant differences among tomato genotypes in feeding damage

score (table 3-27) and number of leaflet surfaces with feeding damage (table 3-32) on day

3 and day 7. Furthermore, statistical differences were found between adaxial and abaxial

surfaces for feeding damage score (table 3-27) and for number of leaflet surfaces with

feeding damage (table 3-32) on day 3 and day 7. Genotype X surface interaction was

significant for only the mite feeding damage score on day 7 (Table 3-27).

The negative controls, W160, and W126 had high mite feeding damage scores,

1.17 and 1.00, respectively on the third day, while the positive control PI127826 and two

backcross hybrids, X71 and X166, displayed no feeding damage (score 0) (Table 3-28).

Similarly, for the negative controls, W129, W160, and W126, feeding damage scores on

the seventh day were high, 2.50, 2.33, and 2.00, respectively, while the backcross hybrid, X155, and wild relative, PI127826 had low feeding damage scores, 0.50 and 0.67, respectively (Table 3-29). The adaxial leaflet surface had higher average mite feeding damage scores than that for the abaxial surface, 0.60 and 0.29 on the third day and 1.44 and 1.18 on the seventh day, respectively (Table 3-30).

For the genotype X surface interaction for mite feeding damage score, which was only significant for day 7 (Table 3-27), scores did not differ between surfaces for most of the plants in the bioassay (Table 3-31). Scores did differ between surfaces for X155, Z58, W75, Z161, and W126. For each of these genotypes, the adaxial surface had higher feeding damage scores than the abaxial surface. Of the five genotypes having a feeding damage score difference, four were hybrids and one, W126, was a negative control. No relationship between surface difference in feeding damage score and susceptibility to feeding damage was noted, because the group of five genotypes included the genotype with the lowest feeding damage score, X155, as well as the other four genotypes were among the lowest for feeding damage score (Table 3-29).

For the number of leaflet surfaces damaged by mite feeding the *S. lycopersicum* susceptible controls, W126, W129, and W160 showed the maximum number of leaflet surfaces damaged by mite feeding 1.83, 1.67, and 1.50 respectively on day 3 (Table 3-33) and the same susceptible genotypes had the highest numbers of surfaces damaged on day 7, 4.50, 4.16, 4.00, respectively (Table 3-34) On the other hand, the *S. habrochaites* positive control, PI127826, and the hybrids, X71 and X166 showed no leaflet surfaces damaged by mite feeding on day 3 (Table 3-33). Similarly, on the seventh day, the positive

control, PI127826, and the hybrid X155 only had 1.00, and 1.17 leaflet surfaces damaged by mite feeding, respectively (Table 3-34). The adaxial leaflet had significantly more surfaces damaged by mite feeding on both the third and seventh day than did the abaxial surface (Table 3-35).

3.4.2.4 Oviposition:

Significant differences among tomato genotypes were found for number of leaflet surfaces with eggs on day 7 and for egg density on day 7 (Table 3-36). Furthermore, statistical differences were found between adaxial and abaxial surfaces for both variables (Table 3-36). The genotype X surface interaction was significant for only egg density on day 7.

The mean of the number of leaflet surfaces infested by mite eggs on day 7 slightly differed among genotypes where the maximum was 5 leaflet surfaces infested by eggs for Z161, Z58, Z116, and Z120 and the minimum was 3.5 leaflet surfaces infested by eggs for PI127826 (Table 3-37).

The *S. lycopersicum* controls, W129, W160, and W126, had the highest mean egg density on day 7 (Table 3-38). Contrarily, the *S. habrochaites* genotype controls, LA2329OH and PI127826, showed the lowest mean egg density (Table 3-38). Average egg density on adaxial leaflet surfaces was higher than that for the abaxial surfaces (Table 3-39).

For the genotype X surface interaction for egg density on day 7, the four positive controls and two hybrids X71, and X155 did not have a significant difference in egg density

between surfaces (Table 3-40). Egg density was lower on abaxial surface compare with adaxial surfaces for the remaining nine genotypes tested.

3.4.3 Correlation of Trichome Density and Zingiberene Content with Behavioral and Biological Mite Variables:

Correlation analyses provided the opportunity to explore potential relationships between densities of type IV glandular trichomes and their exudate contents with spider mite resistance. Genotypes tested in the whole leaf bioassay had a wide range of variability in their foliar zingiberene concentrations (Table 3-2) and glandular type IV trichome densities (Table 3-5). Correlation coefficients (r) between type IV trichome density and all behavioral and biological mite parameters suggested that type IV density was significantly and inversely correlated with spider mite resistance (Table 3-41). In addition, zingiberene contents had a significant negative correlation with all mite response variables except for total mite webbing score on leaflet surfaces on day 7, total leaflet surfaces with webbing on day 7, and total leaflet surfaces infested by mite eggs on day 7. Additionally, there were no significant correlations between the monoterpene content, β-phellandrene, and mite parameters except for total mite webbing score on day 7 which was weak and had a negative association (r = -0.29) as shown in Table 3-41.

3.4.4 Cluster Analysis:

The objective of this analysis was to visualize the extent of the association of phenotypic variability of leaf characteristics and spider mite behavior for the fifteen tested genotypes. The dendrogram obtained by hierarchical cluster analysis for the 15 tomato genotypes showed two main clusters (resistant and susceptible – clusters 1 and

2) among all genotypes evaluated using whole leaf bioassay (Figure 3-3). The cluster of resistant genotypes included the wild *S. habrochaites* LA2329, LA2329OH, and PI127826 and all of the interspecific backcross hybrids W75, X71, X155, X166, Z58, Z70, Z161, Z116, and Z120 (Figure 3-3). The other cluster contained only the susceptible *S. lycopersicum* lines, W126, W129, and W160 (Figure 3-3). Within the resistant genotype cluster, there were four distinct subgroups labeled A, B, C, and D.

Cluster means for type IV trichome density and zingiberene concentration differed dramatically among clusters as did means for some of the mite response variables (Table 3-42). Interestingly, no hybrid was clustered with the susceptible controls in cluster 2 (Figure 3-3) For the four resistant subclusters, 1-A – 1-D, there was considerable variation for zingiberene concentration and fir type IV densities (Table 3-42). For mite responses, means associated with degree of webbing and eggs appeared to be the parameters that differentiated clusters. Hybrids in clusters 1-A and 1-D are of greatest interest because these hybrids clustered with mite resistant wild lines. These two clusters had the high type IV trichome density, High zingiberene concentration, low values for number of surfaces with webbing (especially on day 1), webbing score, and egg density seemed to differentiate clusters 1-A and 1-D from clusters 1-B and 1-C. These observations are consistent with the hypothesis that type IV trichome density and zingiberene concentration are particularly important in determining degree of webbing and egg density in the whole leaf bioassay

The resistant controls LA2329OH and PI127826 were strongly associated with hybrid X166, likely due to high type IV trichome density and high zingiberene production.

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Eight other interspecific backcross hybrids either for one or both resistance features, high density of type IV trichome and zingiberene content, were comparable in to some degree to the wild resistant accessions LA2329, LA2329OH, and PI127826. As a result, the presence of glandular type IV trichomes mediated terpenoids in relation to resistance parameters reflected greater spider mite resistance compared with susceptible tomato varieties.

3.5   Discussion

While investigating zingiberene bioactivity and trichome specific type densities through a whole leaf bioassay, we demonstrated behavioral differences of mites associated with the presence or absence of leaf compounds and trichome densities. The genotypes evaluated in the whole leaf bioassay were selected for wide variation in concentration of zingiberene and type IV trichome density with the expectation that mite responses in the whole leaf bioassay would also widely vary, which they did.

The classification of genotypes based on cluster analysis showed clear dissimilarities among groups based on the presence and magnitude of the allelochemical zingiberene and type IV trichomes and on mite performance. The nine resistant genotypes clustered with the wild parent *S. habrochaites* LA2329 indicating their mite bioassay performance was more similar to that of the donor wild parent than to the *S. lycopersicum* susceptible controls.

In the literature, segregation of zingiberene in interspecific tomato hybrids obtained by crossing wild x cultivated lines was governed by the action of a single gene locus (Rahimi and Carter 1993; Freitas et al. 2002; Lima et al. 2015). Therefore, our

interspecific hybrids created by *S. lycopersicum* x *S. habrochaites* that segregated for presence of zingiberene may be partially explained by this genetic model. The rationale behind choosing backcross hybrids with contrasting leaf traits, high vs. low or absent type IV densities and zingiberene contents was to demonstrate mite responses that may be associated with these characters and to verify transfer of resistance into the hybrids. Based on our cluster analysis, in which all of the hybrids clustered with the wild tomato lines, the positive controls, is strong evidence that resistance has been introgressed into these hybrids.

The introgression of zingiberene from wild species into cultivated tomato has been shown to be related to host-plant resistance. Bleeker et al. (2012) successfully engineered zingiberene synthase from wild to cultivated tomato with the best transgenic hybrids producing about 1.5% of the 7-epizingiberene present in the wild plant *S. habrochaites* PI127826. When one of the transgenic tomato lines, line 2, was evaluated, the number of *T. urticae* eggs were reduced by 81% compared to the wild type control (Bleeker et al. 2012). Also, ninety-nine interspecific backcrosses obtained by crossing *S. habrochaites* LA1363 x S. lycopersicum were screened by Snyder et al. (2005) using the whole leaf bioassay to assess the potential for resistance to tomato spider mites. They evaluated the number of leaflets infested by mites as well as webbing and feeding damage. They reported that this bioassay allowed identification of 16 resistant hybrids having trichomes and 2,3-dihydrofarnesoic acid that had repellent effects on spider mites and also identified hybrids having resistance mechanisms other than repellency.

In our experiment, the hybrid genotypes X116, X71, and W75 are valuable for resistance breeding against *T. urticae*. These genotypes possessed high zingiberene concentrations and high trichome type IV densities, and were in the subclusters containing the wild resistant genotypes. Both factors, zingiberene and type IV trichomes may interact to hinder spider mite performance. Others have reported the advantage of the presence of multiple factors conferring arthropod resistance. Neiva et al. (2019) evaluated fifteen tomato genotypes for whitefly (Bemisia tabaci biotype B) resistance with reference to foliar zingiberene and acylsugar presence. They reported that genotypes having high concentrations of both zingiberene and acylsugars had lower oviposition as compared to genotypes with low concentrations of both allelochemicals or without either allelochemical. Also, the genotypes having leaves producing both zingiberene and acylsugar exhibited a synergistic effect of lowering the number of whitefly eggs compared to genotypes producing only one of these compounds. Sridhar et al. (2019) showed that type IV glandular trichomes with types I and VII were negatively correlated with larval number of *Tuta absoluta* per plant, leaf damage percentage, and adult moth activity while testing different tomato genotypes.

These findings with regard to importance of multiple factors such as trichomes and allelochemicals agree with de Oliveira et al. (2018) who realized that the resistance of tomato genotypes having abundant of glandular trichomes and high zingiberene production was close to the resistance of the wild accession PI127826. Recently, de Oliveira et al. (2018) also bioassayed interspecific crosses between wild *S. habrochaites* PI127826 and *S. lycopersicum* (cv. Redenção) selected based on high vs low zingiberene

production on leaflets to identify *T. urticae* behavior and biology influenced by these genotypes using free- and no-choice tests. The authors reported that genotypes with high zingiberene associated with high glandular trichome densities were significantly less preferred by mites and reduced their fecundity rate, implying harmful impacts on mite behavior and biology.

The hybrid X155 is of particular interest. This hybrid had the highest type IV density among all the hybrids, but lacked zingiberene production. In cluster analysis it did not subcluster with the wild positive controls. However, based on the egg production on this hybrid, it was indistinguishable from the wild positive controls, and from the hybrids that did subcluster with the positive controls. Also, in most of the analysis of mite performance, abaxial surfaces tended to show more resistance than adaxial surfaces. This likely reflects the fact that abaxial surfaces tend to have much higher type IV trichome densities than adaxial surfaces (Antonious 2016), providing additional evidence for the importance of type IV trichomes in the resistance to spider mites. Mite fecundity was inversely and significantly correlated with trichome type IV densities implying the higher the trichome density, the larger the reduction in number of eggs produced by female mites (Carter and Snyder 1985). Alba et al. (2009) found that the density of type IV leaf trichomes releasing allelochemicals such as acylsugars reduced spider mite eggs but increased mite repellency in recombinant inbred lines of tomato.

Three of the hybrids tested, Z70, Z58 and Z161, had zingiberene without type IV trichomes. In terms of resistance as measured in the whole leaf bioassay by egg density, these genotypes were intermediate in resistance. These results suggest that zingiberene

alone can confer a degree of resistance to spider mites. Carter et al. (1989) reported the sesquiterpene hydrocarbon, zingiberene, produced by glandular trichomes of *S. habrochaites* accession PI126445 was toxic to larvae of Colorado potato beetle, *Leptinotarsa decemlineata*, reducing survival percent. Also, Maluf et al. (2001) indicated zingiberene concentrations in leaves of tomato interspecific crosses were correlated positively with high repellency to tobacco spider mite, *T. evansi*,. The F2 populations derived from the interspecific cross between *S. lycopersicum* 'TOM-556' × *S. habrochaites* PI127826 selected for high foliar zingiberene content showed a significant reduction in oviposition and feeding damage of the South American tomato pinworm *Tuta absoluta* (de Azevedo et al. 2003). Moreover, Bleeker et al. (2012) infested intact tomato plants with three female mites then after 45 days found that the foliar 7-epizingiberene produced by the transgenic plant leaves considerably attenuated the mite growth rates in comparison with control susceptible plants.

The whole leaf bioassay may identify potential avoidance resistance mechanism mediated by zingiberene reducing mite oviposition and preventing feeding damage in the tested resistant hybrids. Also, another mechanism indicated that the small number of mites on the five leaf surfaces and reduced webbing are the result of physical impediment rather than avoidance, due to presence of an entrapment mechanism via trichomes. These two mechanisms of plant resistance, defined as antixenosis and antibiosis, have been identified in a few tomato host-pest interaction papers (Antônio et al. 2011; Vijaykumar et al. 2009; Kim et al. 2013; Kamphuis et al. 2013).

Correlations between biological and behavioral mite parameters and densities of type IV trichomes and zingiberene concentration were significantly negative for most parameters. These findings indicate that adverse effects on mite performance e.g. mites on leaf surface, mite webbing, feeding damage, and egg densities were associated with high type IV trichome densities and with abundant zingiberene concentration. These findings were similar to Fernández-Muñoz et al. (2003) who reported that resistance to *T. urticae* was correlated with the density of type IV trichomes in *S. pimpinellifolium* accession 'TO-937' and in the BC_1 hybrids generated by crossing between 'TO-937' and *S. lycopersicum* 'Moneymaker'. In our research, the significant negative association between mite resistance variables and the density of type IV trichomes, suggesting that this resistance mechanism was attributed to higher rates of entrapment via trichomes causing mite starvation, according to Alba et al. (2009).

β-phellandrene did not have significant association with any mite variables except for mite webbing on day 7 which had a marginally significant negative effect. β-phellandrene is generally present on leaves of susceptible *S. lycopersicum* genotypes. Thus, its presence might be associated with mite susceptibility. Some of the hybrids tested in the whole leaf bioassay had concentration of β-phellandrene considerably higher than that found in most *S. lycopersicum* leaves (Snyder, personal communication). This is possible that β-phellandrene, particularly high concentrations of it, may play a role in spider mite resistance. This area needs additional investigation.

3.6   Conclusion

To determine whether spider mite resistance had been successfully introgressed from wild to cultivated tomato, based on selection for zingiberene and type IV trichome density, we evaluated, using a whole leaf bioassay, nine different tomato backcross hybrids with contrasting zingiberene concentrations and type IV trichomes densities. In addition, three positive controls, and three negative controls, were included. Location of mites on the leaf, presence of webbing, and aspects of mite oviposition were evaluated in the bioassay.

Based on results of cluster analysis, the presence of three hybrids that clustered with the highly resistant wild genotype, provided strong evidence that mite resistance had been successfully introgressed into the tested hybrids. Subsequently, more research should center on these valuable tomato hybrids for resistance to other insect pests. The whole leaf bioassay requires limited physical space and labor to detect and characterize plant resistance to herbivorous pests. This bioassay demonstrated behavioral differences of mites associated with the presence/absence of leaf exudates and glandular trichome densities.

Table 3–1: Preliminary observations for type IV trichome rating (Type IV Score) and zingiberene concentration determined by GC-FID as GC area units/cm^2 of leaf area. Type IV Score was scored as 0-3 on the central portion of the abaxial surface on lateral two areas of leaflet vein using Meiji stereo microscope where 0 = none, 3 = density similar to the donor parent, 1= a few type IV trichomes and 2 = density between the 1 and 3 ratings.

Name	Background	Generation	Type IV Score	Zingiberene Concentration
LA2329	Wild	Donor Parent	3	5.3 X 10^7
W126	Fla. 8059	Cultivated tomato	0	0
W129	Maglia Rosa	Cultivated tomato	0	0
W160	ZH2 (Zaofen 2)	Recurrent parent cultivated tomato	0	0
W75	Hybrid	BC$_3$F$_3$	3	2.3 X 10^7
X155	Hybrid	BC$_3$F$_3$	3	0
X166	Hybrid	BC$_3$F$_3$	3	1.8 X 10^7
X71	Hybrid	BC$_3$F$_3$	3	2.6 X 10^7
Z116	Hybrid	BC$_3$F$_3$	3	0
Z120	Hybrid	BC$_3$F$_3$	3	0
Z161	Hybrid	BC$_3$F$_4$	0	1.1 X 10^7
Z58	Hybrid	BC$_3$F$_4$	0	3.7 X 10^7
Z70	Hybrid	BC$_3$F$_4$	0	2.1 X 10^7

Table 3–2: ANOVA model results for leaf compound concentration for 15 tomato genotypes tested in the whole leaf bioassay.

Source of Variation	DF	β-phellandrene		Zingiberene	
		F value	P value	F value	P value
Genotype	14	5.77	<.0001	70.71	<.0001
Error	30				
R^2			0.73		0.97

β-phellandrene and zingiberene concentration determined as GC area unit/cm^2 of leaf area.

Table 3–3: Means of β-phellandrene concentration detected by GC-FID and measured as GC area units/cm^2 of leaf area for 15 tomato genotypes tested in the whole leaf bioassay. Means followed by the same letters are not different based on Tukey's test (P=0.05). Standard error of the mean denoted by SE.

Genotype	Background	Average β-phellandrene (GC area units/cm^2)	±	SE	
Z116	Hybrid	6.6×10^6	±	3.4×10^6	a
X155	Hybrid	6.0×10^6	±	6.4×10^5	ab
W126	Cultivated	2.3×10^6	±	7.3×10^5	abc
Z120	Hybrid	1.8×10^6	±	4.9×10^5	bc
W160	Cultivated	4.8×10^5	±	2.0×10^5	c
W129	Cultivated	3.1×10^5	±	9.5×10^4	c
X71	Hybrid	1.4×10^5	±	1.3×10^4	c
LA2329	Wild relative	0.0	±	0.0	c
W75	Hybrid	0.0	±	0.0	c
X166	Hybrid	0.0	±	0.0	c
PI127826	Wild relative	0.0	±	0.0	c
LA2329OH	Wild relative	0.0	±	0.0	c
Z161	Hybrid	0.0	±	0.0	c
Z58	Hybrid	0.0	±	0.0	c
Z70	Hybrid	0.0	±	0.0	c

Table 3–4: Means of zingiberene concentration detected by GC-FID and measured as GC area units/cm^2 of leaf area for 15 tomato genotypes tested in the whole leaf bioassay. Means followed by the same letters are not different based on Tukey's test (P=0.05). Standard error of the mean denoted by SE.

Genotype	Background	Average zingiberene (GC area units/cm²)	±	SE	
X71	Hybrid	5.7×10^7	±	2.1×10^6	a
LA2329	Wild relative	2.8×10^7	±	3.7×10^6	b
LA2329OH	Wild relative	2.3×10^7	±	4.1×10^6	bc
W75	Hybrid	1.9×10^7	±	2.8×10^6	bcd
Z70	Hybrid	1.4×10^7	±	2.6×10^6	cde
PI127826	Wild relative	1.2×10^7	±	4.1×10^6	def
X166	Hybrid	1.1×10^7	±	1.6×10^6	def
Z58	Hybrid	4.8×10^6	±	2.2×10^5	efg
Z161	Hybrid	2.6×10^6	±	2.6×10^5	gf
W126	Cultivated	0.00	±	0.00	g
X155	Hybrid	0.00	±	0.00	g
Z120	Hybrid	0.00	±	0.00	g
W129	Cultivated	0.00	±	0.00	g
W160	Cultivated	0.00	±	0.00	g
Z116	Hybrid	0.00	±	0.00	g

Table 3–5: ANOVA model results for type IV trichome density (No./mm²) for adaxial and abaxial leaflet surfaces of 15 tomato genotypes tested in the whole leaf bioassay.

	Type IV Density		
Source of Variation	**DF**	**F value**	**P value**
Genotype	14	153.82	<.0001
Surface	1	468.33	<.0001
Genotype X Surface	14	38.13	<.0001
Error	60		
R^2			0.98

Table 3–6: Means of trichome type IV density (No./mm²) for 15 tomato genotypes tested in the whole leaf bioassay. Means followed by the same letters are not different based on Tukey's test (*P*=0.05). Standard error of the mean denoted by SE.

		Average type IV Density			
Genotype	Background	(No./mm²)	±	SE	
PI127826	Wild relative	66.33	±	7.91	a
X155	Hybrid	26.83	±	9.11	b
X71	Hybrid	25.67	±	10.15	b
W75	Hybrid	21.83	±	8.47	bc
X166	Hybrid	21.17	±	8.67	bc
Z116	Hybrid	15.33	±	5.33	cd
LA2329OH	Wild relative	10.67	±	2.09	de
Z120	Hybrid	7.67	±	2.51	e
LA2329	Wild relative	7.00	±	1.39	ef
W160	Cultivated	0.00	±	0.00	f
W129	Cultivated	0.00	±	0.00	f
W126	Cultivated	0.00	±	0.00	f
Z161	Hybrid	0.00	±	0.00	f
Z58	Hybrid	0.00	±	0.00	f
Z70	Hybrid	0.00	±	0.00	f

Table 3–7: Means of trichome type IV Density (No./mm²) for adaxial and abaxial leaflet surfaces of 15 tomato genotypes tested in the whole leaf bioassay. Means followed by the same letters are not different based on Tukey's test (P=0.05). Standard error of the mean denoted by SE.

	Average type IV Density			
Surface	**(No./mm²)**	**±**	**SE**	
Abaxial	21.51	±	3.68	a
Adaxial	5.49	±	1.88	b

Table 3–8: Means of trichome type IV Density (No./mm²) for adaxial (Ad) and abaxial (Ab) leaflet surfaces of the 15 tomato genotypes tested in the whole leaf bioassay. Means within genotypes followed by the same letter are not statistically different as determined by Lsmeans at (*P*=0.05). Standard error of the mean denoted by SE.

			Average type IV Density			
Genotype	**Surface**	**Background**	**(No./mm²)**	**±**	**SE**	
PI127826	Ad	Wild relative	51.33	±	0.88	a
PI127826	Ab	Wild relative	81.33	±	9.35	b
X155	Ad	Hybrid	6.67	±	1.33	a
X155	Ab	Hybrid	47.00	±	2.52	b
X71	Ad	Hybrid	3.00	±	0.58	a
X71	Ab	Hybrid	48.33	±	4.98	b
W75	Ad	Hybrid	3.00	±	0.58	a
W75	Ab	Hybrid	40.67	±	1.86	b
X166	Ad	Hybrid	2.00	±	0.58	a
X166	Ab	Hybrid	40.33	±	2.85	b
Z116	Ad	Hybrid	3.67	±	0.88	a
Z116	Ab	Hybrid	27.00	±	2.31	b

Table 3-8 (continued)

			Average type IV Density			
Genotype	Surface	Background	(No./mm²)	±	SE	
LA2329OH	Ad	Wild relative	6.33	±	0.88	a
LA2329OH	Ab	Wild relative	15.00	±	1.53	b
Z120	Ad	Hybrid	2.33	±	0.33	a
Z120	Ab	Hybrid	13.00	±	1.73	b
LA2329	Ad	Wild relative	4.00	±	0.58	a
LA2329	Ab	Wild relative	10.00	±	0.58	b
W160	Ad	Cultivated	0.00	±	0.00	a
W160	Ab	Cultivated	0.00	±	0.00	a
W129	Ad	Cultivated	0.00	±	0.00	a
W129	Ab	Cultivated	0.00	±	0.00	a
W126	Ad	Cultivated	0.00	±	0.00	a
W126	Ab	Cultivated	0.00	±	0.00	a
Z161	Ad	Hybrid	0.00	±	0.00	a
Z161	Ab	Hybrid	0.00	±	0.00	a
Z58	Ad	Hybrid	0.00	±	0.00	a
Z58	Ab	Hybrid	0.00	±	0.00	a
Z70	Ad	Hybrid	0.00	±	0.00	a
Z70	Ab	Hybrid	0.00	±	0.00	a

Table 3–9: ANOVA model results for number of leaflets infested by mites on day 1 and day 2 for 15 tomato genotypes tested in the whole leaf bioassay.

Source of Variation	DF	Number of leaflets infested by mites on day 1		Number of leaflets infested by mites on day 2	
		F value	P value	F value	P value
Genotype	14	9.87	<.0001	5.14	<.0001
Error	30				
R^2			0.82		0.71

Table 3–10: ANOVA model results for number of leaflet surfaces infested by mites on day 1 and day 2 for adaxial and abaxial leaflet surfaces of 15 tomato genotypes tested in the whole leaf bioassay.

Source of Variations	DF	Number of leaflet surfaces infested by mites on day 1		Number of leaflet surfaces infested by mites on day 2	
		F value	P value	F value	P value
Genotype	14	12.58	<.0001	10.79	<.0001
Surface	1	75.03	<.0001	65.96	<.0001
Genotype X Surface	14	2.31	0.013	2.05	0.028
Error	60				
R^2			0.83		0.80

Table 3–11: Means of number of leaflets infested by mites on day 1 for 15 tomato genotypes tested in the whole leaf bioassay. Means followed by the same letters are not different based on Tukey's test (*P*=0.05). Standard error of the mean denoted by SE.

Genotype	Background	Average number of leaflets infested by mites on day 1			
		No. of Leaflets	±	SE	
X166	Hybrid	1.00	±	0.00	d
PI127826	Wild relative	1.00	±	0.00	d
LA2329OH	Wild relative	1.33	±	0.33	cd
LA2329	Wild relative	2.00	±	0.00	bcd
Z120	Hybrid	2.33	±	0.33	bcd
W75	Hybrid	2.33	±	0.33	bcd
Z70	Hybrid	2.67	±	0.33	abcd
X71	Hybrid	2.67	±	0.33	abcd
X155	Hybrid	2.67	±	0.33	abcd
Z58	Hybrid	3.00	±	0.00	abc
Z116	Hybrid	3.33	±	0.33	ab
Z161	Hybrid	3.67	±	0.67	ab
W160	Cultivated	3.67	±	0.33	ab
W129	Cultivated	3.67	±	0.33	ab
W126	Cultivated	4.33	±	0.33	a

Table 3–12: Means of number of leaflets infested by mites on day 2 for 15 tomato genotypes tested in the whole leaf bioassay. Means followed by the same letters are not different based on Tukey's test (*P*=0.05). Standard error of the mean denoted by SE.

Genotype	Background	No. of Leaflets	±	SE	
		Average number of leaflets infested by mites on day 2			
LA2329OH	Wild relative	1.33	±	0.33	d
PI127826	Wild relative	2.00	±	0.58	cd
X166	Hybrid	2.33	±	0.33	bcd
LA2329	Wild relative	2.33	±	0.33	bcd
X71	Hybrid	3.33	±	0.33	abcd
X155	Hybrid	3.33	±	0.33	abcd
Z70	Hybrid	3.67	±	0.33	abc
Z120	Hybrid	3.67	±	0.88	abc
Z116	Hybrid	4.00	±	0.58	abc
W75	Hybrid	4.00	±	0.58	abc
Z58	Hybrid	4.33	±	0.33	ab
W160	Cultivated	4.33	±	0.33	ab
W129	Cultivated	4.33	±	0.33	ab
W126	Cultivated	4.33	±	0.33	ab
Z161	Hybrid	4.67	±	0.33	a

Table 3–13: Means of the number of leaflet surfaces infested by mites on day 1 for 15 tomato genotypes tested in the whole leaf bioassay. Means followed by the same letters are not different based on Tukey's test (*P*=0.05). Standard error of the mean denoted by SE.

Genotype	Background	Average number of leaflet surfaces infested by mites on day 1			
		No. of Surfaces	±	SE	
PI127826	Wild relative	0.83	±	0.17	g
X166	Hybrid	1.17	±	0.17	fg
LA2329OH	Wild relative	1.33	±	0.21	fg
W75	Hybrid	1.50	±	0.34	efg
Z120	Hybrid	1.83	±	0.31	defg
LA2329	Wild relative	1.83	±	0.17	defg
Z116	Hybrid	2.17	±	0.54	cdef
X71	Hybrid	2.17	±	0.31	cdef
X155	Hybrid	2.17	±	0.54	cdef
Z70	Hybrid	2.33	±	0.33	bcdef
W126	Cultivated	2.67	±	0.33	abcde
Z161	Hybrid	3.00	±	0.37	abcd
W160	Cultivated	3.33	±	0.42	abc
W129	Cultivated	3.50	±	0.43	ab
Z58	Hybrid	3.67	±	0.49	a

Table 3–14: Means of the number of leaflet surfaces infested by mites on day 2 for 15 tomato genotypes tested in the whole leaf bioassay. Means followed by the same letters are not different based on Tukey's test (P=0.05). Standard error of the mean denoted by SE.

Genotype	Background	Average number of leaflet surfaces infested by mites on day 2			
		No. of Surfaces	±	SE	
PI127826	Wild relative	1.33	±	0.33	f
X166	Hybrid	1.67	±	0.33	ef
LA2329OH	Wild relative	2.00	±	0.37	def
LA2329	Wild relative	2.17	±	0.31	cdef
W75	Hybrid	2.33	±	0.49	cdef
Z120	Hybrid	2.83	±	0.54	bcdef
X71	Hybrid	3.17	±	0.60	abcde
X155	Hybrid	3.17	±	0.70	abcde
Z70	Hybrid	3.33	±	0.33	abcd
Z161	Hybrid	3.50	±	0.43	abcd
Z116	Hybrid	3.67	±	0.49	abc
Z58	Hybrid	4.00	±	0.52	ab
W129	Cultivated	4.33	±	0.33	ab
W126	Cultivated	4.33	±	0.33	ab
W160	Cultivated	4.50	±	0.22	a

Table 3–15: Means of the number of surfaces for adaxial and abaxial leaflet surfaces infested by mites on day 1 and day 2 respectively for 15 tomato genotypes tested in the whole leaf bioassay. Means followed by the same letters are not different based on Tukey's test (P=0.05). Standard error of the mean denoted by SE.

Surface	Average number of leaflet surfaces infested by mites on day 1				Average number of leaflet surface infested by mites on day 2			
	Mean	±	SE		Mean	±	SE	
Adaxial	2.78	±	0.18	a	3.73	±	0.19	a
Abaxial	1.69	±	0.12	b	2.44	±	0.18	b

Table 3–16: Means of the number of leaflet surfaces infested by mites for adaxial (Ad) and abaxial (Ab) leaflet surfaces of 15 tomato genotypes tested on day 1 in the whole leaf bioassay. Means within genotypes followed by the same letter are not statistically different as determined by Lsmeans at (*P*=0.05). Standard error of the mean denoted by SE.

Genotype	Surface	Background	Average number of leaflet surfaces infested by mites on day 1		
			Surface No.	± SE	
PI127826	Ad	Wild relative	1.00	± 0.00	a
PI127826	Ab	Wild relative	0.67	± 0.33	a
X166	Ad	Hybrid	1.33	± 0.33	a
X166	Ab	Hybrid	1.00	± 0.00	a
LA2329OH	Ad	Wild relative	1.33	± 0.33	a
LA2329OH	Ab	Wild relative	1.33	± 0.33	a
W75	Ad	Hybrid	2.00	± 0.58	a
W75	Ab	Hybrid	1.00	± 0.00	b
Z120	Ad	Hybrid	2.33	± 0.33	a
Z120	Ab	Hybrid	1.33	± 0.33	b
LA2329	Ad	Wild relative	2.00	± 0.00	a
LA2329	Ab	Wild relative	1.67	± 0.33	a

Table 3-16 (continued)

Genotype	Surface	Background	Average number of leaflet surfaces infested by mites on day 1		
			Surface No.	±	SE
Z116	Ad	Hybrid	3.33	±	0.33 a
Z116	Ab	Hybrid	1.00	±	0.00 a
X71	Ad	Hybrid	2.67	±	0.33 a
X71	Ab	Hybrid	1.67	±	0.33 b
X155	Ad	Hybrid	3.33	±	0.33 a
X155	Ab	Hybrid	1.00	±	0.00 a
Z70	Ad	Hybrid	2.67	±	0.33 a
Z70	Ab	Hybrid	2.00	±	0.58 a
W126	Ad	Cultivated	3.00	±	0.58 a
W126	Ab	Cultivated	2.33	±	0.33 a
Z161	Ad	Hybrid	3.67	±	0.33 a
Z161	Ab	Hybrid	2.33	±	0.33 b
W160	Ad	Cultivated	4.00	±	0.58 a
W160	Ab	Cultivated	2.67	±	0.33 b
W129	Ad	Cultivated	4.33	±	0.33 a
W129	Ab	Cultivated	2.67	±	0.33 b
Z58	Ad	Hybrid	4.67	±	0.33 a
Z58	Ab	Hybrid	2.67	±	0.33 b

Table 3–17: Means of the number of leaflet surfaces infested by mites for adaxial (Ad) and abaxial (Ab) leaflet surfaces of 15 tomato genotypes tested on day 2 in the whole leaf bioassay. Means within genotypes followed by the same letter are not statistically different as determined by Lsmeans at (*P*=0.05). Standard error of the mean denoted by SE.

Genotype	Surface	Background	Surface No.	±	SE	
			Average number of leaflet surfaces infested by mites on day 2			
PI127826	Ad	Wild relative	1.67	±	0.33	a
PI127826	Ab	Wild relative	1.00	±	0.58	a
X166	Ad	Hybrid	2.33	±	0.33	a
X166	Ab	Hybrid	1.00	±	0.00	b
LA2329OH	Ad	Wild relative	2.00	±	0.58	a
LA2329OH	Ab	Wild relative	2.00	±	0.58	a
LA2329	Ad	Wild relative	2.33	±	0.33	a
LA2329	Ab	Wild relative	2.00	±	0.58	a
W75	Ad	Hybrid	3.33	±	0.33	a
W75	Ab	Hybrid	1.33	±	0.33	b
Z120	Ad	Hybrid	3.67	±	0.88	a
Z120	Ab	Hybrid	2.00	±	0.00	b
X71	Ad	Hybrid	4.33	±	0.33	a
X71	Ab	Hybrid	2.00	±	0.58	b

Table 3-17 (continued)

| | | | Average number of leaflet surfaces infested by mites on day 2 | | |
Genotype	Surface	Background	Surface No.	±	SE
X155	Ad	Hybrid	4.67	±	0.33 a
X155	Ab	Hybrid	1.67	±	0.33 b
Z70	Ad	Hybrid	3.67	±	0.33 a
Z70	Ab	Hybrid	3.00	±	0.58 a
Z161	Ad	Hybrid	4.33	±	0.33 a
Z161	Ab	Hybrid	2.67	±	0.33 b
Z116	Ad	Hybrid	4.67	±	0.33 a
Z116	Ab	Hybrid	2.67	±	0.33 b
Z58	Ad	Hybrid	5.00	±	0.00 a
Z58	Ab	Hybrid	3.00	±	0.58 b
W129	Ad	Cultivated	4.67	±	0.33 a
W129	Ab	Cultivated	4.00	±	0.58 a
W126	Ad	Cultivated	4.67	±	0.33 a
W126	Ab	Cultivated	4.00	±	0.58 a
W160	Ad	Cultivated	4.67	±	0.33 a
W160	Ab	Cultivated	4.33	±	0.33 a

Table 3–18: ANOVA model results for average mite webbing score on day 3 and day 7 for adaxial and abaxial leaflet surfaces of 15 tomato genotypes tested in the whole leaf bioassay.

Source of Variations	DF	Mite webbing score on day 3		Mite webbing score on day 7	
		F value	P value	F value	P value
Genotype	14	12.56	<.0001	14.75	<.0001
Surface	1	9.09	0.0038	1.39	0. 243ns
Genotype X Surface	14	0.71	0.751ns	2.22	0. 0169
Error	60				
R^2			0.76		0.80

No significant difference indicated by ns.

Table 3–19: ANOVA model results for the number of leaflet surfaces with mite webbing score on day 3 and day 7 for adaxial and abaxial leaflet surfaces of 15 tomato genotypes tested in the whole leaf bioassay.

Source of Variations	DF	Number of leaflet surfaces with mite webbing on day 3		Number of leaflet surfaces with mite webbing on day 7	
		F value	P value	F value	P value
Genotype	14	11.63	<.0001	8.87	<.0001
Surface	1	4.03	0.049	5.58	0.0214
Genotype X Surface	14	0.96	0.501ns	1.51	0.134ns
Error	60				
R^2		0.75			0.72

No significant difference indicated by ns.

Table 3–20: Means of mite webbing score on day 3 for 15 tomato genotypes tested in the whole leaf bioassay. Means followed by the same letters are not different based on Tukey's test (*P*=0.05). Standard error of the mean denoted by SE.

Genotype	Background	Average mite webbing score on day 3			
		Score (0-3)	±	SE	
PI127826	Wild relative	0.00	±	0.00	d
LA2329OH	Wild relative	0.00	±	0.00	d
LA2329	Wild relative	0.00	±	0.00	d
Z116	Hybrid	0.00	±	0.00	d
X71	Hybrid	0.00	±	0.00	d
X166	Hybrid	0.00	±	0.00	d
Z120	Hybrid	0.17	±	0.17	cd
X155	Hybrid	0.17	±	0.17	cd
W75	Hybrid	0.17	±	0.17	cd
Z161	Hybrid	0.17	±	0.17	cd
Z58	Hybrid	0.67	±	0.21	bcd
Z70	Hybrid	0.67	±	0.21	bcd
W160	Cultivated	0.83	±	0.17	abc
W126	Cultivated	1.33	±	0.21	ab
W129	Cultivated	1.50	±	0.22	a

Table 3–21: Means of mite webbing score on day 7 for 15 tomato genotypes tested in the whole leaf bioassay. Means followed by the same letters are not different based on Tukey's test (*P*=0.05). Standard error of the mean denoted by SE.

Genotype	Background	Average mite webbing score on day 7			
		Score (0-3)	±	SE	
PI127826	Wild relative	0.33	±	0.21	e
X155	Hybrid	0.50	±	0.22	d
LA2329OH	Wild relative	0.67	±	0.21	cde
Z116	Hybrid	0.67	±	0.21	cde
X166	Hybrid	0.67	±	0.21	cde
Z161	Hybrid	1.00	±	0.00	cde
Z120	Hybrid	1.00	±	0.00	cde
Z70	Hybrid	1.00	±	0.00	cde
W75	Hybrid	1.00	±	0.26	cde
LA2329	Wild relative	1.00	±	0.26	cde
X71	Hybrid	1.33	±	0.21	bcd
Z58	Hybrid	1.50	±	0.22	bc
W160	Cultivated	2.00	±	0.26	ab
W129	Cultivated	2.50	±	0.22	a
W126	Cultivated	2.67	±	0.21	a

Table 3–22: Means of mite webbing score on day 3 for adaxial and abaxial leaflet surfaces of 15 tomato genotypes tested in the whole leaf bioassay. Means followed by the same letters are not different based on Tukey's test (P=0.05). Standard error of the mean denoted by SE.

Average mite webbing score on day 3			
Surface	Score (0-3)	±	SE
Adaxial	0.48	±	0.10 a
Abaxial	0.26	±	0.07 b

Table 3–23: Means of mite webbing scores on day 7 for adaxial (Ad) and abaxial (Ab) leaflet surfaces of 15 tomato genotypes tested in the whole leaf bioassay. Means within genotypes followed by the same letter are not statistically different as determined by Lsmeans at (P=0.05). Standard error of the mean denoted by SE.

Genotype	Surface	Background	Score (0-3)	±	SE	
			Average mite webbing score on day 7			
PI127826	Ad	Wild relative	0.33	±	0.33	a
PI127826	Ab	Wild relative	0.33	±	0.33	a
X155	Ad	Hybrid	1.00	±	0.00	a
X155	Ab	Hybrid	0.00	±	0.00	b
LA2329OH	Ad	Wild relative	1.00	±	0.00	a
LA2329OH	Ab	Wild relative	0.33	±	0.33	b
Z116	Ad	Hybrid	1.00	±	0.00	a
Z116	Ab	Hybrid	0.33	±	0.33	b
X166	Ad	Hybrid	0.67	±	0.33	a
X166	Ab	Hybrid	0.67	±	0.33	a
Z161	Ad	Hybrid	1.00	±	0.00	a
Z161	Ab	Hybrid	1.00	±	0.00	a
Z120	Ad	Hybrid	1.00	±	0.00	a
Z120	Ab	Hybrid	1.00	±	0.00	a

Table 3-23 (continued)

Genotype	Surface	Background	Average mite webbing score on day 7		
			Score (0-3)	±	SE
Z70	Ad	Hybrid	1.00	±	0.00 a
Z70	Ab	Hybrid	1.00	±	0.00 a
W75	Ad	Hybrid	1.33	±	0.33 a
W75	Ab	Hybrid	0.67	±	0.33 b
LA2329	Ad	Wild relative	1.33	±	0.33 a
LA2329	Ab	Wild relative	0.67	±	0.33 b
X71	Ad	Hybrid	1.33	±	0.33 a
X71	Ab	Hybrid	1.33	±	0.33 a
Z58	Ad	Hybrid	1.00	±	0.00 a
Z58	Ab	Hybrid	2.00	±	0.00 b
W160	Ad	Cultivated	1.67	±	0.33 a
W160	Ab	Cultivated	2.33	±	0.33 a
W129	Ad	Cultivated	2.33	±	0.33 a
W129	Ab	Cultivated	2.67	±	0.33 a
W126	Ad	Cultivated	2.67	±	0.33 a
W126	Ab	Cultivated	2.67	±	0.33 a

Table 3–24: Means of the number of leaflet surfaces with mite webbing on day 3 for 15 tomato genotypes tested in the whole leaf bioassay. Means followed by the same letters are not different based on Tukey's test (*P*=0.05). Standard error of the mean denoted by SE.

Genotype	Background	Average number of leaflet surfaces with mite webbing on day 3			
		Surface No.	±	SE	
PI127826	Wild relative	0.00	±	0.00	d
LA2329OH	Wild relative	0.00	±	0.00	d
LA2329	Wild relative	0.00	±	0.00	d
Z116	Hybrid	0.00	±	0.00	d
X71	Hybrid	0.00	±	0.00	d
X166	Hybrid	0.00	±	0.00	d
Z120	Hybrid	0.17	±	0.17	cd
X155	Hybrid	0.17	±	0.17	cd
W75	Hybrid	0.17	±	0.17	cd
Z161	Hybrid	0.33	±	0.33	cd
Z58	Hybrid	1.00	±	0.37	bcd
Z70	Hybrid	1.00	±	0.37	bcd
W160	Cultivated	1.33	±	0.33	bc
W126	Cultivated	1.67	±	0.33	ab
W129	Cultivated	2.67	±	0.42	a

Table 3–25: Means of the number of leaflet surfaces with mite webbing on day 7 for 15 tomato genotypes tested in the whole leaf bioassay. Means followed by the same letters are not different based on Tukey's test (*P*=0.05). Standard error of the mean denoted by SE.

Genotype	Background	Average number of leaflet surfaces with mite webbing on day 7			
		Surface No.	±	SE	
PI127826	Wild relative	0.33	±	0.21	d
LA2329OH	Wild relative	0.83	±	0.31	cd
X155	Hybrid	1.17	±	0.65	cd
X166	Hybrid	1.33	±	0.49	cd
LA2329	Wild relative	1.50	±	0.43	cd
W75	Hybrid	1.50	±	0.43	cd
Z116	Hybrid	1.83	±	0.60	cd
Z70	Hybrid	2.17	±	0.40	bcd
Z120	Hybrid	2.33	±	0.33	abc
X71	Hybrid	2.33	±	0.33	abc
Z161	Hybrid	2.33	±	0.33	abc
Z58	Hybrid	2.50	±	0.34	abc
W126	Cultivated	3.83	±	0.30	ab
W160	Cultivated	4.00	±	0.37	ab
W129	Cultivated	4.16	±	0.40	a

Table 3–26: Means of the number of leaflet surfaces with mite webbing on day 3 and day 7 for adaxial and abaxial leaflet surfaces of 15 tomato genotypes tested in the whole leaf bioassay. Means followed by the same letters are not different based on Tukey's test (*P*=0.05). Standard error of the mean denoted by SE.

Surface	Average number of leaflet surfaces with mite webbing on day 3				Average number of leaflet surfaces with mite webbing on day 7			
	Surface No.	±	SE		Surface No.	±	SE	
Adaxial	0.69	±	0.15	a	2.38	±	0.18	a
Abaxial	0.44	±	0.13	b	1.91	±	0.24	b

Table 3–27: ANOVA model results for mite feeding damage score on day 3 and day 7 for adaxial and abaxial leaflet surfaces of 15 tomato genotypes tested in the whole leaf bioassay.

Source of Variations	DF	Mite feeding damage score on day 3		Mite feeding damage score on day 7	
		F value	P value	F value	P value
Genotype	14	4.14	<.0001	10.94	<.0001
Surface	1	10.32	0.0021	8.00	0.0064
Genotype X Surface	14	1.07	0.403ns	4.67	<.0001
Error	60				
R^2			0.58		0.79

No significant difference indicated by ns.

Table 3–28: Means of mite feeding damage score on day 3 for 15 tomato genotypes tested in the whole leaf bioassay. Means followed by the same letters are not different based on Tukey's test (P=0.05). Standard error of the mean denoted by SE.

Genotype	Background	Average mite feeding damage score on day 3			
		Score (0-3)	±	SE	
PI127826	Wild relative	0.00	±	0.00	c
X71	Hybrid	0.00	±	0.00	c
X166	Hybrid	0.00	±	0.00	c
LA2329OH	Wild relative	0.17	±	0.17	bc
Z70	Hybrid	0.17	±	0.17	bc
Z120	Hybrid	0.17	±	0.17	bc
X155	Hybrid	0.17	±	0.17	bc
Z116	Hybrid	0.50	±	0.22	abc
LA2329	Wild relative	0.50	±	0.34	abc
Z58	Hybrid	0.67	±	0.33	abc
Z161	Hybrid	0.67	±	0.21	abc
W75	Hybrid	0.67	±	0.33	abc
W129	Cultivated	0.83	±	0.17	abc
W126	Cultivated	1.00	±	0.00	ab
W160	Cultivated	1.17	±	0.17	a

Table 3–29: Means of mite feeding damage score on day 7 for 15 tomato genotypes tested in the whole leaf bioassay. Means followed by the same letters are not different based on Tukey's test (*P*=0.05). Standard error of the mean denoted by SE.

Genotype	Background	Average mite feeding damage score on day 7			
		Score (0-3)	±	SE	
X155	Hybrid	0.50	±	0.22	f
PI127826	Wild relative	0.67	±	0.21	ef
LA2329OH	Wild relative	0.83	±	0.17	def
X71	Hybrid	0.83	±	0.17	def
X166	Hybrid	0.83	±	0.17	def
Z120	Hybrid	1.00	±	0.00	def
Z116	Hybrid	1.00	±	0.00	def
Z70	Hybrid	1.17	±	0.16	cdef
LA2329	Wild relative	1.33	±	0.21	cdef
Z58	Hybrid	1.50	±	0.22	bcde
W75	Hybrid	1.50	±	0.43	bcde
Z161	Hybrid	1.67	±	0.33	abcd
W126	Cultivated	2.00	±	0.37	abc
W160	Cultivated	2.33	±	0.33	ab
W129	Cultivated	2.50	±	0.22	a

Table 3–30: Means of mite feeding damage score on day 3 and day 7 for adaxial and adaxial leaflet surfaces of 15 tomato genotypes tested in the whole leaf bioassay. Means followed by the same letters are not different based on Tukey's test (P=0.05). Standard error of the mean denoted by SE.

Surface	Average mite feeding damage score on day 3			Average mite feeding damage score on day 7		
	Score (0-3)	±	SE	Score (0-3)	±	SE
Adaxial	0.60	±	0.10 a	1.44	±	0.10 a
Abaxial	0.29	±	0.06 b	1.18	±	0.13 b

Table 3–31: Means of mite feeding damage score for the adaxial (Ad) and abaxial (Ab) leaflet surfaces of 15 tomato genotypes tested on day 7 in the whole leaf bioassay. Means within genotypes followed by the same letter are not statistically different as determined by Lsmeans at (P=0.05). Standard error of the mean denoted by SE.

			Average mite feeding damage score on day 7		
Genotype	Surface	Background	Score (0-3)	±	SE
X155	Ad	Hybrid	1.00	±	0.00 a
X155	Ab	Hybrid	0.00	±	0.00 b
PI127826	Ad	Wild relative	0.67	±	0.33 a
PI127826	Ab	Wild relative	0.67	±	0.33 a
LA2329OH	Ad	Wild relative	1.00	±	0.00 a
LA2329OH	Ab	Wild relative	0.67	±	0.33 a
X71	Ad	Hybrid	1.00	±	0.00 a
X71	Ab	Hybrid	0.67	±	0.33 a
X166	Ad	Hybrid	1.00	±	0.00 a
X166	Ab	Hybrid	0.67	±	0.33 a
Z120	Ad	Hybrid	1.00	±	0.00 a
Z120	Ab	Hybrid	1.00	±	0.00 a
Z116	Ad	Hybrid	1.00	±	0.00 a
Z116	Ab	Hybrid	1.00	±	0.00 a

Table 3-31 (continued)

			Average mite feeding damage score on day 7			
Genotype	**Surface**	**Background**	**Score (0-3)**	**±**	**SE**	
Z70	Ad	Hybrid	1.00	±	0.00	a
Z70	Ab	Hybrid	1.33	±	0.33	a
LA2329	Ad	Wild relative	1.67	±	0.33	a
LA2329	Ab	Wild relative	1.00	±	0.00	a
Z58	Ad	Hybrid	2.00	±	0.00	a
Z58	Ab	Hybrid	1.00	±	0.00	b
W75	Ad	Hybrid	2.33	± 0.33		a
W75	Ab	Hybrid	0.67	± 0.33		b
Z161	Ad	Hybrid	2.33	± 0.33		a
Z161	Ab	Hybrid	1.00	± 0.00		b
W126	Ad	Cultivated	1.33	± 0.33		a
W126	Ab	Cultivated	2.67	± 0.33		b
W160	Ad	Cultivated	2.00	± 0.58		a
W160	Ab	Cultivated	2.67	± 0.33		a
W129	Ad	Cultivated	2.33	± 0.33		a
W129	Ab	Cultivated	2.67	± 0.33		a

Table 3–32: ANOVA model results for the number of leaflet surfaces with mite feeding damage on day 3 and day 7 for adaxial and abaxial leaflet surfaces of 15 tomato genotypes tested in the whole leaf bioassay.

Source of Variations	DF	Number of leaflet surfaces with feeding damage on day 3		Number of leaflet surfaces with feeding damage on day 7	
		F value	P value	F value	P value
Genotype	14	5.75	<.0001	7.99	<.0001
Surface	1	14.05	0.0004	15.80	0.0002
Genotype X Surface	14	1.51	0.137ns	1.48	0.145ns
Error	60				
R^2			0.66		0.71

No significant difference indicated by ns.

Table 3–33: Means of the number of leaflet surfaces with mite feeding damage on day 3 for 15 tomato genotypes tested in the whole leaf bioassay. Means followed by the same letters are not different based on Tukey's test (P=0.05). Standard error of the mean denoted by SE.

		Average number of leaflet surfaces with feeding damage on day 3			
Genotype	Background	Surface No.	±	SE	
PI127826	Wild relative	0.00	±	0.00	d
X71	Hybrid	0.00	±	0.00	d
X166	Hybrid	0.00	±	0.00	d
LA2329OH	Wild relative	0.17	±	0.17	cd
X155	Hybrid	0.17	±	0.17	cd
Z120	Hybrid	0.17	±	0.17	cd
Z70	Hybrid	0.17	±	0.17	cd
LA2329	Wild relative	0.33	±	0.21	bcd
W75	Hybrid	0.67	±	0.33	abcd
Z116	Hybrid	1.00	±	0.52	abcd
Z58	Hybrid	1.17	±	0.48	abcd
Z161	Hybrid	1.17	±	0.48	abcd
W160	Cultivated	1.50	±	0.22	abc
W129	Cultivated	1.67	±	0.49	ab
W126	Cultivated	1.83	±	0.40	a

Table 3–34: Means of the number of leaflet surfaces with mite feeding damage on day 7 for 15 tomato genotypes tested in the whole leaf bioassay. Means followed by the same letters are not different based on Tukey's test (P=0.05). Standard error of the mean denoted by SE.

Genotype	Background	Average number of leaflet surfaces with feeding damage on day 7			
		Surface No.	±	SE	
PI127826	Wild relative	1.00	±	0.37	f
X155	Hybrid	1.17	±	0.54	ef
LA2329OH	Wild relative	1.50	±	0.34	ef
W75	Hybrid	1.83	±	0.48	def
LA2329	Wild relative	2.00	±	0.26	def
Z70	Hybrid	2.00	±	0.26	def
X71	Hybrid	2.17	±	0.65	cdef
X166	Hybrid	2.17	±	0.79	cdef
Z120	Hybrid	2.50	±	0.50	bcdef
Z58	Hybrid	3.00	±	0.37	abcde
Z161	Hybrid	3.00	±	0.26	abcde
Z116	Hybrid	3.67	±	0.42	abcd
W126	Cultivated	4.00	±	0.37	abc
W129	Cultivated	4.17	±	0.31	ab
W160	Cultivated	4.50	±	0.34	a

Table 3–35: Means of the number of leaflet surfaces with mite feeding damage on day 3 and day 7 for adaxial and abaxial leaflet surfaces of 15 tomato genotypes tested in the whole leaf bioassay. Means followed by the same letters are not different based on Tukey's test (*P*=0.05). Standard error of the mean denoted by SE.

Surface	Average number of leaflet surfaces with feeding damage on day 3			Average number of leaflet surfaces with feeding damage on day 7		
	Surface No.	± SE		Surface No.	± SE	
Adaxial	0.93	± 0.16	a	2.97	± 0.19	a
Abaxial	0.40	± 0.10	b	2.18	± 0.23	b

Table 3–36: ANOVA model results for the number of leaflet surfaces with mite eggs and egg density on day 7 for adaxial and abaxial leaflet surfaces of 15 tomato genotypes tested in the whole leaf bioassay.

| Source of Variations | DF | Number of leaflet surfaces infested by mite eggs on day 7 | | Egg density on day 7 (Egg No./cm^2 leaf area) | |
		F value	P value	F value	P value
Genotype	14	2.84	0.0026	68.83	<.0001
Surface	1	0.76	0.387ns	331.45	<.0001
Genotype X Surface	14	0.89	0.575ns	10.40	<.0001
Error	60				
R^2			0.47		0.96

No significant difference indicated by ns.

Table 3–37: Means of the number of leaflet surfaces with mite eggs on day 7 for 15 tomato genotypes tested in the whole leaf bioassay. Means followed by the same letters are not different based on Tukey's test (*P*=0.05). Standard error of the mean denoted by SE.

Genotype	Background	Average number of leaflet surfaces infested by mite eggs on day 7			
		Surface No.	±	SE	
PI127826	Wild relative	3.50	±	0.22	c
LA2329OH	Wild relative	4.00	±	0.37	ab
X166	Hybrid	4.30	±	0.33	ab
X155	Hybrid	4.50	±	0.50	ab
X71	Hybrid	4.50	±	0.34	a
W75	Hybrid	4.67	±	0.21	ab
W129	Cultivated	4.67	±	0.21	a
W160	Cultivated	4.67	±	0.21	a
LA2329	Wild relative	4.67	±	0.21	ab
W126	Cultivated	4.83	±	0.17	a
Z70	Hybrid	4.83	±	0.17	ab
Z120	Hybrid	5.00	±	0.00	a
Z116	Hybrid	5.00	±	0.00	a
Z58	Hybrid	5.00	±	0.00	a
Z161	Hybrid	5.00	±	0.00	a

Table 3–38: Means of egg density (No./cm^2 leaf area) on day 7 for 15 tomato genotypes tested in the whole leaf bioassay. Means followed by the same letters are not different based on Tukey's test (P=0.05). Standard error of the mean denoted by SE.

		Average egg density on day 7			
Genotype	Background	(No./cm^2 leaf area)	±	SE	
PI127826	Wild relative	0.51	±	0.15	e
LA2329OH	Wild relative	0.75	±	0.24	e
X71	Hybrid	1.16	±	0.21	e
X166	Hybrid	1.16	±	0.34	e
W75	Hybrid	1.51	±	0.36	e
LA2329	Wild relative	1.64	±	0.31	e
X155	Hybrid	1.81	±	0.3	e
Z116	Hybrid	3.38	±	0.69	d
Z70	Hybrid	3.64	±	0.87	d
Z161	Hybrid	3.89	±	0.5	cd
Z120	Hybrid	5.12	±	0.92	bc
Z58	Hybrid	5.4	±	1.42	b
W126	Cultivated	5.54	±	0.74	b
W160	Cultivated	6.03	±	1.14	ab
W129	Cultivated	7.16	±	0.85	a

Table 3–39: Means of egg density (No./cm^2 leaf area) on day 7 for adaxial and abaxial leaflet surfaces of 15 tomato genotypes tested in the whole leaf bioassay. Means followed by the same letters are not different based on Tukey's test (P=0.05). Standard error of the mean denoted by SE.

Average egg density on day 7			
Surface	**(No./cm^2 leaf area)**	**±**	**SE**
Adaxial	4.49	±	0.44 a
Abaxial	2.01	±	0.23 b

Table 3–40: Means of egg density (No./cm^2 leaf area) for adaxial (Ad) and abaxial (Ab) leaflet surfaces of 15 tomato genotypes tested on day 7 in the whole leaf bioassay. Means within genotypes followed by the same letter are not statistically different as determined by Lsmeans at (P=0.05). Standard error of the mean denoted by SE.

			Average egg density on day 7			
Genotype	Surface	Background	(No./cm^2 leaf area)	±	SE	
PI127826	Ad	Wild relative	0.82	±	0.15	a
PI127826	Ab	Wild relative	0.21	±	0.05	a
LA2329OH	Ad	Wild relative	1.21	±	0.26	a
LA2329OH	Ab	Wild relative	0.28	±	0.05	a
X71	Ad	Hybrid	1.57	±	0.14	a
X71	Ab	Hybrid	0.74	±	0.14	a
X166	Ad	Hybrid	1.91	±	0.14	a
X166	Ab	Hybrid	0.41	±	0.05	b
W75	Ad	Hybrid	2.16	±	0.48	a
W75	Ab	Hybrid	0.86	±	0.08	b
LA2329	Ad	Wild relative	2.26	±	0.27	a
LA2329	Ab	Wild relative	1.01	±	0.09	b
X155	Ad	Hybrid	2.27	±	0.15	a
X155	Ab	Hybrid	1.35	±	0.47	a
Z116	Ad	Hybrid	4.89	±	0.35	a
Z116	Ab	Hybrid	1.88	±	0.16	b
Z70	Ad	Hybrid	5.32	±	0.89	a
Z70	Ab	Hybrid	1.97	±	0.43	b

Table 3-40 (continued)

Genotype	Surface	Background	Average egg density on day 7			
			(No./cm² leaf area)	±	SE	
Z161	Ad	Hybrid	4.87	±	0.51	a
Z161	Ab	Hybrid	2.90	±	0.10	b
Z120	Ad	Hybrid	7.07	±	0.69	a
Z120	Ab	Hybrid	3.18	±	0.04	b
Z58	Ad	Hybrid	8.57	±	0.28	a
Z58	Ab	Hybrid	2.23	±	0.15	b
W126	Ad	Cultivated	6.99	±	0.63	a
W126	Ab	Cultivated	4.08	±	0.49	b
W160	Ad	Cultivated	8.54	±	0.33	a
W160	Ab	Cultivated	3.51	±	0.25	b
W129	Ad	Cultivated	8.86	±	0.79	a
W129	Ab	Cultivated	5.46	±	0.25	b

Table 3–41: Correlation matrix among total trichome type IV density, zingiberene, and monoterpene parameters and biological and behavioral mite variables (combined by surface) obtained from the whole leaf bioassay.

Variables	Total IV Density	Zingiberene Concentration	β-phellandrene Concentration
Leaflet number infested by mites-day 1	-0.57***	-0.35*	0.19
Leaflet number infested by mites-day 2	-0.45**	-0.35*	0.11
Total number of surfaces infested by mites-day 1	-0.64***	-0.32*	0.01
Total number of surfaces infested by mites-day 2	-0.61***	-0.35*	0.22
Total mite webbing score-day 3	-0.50***	-0.40*	-0.07
Total mite webbing score-day 7	-0.54***	-0.17	-0.29*
Total number of surfaces with webbing-day 3	-0.48***	-0.38*	-0.10
Total number of surfaces with webbing-day 7	-0.62***	-0.27	-0.10
Total mite feeding damage score-day 3	-0.48**	-0.37*	-0.02
Total mite feeding damage score-day 7	-0.58***	-0.33*	-0.22
Total number of surfaces with feeding damage-day 3	-0.48***	-0.44*	-0.01
Total number of surfaces with feeding damage-day 7	-0.56***	-0.39**	-0.02
Total number of surfaces with eggs-day 7	-0.52***	-0.19	0.15
Total egg density-day 7	-0.67***	-0.58***	0.03

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Total IV density= total trichome type IV density (No./mm²), zingiberene and β-phellandrene concentration determined as GC area units/cm^2 of leaf area, total number of surfaces= total number of both adaxial and abaxial leaflet surfaces, total webbing and feeding damage score= total mite webbing and feeding damage score of both adaxial and abaxial leaflet surfaces, and total egg density (No./cm^2 leaf area) on both adaxial and abaxial leaflet surfaces.

Table 3–42: Cluster means for trichome type IV density and zingiberene content in tomato genotypes and mite responses from the whole leaf bioassay. All variables were combined as total by surface except for zingiberene and leaflet number infested by mites on day 1 and 2.

Cluster	Count	Tot IV Density	Zingiberene Content	Leaflet No with mites-day 1	Leaflet No with Mites-Day 2	Total Surface No. with Mites-Day 1	Total Surface No. with Mites-Day 2
1-A	3	18.17	3.5×10^7	2.33	3.22	3.67	5.11
1-B	3	11.50	4.5×10^6	2.56	3.56	4.22	6.22
1-C	3	5.11	2.4×10^6	3.33	4.33	5.89	7.44
1-D	3	32.72	1.5×10^7	1.11	1.89	2.22	3.33
2	3	0.00	0.00	3.89	4.33	6.33	8.78

Table 3-42 (continued)

Cluster	Count	Total Surface No. with Webbing-Day 3	Total Surface No. with Webbing-Day 7	Total Webbing Score-Day 3	Total Webbing Score-Day 7	Total Feeding Damage Score-Day 3
1-A	3	0.11	3.56	0.11	2.22	0.78
1-B	3	0.89	3.78	0.67	1.67	0.33
1-C	3	0.89	4.44	0.56	2.11	1.22
1-D	3	0.00	1.67	0.00	1.11	0.11
2	3	3.78	8.00	2.44	4.78	2.00

Table 3-42 (continued)

Cluster	Count	Total Feeding Damage Score- Day 7	Total Surface No. with Feeding Damage- Day 3	Total Surface No. with Feeding Damage- Day 7	Total Surface No. with Eggs- Day 7	Total Egg Density- Day 7
1-A	3	2.44	0.67	4.00	9.22	2.87
1-B	3	1.78	0.33	3.78	9.56	7.05
1-C	3	2.78	2.22	6.44	10.00	8.45
1-D	3	1.56	0.11	3.11	7.89	1.61
2	3	4.56	3.33	8.44	9.44	12.00

Total IV density= total trichome type IV density (No./mm²), zingiberene concentration determined as GC area units/cm^2 of leaf area, total number of surfaces= total number of both adaxial and abaxial leaflet surfaces, total webbing and feeding damage score= total mite webbing and feeding damage score of both adaxial and abaxial leaflet surfaces, and total egg density (No./cm^2 leaf area) on both adaxial and abaxial leaflet surfaces. Genotypes LA2329, W75, and X71 refer to cluster 1-A. Genotypes X155, Z120, and Z70 refer to cluster 1-B. Genotypes Z116, Z161, and Z58 refer to cluster 1-C. Genotypes LA2329OH, X166, and PI127826 refer to cluster 1-D. Genotypes W126, W160, and W129 refer to cluster 2. Genotypes LA2329, LA2329OH, and PI127826 are *S. habrochaites* accessions while W126, W129, W160 are *S. lycopersicum,* the reminder are interspecific backcross hybrids.

Figure 3–1: Examples of the whole leaf bioassay. A—Array of samples of the whole leaf bioassay set on the illuminated laboratory bench. B—A closeup of a bean leaf infested by spider mites in the detached tomato whole leaf.

Figure 3–2: Two-spotted spider mites *T. urticae* Koch with eggs.

https://agfax.com/2017/08/04/iowa-corn-soybeans-control-options-for-twospotted-spider-mites/
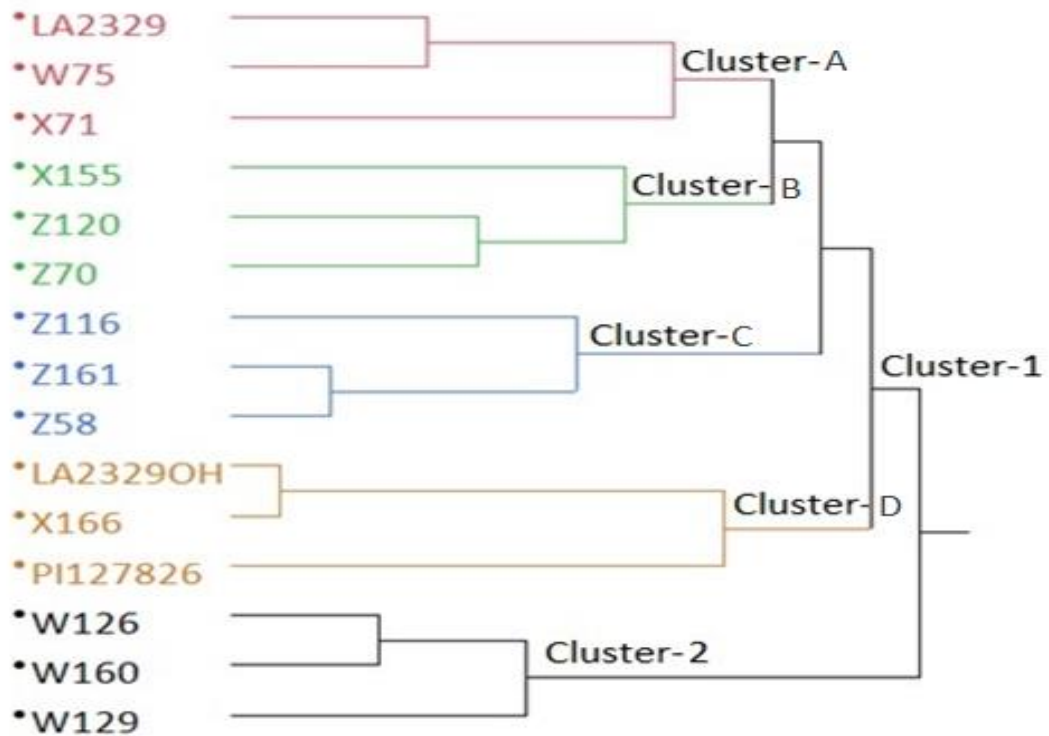
Figure 3–3: Dendrogram resulting from hierarchical cluster analysis (Ward's method) for the 15 tomato genotypes (combined data) involving type IV trichome density (No./mm^2), zingiberene concentrations (area unit of GC/cm^2 leaf area) in association with behavioral and biological variables of spider mite *T. urticae* as follow: leaflet number infested by mites on day 1 and day 2, total number of leaflet surfaces (adaxial and abaxial) infested by mites on day 1 and day 2, total number of leaflet surfaces with mite webbing on day 3 and day 7, total mite webbing score on both leaflet surfaces on day 3 and day 7, total number of leaflet surfaces with feeding damage on day 3 and day 7, total mite feeding damage score on both leaflet surfaces on day 3 and day 7, total number of leaflet surfaces infested by mite eggs on day 7, and total egg density on day 7 (No./cm^2 leaf area) on both leaflet surfaces. Genotypes LA2329, W75, and X71 refer to Cluster 1-A. Genotypes X155, Z120, and Z70 refer to Cluster 1-B. Genotypes Z116, Z161, and Z58 refer to Cluster 1-C. Genotypes LA2329OH, X166, and PI127826 refer to Cluster 1-D. Genotypes W126, W160, and W129 refer to Cluster 2. Genotypes LA2329, LA2329OH, and PI127826 are *S. habrochaites* accessions while W126, W129, W160 are *S. lycopersicum,* the reminder are interspecific backcross hybrids.

CHAPTER 4. Image-Based Spider Mite Thumbtack Bioassays-of Tomato Interspecific Hybrids

4.1   Abstract

Mite response data were obtained by time lapse photography of spider mite thumbtack bioassays. This assay can be used for tomato breeding as a screening technique to measure leaf repellence to spider mites. Interspecific backcross hybrids (BC_3F_2) derived from the cross between the wild tomato relative, *Solanum habrochaites* (LA2329), and the cultivated tomato, *Solanum lycopersicum* (ZH2) were evaluated in thumbtack bioassays. Our objectives for this research were to (a) verify genetic transfer of leaflet repellence from the wild accession to the interspecific hybrids; (b) determine the associations and relative contributions of glandular type IV and VI trichome densities and leaf chemistry to mite behavior over time. (c) evaluate image analysis as a tool for improving the efficiency for evaluating arthropod repellence among different genotypes. Results verified transfer of repellency from wild parent to advanced hybrids.

Type IV and type VI trichome densities as well as zingiberene content had a significant positive correlation with the number of spider mites remaining on the tack for both abaxial and adaxial surfaces across most time intervals. Correlation coefficients of type IV and VI trichome densities as well as zingiberene production with total distance travelled by mites had a significant negative correlation for the abaxial and adaxial surfaces across all time intervals except for type VI trichome density at some time intervals. The results of the number of mites remaining on the tack and total distance traveled by mites significantly differed among the genotypes tested at all sampling

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intervals. Generally, fewer mites remained on thumbtack in bioassays of leaves of the *S. lycopersicum* trial entries than on the interspecific hybrids and the wild donor parent (LA2329). Several backcross hybrids outperformed the wild donor parent, displaying shorter mite distance traveled on the leaves after 15 and 30 min, compared to the donor parent. Stepwise multiple regression analysis found mite repellence was likely mediated by type IV trichome density as the first crucial factor and zingiberene content as a second key factor across most time intervals. *T. urticae* were retained longer on thumbtacks with shorter movement on leaflet surfaces over time durations indicating the presence of arthropod repellence on resistant plant leaves. Altogether, our findings of mite behavior indicate that introgression of resistance from a wild tomato relative into cultivated germplasm has been successfully achieved for spider mite repellence. Image analysis for distance traveled by mite could provide reliable estimates of mite travel and may be a useful tool for digitizing parameters that could be used as one aspect of high throughput phenotypic screening.

4.2   Introduction

A number of wild tomato accessions have great potential as sources of resistance to tomato pests (Vosman et al. 2018). Cultivated tomato varieties, *S. lycopersicum,* experience a broad array of arthropods pests, including the two-spotted spider mite *Tetranychus urticae. T. urticae* can evolve rapidly, due to haplodiploid sex determination and high adaptability of mate competition (Macke et al. 2011). Female spider mites have

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short developmental times (10 to 14 days) beginning from egg to adult stage depending on environmental conditions and host plant (Hance and Van Impe 1999).

There is a need for a substitute to chemical insecticide application for mite control and tomato breeding research oriented toward investigating and developing resistant varieties should be considered as critical piece for integrated management of this pest (de Oliveira et al. 2018). Screening genetic resources of tomato for resistance to arthropods such as two spotted spider mites is needed for a resistance breeding program. Many studies have reported that wild accessions of *S. habrochaites* are remarkably resistant to a wide array of herbivorous pests (Rick 1982; Guo et al. 1993; de Azevedo et al. 2003). Often mite resistance has been associated with trichomes on wild tomato, mostly glandular ones found on leaflet surfaces (Maluf et al. 2001). Type IV trichomes are particularly important and these secretive trichomes are absent in cultivated tomato (Carter and Snyder 1985).

Zingiberene, a sesquiterpene hydrocarbon, secreted by type IV and/or VI glandular trichomes in some accessions of *S. habrochaites* f. *hirsutum*, has been associated with high levels of spider mite resistance (Freitas et al. 2002; Gonçalves et al. 2006; Bleeker et al. 2012). Trichomes may play a role as repellent barriers to small herbivores due to chemical secretions (Guo et al. 1993; Bergau et al. 2015), however, they may also physically hamper insect movement on a leaf surface as mechanical entrapments due to trichome type and density (Baur et al. 1991; Aragão et al. 2000; Simmons and Gurr 2005). Snyder et al. (2005) detected in *S. habrochaites* high levels of the sesquiterpenoid, 2,3-dihydrofarnesoic acid, in the spider mite repellent accession

'LA1363' which was crossed with two susceptible *S. lycopersicum* genotypes, 'EBR1' and 'Summit', to generate the interspecific F_2 and backcross hybrids. The authors presented evidence that the repellency found in the resistant parent, *S. habrochaites,* was transferred to the interspecific hybrids as demonstrated by mite performance in thumbtack bioassays of the hybrids

The interspecific backcross, BPX-368, obtained by crossing *S. lycopersicum* and *S. habrochaites* showed zingiberene concentration and type IV trichome densities were negatively correlated with the distance travelled by *T. evansi* on adaxial leaf surfaces after 20, 40 or 60 minutes in thumbtack bioassays (Maluf et al. 2001). These researchers also reported that type IV trichomes deterred spider mites. According to Alba et al. (2009), who studied mite resistance in hybrids of wild and cultivated tomato, high densities of abaxial type IV trichomes and, especially, high contents of acylsucrose were associated with increased repellence of adult mites. Their conclusions relied on use of stepwise multiple regression. In other work, F_1 interspecific tomato hybrids displayed an intermediate level of resistance compared to their wild parent *S. habrochaites* while the other parent *S. lycopersicum* was susceptible to spider mite (Snyder and Carter 1984).

Screening methods for insect resistance, especially with large numbers of genotypes is laborious and difficult, (Bas et al. 1992). Repellency mechanisms mediated by phytochemicals can be determined by rapid and reliable methods (Weston and Snyder 1990), and can hence be considered as resistance parameters for a broad range of herbivory arthropods (Maluf et al. 2001). Therefore, the thumbtack bioassay, an efficient and rapid screening technique used previously, may be employed to evaluate BC_3F_2

hybrids derived via crossing of wild and domesticated tomato. The feasibility of obtaining digital time lapse images of the bioassays arenas is an important consideration. Digital photography may speedup the research, increase throughput, resolve large statistical differences, and minimize labor and errors compared to manual sampling (Schomaker and Been 1998). The motivation behind the digital imaging of arthropod movement was to optimize the bioassay for achieving reliable and accurate data as mites passed through the leaf arena. Doing so also permitted storage of images that could be retrieved for further research purposes.

Our objectives of this research were to:

(a) determine whether or not indirect selection was conferring spider mite resistance as measured by the thumbtack bioassay.

(b) estimate the relative contributions of glandular type IV and VI trichome densities and leaf allelochemicals to mite behavior over time intervals using multiple regression models.

(c) evaluate image analysis as a tool for recording mite movement with a view toward high throughput phenotyping.

We expected interspecific hybrids of tomato would be more repellent to spider mites than cultivated tomatoes depending on composition and abundance of leaf exudates and on trichome densities of specific types. Results of this study may be applicable to breeding programs for other cash crops and other insect pests. New plant breeding lines may produce toxic or repellent chemicals which will allow them to defend

themselves against certain types of arthropods which turn may lead to elimination or reduction of synthetic pesticide utilization and cost.

4.3 Materials and Methods

4.3.1 Plant Materials:

This study was comprised of ten genotypes maintained in the greenhouse at University of Kentucky, Lexington, KY in June 2016. Two parents, *Solanum habrochaites* LA2329, a wild relative rich in foliar zingiberene and glandular type IV and VI trichomes, and the recurrent parent *Solanum lycopersicum* ZH2 'Zaofen 2', which lacks zingiberene and glandular trichomes especially type IV. Seven backcrosses hybrids (BC$_3$F$_2$) plus an additional cultivated variety, SROMA 'Small Roma', were involved in this experiment. The backcross hybrids were selected for the presence of specific leaf compounds and trichome types since these resistance characteristics are most relevant to arthropod resistance (Maluf et al. 2001). In this research, we selected backcross hybrids with contrasting leaf traits potentially related to mite resistance. Source of seeds, planting, and greenhouse management were mentioned in Chapter 2.

4.3.2 Maintenance of Mite Colony: as previously mentioned in Chapter 2.

4.3.3 Sample Preparation:

A fully expanded leaf from each three-month-old plant was excised from the third node position from the apex. Each plant was replicated three times. Each excised leaf consisting of five leaflets was inserted into 250 ml flask filled with water and was immediately transferred to a laboratory bench. Next, three leaflets were removed from

each leaf for quantification of zingiberene and other compounds by GC-FID and the two remaining leaflets were used for spider mite thumbtack bioassay followed by trichome assessment.

4.3.4 Quantification Chemical Compounds in Tomato Leaflets and Trichome Assessment:

The tips and bases of the three leaflets removed from each leaf were removed by use of scissors and then these center segments were steeped in hexane (2.0 ml). The extract was analyzed by GC-FID to evaluate the abundance of zingiberene and β-phellandrene. GC-FID parameters were previously described in Chapter 2. Extracted leaflet segments were scanned, and the resulting image was used to calculate leaflet area (cm^2). The amount of leaf chemicals detected by GC-FID was divided by leaflet area to establish a leaflet concentration expressed as GC area units/cm^2.

The abaxial and adaxial leaflet surfaces, bioassayed previously, were evaluated for type IV and VI trichome densities with the aid of a Meiji stereo microscope (50X), equipped with 10X10 ocular microgrid (4.3 mm^2), which allowed accurate counting of these trichomes on abaxial and abaxial leaflet surfaces. Two positions away from the tip and base of the leaflet were counted then the average was recorded.

4.3.5 Thumbtack Bioassay:

This bioassay followed a modified procedure as outlined by Weston and Snyder (1990). First, a Styrofoam board (Dow Chemical Co., Midland, MI) was covered with paper just prior to the start of a bioassay which avoided trichome exudate contamination between bioassay setups. A leaf was removed from the flask and the stem end was placed

in a water-filled test tube (10 ml) which was then taped on the Styrofoam board until the bioassay was finished. The two leaflets of each leaf were fixed with a metal thumbtack (diameter 1.27 cm) to the foam board. One leaflet was rotated so that its lower (abaxial) surface was accessible for bioassay. Thumbtacks were washed with hexane prior to use and were inserted through the center of each leaflet. Ten adult female mites were placed onto each thumbtack using a fine paint brush. Mites on a thumbtack were visually inspected during the bioassay to assure the activity of mites (Figure 4-1). Mites that escaped leaflets during the assay were removed from the bioassay arena.

To record the bioassay, an iPad-4 was clamped onto a ring stand at approximately 15 cm above the foam board with bioassay leaflet samples. The iPad was parallel to the foam board. The bioassay was photographed with the rear camera (5-megapixel) using an IOS app called OSnap! (free version). The app was set to take a photographic image every 60 seconds for one hour (1 frame per minute), for a total 60 images for each replication of each genotype. All images were transferred to a computer for image analysis.

4.3.6 Image Analysis, Data Recorded and Statistical Analyses:

Mites remaining on the tack (Whalon et al.) were counted visually at 15, 30, 45, and 60 min. Each image per the designated time interval was manually processed by image analysis software (ImageJ, https://imagej.nih.gov/ij/) using the straight-line tool. Distance was converted from pixels to cm, proportional to the thumbtack head (1.27 cm diameter). Travelled distance was equal to zero for mites that stayed on the thumbtack. The distance traveled by mite that moved onto the leaflet surface was the distance from

the nearest edge of the tack to the mite; these distances were summed. For mites that had left the leaflet, the minimum and maximum distances between the edge of the thumbtack and the leaf margin were determined by image analysis, averaged and then multiplied by the number of mites that had left the leaflet. For each time interval, the distances traveled by mites on each leaflet surface were added to the distance for mites off leaflet surface. This variable, total distance travelled by mite (cm) and is listed as TDTM was statistically analyzed.

This bioassay was comprised of 240 observations, (3 reps x 2 surfaces x 4 intervals x 10 genotypes). Prior to analyses, variables of leaf trichomes and their exudates were log-transformed [$Log_{10}(X + 1)$] based on a recommendation in Oliveira et al. (2009) to normalize the data. Trichome and mite data were submitted to a two-way ANOVA while leaf exudate data were analyzed by one-way ANOVA, all according to completely randomized design using the GLM procedure by SAS v 9.4 (SAS Institute Inc. 2012), with genotype, surface effects and their interaction as sources of variance. Treatment means for main effects were separated by Duncan's new multiple range comparison test (P<0.05), while LSmeans was used for the interaction mean comparisons. Trichome densities and zingiberene contents with mite repellence parameters were submitted to Pearson's correlation analysis using the CORR procedure in SAS. Full model multiple regression analysis was used to determine whether type IV and VI trichome densities and/or compound profiles as independent variables influenced mite repellency for each time interval and surface. Based on full model regression, stepwise regression was then carried out to determine the relative contribution of independent variables giving the

best fitting model for each time interval and surface based on remaining significant terms at P=0.25 and removing nonsignificant terms at P=0.1.

4.4 Results

4.4.1 Tomato Leaflet Extracts and Trichome Densities:

The results of ANOVA analysis of leaflet chemical composition (Table 4-1) indicated that zingiberene and β-phellandrene concentrations per cm^2 of leaf area were significantly different among the tested genotypes (P <.0001). The recurrent parent ZH2 had the highest concentration of β-phellandrene followed by the three hybrids A119, F32, and F51, while six genotypes had no detectable β-phellandrene (Table 4-2). The wild donor LA2329 produced the highest concentration of zingiberene, and zingiberene concentrations for two of the hybrids, C72 and B116 were indistinguishable from that of LA2329. Three hybrids, A119, H21 and H19 produced intermediate concentrations of zingiberene. The remaining four plants including the cultivated controls had no detectable zingiberene production (Table 4-3).

The results of the factorial ANOVA that included genotypes, surfaces, and genotype X surface interactions indicated that all sources of variance were significant for trichome type IV and VI densities (Table 4-4). The backcross hybrid B116 had the most abundant density of type IV trichomes whilst the cultivated tomato varieties as well as two of the hybrids had no type IV trichomes (Table 4-5). The wild species LA2329 had the highest density of type VI trichomes followed by some of the backcross hybrids, however, the backcross hybrid F51 had the lowest type VI trichome density (Table 4-6).

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Type IV trichome densities on abaxial surfaces were higher than on adaxial ones (Table 4-7). In contrast, type VI trichome densities on adaxial surfaces were significantly higher than on abaxial leaflet surfaces (Table 4-7).

Table 4-8 shows the results of the genotype X surface means for type IV trichome density. For the genotypes lacking type IV trichomes, density did not differ between surfaces. However, for genotypes having type IV trichomes, the density of the abaxial surface was always significantly greater than that on the adaxial surface except for genotype A119.

With regard to genotype X surface means for type VI trichomes, the wild relative LA2329, one cultivated plant SROMA, as well as four hybrids, A119, B116, F32 and F51 had more type VI trichomes on their adaxial surfaces compared to their abaxial surfaces. For three hybrids, C72, H19, H21, and the cultivated ZH2, type VI densities were not statistically different between the two surfaces (Table 4-9). Further, the hybrid F51 had the lowest type VI trichome density among all abaxial surfaces (Table 4-9). The extent of difference between the adaxial and abaxial type VI trichome densities depended on genotype.

4.4.2 Mite Performance in Thumbtack Bioassays:

4.4.2.1 Number of Mites Remaining on Thumbtack:

The results of ANOVA analysis indicated significant differences in number of mites remaining on thumbtacks after 15, 30, 45, and 60 min among the genotypes bioassayed, between abaxial and adaxial surfaces, and for the interaction of genotype X surface (Table 4-10). The numbers of mites on the tack was highest on the leaves of the B116 genotype

after 15 min (Table 4-11), on C72 after 30 and 45 min (Tables 4-12 and 4-13), and on the wild parent, LA2329 after 60 min (Tables 4-14). At 60 min hybrids B116, C72, and A119 were indistinguishable from the wild relative LA2329 with regard to the number of mites remaining on the thumbtack (Tables 4-14). The number of mites on the tack was lowest on the genotype SROMA for the 15 min, 30 min and 45 min sample times (Tables 4-11, 4-12, 4-13). Few or no mites remained on the thumbtack at 60 min for the cultivated genotypes, SROMA and ZH2 (Tables 4-14). Other backcross hybrids F32, F51, H19, and H21 had generally intermediate values for mites on the tack means across all time intervals.

Mean number of mites remaining on the tack was significantly higher on the abaxial leaf surfaces than on adaxial surfaces across all time intervals (Table 4-15).

The difference between surfaces for number of mites on the tack within a genotype was statistically significant for two genotypes, C72 and F51 during all time intervals (Tables 4-16, 4-17, 4-18 and 4-19); means for abaxial surfaces were always higher for these two genotypes. At the 15 min sampling period, all genotypes, except C72 and F51 had no significant differences between surfaces of number of mites on the tack (Table 4-16). At the 30 min sampling period, six of the nine genotypes had a difference between surfaces for number of mites on the tack, hybrids C72, B116, A119, F32 and F51 and the wild relative LA2329 (Table 4-17). At the 45 min sampling period, five of the nine genotypes had a difference between surfaces for number of mites on the tack, hybrids C72, B116, F32 and F51 and the wild relative LA2329 (Table 4-18). At the 60 min sampling period, four of the nine genotypes had a difference between surfaces for number of mites

on the tack, hybrids C72, A119, H21 and F51 (Table 4-19). In all cases, except one, where there was a surface difference in the number of mites remaining on the tack, the mean for the abaxial surface was greater than that for the adaxial. The one exception was H21 at the 60 min sampling period, which had more mites on the tack for the adaxial surface, compared to the adaxial.

4.4.2.2 Distance Travelled by Spider Mites:

Time lapse photography of bioassay arenas with an iPad camera was a straightforward technique. Significant differences were found in total distance traveled by mites after 15, 30, 45, and 60 min among the various genotypes bioassayed, between abaxial and adaxial surfaces, as well as for genotype X surface interactions except for the interaction term at 45 min, which was not significant (Table 4-20). Repellence (least distance travelled) was the highest on the genotype B116 after 15 min (Tables 4-21), on C72 after 30 min (Table 4-22), and 45 min (Table 4-23), and on the wild parent genotype (LA2329) after 60 min (Table 4-25). However, in terms of statistical differences, hybrids C72, B116, and A119 were indistinguishable from the wild relative LA2329 at all time intervals (Tables 4-21, 4-22, 4-23, and 4-24). Repellence level was the lowest (most distance traveled) on the genotype SROMA for all time intervals (Tables 4-21, 4-22, 4-23, and 4-24). Generally, repellence was also low, and often statistically indistinguishable from SROMA, for the hybrid F51 and the cultivated ZH2. Surface means indicated that repellence level based on total distance travelled by mites was significantly higher on the abaxial leaf surface than that on adaxial surface across all time intervals (Table 4-25).

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Genotype X surface interaction for total distance travelled by mites was statistically significant for three (15, 30 and 60 min) of the four-time intervals. Hybrid F51 was the only genotype having a surface difference of total distance travelled by mites at all three time periods (Tables 4-26, 4-27, 4-28). Surface differences were also present for hybrid A119 and wild LA2329 at 15 min (Table 4-26), for hybrids H21, H19 and the cultivated SROMA at 30 min (Table 4-27); for genotypes having a surface difference in distance travelled, the distances on abaxial surfaces were significantly less on abaxial surfaces, compared to adaxial surfaces.

4.4.3   Correlation of Trichome Densities and Zingiberene Contents with Mite Repellence Parameters:

The associations of type IV and type VI trichome densities as well as zingiberene content with number of spider mites remaining on the tack showed a significant positive correlation for both abaxial and adaxial surfaces across most time intervals, except for type VI trichome density on adaxial surfaces at 30 min, which was not significant (Table 4-29). The significant correlations between leaf characteristics and mites on tack mean indicated that the number of mites retained on the tack was greater on genotypes with higher type IV and VI trichome densities and with high zingiberene levels. In contrast, correlation coefficients of type IV trichome densities, type VI trichome densities as well as zingiberene content with total distance travelled by mites were significant and negative for both abaxial and adaxial surfaces across all time intervals except for type VI trichome density on the abaxial surface at 30 and 45 min and on the adaxial surface at 15, 30, and 45 min (Table 4-29). Significant negative correlations between leaf characteristics and

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total distance travelled by mites suggested that the total distances traveled by the mites on leaflet surfaces were shorter for genotypes with high trichome densities (types IV and VI) and zingiberene contents.

4.4.4 Multiple Regression Model:

To better understand relationships among trichome types and their exudates with mite responses for these ten tomato genotypes, we carried out multiple regression analyses to diagnose these potential relationships among independent and dependent variables.

For the number of mites remaining on the thumbtack on abaxial surfaces, for any time period analyzed with the full model of multiple regression, only type IV trichome density and zingiberene content were significant independent variables with positive slopes, indicating that higher the type IV density and the higher the zingiberene content, the greater the number of mites remaining on the thumbtack on abaxial leaf surfaces (Table 4-30). Type VI density and B-phellandrene content were not significant regressors in the analyses. Slopes for type IV trichome density tended to increase over time ranging from 1.83 at 15 min to 2.56 at 60 minutes. The slope associated with zingiberene content was remarkably stable, ranging from 0.37 at 60 minutes to 0.49 at 30 minutes. Similarly, the reduced model obtained by stepwise multiple regression analysis reflected that the estimated slopes for type IV trichome density and zingiberene content were positive for mites on tack with the highest relative contribution of trichome IV densities across all time intervals (Table 4-30). Slope estimates were little changed by use of stepwise regression.

For the number of mites remaining on the thumbtack on adaxial surfaces, the only significant predictors included in the full model of multiple regression analysis were trichome type IV densities and zingiberene content with positive slopes for mite on tack means at 15 and 60 min intervals but the only zingiberene content at 30 and 45 min intervals (Table 4-31). Slopes associated with type IV trichome densities had the highest magnitude after 15 and 60 min intervals. In the reduced model obtained by stepwise multiple regression analysis, type IV trichome densities had the highest relative contribution on mites on tack means across most time intervals except for the 45 min interval where only zingiberene content had a positive effect (Table 4-31).

The total distance traveled by mites as a dependent variable in the full model of multiple regression analysis, trichome type IV densities and zingiberene content were the only significant regressors with negative slopes for on abaxial leaf surfaces after 15, 30, and 45 min (Table 4-32). At 60 min, only type IV trichome density was significant (Table 4-32). Type IV trichome densities had the highest relative effect across all time intervals. The reduced model of multiple regression analysis showed that the estimate values for type IV trichome densities and zingiberene content had the negative effects on total distance travelled by mites on abaxial leaflet surface after 15, 30, and 45 min with the highest magnitude for trichome IV densities. However, after 60 min, type IV and VI trichome densities were significantly and negatively associated with total distance travelled by mites and the highest effect was type VI densities (Table 4-32).

Multiple regression analysis for adaxial leaf surface including all predictors in the full model exhibited that trichome type IV density and zingiberene content were the best

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variables for explaining the total distance travelled by mites with negative slope at 15 min. For the 30, 45, and 60 min intervals only the zingiberene content had a significant negative effects on total distance travelled by mites (Table 4-33). The reduced model of multiple regression analysis for adaxial leaf surface indicated that the slopes for type IV trichome density and zingiberene content were significant and negative for total distance travelled by mites after 15 and 60 min with the highest relative contribution of trichome IV densities. However, at 30 and 45 min, only zingiberene content was significantly and negatively associated with total distance travelled by mites (Table 4-33).

4.5 Discussion

The assumption of this bioassay is when more mites remain on tack and/or the mites move less onto a leaflet surface there is a higher degree of mite repellence (Maluf et al. 2007). The backcross genotypes chosen for this bioassay had a broad array of variability for leaf secretions (e.g. β-phellandrene and zingiberene production) and for leaf surface features (e.g. type IV and VI trichome densities), that could potentially influence *T. urticae* behavior. Within the backcross hybrids there were two lines C72 and B116 that exceeded the wild parent LA2329 in type IV trichome densities or were similar in zingiberene contents, both absent in the cultivated parent. Generally, in the thumbtack bioassay, these two lines were as resistant or more resistant than the wild donor parent, LA2329. This observation strongly underpins the conclusion that resistance has been successfully introgressed by selection for type IV trichome density and zingiberene concentration. Prior studies have highlighted the importance of glandular trichomes and

their exudates in resistance of wild tomato accessions (Snyder et al. 2005; Alba et al. 2009; Lucini et al. 2015).

Our results from these bioassays suggested that resistance to the spider mite *T. urticae* in wild parent *S. habrochaites* 'LA2329', and three backcrosses C72, B116, and A119, may be due to high type IV trichome densities and zingiberene production on leaflet surfaces. Resistance was manifested as an increased number of mites remaining on thumbtack plus a reduction in the total distance traveled by mites on abaxial and adaxial surfaces. These results were consistent with other studies that evaluated mite repellence using thumbtack bioassay for tomato genotypes (Snyder et al. 2005; Saeidi and Mallik 2006; Maluf et al. 2007; Resende et al. 2008; Wosula et al. 2009; Murungi et al. 2012; Lima et al. 2016; Maciel et al. 2017; Maciel et al. 2018). Moreover, there could be an interacting role, e.g. synergistic, between trichome densities and repository of chemical secretions (Guo et al. 1993; Maluf et al. 2001).

Imaging of arthropod movement in this bioassay can accelerate transfer of resistance and help with measurement of mite distance, nearly simultaneous determination of distances for multiple mites on leaf surface. There results are similar to those of Hoffmann et al. (2010) who were successful in using image analysis to provide reliable and accurate data associate with insect movement.

Due to the variability of trichome densities on abaxial and adaxial leaflet surfaces among the chosen genotypes, we investigated mite responses for both surfaces. Based on differences of mite on tack and total distance travelled by mites for abaxial and adaxial surfaces, mites were less repelled on the adaxial leaflet surfaces than on abaxial ones.

Generally, genotype X surface interactions for mite repellence across time intervals were different on some genotypes, which may be attributed to the nature of variability of leaf trichome density as a component of resistance with other leaf characters resulting phenotypic differences (Valverde et al. 2001).

In the current study, the correlation results manifested that *T. urticae* behaviors were consistently associated with glandular trichome densities and foliar zingiberene concentrations. Significant positive correlations between certain leaf characteristics and number of mites remaining on thumbtack suggested that higher number of mites on the tack on both abaxial and adaxial surfaces were associated with higher type IV and VI trichome densities and with higher zingiberene contents at all sampling times. Additionally, significant negative correlations between leaf traits and total distance travelled by mites suggested that shorter distances were associated with high trichome densities (types IV and VI) and abundant zingiberene content for most time intervals. Furthermore, the elevated densities of glandular trichomes and/or high concentration of zingiberene were therefore associated with adverse effects on arthropod behavior like deterrence, indicating potential repellency present in the resistant interspecific hybrids. Our findings seemed to generally agree with those of Snyder et al. (2005); Maluf et al. (2007); and Murungi et al. (2012) regarding effects of glandular trichome densities on arthropod behavior.

The repellence parameters involved in the full regression model were significantly associated with the presence of type IV trichome density and/or zingiberene concentration at almost all sample periods. Contrarily, neither type VI trichome density

nor β-phellandrene content were significant contributors to mite performance in the bioassays. In the reduced model using a stepwise regression method, it is noteworthy that trichome type IV densities had an incremental effect to impede the mite movements on the leaflet surface of the genotypes evaluated over sampling times. Similar to analysis of mites on tack means, the slope for type IV trichome density tended to increase with sample time, with estimates ranging from 1.83 at 15 minutes to 2.56 at 60 minutes. The slope for zingiberene concentration changed little over the sampling period, ranging from a low of 0.37 at 60 minutes to 0.49 at 30 minutes. Also, slope estimates were little changed by employment of stepwise regression.

Stepwise regression in the reduced model showed that type IV trichome density was the best single variable model and had a significant impact on mite responses, which agrees with the findings of Carter and Snyder (1985). Our study demonstrated that selection for either high densities of type IV glandular trichomes or high zingiberene production should be appropriate indirect selection parameters for resistance to other tomato pests including spider mites. According to Alba et al. (2009), the high densities of abaxial type IV trichomes and high contents of acylsucrose were associated with increased repellence of two-spotted spider mites, as indicated by stepwise multiple regression, in a population derived from the cross between the wild tomato, *S. pimpinellifolium* 'TO-937' and the cultivated tomato, *S. lycopersicum*. Besides, they found that acylsucrose production showed the best explanatory variable for mite repellence with positive effects among all predictors involved in the multiple regression analysis. When they include trichome types as predictors in the regression model, type IV trichome

density was the only predictor explaining mite repellence parameter with positive slope. The authors also reported significant slopes for type VI trichome density but with opposite sign to the slope for type IV trichome density. Maciel et al. (2017) reported reduced mite displacement on the leaflet surfaces of the wild species *S. pennellii* and mini tomato hybrids associated with high foliar acylsugar over four evaluation times (5, 10, 15, and 20 min). In addition, Maciel et al. (2018), reported that the backcross plant UFU-102- F_2BC_2 #13, which had higher acylsugar content than the recurrent parent (UFU-040) also had shorter distances covered by the mites.

In our experiment, taking together the mean of type IV trichome densities and zingiberene abundance significantly improved mite repellence in several interspecific hybrids compared to the negative control (cultivated genotypes), whose leaves lack specific type of glandular trichomes and zingiberene. It is notable that some backcross hybrids rich in trichome type IV and zingiberene production could be lines having resistance to other arthropods or insects.

Conversely, the other trichome exudate component, β-phellandrene as well as type VI trichome density did not show significant association with mite deterrence except for trichome type VI density which had a negative impact, same as trichome IV density, on the mite movements on abaxial leaf surface after 60 min in the reduced model.

4.6 Conclusion

For the repellent parameters measured, e.g. mites on tack and total distance travelled by mites, mite resistance was mainly associated with type IV trichome densities and foliar zingiberene production and marginally associated with type VI trichome

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densities. Interestingly, type IV trichome density is the most crucial factor in mite deterrence while zingiberene seemed to be a second key factor across most of time durations for both surfaces, but both factors could have synergistic effect, particularly on the abaxial leaf surface. In other words, a low level of zingiberene with the presence of glandular type IV trichomes was sufficient to realize strong resistance to arthropods. This conclusion is similar to that of Lucatti et al. (2013), who suggested that a low content of acylsugars accompanied by the presence of type IV trichomes on plant leaves was a prerequisite for attaining a durable resistance phenotype. The evidence presented herein also indicated that the degree of repellency may be different between the abaxial and adaxial leaflet surface due to the variability of trichome density and chemical profile. A high number of *T. urticae* remaining on thumbtacks as well as shorter mite movement on leaflet surfaces over all sampling times indicated a degree of arthropod repellence on plant leaves. It is worth mentioning that thumbtack bioassay is a tested technique for repellency to arthropods among diverse tomato genotypes as a model. However, it may also be a quick and efficient method for testing a small number of plant samples. Results of this study could be utilized by tomato breeding programs that require better knowledge of the impact of specific trichome types on performance of arthropod pests.

Table 4–1: ANOVA model results for β-phellandrene and zingiberene concentration of leaflets for 10 tomato genotypes tested in the thumbtack bioassay.

| Source of Variations | DF | β-phellandrene | | Zingiberene | |
		F Value	P Value	F Value	P Value
Genotype	9	1148.54	<.0001	1681.6	<.0001
Error	20				
R^2		0.99		0.99	

β-phellandrene and zingiberene determined as GC area unit/cm^2 of leaf area were transformed data $\log_{10}(X+1)$ prior to analysis.

Table 4–2: Means of β-phellandrene concentration (GC area units/cm^2 of leaf area) and means of transformed data log$_{10}$(X+1) for 10 tomato genotypes tested in the thumbtack bioassay. Means followed by the same letter(Breeden and Coates) are not significantly different (*P* = 0.05) as determine by Duncan's multiple range test. Standard error of the mean denoted by SE.

Genotype	Background	Average β-phellandrene (GC area units/cm^2)	Transformed (Log$_{10}$(X+1))	±	SE	
ZH2	Cultivated	5.9 X 10^6	6.76	±	0.07	a
A119	Hybrid	2.4 X 10^6	6.33	±	0.15	b
F32	Hybrid	2.0 X 10^6	6.15	±	0.24	*bc*
F51	Hybrid	1.0 X 10^6	5.99	±	0.09	c
C72	Hybrid	0.00	0.00	±	0.00	d
H19	Hybrid	0.00	0.00	±	0.00	d
H21	Hybrid	0.00	0.00	±	0.00	d
B116	Hybrid	0.00	0.00	±	0.00	d
LA2329	Wild relative	0.00	0.00	±	0.00	d
SROMA	Cultivated	0.00	0.00	±	0.00	d

Table 4–3: Means of zingiberene concentration (GC area units/cm^2 of leaf area) and means of transformed data $\log_{10}(X+1)$ for 10 tomato genotypes tested in the thumbtack bioassay. Means followed by the same letter are not significantly different ($P = 0.05$) as determine by Duncan's multiple range test. Standard error of the mean denoted by SE.

		Average zingiberene				
Genotype	Background	(GC area units/cm^2)	Transformed ($\log_{10}(X+1)$)	±	SE	
LA2329	Wild relative	4.3 X 10^7	7.63	±	0.04	a
C72	Hybrid	4.2 X 10^7	7.62	±	0.06	a
B116	Hybrid	2.4 X 10^7	7.38	±	0.04	a
A119	Hybrid	9.0 X 10^6	6.85	±	0.25	b
H21	Hybrid	5.1 X 10^6	6.70	±	0.05	b
H19	Hybrid	4.3 X 10^6	6.61	±	0.12	b
F32	Hybrid	0.00	0.00	±	0.00	c
F51	Hybrid	0.00	0.00	±	0.00	c
ZH2	Cultivated	0.00	0.00	±	0.00	c
SROMA	Cultivated	0.00	0.00	±	0.00	c

Table 4–4: ANOVA results for types IV and VI trichome densities on abaxial and adaxial surfaces for 10 tomato genotypes tested in the thumbtack bioassay.

Source of Variation	DF	IV Density[1]		VI Density [1]	
		F-Value	P-Value	F-Value	P-Value
Genotype	9	246.88	<.0001	17.53	<.0001
Surface	1	258.34	<.0001	28.14	<.0001
Genotype X Surface	9	39.01	<.0001	4.37	<.0005
Error	40				
R^2		0.99		0.85	

[1]Type IV and VI Density= trichome type IV and VI Density (No./mm^2) were transformed data $\log_{10}(X+1)$ prior to analysis.

Table 4–5: Means of type IV trichome density (No./mm^2) and means of transformed data log$_{10}$(X+1) for 10 tomato genotypes tested in the thumbtack bioassay. Means followed by the same letter are not significantly different ($P = 0.05$) as determine by Duncan's multiple range test. Standard error of the mean denoted by SE.

Genotype	Background	(No./mm²)	Average type IV Density			
			Transformed (Log$_{10}$(X+1))	±	SE	
B116	Hybrid	34.00	1.51	±	0.08	a
A119	Hybrid	15.33	1.21	±	0.04	b
F32	Hybrid	16.17	1.09	±	0.17	c
LA2329	Wild relative	12.33	1.08	±	0.10	c
C72	Hybrid	20.00	1.04	±	0.25	c
F51	Hybrid	11.33	0.73	±	0.29	d
H19	Hybrid	0.00	0.00	±	0.00	e
H21	Hybrid	0.00	0.00	±	0.00	e
ZH2	Cultivated	0.00	0.00	±	0.00	e
SROMA	Cultivated	0.00	0.00	±	0.00	e

Table 4–6: Means of type VI trichome density (No./mm^2) and means of transformed data log$_{10}$(X+1) for 10 tomato genotypes tested in the thumbtack bioassay. Means followed by the same letter(s) are not significantly different (P = 0.05) as determine by Duncan's multiple range test. Standard error of the mean denoted by SE.

		Average type VI Density				
Genotype	Background	(No./mm^2)	Transformed (Log$_{10}$(X+1))	±	SE	
LA2329	Wild relative	21.00	1.29	±	0.10	a
A119	Hybrid	9.50	0.98	±	0.08	b
C72	Hybrid	5.83	0.81	±	0.07	bc
H19	Hybrid	4.67	0.74	±	0.05	cd
H21	Hybrid	4.00	0.69	±	0.05	cde
ZH2	Cultivated	3.83	0.67	±	0.05	cde
B116	Hybrid	3.50	0.60	±	0.10	cde
SROMA	Cultivated	3.50	0.57	±	0.12	de
F32	Hybrid	3.50	0.49	±	0.18	e
F51	Hybrid	1.17	0.28	±	0.10	f

Table 4–7: Means of trichome type IV and VI Density (No./mm^2) on adaxial and abaxial leaflet surfaces and means of transformed data $\log_{10}(X+1)$ for 10 tomato genotypes tested in the thumbtack bioassay. Means followed by the same letter are not significantly different (P = 0.05) as determine by Duncan's multiple range test. Standard error of the mean denoted by SE.

Surface	Average type IV Density				Average type VI Density			
	(No./ mm²)	Transformed ($\log_{10}(X+1)$)	± SE		(No./ mm²)	Transformed ($\log_{10}(X+1)$)	± SE	
Abaxial	16.9	0.86	± 0.13	a	4.2	0.60	± 0.06	a
Adaxial	4.93	0.47	± 0.09	b	7.9	0.82	± 0.05	b

Table 4–8: Means of trichome type IV density (No./mm^2) and for adaxial (Ad) and abaxial (Ab) leaflet surfaces of 10 tomato genotypes tested in the thumbtack bioassay. Data were transformed prior to analysis (log$_{10}$(X+1)). Means of transformed data within a genotype followed by the same letter are not significantly different as determined by LSmeans (P = 0.05). Standard error of the mean denoted by SE.

				Average type IV Density			
Genotype	Surface	Background	(No./mm²)	Transformed (Log$_{10}$(X+1))	±	SE	
B116	Ad	Hybrid	21.67	1.35	±	0.04	a
B116	Ab	Hybrid	46.33	1.67	±	0.04	b
A119	Ad	Hybrid	13.33	1.15	±	0.06	a
A119	Ab	Hybrid	17.33	1.26	±	0.03	a
F32	Ad	Hybrid	4.67	0.72	±	0.13	a
F32	Ab	Hybrid	27.67	1.46	±	0.02	b
LA2329	Ad	Wild relative	7.00	0.89	±	0.08	a
LA2329	Ab	Wild relative	17.67	1.27	±	0.05	b
C72	Ad	Hybrid	2.33	0.49	±	0.12	a
C72	Ab	Hybrid	37.67	1.59	±	0.03	b
F51	Ad	Hybrid	0.33	0.10	±	0.10	a
F51	Ab	Hybrid	22.33	1.36	±	0.05	b
H19	Ad	Hybrid	0.00	0.00	±	0.00	a
H19	Ab	Hybrid	0.00	0.00	±	0.00	a
H21	Ad	Hybrid	0.00	0.00	±	0.00	a
H21	Ab	Hybrid	0.00	0.00	±	0.00	a
ZH2	Ad	Cultivated	0.00	0.00	±	0.00	a
ZH2	Ab	Cultivated	0.00	0.00	±	0.00	a
SROMA	Ad	Cultivated	0.00	0.00	±	0.00	a
SROMA	Ab	Cultivated	0.00	0.00	±	0.00	a

Table 4–9: Means of trichome type VI density (No./mm^2) and for adaxial (Ad) and abaxial (Ab) leaflet surfaces of 10 tomato genotypes tested in the thumbtack bioassay. Data were transformed prior to analysis (log$_{10}$(X+1)). Means of transformed data within a genotype followed by the same letter are not significantly different as determined by LSmeans (*P* = 0.05). Standard error of the mean denoted by SE.

Genotype	Surface	Background	(No./mm²)	Transformed (Log$_{10}$(X+1))	±	SE	
				Average type VI Density			
LA2329	Ad	Wild relative	29.67	1.46	±	0.10	a
LA2329	Ab	Wild relative	12.33	1.12	±	0.06	b
A119	Ad	Hybrid	11.33	1.03	±	0.17	a
A119	Ab	Hybrid	7.67	0.94	±	0.02	b
C72	Ad	Hybrid	7.33	0.91	±	0.08	a
C72	Ab	Hybrid	4.33	0.70	±	0.10	a
H19	Ad	Hybrid	6.00	0.84	±	0.04	a
H19	Ab	Hybrid	3.33	0.63	±	0.03	a
H21	Ad	Hybrid	4.67	0.75	±	0.03	a
H21	Ab	Hybrid	3.33	0.62	±	0.09	a
ZH2	Ad	Cultivated	3.67	0.64	±	0.11	a
ZH2	Ab	Cultivated	4.00	0.69	±	0.05	a
B116	Ad	Hybrid	2.00	0.46	±	0.09	a
B116	Ab	Hybrid	5.00	0.74	±	0.13	b
SROMA	Ad	Cultivated	6.00	0.83	±	0.07	a
SROMA	Ab	Cultivated	1.00	0.30	±	0.00	b
F32	Ad	Hybrid	6.33	0.83	±	0.13	a
F32	Ab	Hybrid	0.67	0.16	±	0.16	b
F51	Ad	Hybrid	2.00	0.46	±	0.09	a
F51	Ab	Hybrid	0.33	0.10	±	0.10	b

Table 4–10: ANOVA results for the number of mites remaining on thumbtack after 15, 30, 45, and 60 min for adaxial and abaxial surfaces of 10 tomato genotypes tested in the thumbtack bioassay.

		15 min		30 min	
Source of Variations	DF	F Value	P Value	F Value	P Value
Genotype	9	28.93	<.0001	37.58	<.0001
Surface	1	16.78	0.0002	42.25	<.0001
Genotype X Surface	9	4.00	0.0011	3.33	0.004
Error	40				
R^2		0.89		0.91	

Table 4-10 (continued)

		45 min		60 min	
Source of Variations	DF	F Value	P Value	F Value	P Value
Genotype	9	38.01	<.0001	30.48	<.0001
Surface	1	32.55	<.0001	15.01	0.0004
Genotype X Surface	9	4.59	0.0003	3.96	0.0011
Error	40				
R^2		0.91		0.89	

Table 4–11: Means of number of mites remaining on thumbtack after 15 min for 10 tomato genotypes tested in the thumbtack bioassay. Means followed by the same letter(s) are not significantly different (P = 0.05) based on Duncan's multiple range test. Standard error of the mean denoted by SE.

Genotype	Background	Average mites remaining on thumbtack			
		Mite No.	±	SE	
B116	Hybrid	9.33	±	0.33	a
LA2329	Wild relative	9.17	±	0.31	ab
C72	Hybrid	8.67	±	0.67	abc
A119	Hybrid	7.83	±	0.40	bcd
H19	Hybrid	7.33	±	0.67	cd
F32	Hybrid	7.00	±	0.37	d
H21	Hybrid	6.50	±	0.50	d
ZH2	Cultivated	4.50	±	0.50	e
F51	Hybrid	4.33	±	1.28	e
SROMA	Cultivated	1.83	±	0.48	f

Table 4–12: Means of number of mites remaining on thumbtack after 30 min for 10 tomato genotypes tested in the thumbtack bioassay. Means followed by the same letter(s) are not significantly different (P = 0.05) based on Duncan's multiple range test. Standard error of the mean denoted by SE.

Genotype	Background	Mite No.	±	SE	
		Average mites remaining on thumbtack			
C72	Hybrid	8.67	±	0.61	a
B116	Hybrid	8.17	±	0.54	ab
LA2329	Wild relative	7.67	±	0.71	ab
A119	Hybrid	7.00	±	0.58	bc
H19	Hybrid	6.33	±	0.33	c
F32	Hybrid	6.00	±	0.45	cd
H21	Hybrid	4.83	±	0.40	de
F51	Hybrid	3.67	±	1.31	ef
ZH2	Cultivated	3.33	±	0.42	f
SROMA	Cultivated	0.33	±	0.33	g

Table 4–13: Means of number of mites remaining on thumbtack after 45 min for 10 tomato genotypes tested in the thumbtack bioassay. Means followed by the same letter(s) are not significantly different (P = 0.05) based on Duncan's multiple range test. Standard error of the mean denoted by SE.

Genotype	Background	Mite No.	±	SE	
C72	Hybrid	8.17	±	0.79	a
LA2329	Wild relative	7.83	±	0.75	a
B116	Hybrid	7.00	±	0.63	ab
A119	Hybrid	6.50	±	0.43	bc
H19	Hybrid	5.67	±	0.49	cd
F32	Hybrid	5.00	±	0.63	d
H21	Hybrid	5.00	±	0.52	d
ZH2	Cultivated	3.00	±	0.45	e
F51	Hybrid	2.17	±	1.11	e
SROMA	Cultivated	0.00	±	0.00	f

Table 4–14: Means of number of mites remaining on thumbtack after 60 min for 10 tomato genotypes tested in the thumbtack bioassay. Means followed by the same letter(s) are not significantly different (P = 0.05) based on Duncan's multiple range test. Standard error of the mean denoted by SE.

Genotype	Background	Average mites remaining on thumbtack			
		Mite No.	±	SE	
LA2329	Wild relative	7.50	±	0.56	a
B116	Hybrid	6.83	±	0.48	a
C72	Hybrid	6.67	±	0.76	a
A119	Hybrid	6.17	±	0.70	a
F32	Hybrid	4.67	±	0.33	b
H19	Hybrid	4.50	±	0.56	b
H21	Hybrid	4.33	±	0.61	b
F51	Hybrid	2.17	±	1.11	c
ZH2	Cultivated	1.17	±	0.48	cd
SROMA	Cultivated	0.00	±	0.00	d

Table 4–15: Means of number of mites remaining on thumbtack after 15, 30, 45, and 60 min on adaxial and abaxial leaflet surfaces for 10 tomato genotypes tested in the thumbtack bioassay. Means followed by the same letter are not significantly different (P = 0.05) based on Duncan's multiple range test. Standard error of the mean denoted by SE.

Surface	Average mites remaining on thumbtack After 15 min				Average mites remaining on thumbtack After 30 min			
	Mite No.	±	SE		Mite No.	±	SE	
Abaxial	7.23	±	0.48	a	6.47	±	0.52	a
Adaxial	6.07	±	0.49	b	4.73	±	0.47	b

Table 4-15 (continued)

Surface	Average mites remaining on thumbtack After 45 min				Average mites remaining on thumbtack After 60 min			
	Mite No.	±	SE		Mite No.	±	SE	
Abaxial	5.80	±	0.55	a	4.97	±	0.54	a
Adaxial	4.27	±	0.47	b	3.83	±	0.47	b

Table 4–16: Means of number of mites remaining on thumbtack after 15 min for abaxial (Ab) and adaxial (Ad) leaflet surfaces of 10 tomato genotypes tested in the thumbtack bioassay. Means for the two surfaces of a genotype followed by the same letter are not significantly different as determined by LSmeans ($P = 0.05$). Standard error of the mean denoted by SE.

Genotype	Surface	Background	Average mites remaining on thumbtack			
			Mite No.	±	SE	
B116	Ab	Hybrid	9.67	±	0.33	a
B116	Ad	Hybrid	9.00	±	0.58	a
LA2329	Ab	Wild relative	9.67	±	0.33	a
LA2329	Ad	Wild relative	8.67	±	0.33	a
C72	Ab	Hybrid	10.00	±	0.00	a
C72	Ad	Hybrid	7.33	±	0.67	b
A119	Ab	Hybrid	8.33	±	0.67	a
A119	Ad	Hybrid	7.33	±	0.33	a
H19	Ab	Hybrid	7.00	±	1.15	a
H19	Ad	Hybrid	7.67	±	0.88	a
F32	Ab	Hybrid	7.33	±	0.67	a
F32	Ad	Hybrid	6.67	±	0.33	a
H21	Ab	Hybrid	6.67	±	0.67	a
H21	Ad	Hybrid	6.33	±	0.88	a
ZH2	Ab	Cultivated	5.33	±	0.67	a
ZH2	Ad	Cultivated	3.67	±	0.33	a
F51	Ab	Hybrid	7.00	±	0.58	a
F51	Ad	Hybrid	1.67	±	0.88	b
SROMA	Ab	Cultivated	1.33	±	0.33	a
SROMA	Ad	Cultivated	2.33	±	0.88	a

Table 4–17: Means of number of mites remaining on thumbtack after 30 min for abaxial (Ab) and adaxial (Ad) leaflet surfaces of 10 tomato genotypes tested in the thumbtack bioassay. Means for the two surfaces of a genotype followed by the same letter are not significantly different as determined by LSmeans (P = 0.05). Standard error of the mean denoted by SE.

Genotype	Surface	Background	Average mites remaining on thumbtack			
			Mite No.	±	SE	
C72	Ab	Hybrid	9.67	±	0.33	a
C72	Ad	Hybrid	7.67	±	0.88	b
B116	Ab	Hybrid	9.33	±	0.33	a
B116	Ad	Hybrid	7.00	±	0.00	b
LA2329	Ab	Wild relative	8.67	±	0.88	a
LA2329	Ad	Wild relative	6.67	±	0.88	b
A119	Ab	Hybrid	8.00	±	0.58	a
A119	Ad	Hybrid	6.00	±	0.58	b
H19	Ab	Hybrid	6.33	±	0.67	a
H19	Ad	Hybrid	6.33	±	0.33	a
F32	Ab	Hybrid	7.00	±	0.00	a
F32	Ad	Hybrid	5.00	±	0.00	b
H21	Ab	Hybrid	5.33	±	0.67	a
H21	Ad	Hybrid	4.33	±	0.33	a
F51	Ab	Hybrid	6.33	±	1.20	a
F51	Ad	Hybrid	1.00	±	0.00	b
ZH2	Ab	Cultivated	3.33	±	0.67	a
ZH2	Ad	Cultivated	3.33	±	0.67	a
SROMA	Ab	Cultivated	0.67	±	0.67	a
SROMA	Ad	Cultivated	0.00	±	0.00	a

Table 4–18: Means of number of mites remaining on thumbtack after 45 min for abaxial (Ab) and adaxial (Ad) leaflet surfaces of 10 tomato genotypes tested in the thumbtack bioassay. Means for the two surfaces of a genotype followed by the same letter are not significantly different as determined by LSmeans (P = 0.05). Standard error of the mean denoted by SE.

Genotype	Surface	Background	Average mites remaining on thumbtack			
			Mite No.	±	SE	
C72	Ab	Hybrid	9.67	±	0.33	a
C72	Ad	Hybrid	6.67	±	0.88	b
LA2329	Ab	Wild relative	9.33	±	0.33	a
LA2329	Ad	Wild relative	6.33	±	0.67	b
B116	Ab	Hybrid	8.33	±	0.33	a
B116	Ad	Hybrid	5.67	±	0.33	b
A119	Ab	Hybrid	7.33	±	0.33	a
A119	Ad	Hybrid	5.67	±	0.33	a
H19	Ab	Hybrid	5.00	±	0.00	a
H19	Ad	Hybrid	6.33	±	0.88	a
F32	Ab	Hybrid	6.00	±	1.00	a
F32	Ad	Hybrid	4.00	±	0.00	b
H21	Ab	Hybrid	4.67	±	0.67	a
H21	Ad	Hybrid	5.33	±	0.88	a
ZH2	Ab	Cultivated	3.33	±	0.67	a
ZH2	Ad	Cultivated	2.67	±	0.67	a
F51	Ab	Hybrid	4.33	±	1.20	a
F51	Ad	Hybrid	0.00	±	0.00	b
SROMA	Ab	Cultivated	0.00	±	0.00	a
SROMA	Ad	Cultivated	0.00	±	0.00	a

Table 4–19: Means of number of mites remaining on thumbtack after 60 min for abaxial (Ab) and adaxial (Ad) leaflet surfaces of 10 tomato genotypes tested in the thumbtack bioassay. Means for the two surfaces of a genotype followed by the same letter are not significantly different as determined by LSmeans (P = 0.05). Standard error of the mean denoted by SE.

			Average mites remaining on thumbtack			
Genotype	Surface	Background	Mite No.	±	SE	
LA2329	Ab	Wild relative	8.33	±	0.88	a
LA2329	Ad	Wild relative	6.67	±	0.33	a
B116	Ab	Hybrid	7.67	±	0.33	a
B116	Ad	Hybrid	6.00	±	0.58	a
C72	Ab	Hybrid	8.00	±	0.58	a
C72	Ad	Hybrid	5.33	±	0.88	b
A119	Ab	Hybrid	7.33	±	0.88	a
A119	Ad	Hybrid	5.00	±	0.58	b
F32	Ab	Hybrid	5.00	±	0.58	a
F32	Ad	Hybrid	4.33	±	0.33	a
H19	Ab	Hybrid	4.00	±	1.00	a
H19	Ad	Hybrid	5.00	±	0.58	a
H21	Ab	Hybrid	3.33	±	0.67	a
H21	Ad	Hybrid	5.33	±	0.67	b
F51	Ab	Hybrid	4.33	±	1.20	a
F51	Ad	Hybrid	0.00	±	0.00	b
ZH2	Ab	Cultivated	1.67	±	0.88	a
ZH2	Ad	Cultivated	0.67	±	0.33	a
SROMA	Ab	Cultivated	0.00	±	0.00	a
SROMA	Ad	Cultivated	0.00	±	0.00	a

Table 4–20: ANOVA results for total distance travelled by spider mites (cm) after 15, 30, 45, and 60 min for adaxial and abaxial surfaces of 10 tomato genotypes tested in the thumbtack bioassay.

Source of Variations	DF	15 min		30 min	
		F Value	P Value	F Value	P Value
Genotype	9	27.23	<.0001	15.66	<.0001
Surface	1	11.65	0.0015	22.84	<.0001
Genotype X Surface	9	6.10	<.0001	2.97	0.0084
Error	40				
R^2		0.89		0.83	

Table 4-20 (continued)

Source of Variations	DF	45 min		60 min	
		F Value	P Value	F Value	P Value
Genotype	9	12.48	<.0001	19.97	<.0001
Surface	1	13.01	0.0009	9.35	0.004
Genotype X Surface	9	1.85	0.0882ns	3.35	0.0039
Error	40				
R^2		0.78		0.85	

Table 4–21: Means of total distance travelled by spider mites (cm) after 15 min for 10 tomato genotypes tested in the thumbtack bioassay. Means followed by the same letter are not significantly different (P = 0.05) based on Duncan's multiple range test. Standard error of the mean denoted by SE.

		Average total distance travelled by mites			
Genotype	Background	Distance (cm)	±	SE	
B116	Hybrid	0.29	±	0.19	a
C72	Hybrid	0.42	±	0.24	a
A119	Hybrid	1.12	±	0.37	a
LA2329	Wild relative	1.29	±	0.66	ab
F32	Hybrid	1.51	±	0.45	ab
H19	Hybrid	2.03	±	0.27	ab
H21	Hybrid	2.93	±	0.94	b
F51	Hybrid	6.60	±	1.96	c
ZH2	Cultivated	6.84	±	0.77	c
SROMA	Cultivated	8.15	±	0.71	c

Table 4–22: Means of total distance travelled by spider mites (cm) after 30 min for 10 tomato genotypes tested in the thumbtack bioassay. Means followed by the same letter are not significantly different (P = 0.05) based on Duncan's multiple range test. Standard error of the mean denoted by SE.

		Average total distance travelled by mites			
Genotype	Background	Distance (cm)	±	SE	
C72	Hybrid	0.62	±	0.37	a
B116	Hybrid	2.00	±	0.59	ab
A119	Hybrid	2.09	±	0.59	ab
LA2329	Wild relative	3.18	±	1.41	abc
F32	Hybrid	3.34	±	0.83	bc
H21	Hybrid	4.90	±	1.07	c
H19	Hybrid	4.98	±	0.69	c
F51	Hybrid	8.41	±	2.54	d
ZH2	Cultivated	8.60	±	0.67	d
SROMA	Cultivated	10.70	±	0.81	d

Table 4–23: Means of total distance travelled by spider mites (cm) after 45 min for 10 tomato genotypes tested in the thumbtack bioassay. Means followed by the same letter are not significantly different (P = 0.05) based on Duncan's multiple range test. Standard error of the mean denoted by SE.

		Average total distance travelled by mites			
Genotype	Background	Distance (cm)	±	SE	
C72	Hybrid	1.75	±	0.92	a
LA2329	Wild relative	2.81	±	1.18	ab
B116	Hybrid	3.15	±	0.84	ab
F32	Hybrid	4.47	±	1.35	ab
A119	Hybrid	4.60	±	0.32	ab
H19	Hybrid	6.06	±	1.06	b
H21	Hybrid	6.30	±	1.98	b
ZH2	Cultivated	10.21	±	0.52	c
F51	Hybrid	10.69	±	2.52	c
SROMA	Cultivated	14.42	±	1.41	d

Table 4–24: Means of total distance travelled by spider mites (cm) after 60 min for 10 tomato genotypes tested in the thumbtack bioassay. Means followed by the same letter are not significantly different (P = 0.05) based on Duncan's multiple range test. Standard error of the mean denoted by SE.

		Average total distance travelled by mites			
Genotype	Background	Distance (cm)	±	SE	
LA2329	Wild relative	2.50	±	0.87	a
C72	Hybrid	2.93	±	1.07	a
B116	Hybrid	3.94	±	0.68	a
F32	Hybrid	4.82	±	0.43	a
A119	Hybrid	4.91	±	1.45	a
H21	Hybrid	8.61	±	1.73	b
H19	Hybrid	8.89	±	1.09	b
F51	Hybrid	12.01	±	2.66	c
ZH2	Cultivated	12.17	±	1.11	c
SROMA	Cultivated	17.17	±	1.23	d

Table 4–25: Means of total distance travelled by spider mites (cm) after 15, 30, 45, and 60 min on adaxial and abaxial leaflet surfaces for 10 tomato genotypes tested in the thumbtack bioassay. Means followed by the same letter are not significantly different (P = 0.05) based on Duncan's multiple range test. Standard error of the mean denoted by SE.

Surface	Average total distance travelled by mites After 15 min				Average total distance travelled by mites After 30 min			
	Distance (cm)	±	SE		Distance (cm)	±	SE	
Abaxial	2.51	±	0.65	a	3.61	±	0.63	a
Adaxial	3.73	±	0.57	b	6.15	±	0.79	b

Table 3-25 (continued)

Surface	Average total distance travelled by mites After 45 min				Average total distance travelled by mites After 60 min			
	Distance (cm)	±	SE		Distance (cm)	±	SE	
Abaxial	5.13	±	0.82	a	6.75	±	0.97	a
Adaxial	7.76	±	0.93	b	8.84	±	1.04	b

Table 4–26: Means of total distance travelled by spider mites (cm) after 15 min for abaxial (Ab) and adaxial (Ad) leaflet surfaces of 10 tomato genotypes tested in the thumbtack bioassay. Means for the two surfaces of a genotype followed by the same letter are significantly different as determined by LSmeans (*P* = 0.05). Standard error of the mean denoted by SE.

			Average total distance travelled by mites			
Genotype	Surface	Background	Distance (cm)	±	SE	
B116	Ab	Hybrid	0.06	±	0.06	a
B116	Ad	Hybrid	0.51	±	0.35	a
C72	Ab	Hybrid	0.00	±	0.00	a
C72	Ad	Hybrid	0.84	±	0.33	a
A119	Ab	Hybrid	0.55	±	0.29	a
A119	Ad	Hybrid	1.69	±	0.53	b
LA2329	Ab	Wild relative	0.04	±	0.04	a
LA2329	Ad	Wild relative	2.54	±	0.79	b
F32	Ab	Hybrid	1.28	±	0.62	a
F32	Ad	Hybrid	1.75	±	0.75	a
H19	Ab	Hybrid	1.98	±	0.42	a
H19	Ad	Hybrid	2.08	±	0.44	a
H21	Ab	Hybrid	3.29	±	1.79	a
H21	Ad	Hybrid	2.56	±	1.05	a
F51	Ab	Hybrid	2.31	±	0.72	a
F51	Ad	Hybrid	10.89	±	0.50	b
ZH2	Ab	Cultivated	6.81	±	0.90	a
ZH2	Ad	Cultivated	6.88	±	1.46	a
SROMA	Ab	Cultivated	8.79	±	0.58	a
SROMA	Ad	Cultivated	7.52	±	1.34	a

Table 4–27: Means of total distance travelled by spider mites (cm) after 30 min for abaxial (Ab) and adaxial (Ad) leaflet surfaces of 10 tomato genotypes tested in the thumbtack bioassay. Means for the two surfaces of a genotype followed by the same letter are not significantly different as determined by LSmeans (P = 0.05). Standard error of the mean denoted by SE.

			Average total distance travelled by mites			
Genotype	Surface	Background	Distance (cm)	±	SE	
C72	Ab	Hybrid	0.05	±	0.05	a
C72	Ad	Hybrid	1.18	±	0.60	a
B116	Ab	Hybrid	1.00	±	0.55	a
B116	Ad	Hybrid	3.00	±	0.64	a
A119	Ab	Hybrid	1.21	±	0.68	a
A119	Ad	Hybrid	2.96	±	0.72	a
LA2329	Ab	Wild relative	1.98	±	1.87	a
LA2329	Ad	Wild relative	4.37	±	2.24	a
F32	Ab	Hybrid	2.30	±	0.92	a
F32	Ad	Hybrid	4.38	±	1.22	a
H21	Ab	Hybrid	3.79	±	1.76	a
H21	Ad	Hybrid	6.00	±	1.18	b
H19	Ab	Hybrid	4.15	±	0.32	a
H19	Ad	Hybrid	5.81	±	1.27	b
F51	Ab	Hybrid	3.28	±	1.22	a
F51	Ad	Hybrid	13.54	±	2.11	b
ZH2	Ab	Cultivated	9.07	±	0.87	a
ZH2	Ad	Cultivated	8.14	±	1.12	a
SROMA	Ab	Cultivated	9.26	±	0.79	a
SROMA	Ad	Cultivated	12.14	±	0.79	b

Table 4–28: Means of total distance travelled by spider mites (cm) after 60 min for abaxial (Ab) and adaxial (Ad) leaflet surfaces of 10 tomato genotypes tested in the thumbtack bioassay. Means for the two surfaces of a genotype followed by the same letter are not significantly different as determined by LSmeans (P = 0.05). Standard error of the mean denoted by SE.

Genotype	Surface	Background	Average total distance travelled by mites			
			Distance (cm)	±	SE	
LA2329	Ab	Wild relative	1.26	±	0.90	a
LA2329	Ad	Wild relative	3.73	±	1.20	a
C72	Ab	Hybrid	1.37	±	0.73	a
C72	Ad	Hybrid	4.48	±	1.68	a
B116	Ab	Hybrid	3.04	±	0.49	a
B116	Ad	Hybrid	4.84	±	1.13	a
F32	Ab	Hybrid	4.94	±	0.76	a
F32	Ad	Hybrid	4.69	±	0.59	a
A119	Ab	Hybrid	2.89	±	1.29	a
A119	Ad	Hybrid	6.93	±	2.18	a
H21	Ab	Hybrid	10.68	±	1.87	a
H21	Ad	Hybrid	6.55	±	2.67	a
H19	Ab	Hybrid	9.33	±	2.05	a
H19	Ad	Hybrid	8.44	±	1.22	a
F51	Ab	Hybrid	6.52	±	1.44	a
F51	Ad	Hybrid	17.50	±	1.76	b
ZH2	Ab	Cultivated	10.50	±	1.02	a
ZH2	Ad	Cultivated	13.83	±	1.53	a
SROMA	Ab	Cultivated	16.93	±	2.62	a
SROMA	Ad	Cultivated	17.41	±	0.77	a

Table 4–29: Correlation coefficients among trichome type IV density, trichome type VI density, zingiberene content and mite repellence variables obtained from the thumbtack bioassay of two surfaces of 10 tomato genotypes. N=30 for each leaflet surface and time interval.

Variables	15 min Abaxial Surface		30 min Abaxial Surface		45 min Abaxial Surface		60 min Abaxial Surface	
	MOT	TDTM	MOT	TDTM	MOT	TDTM	MOT	TDTM
IV Density	0.69***	-0.72***	0.75***	-0.72***	0.71***	-0.68***	0.76***	-0.78***
VI Density	0.49**	-0.37*	0.43*	-0.33	0.52**	-0.34	0.49**	-0.41*
Zingiberene	0.66***	-0.63***	0.65***	-0.59**	0.69***	-0.59**	0.65***	-0.50**

Variables	Adaxial Surface		Adaxial Surface		Adaxial Surface		Adaxial Surface	
	MOT	TDTM	MOT	TDTM	MOT	TDTM	MOT	TDTM
IV Density	0.64***	-0.58**	0.58**	-0.54**	0.46**	-0.51**	0.59**	-0.57**
VI Density	0.37*	-0.30	0.32	-0.35	0.37*	-0.32	0.46**	-0.41*
Zingiberene	0.77***	-0.71***	0.79***	-0.67***	0.84***	-0.66***	0.84***	-0.68***

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. IV Density= trichome type IV Density (No./mm^2), VI Density= trichome type VI Density (No/mm^2), Zingiberene determined as GC area units/cm^2 of leaf area, MOT= Number of mites remaining on tack, TDTM=total distance travelled by mites.

Table 4–30: Parameter estimates (ParEst), standard errors (SE), and P-values for multiple regression of number of mites remaining on thumbtack on the independent variables of zingiberene and β-phellandrene content and type IV and type VI trichome densities for abaxial leaf surfaces of 10 tomato genotypes evaluated at 15, 30, 45 and 60 in the thumbtack bioassay. Independent variables were transformed to $\log_{10}(X+1)$ prior to analysis. ns = non-significant.

	After 15 min						
	Full Model				**Reduced Model**		
Variable	**ParEst**	**SE**	**P Value**		**ParEst**	**SE**	**P Value**
Intercept	2.86	0.66	0.0002		3.01	0.61	<.0001
IV Density	1.83	0.40	0.0001		1.76	0.38	<.0001
VI Density	0.72	1.16	0.543ns				
Zingiberene	0.45	0.14	0.0050		0.51	0.10	<.0001
β-phellandrene	0.18	0.11	0.123ns		0.21	0.11	0.061ns

	After 30 min						
	Full Model				**Reduced Model**		
Variable	**ParEst**	**SE**	**P Value**		**ParEst**	**SE**	**P Value**
Intercept	2.01	0.65	0.0049		2.59	0.45	<.0001
IV Density	2.29	0.39	<.0001		2.50	0.35	<.0001
VI Density	-0.05	1.14	0.968ns				
Zingiberene	0.49	0.14	0.0017		0.40	0.07	<.0001
β-phellandrene	0.16	0.11	0.182ns				

Table 4-30 (continued)

After 45 min

Variable	Full Model				Reduced Model		
	ParEst	SE	P Value		ParEst	SE	P Value
Intercept	0.98	0.66	0.1555		1.71	0.46	0.0010
IV Density	2.31	0.41	<.0001		2.39	0.36	<.0001
VI Density	1.01	1.17	0.395ns				
Zingiberene	0.46	0.14	0.0038		0.47	0.07	<.0001
β-phellandrene	0.09	0.11	0.402ns				

After 60 min

Variable	Full Model				Reduced Model		
	ParEst	SE	P Value		ParEst	SE	P Value
Intercept	0.20	0.64	0.7530		0.94	0.45	0.0459
IV Density	2.56	0.39	<.0001		2.60	0.35	<.0001
VI Density	1.27	1.13	0.269ns				
Zingiberene	0.37	0.14	0.0126		0.42	0.07	<.0001
β-phellandrene	0.08	0.11	0.494ns				

Table 4–31: Parameter estimates (ParEst), standard errors (SE), and P-values for multiple regression of number of mites remaining on thumbtack on the independent variables of zingiberene and β-phellandrene content and type IV and type VI trichome densities for adaxial leaf surfaces of 10 tomato genotypes evaluated at 15, 30, 45 and 60 in the thumbtack bioassay. Independent variables were transformed to $\log_{10}(X+1)$ prior to analysis. ns = non-significant.

| | After 15 min | | | | | | |
| | Full Model | | | | Reduced Model | | |
Variable	ParEst	SE	P Value		ParEst	SE	P Value
Intercept	2.96	0.97	0.0054		3.24	0.45	<.0001
IV Density	1.92	0.69	0.0100		1.84	0.61	0.0055
VI Density	0.67	0.99	0.502ns				
Zingiberene	0.41	0.13	0.0037		0.46	0.09	<.0001
β-phellandrene	-0.04	0.12	0.746ns				

Table 4-31 (continued)

After 30 min

Variable	Full Model			Reduced Model		
	ParEst	SE	P Value	ParEst	SE	P Value
Intercept	1.26	0.97	0.2037	2.03	0.46	0.0001
IV Density	1.02	0.68	0.149ns	1.38	0.62	0.0338
VI Density	0.13	0.98	0.894ns			
Zingiberene	0.58	0.13	0.0001	0.48	0.09	<.0001
β-phellandrene	0.16	0.12	0.215ns			

After 45 min

Variable	Full Model			Reduced Model		
	ParEst	SE	P Value	ParEst	SE	P Value
Intercept	0.58	0.92	0.5341	1.66	0.42	0.0004
IV Density	0.15	0.65	0.817ns			
VI Density	0.52	0.93	0.578ns			
Zingiberene	0.66	0.12	<.0001	0.61	0.08	<.0001
β-phellandrene	0.15	0.12	0.222ns			

After 60 min

Variable	Full Model			Reduced Model		
	ParEst	SE	P Value	ParEst	SE	P Value
Intercept	0.14	0.83	0.8649	1.00	0.39	0.0147
IV Density	1.27	0.59	0.0390	1.27	0.54	0.0258
VI Density	1.36	0.84	0.118ns			
Zingiberene	0.47	0.11	0.0002	0.52	0.08	<.0001
β-phellandrene	-0.01	0.11	0.899ns			

Table 4–32: Parameter estimates (ParEst), standard errors (SE), and P-values for multiple regression of total distance travelled by spider mites (cm) on the independent variables of zingiberene and β-phellandrene content and type IV and type VI trichome densities for abaxial leaf surfaces of 10 tomato genotypes evaluated at 15, 30, 45 and 60 in the thumbtack bioassay. Independent variables were transformed to $\log_{10}(X+1)$ prior to analysis. ns = non-significant.

After 15 min

	Full Model				Reduced Model		
Variable	ParEst	SE	P Value		ParEst	SE	P Value
Intercept	7.07	0.78	<.0001		6.55	0.55	<.0001
IV Density	-2.19	0.47	0.0001		-2.59	0.43	<.0001
VI Density	1.27	1.37	0.363ns				
Zingiberene	-0.66	0.17	0.0006		-0.42	0.09	<.0001
β-phellandrene	-0.25	0.13	0.081ns				

After 30 min

	Full Model				Reduced Model		
Variable	ParEst	SE	P Value		ParEst	SE	P Value
Intercept	8.16	0.95	<.0001		7.97	0.64	<.0001
IV Density	-2.63	0.57	0.0001		-2.92	0.50	<.0001
VI Density	1.27	1.66	0.448ns				
Zingiberene	-0.62	0.20	0.0058		-0.43	0.10	0.0003
β-phellandrene	-0.16	0.16	0.329ns				

After 45 min

	Full Model				Reduced Model		
Variable	ParEst	SE	P Value		ParEst	SE	P Value
Intercept	10.46	1.37	<.0001		10.67	0.91	<.0001
IV Density	-3.46	0.83	0.0003		-3.56	0.72	<.0001
VI Density	1.01	2.40	0.677ns				
Zingiberene	-0.67	0.30	0.0327		-0.58	0.15	0.0005
β-phellandrene	-0.04	0.24	0.883ns				

Table 4-32 (continued)   **After 60 min**

Variable	Full Model			Reduced Model		
	ParEst	SE	P Value	ParEst	SE	P Value
Intercept	15.76	1.31	<.0001	14.96	1.18	<.0001
IV Density	-4.81	0.79	<.0001	-5.57	0.71	<.0001
VI Density	-2.86	2.29	0.225ns	-5.70	1.52	0.0009
Zingiberene	-0.52	0.28	0.080ns			
β-phellandrene	-0.37	0.23	0.112ns			

Table 4–33: Parameter estimates (ParEst), standard errors (SE), and P-values for multiple regression of total distance travelled by spider mites (cm) on the independent variables of zingiberene and β-phellandrene content and type IV and type VI trichome densities for adaxial leaf surfaces of 10 tomato genotypes evaluated at 15, 30, 45 and 60 in the thumbtack bioassay. Independent variables were transformed to $\log_{10}(X+1)$ prior to analysis. ns = non-significant.

After 15 min

Variable	Full Model			Reduced Model		
	ParEst	SE	P Value	ParEst	SE	P Value
Intercept	7.07	1.54	0.0001	7.15	0.71	<.0001
IV Density	-2.35	1.09	0.0415	-2.16	0.96	0.0327
VI Density	-0.42	1.57	0.789ns			
Zingiberene	-0.49	0.20	0.0232	-0.56	0.14	0.0004
β-phellandrene	0.08	0.19	0.674ns			

After 30 min

Variable	Full Model			Reduced Model		
	ParEst	SE	P Value	ParEst	SE	P Value
Intercept	12.34	1.94	<.0001	10.07	0.92	<.0001
IV Density	-1.71	1.38	0.225ns	-2.42	1.25	0.064ns
VI Density	-1.44	1.98	0.473ns			
Zingiberene	-0.81	0.26	0.0044	-0.65	0.18	0.0014
β-phellandrene	-0.30	0.25	0.245ns			

Table 4-33 (continued)

After 45 min

Variable	Full Model			Reduced Model		
	ParEst	SE	P Value	ParEst	SE	P Value
Intercept	14.87	2.38	<.0001	14.30	1.69	<.0001
IV Density	-1.63	1.69	0.343ns			
VI Density	-1.28	2.43	0.602ns			
Zingiberene	-0.99	0.31	0.0040	-1.23	0.24	<.0001
β-phellandrene	-0.40	0.30	0.202ns	-0.53	0.27	0.065ns

After 60 min

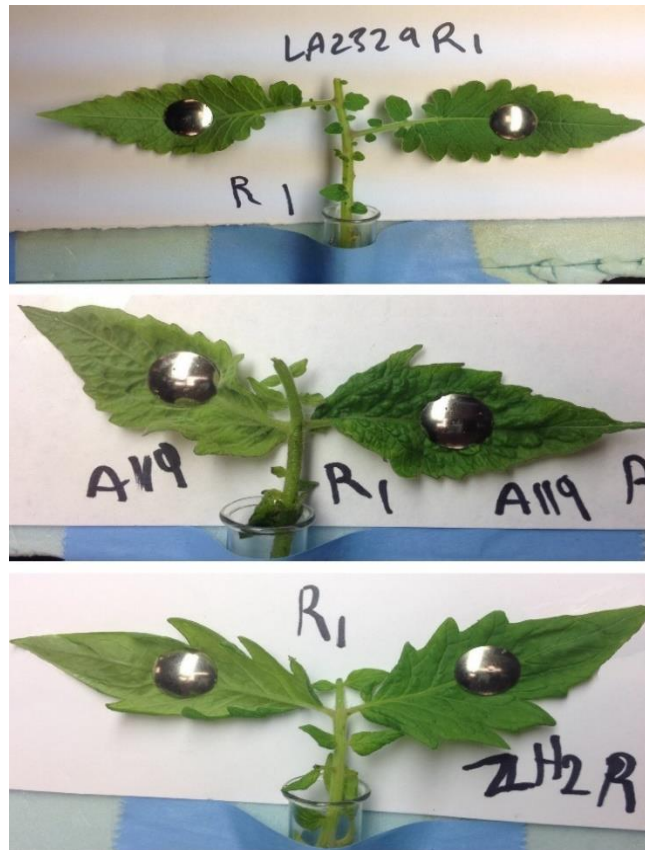| Variable	Full Model			Reduced Model		
	ParEst	SE	P Value	ParEst	SE	P Value
Intercept	16.79	2.48	<.0001	14.14	1.17	<.0001
IV Density	-3.24	1.76	0.077ns	-3.57	1.59	0.0332
VI Density	-3.14	2.53	0.225ns			
Zingiberene	-0.83	0.33	0.0182	-0.85	0.23	0.0011
β-phellandrene	-0.12	0.32	0.707ns			

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Figure 4–1: Examples of the thumbtack bioassay showing three genotypes setup on the Styrofoam board under laboratory conditions where ten adult female mites were placed onto each thumbtack.

CHAPTER 5. Implications and Future Perspectives

This research provides considerable insights into the resistance of tomato to two-spotted spider mites *T. urticae*. The wild tomato species *S. habrochaites* produces the bioactive compound, zingiberene alcohol, conferring acaricidal activity towards *T. urticae*. Based on research presented herein, mite resistance mediated by type IV trichome density and zingiberene have been successfully transferred from the wild tomato species into advanced interspecific hybrids.

The discoveries associated with characterizing the acaricidal properties of the newly discovered zingiberene alcohol, accompanied by the study of the comparative activities of zingiberene stereoisomers, should motivate breeders and natural product chemists to further discover and exploit powerful allelochemicals. The efficacy of zingiberene alcohol against mites suggests it could be employed as natural acaricide, replacing synthetic pesticides. Thus, tomato breeders should consider introgression of the genes responsible for zingiberene alcohol from the wild species, such as LA2329OH or PI127826, into cultivated tomato lines to improve resistance against a broad spectrum of herbivorous pests. Future studies could aim at combining multiple advanced backcross hybrids of tomato to develop recombinant inbred lines (RILs) that would facilitate studying spider mite resistance characters while possibly avoiding undesirable linked or epistatic plant traits.

The hybrids that were tested in this dissertation in different bioassays are promising with regard to progress of the tomato-breeding program, which targets release of tomato cultivars with reliable resistance to arthropod pests combined with desirable characteristics for agriculture production. Introgression of leaf chemical compounds and specific trichomes from wild into cultivated advanced-backcross lines has proven effective in producing lines resistant to mites, especially in these early generations, BC_3 and BC_4. Nonetheless, integrative procedures that adopt conventional breeding and molecular biotechnology methods, e.g. marker-assisted backcross, could significantly accelerate breeding programs and recover more genetic resources responsible for unique biological features (Monforte and Tanksley 2000). Also, marker-assisted selection (MAS) may be useful to reduce linkage drag while recovering the desirable phenotype from the recurrent parent genotype (Tanksley et al. 1989).

One of the breeding objectives is to produce arthropod-resistant lines and make them affordable and accessible for future studies. This may permit breeders and geneticists to discover other factors associated with resistance. These advanced lines may also be candidates for gene editing that would target defects like nonfertility, slow germination, and fruit size. Gene editing to promote the synthesis of zingiberene alcohol could be an additional and appropriate technology to improve tomato resistance.

The bioassays used in this research are rapid methods especially when using small number of genotypes. However, their application to large populations will be difficult and costly. However, there may be potential for direct selection for arthropod resistance using

the whole leaf bioassay based on density of webbing and oviposition measured after few days.

The image-based thumbtack bioassay is one aspect of high throughput phenotyping for resistance to arthropods. Distance traveled by mites was successfully evaluated from images obtained during the bioassay. Bioassays aided by image analysis could allow capture of extra parameters such as mite webbing and egg density in a quick and accurate manner, reducing reliance on less reliable and less objective methods such as visual rating scales. Photography of multiple live insects on plant surfaces accompanied by image processing for measuring insect movement provided reliable and accurate data (Hoffmann et al. 2010), a finding reiterated in this research. Although capturing images for our thumbtack bioassay was quick and straightforward, it was laborious to measure distance traveled by mites on tomato leaf surfaces. However, with additional research, it may be possible to automate, or partially automate the image analysis. Regardless, using image analysis for assessment of mite movement would be a component of high throughput phenotyping and would provide useful research information in the era of omics.

The reader should keep in mind that the hybrids evaluated in my bioassays might not have the same level of resistance to spider mite under other conditions, for example, under field conditions. In other words, do the tested hybrids have really adequate and usable resistance useful for production of productive and adapted tomato varieties? Therefore, determining the relationship between responses observed in short term laboratory bioassays and responses under field conditions is the next step. If backcross

hybrids are advanced by inbreeding until completely inbred lines have been obtained, we do not know whether these inbreds would have adequate levels of resistance to spider mites. Resistance testing will need to be expanded to verify the utility of my initial results.

The successful use of two-spotted spider mites in this research was partly related to their short reproduction period and the ease of rearing them under lab conditions. It is likely that mites can be considered as a model of the interaction of tomato with other small herbivorous attackers like aphid, pinworm, and whitefly. Thus, it would not be surprising that the mite resistant hybrids identified in this research would also be resistant to other arthropods.

REFERENCES

Alba JM, Montserrat M, Fernandez-Munoz R (2009) Resistance to the two-spotted spider mite (*Tetranychus urticae*) by acylsucroses of wild tomato (*Solanum pimpinellifolium*) trichomes studied in a recombinant inbred line population. Exp Appl Acarol 47 (1):35-47

Andrade MC, da Silva AA, Carvalho RdC, de Andrade Santiago J, de Oliveira AMS, Francis DM, Maluf WR (2018) Quantitative trait loci associated with trichomes in the *Solanum galapagense* accession LA1401. Genetic Resources and Crop Evolution 65 (6):1671-1685

Andrade MC, da Silva AA, Neiva IP, Oliveira IRC, De Castro EM, Francis DM, Maluf WR (2017) Inheritance of type IV glandular trichome density and its association with whitefly resistance from *Solanum galapagense* accession LA1401. Euphytica 213 (2):52

Antônio ADC, Silva DJHD, Picanço MC, Santos NT, Fernandes MES (2011) Tomato plant inheritance of antixenotic resistance to tomato leafminer. Pesquisa Agropecuária Brasileira 46 (1):74-80

Antonious GF (2016) Exploring wild tomato leaf extracts in pesticide formulations. Journal of Environmental & Analytical Toxicology 06 (01)

Antonious GF, Kamminga K, Snyder JC (2014) Wild tomato leaf extracts for spider mite and cowpea aphid control. J Environ Sci Health B 49 (7):527-531

Antonious GF, Snyder JC (2006) Natural products: repellency and toxicity of wild tomato leaf extracts to the two-spotted spider mite, *Tetranychus urticae* Koch. J Environ Sci Health B 41 (1):43-55

Antonious GF, Snyder JC (2008) Tomato leaf crude extracts for insects and spider mite control. In: V. R. Preedy, Watson RR (eds) Tomatoes and tomato products: nutritional, medicinal and therapeutic properties. Department of Nutrition and Dietetics, King's College London; Science Publishers London, pp 269-297

Antonious GF, Snyder JC (2015) Repellency and oviposition deterrence of wild tomato leaf extracts to spider mites, *Tetranychus urticae* Koch. J Environ Sci Health B 50 (9):667-673

Aragão CA, Dantas BF, Benites FRG (2000) Tricomas foliares em tomateiro com teores contrastantes do aleloquímico 2-Tridecanona. Scientia Agricola 57 (4):813-816

Azevedo SM, Maluf WR, Faria MV, Oliveira ACB, Ribeiro CA, R.C. G-G, Santa-Cecília LVC (1999) Resistência à traça (*Tuta absoluta*) em genótipos de tomateiro com diferentes teores de sesquiterpenos nos folíolos. In: CONGRESSO BRASILEIRO DE OLERICULTURA Resumo Tubarão: SOB 39 (1999):38

Aznar-Fernández T, Rubiales D (2018) Identification and characterisation of antixenosis and antibiosis to pea aphid (*Acyrthosiphon pisum*) in *Pisum* spp. germplasm. Annals of Applied Biology 172 (3):268-281

Baldwin IT, Halitschke R, Kessler A, Schittko U (2001) Merging molecular and ecological approaches in plant–insect interactions. Current Opinion in Plant Biology 4 (4):351-358

Barczak-Brzyzek AK, Kielkiewicz M, Gawronski P, Kot K, Filipecki M, Karpinska B (2017) Cross-talk between high light stress and plant defence to the two-spotted spider mite in *Arabidopsis thaliana*. Exp Appl Acarol 73 (2):177-189

Bas N, Mollema C, Lindhout P (1992) Resistance in *Lycopersicon hirsutum* f. *glabratum* to the greenhouse whitefly (*Trialeurodes vaporariorum*) increases with plant age. Euphytica 64 (3):189-195

Baur R, Binder S, Benz G (1991) Nonglandular leaf trichomes as short-term inducible defense of the grey alder, *Alnus incana* (L.), against the chrysomelid beetle, *Agelastica alni* L. Oecologia 87 (2):219-226

Bergau N, Bennewitz S, Syrowatka F, Hause G, Tissier A (2015) The development of type VI glandular trichomes in the cultivated tomato *Solanum lycopersicum* and a related wild species *S. habrochaites*. BMC Plant Biol 15:289

Bergougnoux V (2014) The history of tomato: from domestication to biopharming. Biotechnol Adv 32 (1):170-189

Bleeker PM, Diergaarde PJ, Ament K, Schutz S, Johne B, Dijkink J, Hiemstra H, de Gelder R, de Both MT, Sabelis MW, Haring MA, Schuurink RC (2011) Tomato-produced 7-epizingiberene and R-curcumene act as repellents to whiteflies. Phytochemistry 72 (1):68-73

Bleeker PM, Mirabella R, Diergaarde PJ, VanDoorn A, Tissier A, Kant MR, Prins M, de Vos M, Haring MA, Schuurink RC (2012) Improved herbivore resistance in cultivated tomato with the sesquiterpene biosynthetic pathway from a wild relative. Proc Natl Acad Sci U S A 109 (49):20124-20129

Breeden DC, Coates RM (1994) 7-Epizingiberene, A novel bisabolane sesquiterpene from wild tomato leaves. Tetrahedron 50 (38):11123-11132

Carter CD, Sacalis JN, Gianfagna TJ (1989) Zingiberene and resistance to Colorado potato beetle in *Lycopersicon hirsutum* f. *hirsutum*. J of Agricultural and Food Chemistry 37 (1):206-210

Carter CD, Snyder JC (1985) Mite responses in relation to trichomes of *Lycopersicon esculentum* x *L. hirsutum* F2 hybrids. Euphytica 34 (1):177-185

Channarayappa C, Shivashankar G, Muniyappa V, Frist R (1992) Resistance of *Lycopersicon* species to *Bemisia tabaci*, a tomato leaf curl virus vector. Canadian Journal of Botany 70 (11):2184-2192

de Azevedo SM, Ventura Faria M, Maluf WR, Barneche de Oliveira AC, de Freitas JA (2003) Zingiberene-mediated resistance to the South American tomato pinworm derived from *Lycopersicon hirsutum* var. *hirsutum*. Euphytica 134 (3):347-351

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

Dobzhansky T (1937) Genetics and the origin of species. Columbia University Press, New York.

Egas M, Norde D, Sabelis M (2003) Adaptive learning in arthropods: spider mites learn to distinguish food quality. Experimental and Applied Acarology 30 (4):233-247

Eigenbrode SD, Trumble JT, Millar JG, White KK (1994) Topical toxicity of tomato sesquiterpenes to the Beet Armyworm and the role of these compounds in resistance derived from an Accession of *Lycopersicon hirsutum* f. *typicum*. J of Agricultural and Food Chemistry 42 (3):807-810

EP Patent No. EP3178313A1 (2017) Gene and protein for the synthesis of oxidized zingiberene derivatives.

Falara V, Akhtar TA, Nguyen TT, Spyropoulou EA, Bleeker PM, Schauvinhold I, Matsuba Y, Bonini ME, Schilmiller AL, Last RL, Schuurink RC, Pichersky E (2011) The tomato terpene synthase gene family. Plant Physiol 157 (2):770-789

FAO (2017) FAOSTAT Statistical yearbook. Statistical Division Food and Agriculture Organization of the United Nations. http://www.fao.org/faostat/en/#home.

Fernández-Muñoz R, Salinas M, M Álvarez, Cuartero J (2003) Inheritance of resistance to two-spotted spider mite and glandular leaf trichomes in wild tomato *Lycopersicon pimpinellifolium* (Jusl.) Mill. J Am Soc Hortic Sci 128 (2):188-195

Freitas JA, Maluf WR, das Graças Cardoso M, Gomes LAA, Bearzotti E (2002) Inheritance of foliar zingiberene contents and their relationship to trichome densities and whitefly resistance in tomatoes. Euphytica 127 (2):275-287

Gianfagna TJ, Carter CD, Sacalis JN (1992) Temperature and photoperiod influence trichome density and sesquiterpene content of *Lycopersicon hirsutum* f. *hirsutum*. Plant Physiology 100 (3):1403-1405

Gonçalves LD, Maluf WR, Cardoso MdG, Resende JTVd, Castro EMd, Santos NM, Nascimento IRd, Faria MV (2006) Relação entre zingibereno, tricomas foliares e repelência de tomateiros a *Tetranychus evansi*. Pesquisa Agropecuária Brasileira 41 (2):267-273

Gonçalves LD, Maluf WR, das Graças Cardoso M, Gomes LAA, do Nascimento IR (2007) Herança de acilaçúcares em genótipos de tomateiro provenientes de cruzamento interespecífico. Pesquisa Agropecuária Brasileira 42 (5):699-705

Govindaraj M, Vetriventhan M, Srinivasan M (2015) Importance of genetic diversity assessment in crop plants and its recent advances: an overview of its analytical perspectives. Genet Res Int 2015:431487

Grbic M, Van Leeuwen T, Clark RM, Rombauts S, Rouze P, Grbic V, Osborne EJ, Dermauw W, Ngoc PC, Ortego F, Hernandez-Crespo P, Diaz I, Martinez M, Navajas M, Sucena E, Magalhaes S, Nagy L, Pace RM, Djuranovic S, Smagghe G, Iga M, Christiaens O, Veenstra JA, Ewer J, Villalobos RM, Hutter JL, Hudson SD, Velez M, Yi SV, Zeng J, Pires-daSilva A, Roch F, Cazaux M, Navarro M, Zhurov V, Acevedo G, Bjelica A, Fawcett JA, Bonnet E, Martens C, Baele G, Wissler L, Sanchez-Rodriguez A, Tirry L, Blais C, Demeestere K, Henz SR, Gregory TR, Mathieu J, Verdon L, Farinelli L, Schmutz J, Lindquist E, Feyereisen R, Van de Peer Y (2011) The genome of *Tetranychus urticae* reveals herbivorous pest adaptations. Nature 479 (7374):487-492

Guo Z, Weston PA, Snyder JC (1993) Repellency to two-spotted spider mite, *Tetranychus urticae* Koch, as related to leaf surface chemistry of *Lycopersicon hirsutum* accessions. Journal of chemical ecology 19 (12):2965-2979

Hance T, Van Impe G (1999) The influence of initial age structure on predator–prey interaction. Ecological Modelling 114 (2):195-211

Hoffmann WC, Jank PC, Klun JA, Fritz BK (2010) Quantifying the movement of multiple insects using an optical insect counter. Journal of the American Mosquito Control Association 26 (2):167-172

Huang M, Zhang H, Wang Z, Niu D, Li Y, Cui H (2018) Comparative studies of leaf surface chemical biosynthesis in different tobacco cultivars. Acta Physiologiae Plantarum 40 (4): 67

ID-36. http://www2.ca.uky.edu/agcomm/pubs/id/id36/id36.pdf.

JMP® version 12.1.0 SAS Institute Inc, Cary, NC, USA. Statistical Analysis System Institute.

Júnior JF, Resende JTV, da Silva AA, Gabriel A, Zeist AR, Favaro R, Nascimento DA, Zeist RA, Camargo CK (2018) Resistance to *Helicoverpa armigera* mediated by zingiberene and glandular trichomes in tomatoes for industrial processing. Genetics and Molecular Research 17 (2)

Kamphuis LG, Lichtenzveig J, Peng K, Guo SM, Klingler JP, Siddique KH, Gao LL, Singh KB (2013) Characterization and genetic dissection of resistance to spotted alfalfa aphid (*Therioaphis trifolii*) in *Medicago truncatula*. J Exp Bot 64 (16):5157-5172

Kang JH, Liu G, Shi F, Jones AD, Beaudry RM, Howe GA (2010) The tomato odorless-2 mutant is defective in trichome-based production of diverse specialized metabolites and broad-spectrum resistance to insect herbivores. Plant Physiol 154 (1):262-272

Kennedy GG (2003) Tomato, pests, parasitoids, and predators: tritrophic interactions involving the genus *Lycopersicon*. Annu Rev Entomol 48:51-72

Khrimian A, Zhang A, Weber DC, Ho HY, Aldrich JR, Vermillion KE, Siegler MA, Shirali S, Guzman F, Leskey TC (2014) Discovery of the aggregation pheromone of the brown marmorated stink bug (*Halyomorpha halys*) through the creation of stereoisomeric libraries of 1-bisabolen-3-ols. J Nat Prod 77 (7):1708-1717

Kim H, Xing G, Wang Y, Zhao T, Yu D, Yang S, Li Y, Chen S, Palmer RG, Gai J (2013) Constitution of resistance to common cutworm in terms of antibiosis and antixenosis in soybean RIL populations. Euphytica 196 (1):137-154

Kortbeek RW, Xu J, Ramirez A, Spyropoulou E, Diergaarde P, Otten-Bruggeman I, de Both M, Nagel R, Schmidt A, Schuurink RC, Bleeker PM (2016) Engineering of tomato glandular trichomes for the production of specialized metabolites. In: O'Connor SE (ed) Methods in enzymology, 576. Academic Press, pp 305-331

Labate JA, Grandillo S, Fulton T, Muños S, Caicedo AL, Peralta I, Ji Y, Chetelat RT, Scott JW, Gonzalo MJ, Francis D, Yang W, van der Knaap E, Baldo AM, Smith-White B, Mueller LA, Prince JP, Blanchard NE, Storey DB, Stevens MR, Robbins MD, Wang J-F, Liedl BE, O'Connell MA, Stommel JR, Aoki K, Iijima Y, Slade AJ, Hurst SR, Loeffler D, Steine MN, Vafeados D, McGuire C, Freeman C, Amen A, Goodstal J, Facciotti D, Van Eck J, Causse M (2007) Tomato. In: Kole C (ed) Vegetables. Springer Berlin Heidelberg, Berlin, Heidelberg, pp 1-125

Leckie BM, Halitschke R, De Jong DM, Smeda JR, Kessler A, Mutschler MA (2014) Quantitative trait loci regulating the fatty acid profile of acylsugars in tomato. Molecular Breeding 34 (3):1201-1213

Liedl B, McCormick S, Mutschler M (1996) Unilateral incongruity in crosses involving *Lycopersicon pennellii* and *L. esculentum* is distinct from self-incompatibility in expression, timing and location. Sexual Plant Reproduction 9 (5):299-308

Liedl BE, Lawson DM, White KK, Shapiro JA, Cohen DE, Carson WG, Trumble JT, Mutschler MA (1995) Acylsugars of wild tomato *Lycopersicon pennellii* alters settling and reduces oviposition of *Bemisia argentifolii* (Homoptera: Aleyrodidae). Journal of Economic Entomology 88 (3):742-748

Lima IP, Resende JT, Oliveira JR, Faria MV, Resende NC, Lima Filho RB (2015) Indirect selection of industrial tomato genotypes rich in zingiberene and resistant to *Tuta absoluta* Meyrick. Genet Mol Res 14 (4):15081-15089

Lima IP, Resende JTV, Oliveira JRF, Faria MV, Dias DM, Resende NCV (2016) Selection of tomato genotypes for processing with high zingiberene content, resistant to pests. Horticultura Brasileira 34 (3):387-391

Lindhout P (2005) Genetics and breeding. In: Heuvelink E (ed) Tomatoes. CABI Publishing, Wallingford, UK:21–52

Lowry DB, Modliszewski JL, Wright KM, Wu CA, Willis JH (2008) Review. The strength and genetic basis of reproductive isolating barriers in flowering plants. Philos Trans R Soc Lond B Biol Sci 363 (1506):3009-3021

Lucatti AF, van Heusden AW, de Vos RCH, Visser RGF, Vosman B (2013) Differences in insect resistance between tomato species endemic to the Galapagos Islands. BMC Evolutionary Biology 13 (1):175

Lucini T, Faria MV, Rohde C, Resende JTV, de Oliveira JRF (2015) Acylsugar and the role of trichomes in tomato genotypes resistance to *Tetranychus urticae*. Arthropod-Plant Interactions 9 (1):45-53

Lucini T, Resende JT, Oliveira JR, Scabeni CJ, Zeist AR, Resende NC (2016) Repellent effects of various cherry tomato accessions on the two-spotted spider mite *Tetranychus urticae* Koch (Acari: Tetranychidae). Genet Mol Res 15 (1)

Luckwill LC (1943) The genus Lycopersicon an historical, biological, and taxonomic survey of the wild and cultivated tomatoes. Aberdeen University Studies No. 120, Aberdeen University Press

Lundgren L, Norelius C, Stenhagen G (1985) Leaf volatiles from some wild tomato species. Nordic Journal of Botany 5 (4):315-320

Maciel GM, Almeida RS, da Rocha JP, Andalo V, Marquez GR, Santos NC, Finzi RR (2017) Mini tomato genotypes resistant to the silverleaf whitefly and to two-spotted spider mites. Genet Mol Res 16 (1):1-9

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

Macke E, Magalhães S, Bach F, Olivieri I (2011) Experimental evolution of reduced sex ratio adjustment under local mate competition. Science (New York, NY) 334 (6059):1127-1129

Maluf WR, Campos GA, das Graças Cardoso M (2001) Relationships between trichome types and spider mite (*Tetranychus evansi*) repellence in tomatoes with respect to foliar zingiberene contents. Euphytica 121 (1):73-80

Maluf WR, Inoue IF, Ferreira RdPD, Gomes LAA, Castro EMd (2007) Higher glandular trichome density in tomato leaflets and repellence to spider mites. Pesquisa Agropecuaria Brasileira 42 (9):1227-1235

Maria IGM, Antonio EGSA, Felipe MTV, Wbyratan LS, Liziane ML, Reginaldo C, Pericles AMF, Roseane CS (2016) Bioactivity of basil (*Ocimum basilcum* L.) on control of the spider mite (*Tetranychus urticae* Koch.) in peanut. African Journal of Biotechnology 15 (30):1597-1607

Meck ED, Kennedy GG, Walgenbach JF (2013) Effect of *Tetranychus urticae* (Acari: Tetranychidae) on yield, quality, and economics of tomato production. Crop Protection 52:84-90

Meck ED, Walgenbach JF, Kennedy GG (2009) Effect of vegetation management on autumn dispersal of *Tetranychus urticae* (Acari: Tetranychidae) from tomato. Journal of Applied Entomology 133 (9-10):742-748.

Meck ED, Walgenbach JF, Kennedy GG (2012) Association of *Tetranychus urticae* (Acari: Tetranychidae) feeding and gold fleck damage on tomato fruit. Crop Protection 42:24-29

Monforte AJ, Tanksley SD (2000) Development of a set of near isogenic and backcross recombinant inbred lines containing most of the *Lycopersicon hirsutum* genome in a *L. esculentum* genetic background: a tool for gene mapping and gene discovery. Genome 43 (5):803-813

Muigai SG, Schuster DJ, Snyder JC, Scott JW, Bassett MJ, McAuslane HJ (2002) Mechanisms of resistance in *Lycopersicon* germplasm to the whitefly *Bemisia argentifolii*. Phytoparasitica : Journal of Plant Protection Sciences 30 (4):347-360

Murungi LK, Knapp M, Salifu D, Wesonga J, Nyende A, Masinde P, Torto B (2012) Differential effects of various African nightshade species on the fecundity and movement of *Tetranychus evansi* (Acari: Tetranychidae). International Journal of Tropical Insect Science 31 (04):269-276

Nakazato T, Housworth EA (2011) Spatial genetics of wild tomato species reveals roles of the Andean geography on demographic history. American journal of botany 98 (1):88-98

Neiva IP, Andrade Júnior VCd, Maluf WR, Oliveira CM, Maciel GM (2013) Role of allelochemicals and trichome density in the resistance of tomato to whitefly. Ciência e agrotecnologia 37 (1):61-67

Neiva IP, Silva AAD, Resende JF, Carvalho RDC, Oliveira AMSD, Maluf WR (2019) Tomato genotype resistance to whitefly mediated by allelochemicals and Mi gene. Chilean journal of agricultural research 79 (1):124-130

Oku K, Magalhaes S, Dicke M (2009) The presence of webbing affects the oviposition rate of two-spotted spider mites, *Tetranychus urticae* (Acari: Tetranychidae). Exp Appl Acarol 49 (3):167-172

Oliveira H, Fadini MA, Venzon M, Rezende D, Rezende F, Pallini A (2009) Evaluation of the predatory mite *Phytoseiulus macropilis* (Acari: Phytoseiidae) as a biological control agent of the two-spotted spider mite on strawberry plants under greenhouse conditions. Exp Appl Acarol 47 (4):275-283

Peralta IE, Spooner DM, Knapp S (2008) Taxonomy of wild tomatoes and their relatives (*Solanum* Sect. *Lycopersicoides*, Sect. *Juglandifolia*, Sect. *Lycopersicon*; Solanaceae). Systematic Botany Monographs 84 (1-186)

Pokle PP, Shukla A (2015) Population dynamics of two spotted spider mite, *Tetranychus urticae* (Koch) (Acari: Tetranychidae) on tomato under polyhouse condition. J Appl Biosci 41 (2):148-151

Rahimi FR, Carter CD (1993) Inheritance of zingiberene in *Lycopersicon*. Theoretical and Applied Genetics 87 (5):593-597

Rakha M, Bouba N, Ramasamy S, Regnard J-L, Hanson P (2016) Evaluation of wild tomato accessions (*Solanum* spp.) for resistance to two-spotted spider mite (*Tetranychus urticae* Koch) based on trichome type and acylsugar content. Genetic Resources and Crop Evolution 64 (5):1011-1022

Rakha M, Zekeya N, Sevgan S, Musembi M, Ramasamy S, Hanson P, Havey M (2017) Screening recently identified whitefly/spider mite-resistant wild tomato accessions for resistance to *Tuta absoluta*. Plant Breeding 136 (4):562-568

Resende JTVD, Maluf WR, Cardoso MDG, Faria MV, Gonçalves LD, Nascimento IRD (2008) Resistance of tomato genotypes with high level of acylsugars to *Tetranychus evansi* Baker & Pritchard. Scientia Agricola 65 (1):31-35

Rick CM (1982) The potential of exotic germplasm for tomato improvement. In: Plant improvement and somatic cell genetics. Edited by I.K. Vasil, W.R. Scowcroft, and K.J. Frey. Academic Press, NY:1–28

Rieseberg LH, Willis JH (2007) Plant speciation. Science 317 (5840):910-914

Rodríguez-López MJ, Garzo E, Bonani JP, Fereres A, Fernández-Muñoz R, Moriones E (2011) Whitefly resistance traits derived from the wild tomato *Solanum pimpinellifolium* affect the preference and feeding behavior of *Bemisia tabaci* and reduce the spread of tomato yellow leaf curl virus. Phytopathology 101 (10):1191-1201

Saeidi Z, Mallik B (2006) In vitro screening of 67 *Lycopersicon* accessions/cultivars for resistance to two-spotted spider mite. Journal of Biological Science 6:847–853

SAS Institute Inc. (2012) Version 9.4, Statistical Analysis System Institute, Cary, North Carolina, USA

Schilmiller A, Shi F, Kim J, Charbonneau AL, Holmes D, Daniel Jones A, Last RL (2010) Mass spectrometry screening reveals widespread diversity in trichome specialized metabolites of tomato chromosomal substitution lines. Plant J 62 (3):391-403

Schomaker CH, Been TH (1998) Errors due to subsampling of soil samples with *Globodera rostochiensis* and *G. pallida*, and to other laboratory procedures. In: Been TH, Schomaker CH (eds) quantitative studies on the management of potato cyst nematodes (*Globodera* Spp.) in The Netherlands. 37–70

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

Snyder JC, Carter CD (1984) Leaf trichomes and resistance of *Lycopersicon hirsutum* and *Lycopersicon esculentum* to spider mites. Journal of the American Society for Horticultural Science 109:837–843

Snyder JC, Carter CD (1985) Trichomes on leaves of *Lycopersicon hirsutum*, *Lycopersicon esculentum* and their hybrids. Euphytica 34:53-64

Snyder JC, Thacker RR, Zhang X (2005) Genetic transfer of a twospotted spider mite (Acari: Tetranychidae) repellent in tomato hybrids. J Econ Entomol 98 (5):1710-1716

Sridhar V, Sadashiva AT, Keshava Rao V, Swathi P, Gadad HS (2019) Trichome and biochemical basis of resistance against *Tuta absoluta* in tomato genotypes. Plant Genetic Resources: Characterization and Utilization:1-5

Tan H, Xiao L, Gao S, Li Q, Chen J, Xiao Y, Ji Q, Chen R, Chen W, Zhang L (2015) Trichome and artemisinin regulator 1 is required for trichome development and artemisinin biosynthesis in *Artemisia annua*. Mol Plant 8 (9):1396-1411

Tanksley SD, Young ND, Paterson AH, Bonierbale MW (1989) RFLP mapping in plant breeding: New tools for an old science. Nature biotechnology 7 (3):257-264.

Valverde PL, Fornoni J, Nunez-Farfan J (2001) Defensive role of leaf trichomes in resistance to herbivorous insects in *Datura stramonium*. Journal of Evolutionary Biology 14 (3):424-432

Van Leeuwen T, Vontas J, Tsagkarakou A, Dermauw W, Tirry L (2010) Acaricide resistance mechanisms in the two-spotted spider mite *Tetranychus urticae* and other important Acari: a review. Insect Biochem Mol Biol 40 (8):563-572

Vandenborre G, Groten K, Smagghe G, Lannoo N, Baldwin IT, Van Damme EJ (2010) *Nicotiana tabacum* agglutinin is active against Lepidopteran pest insects. J Exp Bot 61 (4):1003-1014

Vijaykumar L, Chakravarthy AK, Patil SU, Rajanna D (2009) Resistance mechanism in rice to the midge *Orseolia oryzae* (Diptera: Cecidomyiidae). Journal of Economic Entomology 102 (4):1628-1639

Vosman B, van't Westende WPC, Henken B, van Eekelen HDLM, de Vos RCH, Voorrips RE (2018) Broad spectrum insect resistance and metabolites in close relatives of the cultivated tomato. Euphytica 214 (3)

Weston PA, Johnson DA, Burton HT, Snyder JC (1989) Trichome secretion composition, trichome densities, and spider mite resistance of ten accessions of *Lycopersicon hirsutum*. Journal of the American Society for Horticultural Science 114 (3):492-498

Weston PA, Snyder JC (1990) Thumbtack bioassay a quick method for measuring plant resistance to twospotted spider mites. J Econ Entomol 83 (2):500-504

Whalon ME, Mota-Sanchez RM, Hollingworth RM (2016) Arthropods resistant to pesticides database (ARPD). Available online: http://wwwpesticideresistanceorg

Wosula EN, Knapp M, Agong SG (2009) Resistance to *Tetranychus evansi* in *Lycopersicon esculentum* L. *hirsutum* var. *glabratum* hybrids. The Journal of Horticultural Science and Biotechnology 84 (3):360-364

Zhang W, McAuslane HJ, Schuster DJ (2004) Repellency of ginger oil to *Bemisia argentifolii* (Homoptera: Aleyrodidae) on tomato. Journal of economic entomology 97 (4):1310-1318

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   http://tgc.ifas.ufl.edu/2013/2018%20Presentations.htm