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Arthropod Resistant Tomatoes: Screening Tools, Yield and Nutritional Quality of Interspecific Hybrids

Mohammad Hasan Salman Ali Dawood
University of Kentucky, msa249@uky.edu
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Mohammad Hasan Salman Ali Dawood, Student

Dr. John C. Snyder, Major Professor

Dr. Mark Coyne, Director of Graduate Studies

Arthropod Resistant Tomatoes: Screening Tools, Yield and Nutritional Quality of Interspecific Hybrids

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DISSERTATION

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
Mohammad Hasan Salman Ali Dawood
Lexington, Kentucky
Director: Dr. John C Snyder, Associate Professor
Lexington, Kentucky
2020

ABSTRACT OF DISSERTATION

Arthropod Resistant Tomatoes: Screening Tools and Yield and Nutritional Quality of Interspecific Hybrids

Tomato (*Solanum lycopersicum*) is one of the most economically important vegetable crops grown around globe but is a host for numerous pests and pathogens. In the future, tomato breeders will have to focus on increasing fruit quantity and on enhancing pest resistance. Many accessions of the wild relative of tomato*, S. habrochaites* display high levels of resistance towards arthropods such as spider mites. The presence of the sesquiterpene hydrocarbon, 7-epi-zingiberene, found in *S. habrochaites* type IV trichomes has been associated with arthropod resistance. However, the presence of other compounds in its trichome secretions may also be related to arthropod resistance. One goal of this research was to evaluate the potential for using a spectrophotometer to enable accurate selection for 7-epi-zingiberene content by breeders. Another objective was to identify and evaluate the relative antixenotic activities on spider mites of major components present in the trichome secretions of a wild tomato. The third objective was to evaluate yield, 7-epi-zingiberene content and fruit nutrient quality in advanced interspecific hybrid lines to demonstrate that high yield and high zingiberene content have been successfully combined, and also to evaluate nutritional aspects of fruit quality of these hybrids such as phenolic content, lycopene, soluble solids, and ascorbic acid. Results for the first objective included identification of two novel compounds present in wild tomato trichome secretions as hydroxy-zingiberene and 9-hydroxy-10,11-epoxy-zingiberene. The spider mite repellency of each of these compounds was at least five times greater than that of 7-epi-zingiberene. The results for objective two showed that a spectrophotometer could be a very valuable tool for aiding selection of plants having high levels of 7-epi-zingiberene on their leaves and having low levels of other compounds such as monoterpenes, which are present on arthropod –susceptible tomato plants. Completion of the third objective indicated that high yield has been successfully combined with high 7-epi-zingiberene

concentration and that the nutritional value of the fruit in the hybrids is at least equal to the recurrent parent and in some cases, the interspecific hybrids may be useful for improving tomato fruit nutritional content including: phenolics, lycopene, soluble solids, and ascorbic acid. In general, the phenolic content of interspecific hybrid tomatoes ranged from 325 to 427 μg/g fresh fruit, lycopene content ranged from 31 to 66 μg/g and soluble solids ranged from 4 to 7.8%, whereas those characteristics were lower in cultivated tomato hybrids. Ascorbic acid typically ranged between 483 and 498 μg/g fresh fruit in interspecific hybrids and was higher than that in cultivated tomato hybrids (337 μg/g). For future prospects, it may be possible to breed genotypes that have high yield, have improved nutritional value and are also resistant to arthropod pests.

KEYWORDS: Tomato, spider mites, repellency, 7-epi-zingiberene, 9-hydroxy-zingiberene, yield, phenolic, UV-absorbance, *Solanum habrochaites*

Mohammad Hasan Salman Ali Dawood

04/25/2020

Arthropod Resistant Tomatoes: Screening Tools and Yield and Nutritional Quality of Interspecific Hybrids

By
Mohammad Hasan Salman Ali Dawood

Dr. John C. Snyder
Director of Dissertation

Dr. Mark Coyne
Director of Graduate Studies

04-25-2020
Date

This Dissertation is dedicated to the memory of a brave and humble soldier, my beloved cousin *Arjan Elias Dawood*. In June 2014, he was killed by terrorists in Mosul city, North Iraq.

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CHAPTER 1. INTRODUCTION

Tomato, *Solanum lycopersicum,* is one of the most produced and the second significant vegetable grown crop worldwide, next to potato. World production today amounts to around 200 million tons on 12 million acres (FAOSTAT 2017). In 2006 more than 100 million tons were produced (http://faostat.fao.org), so production has doubled in over a period of 11 years, a very rapid increase. China, United States, and Turkey are the three main production areas in the world. Tomatoes (wild and cultivated) originated from the Andes in South America and include thirteen closely related species, containing *Solanum* sect. *Lycopersicum*, formerly known as the genus *Lycopersicum* (Peralta, Spooner et al. 2008, Nakazato and Housworth 2011). Tomato consumption is increasing because of increasing human population around the world, and it significantly varies per capita among countries(Rubatzky and Yamaguchi 2012).

Cultivated tomatoes have low genetic diversity although they contain a high morphological diversity because their genetic base during domestication was narrow outside their native South American region, resulting in a genetic bottleneck(Miller and Tanksley 1990, Bauchet and Causse 2012). New (mostly hybrid) tomato varieties have been developed through screening and breeding by scientists and breeders worldwide for all types, colors, shapes and sizes of fruit (Bai and Lindhout 2007). The first step in domestication was partly accomplished through the selection of desired genotypes in the existing narrow germplasm (Bai and Lindhout 2007). Usually horticultural plants such as tomatoes are grown with a limited numbers of plants (Bai and Lindhout 2007). Genetic variation continues to decline in a primarily inbreeding population, such as cultivated

tomatoes, even without the use of selection. enetic drift is therefore a major mechanism which decreases genetic diversity during domestication and breeding (Bauchet and Causse 2012).

The genetic diversity of wild tomatoes is large, in particular within individual species like *Solanum chilense* and *Solanum peruvianum,* compared to the cultivated tomato (Rick 1984). In modern breeding programs, genetic variation in wild tomato relatives could be a great resource that has been utilized by breeders to develop hybrids associated with arthropod resistance (Rick and Chetelat 1995). The genetic variation in wild species has been thoroughly studied for specific characteristics and used in tomato breeding program (Walter 1967). Therefore, it is important to expand the skills and details on the nature and value of the associations between characteristics of interest. In some cases, by selecting a specific feature in the breeding program based on the genetic association between them, a breeder can reduce or increase the expression of desirable traits (de Souza, Melo et al. 2012, Topwal and Singh 2018).

Since most cultivated tomatoes are susceptible to pests, chemical control by insecticide and acaricide spraying is still the main approach used to control tomato crop pests (Letourneau and Goldstein 2001). However, the use of these products as the main management method can cause severe damage to the environment, such as biological imbalance, deleterious effects on rural and consumer health, as well as increased production costs (Pingali 1995, Pretty 2008, Popp, Pető et al. 2013).

The objective of the first study was to evaluate the potential for using a spectrophotometer to enable accurate selection for 7-epi-zingiberene content by breeders.

The objective of the second study was investigate antixenotic activities on spider mites of major components present in the trichome secretions of a wild tomato *Solanum habrochaites*, LA2329. The third objective of this study was to evaluate yield, 7-epi-zingiberene content and fruit nutrient quality in advanced interspecific hybrid lines to assess whether high yield and high zingiberene content were successfully combined, and also to evaluate nutritional aspects of fruit quality of these hybrids such as phenolic, lycopene, soluble solids, and ascorbic acid contents.

One of the greatest problems facing tomato growers around the globe is the lack of tomato varieties that are resistant to most devastating pests and pathogens. Based on the literature, more than 200 types of pests and pathogens attack cultivated tomato, which can cause significant economic losses (Lange and Bronson 1981). One of the pests that attack many crop families such as *Solanaceae* and *Cucurbitaceae* is two-spotted spider mite, *Tetranychus urticae,* because it can cause severe damage, expressed as slow growth, leaf wilt, yield loss and plant death,, and can lead to severe loss of the tomato crop (Brødsgaard and Albajes 1999, Gerson and Weintraub 2007, Muniappan 2012). It is found in greenhouses in the United States and can survive the winter beyond its natural constraints (Tuttle and Baker 1968). One major reason the spider mite is an omnipresent pest species is probably due to its biologically variable species complex (Bolland, Gutierrez et al. 1998). Hessey and Parr (1963) investigating spider mites found they caused significant reductions in yield when invading cucumber plants by reducing the chlorophyll content of the leaves, thus resulting in a decrease in photosynthesis. Luczynski, Isman et al. (1990) stated that the efficiency of photosynthesis and the transpiration of the leaves decreases when *T. urticae* feeds on the surface of the foliage.

Due to its short life span, high productivity and increased resistance to numerous acaricides it has become extremely difficult to control this pest with chemical pesticides (Sances, Wyman et al. 1979).

Sesquiterpene hydrocarbon (7-epi-zingiberene)

The sesquiterpene hydrocarbon, 7-epi-zingiberene, is a semi-volatile compound synthesized by the species *S. habrochaites*, a wild relative of cultivated tomato. 7-epi-zingiberene is one of the main anti-insect chemicals present in its leaf trichomes (Weston and Snyder 1990). Therefore introgressing this bioactive compound from wild types into cultivated tomatoes may improve tomato insect resistance due to its toxicity and repellence (Weston and Snyder 1990, Maluf, Campos et al. 2001, Zhang, McAuslane et al. 2004). Bleeker, Diergaarde et al. (2011) have confirmed that purified 7-epi-zingiberene from *Solanum habrochaites* (PI127826) repelled whiteflies (*Bemisia tabaci*) when applied to the headspace of vulnerable cultivated tomatoes. de Azevedo, Faria et al. (2003) recommended that indirect selection of high-foliar zingiberene content originating from *Solanum habrochaites*Hirsutum (PI-127826) be an effective method for breeding tomatoes resistant to the South American tomato pinworm. Maluf, Campos et al. (2001) reported that selection for high-foliar zingiberene was efficient in improving tomato resistance to the spider mite. Neiva, Silva et al. (2019) reported that the combination of high foliar contents of bioactive compounds such as acylsugar and 7-epi-zingiberene in wild tomato plants associated with the Mi gene may lead to higher resistance to whitefly. The objective of the first study was to identify and evaluate the relative antixenotic

activities on spider mites of major components present in the trichome secretions specifically 7-epi-zingiberene of a wild tomato *S. habrochaites*, LA2329 accessions.

Selection during breeding that relies on quantification of foliage compounds may be a useful approach for the development of new arthropod-resistant cultivars (Snyder, Simmons et al. 1998, Maluf, de Fátima Silva et al. 2010, Dias, Resende et al. 2013, Dawood and Snyder 2020). This can be achieved through transferring some compounds such as 7-epi-zingiberene and others that are present in trichomes of wild relatives into cultivated tomato (Bleeker, Mirabella et al. 2012).

Quantification of 7-epi-zingiberene

There are several methods for measuring 7-epi-zingiberene. One of the options is using gas chromatography and mass spectrometry (GC/MS) or using gas chromatography-flame ionization detector (GC-FID) (Lin, Trumble et al. 1987, Carter, Gianfagna et al. 1989, Chatzivasileiadis, Boon et al. 1999, Antonious and Kochhar 2003, Snyder, Thacker et al. 2005). However, this method is expensive, relatively slow, and may not be accessible or affordable in all plant breeding programs. Quantification of 7-epi-zingiberene using a spectrophotometric procedure could be a convenient alternative approach that might be used by plant breeders to obtain pure lines and/or hybrids of tomato that have high levels of zingiberene and may consequently be resistant to arthropods (de Freitas, Maluf et al. 2000, de Sena Fernandes, Fernandes et al. 2014). In fact, Freitas, Maluf et al. (2002) reported selecting genotypes with high concentrations of 7-epi-zingiebrene using a spectrophotometer. However, it appears that this proposed method has not been fully evaluated for effects of interfering substances or for its ability to estimate actual

concentrations of zingiberene. For example, in studies involving the introgression of zingiberene based on spectrophotometric readings, parental levels of zingiberene were never recovered in any of the interspecific hybrids (Resende, da Silva et al. 2018, Valadares, Melo et al. 2018). Contrarily, Snyder, Dawood et al. (2018), have consistently recovered parental levels of zingiberene in interspecific offspring when the selection was based on GC-FID.

Oxygenated sesquiterpenoids, mainly carboxy acids of sesquiterpenes, have been documented in *Solanum habrochaites,* often as the predominate components of trichome secretions (Coates, Denissen et al. 1988, Snyder, Guo et al. 1993). The wild tomato accession LA2329 has three major allelochemical components (7-epi-zingiberene, 9-hydroxy-zingiberene, and 9-hydroxy-10,11-epozy-zingiberene) in its trichome secretion. These three major compounds contain conjugated double bonds in their chemical structures (Dawood and Snyder 2020). Since 7-epi-zingiberene absorbs light in spectrophotometer UV-region, it is possible that the oxygenated sesquiterpenoids also absorb light at the same UV-region.

Screening for 7-epi-zingiberene content in leaf tissue of interspecific hybrid populations is a major undertaking for many tomato breeders around the world (Lima, Resende et al. 2015, Lima, Resende et al. 2016). Consequently, development of a simple and inexpensive approach for faster screening of 7-epi-zingiberene content is essential. The current research was aimed at developing a method to estimate 7-epi-zingiberene contents on the leaves of wild tomato accessions and interspecific hybrid plants that can be used for selective breeding of resistant tomatoes. However, use of a spectrophotometer could be

problematic when genotypes have more than one UV-absorbing substance in their trichome secretions, since their presence could interfere with zingiberene quantitation. In the absence of interfering substances or in the presence of the ability to measure interference, use of a spectrophotometer to estimate foliage zingiberene content could be a cheaper and faster technique for selecting tomato plants containing high levels of 7-epi-zingiberene. Therefore, the objective of this experiment was to evaluate the use of a spectrophotometer as a selection tool for identifying high levels of 7-epi-zingiberene in the wild parent and in interspecific populations of tomato that are segregating for presence and abundance of 7-epi-zingiberene as well as for other potentially interfering substances.

Tomato Consumption and Important Nutritional Compounds

Consumption of tomatoes (*Solanum lycopersicum*) is an important part of the human diet (Borguini and Ferraz da Silva Torres 2009) because it contains phytochemicals such as ascorbic acid (vitamin C), carotenoids, lycopene and phenolics. For people with poor nutritional diets, tomatoes can be a source of pro-vitamin A, carotenoids and vitamin C (Abushita, Hebshi et al. 1997, Agarwal and Rao 2000, Kaur, Savage et al. 2002). Carotenoids such as β-carotene and lycopene in the tomatoes may prevent oxidation of lipoproteins and cell membranes. Some epidemiological studies have shown that carotenoids and foods rich in antioxidants can reduce cardiovascular risk (Mayne 1996). Many of the nutrients present in tomatoes are known antioxidants, some of which work together to improve the status of oxidation (Toor and Savage 2005). Consumption of tomato fruit is associated with cardiovascular protection (Böhm 2018). It

is therefore wise to identify and consume food such as tomatoes as a source of cardiovascular protection, providing a combination of these antioxidants.

Phenolic compounds such as flavonoids, phenolic acids, and tannins are ubiquitous in plants and are of considerable interest due to their antioxidant properties, one of the major groups of bioactive components and secondary metabolites of dietary phytochemicals found in fruits, vegetables and grains (Balasundram, Sundram et al. 2006, Garcia-Salas, Morales-Soto et al. 2010). Besides lycopene, the red pigment in the fruit, tomato also contains several phenolic acids that can provide an essential part of the human diet and act as antioxidants (Bahorun, Luximon-Ramma et al. 2004, Naczk and Shahidi 2006). Phenolics may also be involved in plant growth and reproduction and may provide resistance against pathogens (Lattanzio, Lattanzio et al. 2006).

Tomato fruit based on literature reviews contain several major types of phenols such as chlorogenic acids, 5-caffeoyl quinic acid, ferulic acid, and caffeic acid (Naczk and Shahidi 2006, Tulipani, Huelamo et al. 2012). Multiple polyphenol content has been reported in components of tomato fruit, such as skin and pulp (Wu and Burrell 1958, Rivas and Luh 1968, Clifford 1985). Walker (1962) has identified three major phenolic compounds in tomatoes: ferulic, caffeic and chlorogenic acids. Another important compound, P-coumaric acid, was discovered later in the skin of tomato fruit (Fleuriet and Macheix 1985). During extracting and processing steps, some researchers detected sinapic acid in green tomato fruits (Fleuriet and Macheix 1976). The level of polyphenolic content differed in the ripening period of tomato fruits. In placental and epidermal tissues, the more concentrated level was found (Le Gall, DuPont et al. 2003,

Bhagwat, Haytowitz et al. 2014); and the most abundant type of phenol in tomato fruit was chlorogenic acid, with the highest level found at the red ripe fruit stage (Salunkhe, Jadhav et al. 1974). Phenolic types therefore vary in tissue and maturity stages of fruits.

Tomato plants grown under low tunnel conditions accumulate higher total phenolic content in the fruit than that in fruits produced in the field (Ilic and Milenkovic 2012). These high levels of phenolic content may be associated with high levels of UV, temperature, and no rainfall (Marsic, Gasperlin et al. 2011). Rivero, Ruiz et al. (2003) reported that more polyphenolic components accumulated when tomato plants were grown between 33-35° C due to enhanced mechanisms against high temperature stress.. Macheix, Fleuriet et al. (1990) identified both genetic and environmental conditions can contribute to phenolic accumulation in vegetables. Raffo, La Malfa et al. (2006) reported that, by increasing UV solar radiation, the total phenolic content of cherry tomatoes increased. In conclusion, changes in light intensity, temperature and relative humidity can affect the plant's output and partition of photo-assimilates and, therefore, the composition of the fruit produced.

Phenolic components were evaluated in fruit of tomato varieties from different areas of Mauritius (Bahorun, Luximon-Ramma et al. 2004). Total phenolic components has been extensively reported in cultivated tomatoes (Buta and Spaulding 1997, Martínez-Valverde, Periago et al. 2002, Van der Rest, Danoun et al. 2006). Typically, phenolic compounds are connected to plant defense mechanisms. Nevertheless, polyphenolic metabolites play a major role in other processes, particularly in attracting pollinators to speed up pollination and protect against arthropods, fungi, and bacterial diseases (Bravo

1998, Alasalvar, Grigor et al. 2001, Cheynier 2005). However, phenolic content in fruit and leaves of interspecific hybrid tomatoes has not been evaluated. One objective of my research was to estimate total phenolic contents in leaves and fruit of interspecific hybrid tomato compared to commonly cultivated tomato (Dawood, Snyder et al.).

Duffey and Isman (1981) suggested that certain types of phenolic components such as chlorogenic acid found in tetra-cellular glandular trichomes on tomato leaves could have a significant impact on the antibiotic effect of the leaf on *Heliothis zea*, the tomato fruit worm. They also found that rutin or chlorogenic acid substratum inhibited the early larval growth of *H. zea* on tomato leaves (Isman and Duffey 1982).

Tomato Fruits and Ascorbic Acid

Fruits and vegetables are the main sources of nearly all ascorbic acid in the human diet. However, ascorbic acid content in fruit and vegetables varies greatly (Goddard and Matthews 1979, Achinewhu 1983). In most cases, leafy vegetables, citrus and some tropical fruits contain high levels of ascorbic acid. Excellent ascorbic acid sources are tomatoes and bell pepper (Yahia, Contreras-Padilla et al. 2001, Lim, Lim et al. 2007). Tomato fruits do not contain a high level of ascorbic acid compared to other fruits and vegetables, but they are important because of their abundant use in the human diet (Klein and Perry 1982, Borguini and Ferraz da Silva Torres 2009). Due to the importance of ascorbic acid in our life, the amount of ascorbic acid in interspecific hybrid populations compared with cultivated tomatoes is essential for evaluation and selection in breeding program. The phytonutrients and health benefits of ascorbic acid are also essential in people's diets to avoid the disease scurvy, to reduce stress, and to increase antioxidants in

the body. In amino acid biosynthesis, adrenaline development and in liver detoxification, ascorbic acid is essential (Smirnoff 1996).

Tomato growers and breeders are looking for approaches to boost yields. World production today amounts to around 200 million tons on 12 million acres (FAOSTAT 2017). In modern breeding programs genetic variation available in wild tomato relatives has often been the source of characteristics used to breed for enhanced yield (Rick and Chetelat 1995). Yield is a genetically complex character and genetic selection for yield requires tremendous attention by the breeder. An increase in yield and quality of self-pollinated crops such as tomato is usually accomplished by choosing those genotypes that have the desired combination of phenotypic characters (de Souza, Melo et al. 2012). It is extremely important to understand the extent of genetic diversity available to improve the yields of tomatoes (Bhattarai, Louws et al. 2016). Due to ease of application, morphological features have often been utilized to estimate genetic diversity (Fufa, Baenziger et al. 2005).

Tomato Fruits and Lycopene

Tomato is the primary source of lycopene, a red carotenoid associated with multiple health benefits (Østerlie and Lerfall 2005). Because lycopene has benefits as a phytonutrient, most breeders would like to increase the content of lycopene in their breeding lines, and producers want to employ techniques to boost the content of lycopene (Scott 2005).

improvement of tomato production involves the use of improved cultivars that are resistant to arthropods, obtaining high yield, and have a reasonable level of nutrition. In order to obtain pure line of tomato cultivars having a 7-epi-zingiberene content in their leaf secretion, producing high yield, and having an appropriate level of nutrition in their fruits, therefore, the objectives of my project were: First, to evaluate the potential for using a spectrophotometer to enable accurate selection for 7-epi-zingiberene content by breeders. Second, to identify and evaluate the relative antixenotic activities on spider mites of major components present in the trichome secretions of a wild tomato. Third, to evaluate yield, 7-epi-zingiberene content and fruit nutrient quality in advanced interspecific hybrid lines to demonstrate that high yield and high zingiberene content have been successfully combined, and also to evaluate nutritional aspects of fruit quality of these hybrids such as phenolic content, lycopene, soluble solids, and ascorbic acid.

CHAPTER 2. CAN A SPECTROPHOTOMETER BE USED TO ACCURATELY MEASURE ZINGIBERENE IN TOMATO LEAF EXTRACTS?

2.1 Abstract

Tomato (*Solanum lycopersicum*) is one of the most important vegetable crops in the globe but is a host for numerous pests and pathogens. Tomato breeders have focused on increasing fruit quantity and quality with little focus on enhancing arthropod resistance. Many accessions of the wild relative *Solanum habrochaites* display a high resistance towards arthropods such as spider mites. The presence of the sesquiterpene hydrocarbon 7-epi-zingiberene found in *S. habrochaites* trichomes is associated with arthropod resistance. However, the presence of other compounds in trichome secretions may also be related to arthropod resistance. Because 7-epi-zingiberene absorbs ultraviolet light, UV-spectrophotometry has been suggested as a method for its quantitation. However, such a suggestion does not consider the potential for the presence of other compounds that could interfere with 7-epi-zingiberene quantitation by UV absorbance and its validity has never been demonstrated in segregating, interspecific populations. This study aimed to evaluate the use of UV-absorbance as a method for quantitation of 7-epi-zingiberene in distinct generations of an interspecific hybrid breeding population (*S. lycopersicum* × *S. habrochaites*) and in the wild donor accession LA2329. The latter produces 7-epi-zingiberene and also produces the oxygenated forms such as 9-hydroxy-zingiberene. The study also examined the accuracy of the Maluf, Campos et al. (2001) procedure to quantify 7-epi-zingiberene content in hybrids. Based on gas chromatographic analysis of the 7-epi-zingiberene content of interspecific hybrids and wild accessions, the plants having high concentrations of 7-epi-zingiberene demonstrated high absorbance values;

plants having no 7-epi-zingiberene had low, but non-zero absorbance values indicating the presence of interfering compounds. β-phellandrene, a monoterpene hydrocarbon likely contributed this interference, but its contribution was minimal (0.25-0.55 absorbance units) and this interference was more pronounced at low 7-epi-zingiberene concentrations. Regression analysis indicated a strong linear association between 7-epi-zingiberene content and UV-absorbance value at 270 nm. In general, by increasing 7-epi-zingiberene content, the UV absorption reading increased (R^2=0.922). Other compounds such as 9-hydroxy-zingiberene present in trichome secretions of the wild tomato accession LA2329, caused high interference in determining the 7-epi-zingiberene concentration. Thus, 7-epi-zingiberene content can be estimated by using UV-spectrophotometer when the plant tested did not contain additional major allelochemical inconsistences in their trichome secretions. Without interfering compounds, this technique offers a faster, cost-efficient technique to select plants that contain high levels of 7-epi-zingiberene, that consequently may repel various arthropods. Measuring multiple wavelengths in the UV range, backstopped by GC-FID could be useful for determining multiple compounds in tomato interspecific hybrids. trichome

Keywords: Tomato, 7-epi-zingiberene, 9-hydroxy-zingiberene, spectrophotometer, absorbance.

2.2 Introduction

7-epi-zingiberene (Figure 2-1) is an important allelochemical and natural volatile compound (Blázquez 2014). It is a monocyclic sesquiterpene (15 C) with toxic and repellent properties on arthropods (Choi, Park et al. 2002, Reis, Mantello et al. 2016). 7-epi-zingiberene has three double bonds and the two in the ring are conjugated. These conjugated systems have a large impact on spectral absorbance modifying both wavelength of maximum (λ_{max}) absorption and intensity especially in UV range (Hu, Yu et al. 2010, Butu, Butnariu et al. 2014).

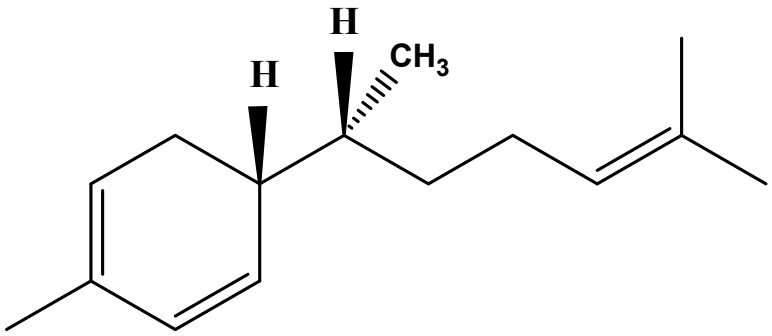

Figure 2-1. Chemical structure of 7-epi-zingiberene ($C_{15}H_{24}$) showing the two conjugated double bonds in the ring.

7-epi-zingiberene is found in types IV and VI glandular trichomes of *Solanum habrochaites* (Guo, Weston et al. 1993, Antonious and Kochhar 2003, Antonious and Snyder 2006). This natural compound is formed via the isoprenoid pathway from farnesyl pyrophosphate (FPP) (Chatzivasileiou 2019). 7-epi-zingiberene is of interest because presence of this specific allelochemical in tomato trichomes has been associated with resistance against arthropods, e.g. spider mites, aphids, and whitefly (Weston and Snyder

1990, Aragão, Dantas et al. 2000, Maluf, Campos et al. 2001, Freitas, Maluf et al. 2002, Gonçalves, Maluf et al. 2006, Bleeker, Diergaarde et al. 2011). Furthermore, 7-epi-zingiberene has been identified as a medicinal agent (Bhatt, Naidoo et al. 2010, Khatun, Cakilcioglu et al. 2011, da Silveira Vasconcelos, Mota et al. 2019).

Tomato (*Solanum lycopersicum*) is a host for numerous pests and pathogens (Lange and Bronson 1981). Tomato breeders have focused on increasing fruit quantity and quality with little focus on enhancing resistance to arthropods (Zeist, da Silva et al. 2018). As a result, tomato plants are vulnerable to pest attacks throughout the crop cycle; thus, it is necessary to find a way to avoid damages that can reduce yield. Insecticide sprays are the predominant method used for pest control. The use of chemical pest control can however, cause serious damage and deleterious effects on growers, the health of consumers and can increase in production costs (Weaver, Evans et al. 1992, Racke 2013, Ansari, Moraiet et al. 2014).

Certain accessions of the wild tomato *Solanum habrochaites* are resistant to a wide range of arthropods in part because the foliage of these accessions contains high levels of allelochemicals. These allelochemicals have been tested against a wide assortment of arthropods (Alba, Montserrat et al. 2009, Firdaus, van Heusden et al. 2012). Lucini, Faria et al. (2015) verified that the selected genotypes of tomato plants with high acylsugar content had a level of resistance to spider mites similar to that of wild accession *S. pennellii* LA716, while de Oliveira, de Resende et al. (2018) suggested that genotypes selected for high 7-epi-zingiberene content are great sources of resistant genes against spider mites and conceivably against other arthropods. Neiva, Silva et al. (2019)

have indicated that high acylsugar and 7-epi-zingiberene contents are associated with higher resistance to whitefly in tomato.

A procedure that can quantify foliage compounds responsible for deterring pests could be a viable approach for the development of new arthropod-resistant cultivars (Snyder, Simmons et al. 1998, Maluf, de Fátima Silva et al. 2010, Dias, Resende et al. 2013, Dawood and Snyder 2020). The ultimate aim would be to transfer the responsible compounds present in trichomes of wild relatives to cultivated tomato (Bleeker, Mirabella et al. 2012).

There are several methods for measuring 7-epi-zingiberene. One of the options is using gas chromatography and mass spectrometry (GC/MS) or GC-FID (Lin, Trumble et al. 1987, Carter, Gianfagna et al. 1989, Chatzivasileiadis, Boon et al. 1999, Antonious and Kochhar 2003, Snyder, Thacker et al. 2005). However, this method is expensive, slow and may not be accessible or affordable in plant breeding programs. Quantification of 7-epi-zingiberene using a spectrophotometric procedure could be a convenient approach that might be used by plant breeders to transfer this trait to cultivated tomato which may result in tomato cultivars that are resistant to arthropods (de Freitas, Maluf et al. 2000, de Sena Fernandes, Fernandes et al. 2014). In fact, Freitas, Maluf et al. (2002) have reported the use of a spectrophotometer to aid selection of genotypes having high concentrations of 7-epi-zingiberene. However, it appears that this proposed method has not been fully evaluated for effects of interfering substances or for its ability to estimate actual concentrations of zingiberene. For example, in tomato breeding studies involving introgression of zingiberene based on spectrophotometric readings, parental levels of

zingiberene have never been recovered in any of the interspecific hybrids (Lima, Resende et al. 2016, Zeist, da Silva et al. 2018). Contrarily, Snyder, Dawood et al. (2018), have consistently recovered parental levels of zingiberene in interspecific offspring when the selection was based on GC-FID.

Oxygenated sesquiterpenoids, mainly carboxy acids of sesquiterpenes are also documented in *S. habrochaites* often as predominate components of trichome secretions (Coates, Denissen et al. 1988, Snyder, Guo et al. 1993). The wild tomato accession LA2329 has three major allelochemical components (7-epi-zingiberene, 9-hydroxy-zingiberene, and 9-hydroxy-10,11-epozy-zingiberene) in its trichome secretions. secretion. Each of these compounds contain conjugated double bonds in their chemical structure (Dawood and Snyder 2020). Since 7-epi-zingiberene absorbs light in the UV region, it is possible these oxygenated sesquiterpenoids will also absorb UV light.

Screening for 7-epi-zingiberene content in the leaf tissue of interspecific hybrid populations is a major undertaking for many tomato breeders around the world (Lima, Resende et al. 2015, Lima, Resende et al. 2016). Consequently, development of a simple and inexpensive approach for faster screening of 7-epi-zingiberene content is essential. The current research was intended assist in developing a method to estimate 7-epi-zingiberene contents on leaves of interspecific tomato hybrids and their progeny that can be used for breeding of tomato with arthropod resistance. However, use of a spectrophotometer could be problematic when genotypes have more than one UV-absorbing substance in their trichome secretions since their presence could interfere with zingiberene quantitation. In the absence of interfering substances or in the presence of the

ability to measure interference, use of a spectrophotometer to estimate foliage zingiberene content could be a cheaper and faster technique for selecting tomato plants containing a high content of 7-epi-zingiberene.

The biosynthetic pathways of organic compounds that are present in the leaf secretion of interspecific hybrid plants are incredibly important in developing resistant tomato lines in a breeding program. This work will assess the interference of compounds other than the target compound that are present in the interspecific hybrid populations obtained via crossing the commercial tomato, *Solanum lycopersicum* with the wild tomato (*Solanum habrochaites*). Two major compounds, 7-epi-zingibrene and β-phellandrene are often present in the trichome secretions of these interspecific hybrids (Snyder – personal communication). β-phellandrene (Figure 2-2) is a water-insoluble and volatile organic compound (Lange, Rios-Estepa et al. 2012, Aubin 2019). It is an endocyclic monoterpene (10 C) and has conjugated double-bonds that absorb UV-light (Melis, Davies et al. 2018).

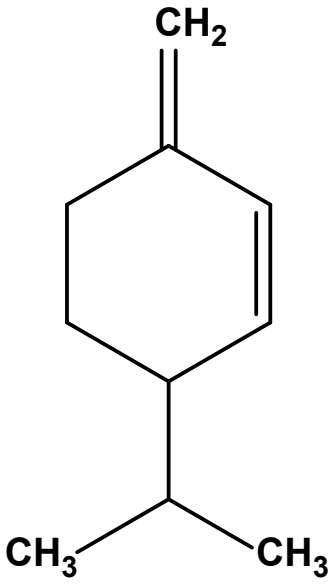

Figure 2-2. Chemical structure of β-phellandrene ($C_{10}H_{16}$) showing the conjugated double bonds.

7-epi-zingiberene (sesquiterpene) and β-phellandrene (monoterpene) in plants are derived from two biosynthetic pathways, the plastidic mevalonate pathway, also known as methylerythritol 4-phosphate (MEP) and the cytoplasmic mevalonate (MVA) pathway. Both biosynthetic pathways produce isopentenyl pyrophosphate (IPP) and dimethylallyl pyrophosphate (DMAPP), which are consequently exploited to produce prenyl diphosphates through prenyltransferases. Geranyl diphosphate, also known as geranyl pyrophosphate (GPP) and monoterpenes (10 C) are synthesized in the plastids, whereas farnesyl pyrophosphate, also known as farnesyl diphosphate (FPP) and sesquiterpenes (15 C) are produced in the cytosol (Klingler, Frosch et al. 1991, Gutensohn, Nagegowda et al. 2012, Melis, Davies et al. 2018).

Therefore, the objective of this research was to evaluate the use of a spectrophotometer as a selection tool for identifying and quantifying high levels of 7-epi-

zingiberene in the wild parent and interspecific hybrids and populations that are segregating for presence and abundance of 7-epi-zingiberene as well as for other potentially interfering substances.

2.3 Materials and Methods

2.3.1 Plant material

This experiment was conducted at the Horticulture Department, University of Kentucky and at the Horticulture Research Farm, Lexington, KY. Plants used in this experiment were interspecific hybrid populations (BC3F5, BC4F2 and $BC5F_1$), the wild accession *Solanum habrochaites* LA2329 (formerly known as *Lycopersicum hirsutum* LA2329) and the recurrent parent 'Zaofen 2'.

The plants sampled for the BC4F2 were field grown. Seeds were germinated on moist filter paper in an incubator (27ºC). After radicle emergence, seeds were planted in 72-cell trays containing ProMix BX. Six weeks later, seedlings were transplanted into the field at the Horticulture Research Farm, Lexington, KY. Cultural methods for transplant and field production followed those recommended in ID-36 (http://www2.ca.uky.edu/agcomm/pubs/id/id36/id36.pdf).

Cuttings of interspecific hybrid populations of (BC3F5 and $BC5F_1$) were derived from crosses between a commercial tomato variety, *Solanum lycopersicum* and the wild tomato (*Solanum habrochaites*). The cuttings after rooting were transplanted into pots filled with ProMix BX and auto-fertigated every day using a fertilizer solution containing

Peter's Professional 5-11-26 (ICL SF USA & Canada, Dublin, OH) plus $CaNO_3$ (Viking Yara, Tampa, FL) to provide 180 ppm of nitrogen. Plants were grown under normal daylength conditions, during spring, summer, and fall. Throughout this time, the average greenhouse temperature and relative humidity were 24±2 ° C and 67% RH, respectively. Three weeks after transplanting, the plants were ready for the lab experiments. Seeds of the wild tomato accession, *S. habrochaites* LA2329, originally obtained from the Tomato Genetics Resource Center, University of California, Davis were germinated at 27° C on moist filter paper in an incubator. All germinated seeds were transferred into 72-cell trays filled with ProMix BX and watered daily. After four weeks, all seedlings were transplanted to the greenhouse and then grown in the same fashion as the plants from cuttings.

2.3.2 Gas Chromatography

Gas chromatography with flame ionization detection (GC-FID) was used to quantify compounds of interest in this research. Conditions for GC-FID were as follows: RTX-5 column (5% diphenyl 95% dimethyl polysiloxane, 15 m, 0.53-mmID, 0.5 μm) (Restek Corporation, Bellefonte, PA, USA). Temperatures were as follows: injector 250°C, detector 300°C, oven initial temperature 50ºC for 1 min, then increasing at 20°C/min to 260 ºC. The gas chromatograph used was a Hewlett Packard 5890 Series II. To allow quantitation of compounds detected by GC-FID, a standard curve using tetradecane was constructed over the range of 0 to 100 ppm (0 to 100 ng/μL).

2.3.3 Spectrophotometry

For spectrophotometry, a Thermo Scientific Evolution 60S UV-Visible scanning spectrophotometer was employed. In some cases, as explained below, absorbance was measured at a single wavelength, 270 nm. When samples were scanned, they were scanned from 190 to 600 nm wavelengths.

2.3.4 Methods for qualitative analysis

Leaflet washes from the donor and recurrent parents were used for qualitative characterization. The leaflet wash from the recurrent parent *Solanum lycopersicum* cv. Zoafen-2 was known to have β-phellandrene as a major compound. The leaflet wash from the wild donor parent *Solanum habrochaites* LA2329, occurs as two chemotypes. One chemotype of LA2329 contained three major compounds in its *n*-hexane leaflet wash, 7-epi-zingiberene, 9-hydroxy-zingiberene, and 9-hydroxy-10,11-epoxy-zingiberene, and was identified as LA2329-A. In contrast, the second chemotype of LA2329 had only 7-epi-zingiberene as the major compound in its *n*-hexane leaflet wash and was identified as LA2329-B (Dawood and Snyder 2020).

To determine the absorbance of major compounds, present in the parents, leaflet washes of the recurrent parent, 'Zaofen 2' and the donor parent LA2329-A and LA2329-B were prepared. Leaflet washes were prepared by placing leaflets in *n*-hexane (~2 ml/leaflet), vortexing and then removing the leaflets. Leaflet washes were then scanned with the spectrophotometer, and compounds of interested were quantified by GC-FID. Purified 7-epi-zingiberene and 9-hydroxy-zingiberene from the leaflet wash of LA2329-

A were obtained by open column chromatography on silica gel as described by Dawood and Snyder (2020). β-phellandrene was purified in a similar fashion. Purity of the fractions was determined by GC-FID. Fractions of the highest purity were scanned with the spectrophotometer in the usual fashion.

2.3.5 Methods for quantitative analysis

2.3.5.1 BC4F2 generation

To evaluate the use of the spectrophotometer for quantitation of 7-epi-zingiberene a widely segregating population, an interspecific BC4F2 population was evaluated. The entire population had previously been evaluated by GC-FID to determine concentrations of 7-epi-zingiberene and β-phellandrene. 38 plants were identified that represented the range of concentrations of these two compounds that were present in the BC4F2 population. Leaflet washes were obtained by taking the middle 1/3 to ¼ of each of three leaflets from the third or fourth leaf position of each plant, approximately 10 cm^2 foliage, and placing the leaflet tissue in 4 ml of *n*-hexane. After vortexing and removing the leaflet tissue, absorbance of the samples at 270 nm was determined spectrophotometrically and concentrations of 7-epi-zingiberene and β-phellandrene were determined by GC-FID.

2.3.5.2 BC3F5 generation

To evaluate the BC3F5 generation, an interspecific hybrid population that contained one major compound (7-epi-zingiberene) in its leaflet washes based on GC-

FID, two sampling procedures (A and B) were used. This was done to explore the accuracy of the spectrophotometric analysis for 7-epi-zingiberene determination, especially with regard to ratio of leaflet tissue to *n*-hexane in the leaflet wash. The same twenty-five genotypes of the BC3F5 interspecific hybrid population were used for each procedure.

In procedure A, three intact leaflets were taken (average = 28.0 cm^2, range= 21.7–37.3 cm^2), and the leaflets of each genotype were placed in a 20 mL disposable scintillation vials containing 4 mL of *n*-hexane. With procedure B, the middle part (center 1/3 to 1/4 of the leaflet) from each of three leaflets (average = 14.4 cm^2, range= 9.5 – 20.5.3 cm^2) were taken and placed in a 20 mL disposable scintillation vials containing 4 mL of *n*-hexane. For both procedures, vials were vortexed, and then all samples were scanned with the spectrophotometer. 7-epi-zingiberene concentrations in theses leaflet washes were determined by GC-FID.

2.3.5.3 BC5F$_1$ generation

The BC5F$_1$ interspecific hybrid population had low 7-epi-zingiberene concentrations and variable β-phellandrene concentrations. Ten cm^2 of foliage from the third and fourth position of each of 10 plants was taken in duplicate (10 plants × 2 reps). Leaflet washes were prepared in the usual fashion with *n*-hexane and then evaluated by spectrophotometer and by GC-FID.

2.3.5.4 Purified β-Phellandrene

To prepare crude extracts of recurrent parent (Zaofen 2) that contained two main compounds: β-phellandrene and other unknown compounds, half kg of foliage was collected in a 5 L beaker. leaves were soaked in an excess of *n*-hexane. Subsequent steps of extracts were followed in the same fashion as for LA2329-A extracts. Open column chromatography was used to obtain pure β-phellandrene of the crude extract. A glass column (7 X 0.5 cm) was filled with SiO_2. 10 mg of crude extract was loaded onto the column, which was then eluted with *n*-hexane: methyl-tert-butyl ether (MTBE) with ratio of 99 % *n*-hexane:1% MTBE. Two ml fractions were collected, and elution was monitored by spotting one drop of each fraction onto a thin TLC plate followed by illumination with 254 nm ultraviolet light. Pure β-phellandrene in fractions 3-5 at 80% purity was obtained by silica gel chromatography and determined by GC-FID.

2.3.6 Statistical Analysis

Statistical analysis was performed via the SAS 9.4 statistics package (SAS Institute Inc., 2016) and Excel (Microsoft 365, 2019). Regression analysis was used to investigate the association between the spectrophotometer reading value and major compounds in interspecific hybrid populations and in the wild accession (LA2329). Correlation analysis between major components such as 7-epi-zingiberene and β-phellandrene in the *n*-hexane leaflet wash solution and their UV-absorbance was accomplished using Pearson correlation.

2.4 Results and Discussion

2.4.1 UV absorbance of tomato terpenoids -- qualitative aspects

Because the interspecific hybrid populations were obtained through crossing the cultivated tomato, 'Zaofen 2', *Solanum lycopersicum* that had a single major trichome secretion compound, β-phellandrene, and *Solanum habrochaites* LA2329 which had two chemotypes, the analysis was complex. One chemotype for LA2329, identified as LA2329-A, possessed three major compounds, 7-epi-zingiberene, 9-hydroxy-zingiberene, and 9-hydroxy-10,11-epoxy-zingiberene, in the *n*-hexane leaf washes. In contrast the second chemotype, LA2329-B, had only one major compound, 7-epi-zingiberene in its trichome secretions (Dawood and Snyder 2020). All the chromatogram and UV/visible scans for each parent and generation of interspecific hybrid populations were obtained using GC-FID and spectrophotometric methods, respectively.

2.4.1.1 Sesquiterpenoids

Figure 2-3 depicts the high purity of 7-epi-zingiberene purified from leaf washes of LA2329-A, having a retention time of 6.00 min when chromatographed by GC-FID and Figure 2-4 provides a UV/visible spectrophotometric scan of this purified 7-epi-zingiberene demonstrating a λ_{max} at 270 nm. Similarly, Figure 2-5 is a chromatogram (GC-FID) of purified 9-hydroxy-zingiberene with a retention time of 6.91 min. Figure 2-6 contains a UV/visible spectrophotometric scan of the purified 9-hydroxy-zingiberene displaying a λ_{max} of 270 nm. Figure 2-7 is a GC-FID chromatogram of the *n*-hexane leaflet wash of the chemotype LA2329-A illustrating two major peaks at retention times

of 6.00 min which corresponds with 7-epi-zingiberene and 6.91 min for 9-hydroxy-zingiberene.

The UV/visible spectrophotometric scan of the same *n*-hexane leaflet wash (Figure 2-8) had a λ_{max} of 270 nm, which also aligns with the λ_{max} observed for purified 7-epi-zingiberene (Figure 2-4). Figure 2-9 is a GC-FID chromatogram of the *n*-hexane leaflet wash of the chemotype LA2329-B demonstrating one major peak at a retention time of 6.00 min, which corresponds with 7-epi-zingiberene. Figure 2-10 is a UV/visible spectrophotometric scan of the leaflet wash of chemotype LA2329-A from 200-600 nm. Even though this extract contains two major compounds, it showed a single absorption peak at 270 nm. These outcomes from the that UV/visible spectrophotometric scan and GC-FID indicate that the second compound (9-hydroxy-zingiberene) entirely interferes with 7-epi-zingiberene because both compounds before and/after purified had a λmax of 270 nm. This also indicates that the presence of both compounds interferes with each other and makes the precise detection of 7-epi-zingiberene at 270 nm more difficult.

Monoterpenes

Figure 2-11 provides a chromatogram (GC-FID) of the *n*-hexane leaflet wash of the recurrent parent Zaofen-2 with a major peak at 2.37 min corresponding with beta-phellandrene, while figure 2-12 provides a UV/visible spectrophotometric scan of purified β-phellandrene demonstrating a λ_{max} of 232 nm. Figure 2-13 shows a UV/visible scan of the *n*-hexane leaflet wash 'Zaofen 2' having a λmax of 232 nm. These outcomes from the that UV/visible spectrophotometric scan and GC-FID imply that the presence of

other unknown compounds in the *n*-hexane wash of Zoafen-2 do not interfere with β-phellandrene because the β-phellandrene before and/after purified had a λmax of 232 nm.

2.4.1.2 Summary and implications of qualitative spectrophotometry

Zingiberene has a λmax of 270 as do it oxygenated forms. Thus, the presence of the oxygenated forms will interfere with quantitation of 7-epi-zingiberene. Based on the finding of similar λmax for purified and leaflet wash samples, there seems to be little interference caused by the presence of compounds other than zingiberene and its derivatives in the wild donor parent LA2329 with regard to the λmax at 270 nm. β-phellandrene has a λmax of 232 nm, and based on the similar λmax for purified β-phellandrene and the leaflet wash sample from the recurrent parent Zaofen-2, the presence of other compounds in the leaflet wash of the recurrent parent did not shift

2.4.2 UV-Absorbance of tomato terpenoids quantitative aspects

2.4.2.1 Tetradecane as a standard to permit calculation of mass of terpenes

In order to quantify the concentrations of 7-epi-zingiberene and other compounds in the interspecific hybrid populations and in wild accessions, the GC-FID detector response needed to be converted into quantifiable understandable units of mass. Thus, the standard curve using tetradecane was obtained (Figure 2-14. Tetradecane is the preferred method of calibration for accurate quantitative analysis since its concentration remain unchanged during GC-FID runs. Also, because it has a higher boiling degree than extracted analytes and do not readily evaporate (Kokosa, Przyjazny et al. 2009).

2.4.2.2 Absorbance vs. concentration of zingiberene, zingiberene alcohol and zingiberene epoxy alcohol

Standard curves were obtained for purified 7-epi-zingiberene and 9-hydroxy-zingiberene (Figure 2-15). Doing so allowed us to determine whether similar masses of compounds would provide similar optical density values. Both compounds displayed strong linear responses of optical density to concentration but the slope for purified 9-hydroxy-zingiberene (y = 6E07x + 0.015) was greater than that for 7-epi-zingiberene (y = 1E07x + 0.06).This indicates that the extinction coefficient for 9-hydroxy-zingiberene is much higher than that for 7-epi-zingiberene (Figure 2-15).

2.4.2.3 Absorbance at 270 in a BC4F2 population

In interspecific hybrid population, a BC4F2 was segregating for presence and abundance of zingiberene and β-phellandrene, I took a sub (56) subsample of this BC4F2 population to compare absorbance values at 270 nm and zingiberene concentration as determined by GC-FID.GC-FID. The 37 subsamples used had an average 7-epi-zingiberene content of 36.34±37.06 µg/cm² ranging from 0 to 176.51 µg/cm² leaf area. The average β-phellandrene content for the 19 subsamples was 50.33±38 µg/cm² and ranged from 0 to 115.51 µg/cm² of leaf area (Table 2-1). No individual in the subsample produced any oxygenated forms of zingiberene. An ANOVA of the regression of absorbance at 270 nm on the concentration of 7-epi-zingiberene measured by GC-FID detector response (Table 2-22) was highly significant (P=0.0000; 1, 36 df). For this subsample of plants there was a strong association (R^2=0.92) between 7-epi-zingiberene content and absorbance at 270 nm (Figure 2-16). Interestingly, the intercept for the regression of this set of data was 0.36 absorbance units, supporting the idea that compounds other than zingiberene may

have been present in some or all of the samples, and these unidentified compounds contributed to absorbance at 270 nm (Figure 2-17).

To better understand the contribution of compounds other than zingiberene to absorbance at 270 nm, the concentration of β-phellandrene as measured by GC-FID was regressed on absorbance at 270 nm for the samples that did not contain zingiberene (n=19) The results of this regression ($Y=0.2454 + 1 \times 10^6X$, $R^2 = 0.59$) indicated a significant positive slope and a non-zero intercept. Furthermore, the samples having low concentrations of β-phellandrene (those nearest the y-axis) had absorbances ranging from ~0.05 to 0.38. These results support the idea that β-phellandrene may have contributed to absorbance at 270 nm, but there were other, unmeasured components present in the leaf washes that contributed absorbance at this wavelength. Unfortunately, absorbance of these samples was only determined at 270 nm, i.e. they were not scanned, so relationships between absorbance at wavelengths other than 270 nm could not be investigated. That said, the evidence supports the idea that substances other than zingiberene and β-phellandrene contributed to absorbance at 270 nm in this set of samples.

2.4.2.4 Relationship between absorbance values at several wavelengths and zingiberene concentration in a BC3F5 population

To better understand the contribution of compounds other than zingiberene and β-phellandrene to the UV absorbance of leaflet washes, a BC3F5 population was evaluated. The major terpenoid produced by this population was 7-epi-zingiberene, and β-phellandrene was not detected in any plant (Table 2-1). (Table 2-5). All samples were scanned for absorbance from 200 to 600 nm and zingiberene was quantified by GC-FID.

Two sampling procedures (designated A and B) were employed and the main difference between the two was the ratio of leaf tissue to *n*-hexane was greater in the A samples, compared to the B samples. Consequently, interference of unidentified substances should be more apparent in the A samples, compared to the B samples. The average of 7-epi-zingiberene in interspecific hybrids BC3F5 subpopulation for procedure A was 43±4.37 μg/cm² and ranged from 4.02 to 143.12 μg/cm² of foliage. For the B procedure the 7-epi-zingiberene content averaged 21.00±13.19 μg/cm² and ranged from 3.20 to 69.20 μg/cm² of foliage. An ANOVA of 7-epi-zingiberene content of a subpopulation of BC3F5 interspecific hybrid plants for the A and B procedures found the F-test for the association between 7-epi-zingiberene and absorbance at 270 nm was highly significant (P=0.0000; 1, 25 df).

To better understand the contribution of compounds other than 7-epi-zingiberene and nature of β-phellandrene to variation of UV absorbance, I explored this by use of stepwise regression, using selected wavelengths as independent variables and zingiberene concentration as the dependent variable. Also, A and B samples were separately analyzed. To choose wavelengths for inclusion as independent variables, all of the UV absorbance scans were inspected visually. A representative sample of these scans is provided in Figure (2-20). In the scans, in addition to the 270 nm region of the scans, three other regions appeared to vary considerably among samples. The regions so identified were 230, 250 and 300 nm of the UV scans (Figure 2-20). Consequently, the regions of 230, 250, 270 and 300 nm were chosen as independent variables for the stepwise regression analysis.

As expected, 270 nm was selected as the best independent variable for the A and B sampling procedures (Table 2-5). However, based on the R^2 values for these two single variable regressions, procedure B was more consistent or precise than procedure A for determining concentrations. Nevertheless, estimated slopes and intercepts for these two regressions were very similar supporting the idea that the accuracy of 7-epi-zingiberene prediction was similar between the two sampling procedures, A and B. β-phellandrene was not present in any of these samples so variation in β-phellandrene did not contribute to imprecision of the regression. However, the ratio of leaf tissue to *n*-hexane was greater in the A samples compared to the B samples also means that concentrations of compounds other than 7-epi-zingiberene were also likely greater in the A samples than the B samples. Thus, it is likely that variation of these other, unmeasured compounds likely contributed to the reduced precision of the A sampling procedure.

When stepwise multiple regression was employed to examine relationships among absorption at four wavelengths (230, 250, 270 and 300 nm) and zingiberene concentration as the dependent variable, the best two variable model selected the wavelength at 230 nm as the second variable for both A and B sampling procedures (Table 2-5). Slope estimates for the 230 nm wavelength were negative and of a magnitude similar to the slope for 270 nm. Thus, the impact of the absorbance at 230 nm on estimation of zingiberene concentration was considerable and, in a direction opposite to that associated with absorbance at 270 nm. In other words, the presence of absorbance at 230 nm would tend to lead to overestimation of zingiberene in these sample, if not taken into account. Inclusion of absorbance at 230 nm as the second variable in the regression provided minor improvement of the R^2 for the B sample model, from 0.92 to

0.96. However, for the A sample model, inclusion of this absorbance at 230 resulted in a very significant improvement of R^2 from 0.78 to 0.94. The resulting two variable regression equations, with regard to R^2 values, and estimated intercepts, and slopes associated with absorbances at 230 and 270 nm were very similar to each other, likely indicating that inclusion of absorbance at 230 nm made the two sampling procedures A and B, equivalent with regard to precision of 7-epi-zingiberene concentration. Adding additional independent variables to the model did not significantly improve R^2 values, so measuring absorbance at these wavelengths likely has little value for improving precision of the estimates.

2.4.2.5 Evaluation of a $BC5F_1$ population having low levels of zingiberene and β-phellandrene in its leaflet washes

The terpene composition of leaf washes of the $BC5F_1$ population presented a somewhat different scenario b because this population was an F_1 population where all the plants contained β-phellandrene and 7-epi-zingiberene and average levels of zingiberene was considerably lower than its concentration in the BC3F5 and BC4F2 populations Average β-phellandrene was also lower than that observed in the BC4F2 population. The average 7-epi-zingiberene concentration in the $BC5F_1$ subpopulation was 14.27±7.18 µg/cm² and ranged from 0.14 to 29.19 µg/cm² of foliage. On the other hand, the average β-phellandrene content the subpopulation was 9.06±±4.37 µg/cm² and ranged from 2.04 to 16.67 µg/cm² of foliage (Table 2-1). In this subpopulation, absorbance at 270 was highly correlated with 7-epi-zingiberene concentration ($r=0.91$, $P<0.05$). Regression analysis (Figure 2-18) indicated a non-zero intercept of 0.12 and a slope of 8×10^6,

results very similar to the relationship between 7-epi-zingiberene and absorbance at 270 nm for the BC4F2 (Figure 2-16).β-phellandrene and UV-absorption at 232 nm were strongly correlated (R=0.93), (Table 2-6).

In all interspecific hybrid subpopulations (BC3F5, BC4F2 and $BC5F_1$) the association between 7-epi-zingiberene and absorbance of UV-light at 270 nm was strong and are in agreement with Maluf, Campos et al. (2001) who listed 270 nm as a reasonable λmax for 7-epi-zingiberene via spectrophotometer.

The explanation of why these allelochemical compounds are present in the trichome secretions of interspecific hybrids is likely due to the presence of members of the terpene synthase (TPS) family which includes enzymes that synthesize the backbone of the β-phellandrene, which is a monoterpenes (C10), and the sesquiterpenes such as 7-epi-zingiberene (C15) (Bohlmann, Meyer-Gauen et al. 1998, Falara, Akhtar et al. 2011, Pichersky 2020). Researchers have reported that the TPS-a clade has been identified in the tomato and is the largest clade of functional TPS genes, particularly on chromosomes 1, 2, 6, 8, and 10.

The outcome of spectrophotometric analysis of the leaflet washes from the two chemotypes of the wild accessions LA2329 suggest that 7-epi-zingiberene and 9-hydroxy-zingiberene absorbed UV-light at 270 nm wavelength. The conclusion is strongly supported by the spectrophotometric analysis of purified 7-epi-zingiberene and 9-hydroxy-zingiberene with each compound sharing a λ_{max} of 270 nm. Thus, UV absorbance at 270 can only be used to measure only when oxygenated forms of 7-epi-zingberene are not present in the leaflet wash. -Spectrophotometric analysis cannot be

used with genotypes similar to the LA2329-A chemotype that had 7-epi-zingiberene and 9-hydroxy-zingiberene. These observations also mean oxygenated zingiberene completely interferes with 7-epi-zingiberene because both compounds before and/after purified had a λmax of 270 nm. This implies also that the presence of both compounds interfere with each other and makes the precise detection of 7-epi-zingiberene more difficult in breeding program. Maluf, Campos et al. (2001) did not mention this interference and it is this interference that likely explains why they have not recovered parental absorbance values in their segregating generations. Thus, this study indicated that 7-epi-zingiberene can only be quantified in the absence of it oxygenated forms. However, this does not preclude use of measuring absorbance of leaf washes at 270 nm to estimate total zingiberene-related compounds because all of these compounds have a λmax of 270 nm. But, this approach needs additional research, because based on my results, it appears that the extinction coefficients differ for 7-epi-zingiberene and 9-hydroxy-zingiberene, with the extinction coefficient for the latter being considerably higher than that for 7-epi-zingiberene.

2.5 Conclusion

In conclusion, genotypes from interspecific hybrid populations could be selected for high concentrations of 7-epi-zingiberene demonstrated by correspondingly high absorbance values using a UV-vis spectrophotometer, while genotypes that had no 7-epi-zingiberene had lower absorbance values in genotypes that had β-phellandrene only. At the same time, non-zero absorbance values for these genotypes having no 7-epi-zingiberene indicated that β-phellandrene could interfere with quantification of 7-epi-

zingiberene. Based on the results obtained for the BC3F5 generation, it is clear that compounds other than β-phellandrene that absorb in the 230 nm region also contribute to inaccuracy of determining 7-epi-zingiberene concentration by measuring absorbance at 270 nm. Thus, measuring absorbance of leaflet washes at multiple wavelengths such as 230 and 270 nm may allow for accurate determination of 7-epi-zingiberene concentrations in leaf washes of segregating, interspecific hybrid populations. Such an approach may be useful for identifying lines that are homozygous and/or heterozygous for 7-epi-zingiberene and β-phellandrene. From a plant breeding perspective , using a UV-spectrophotometer and GC-FID as techniques for selecting high levels of 7-epi-zingiberene and 9-hydroxy-zingiberene in interspecific tomato populations may lead to improved resistance of tomato to arthropods and perhaps, to a crop that provides a source of natural pesticide for use in pest management. Furthermore, 7-epi-zingiberene and 9-hydroxy-zingiberene may have medicinal value and selecting for high levels of 7-epi-zingiberene and 9-hydroxy-zingiberene contents on leaves of interspecific hybrid tomatoes might be exploited as biorational source of these two essential substrates. Future work could be to investigate plants that are homozygous for 7-epi-zingiberene when β-phellandrene is not present in the foliage secretion, compared to plants heterozygous that are heterozygote for 7-epi-zingiberene when it contains for two main compounds (7-epi-zingiebrene and β-phellandrene). More research in the future also need to address the relationship between β-phellandrene and 7-epi-zingiberene in these interspecific hybrid generations. Also, the quantification of 7-epi-zingiberene in complex hybrids by spectrophotometric analysis needs additional research.

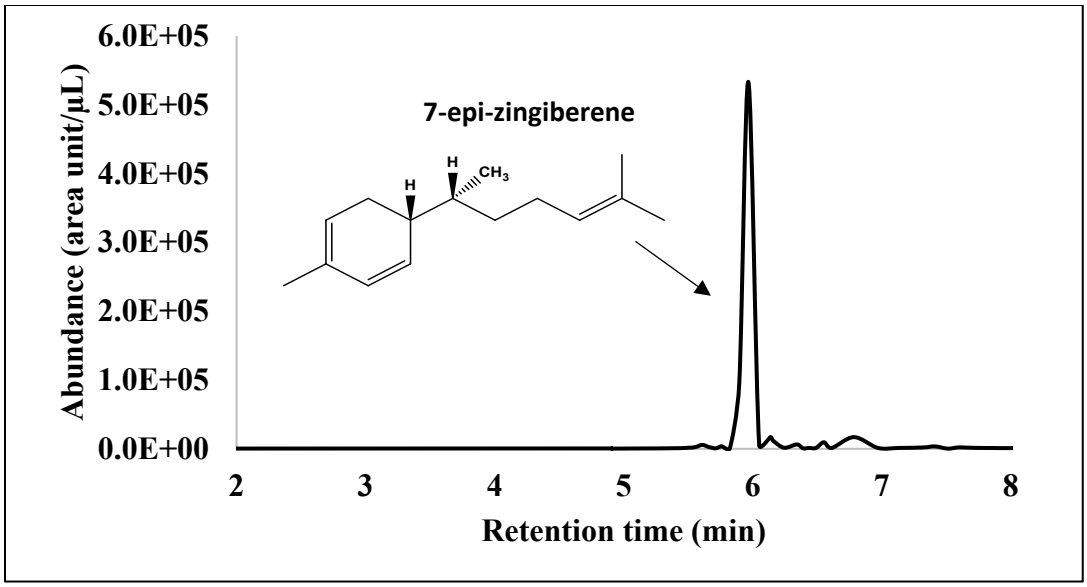

Figure 2-3 Chromatogram (GC-FID) of purified 7-epi-zingiberene from LA2329-A chemotype accession showing a retention time of 6.00 min.

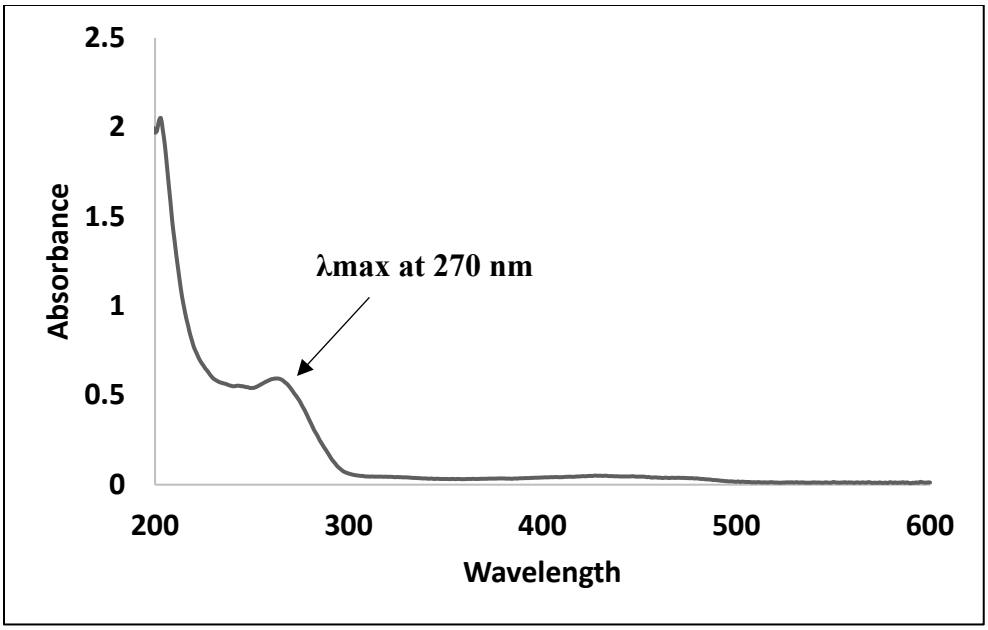

Figure 2-4 UV/visible scan (200-600 nm) of purified 7-epi-zingiberene with a λmax of 270 nm.

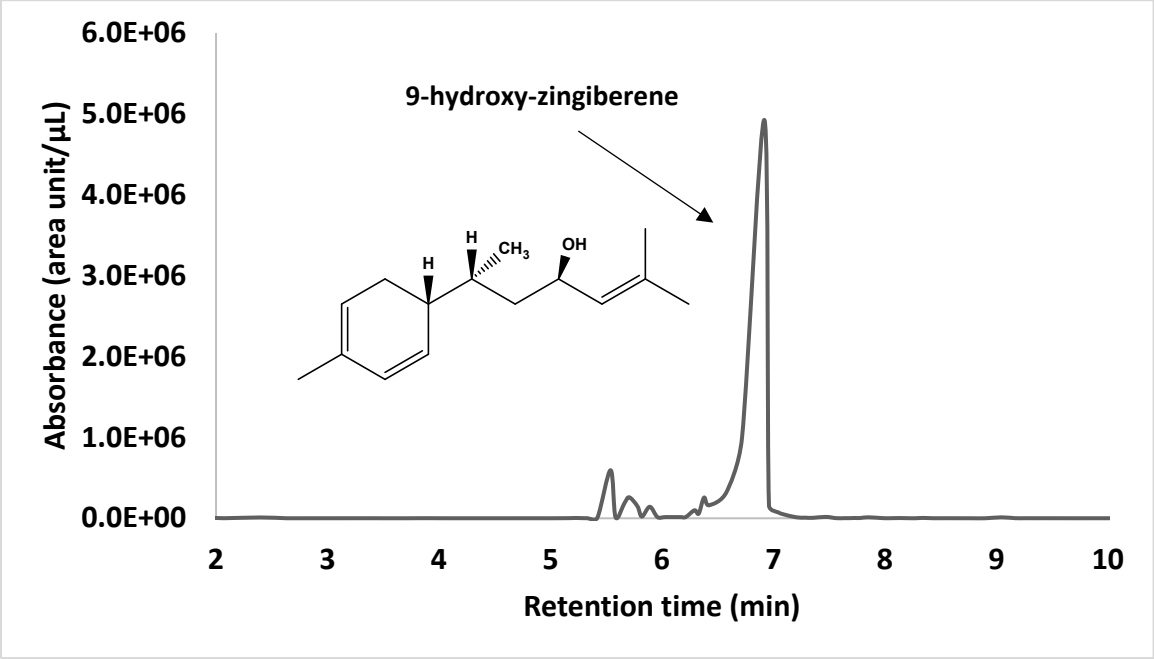

Figure 2-5 Chromatogram (GC-FID) of purified 9-hydroxy-zingiberene from showing a retention time of 6.91 min.

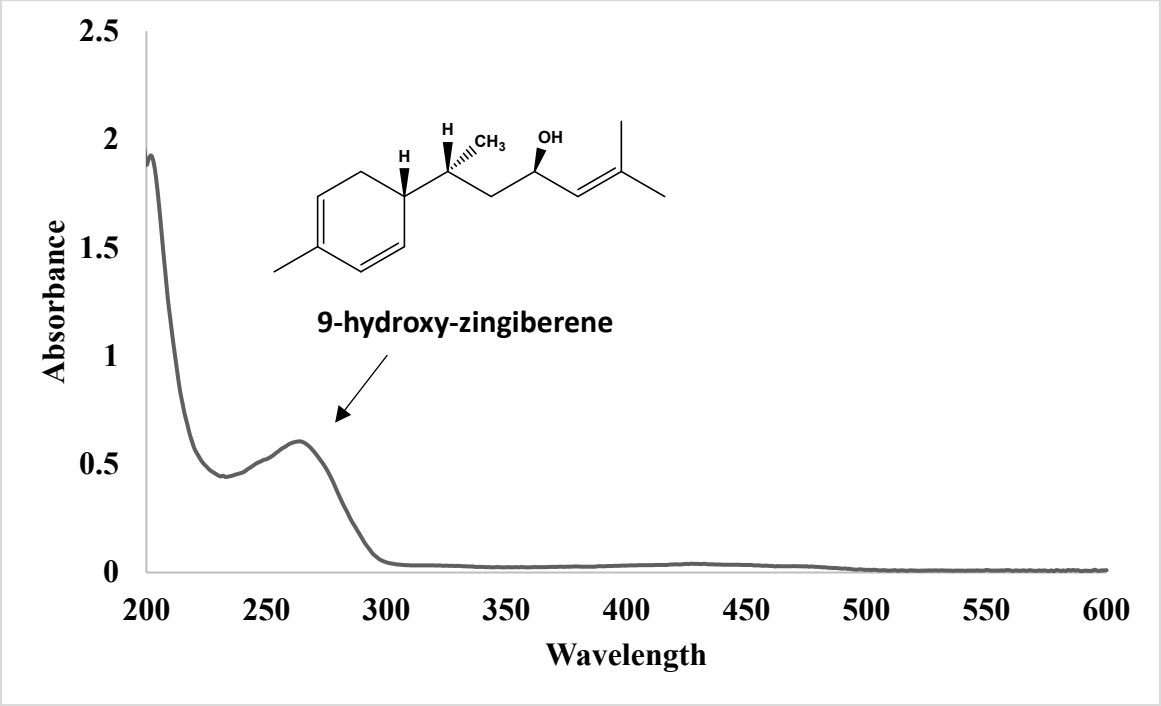

Figure 2-6 UV/visible scan (200-600 nm) of purified 9-hydroxy-zingiberene with a λmax of 270 nm.

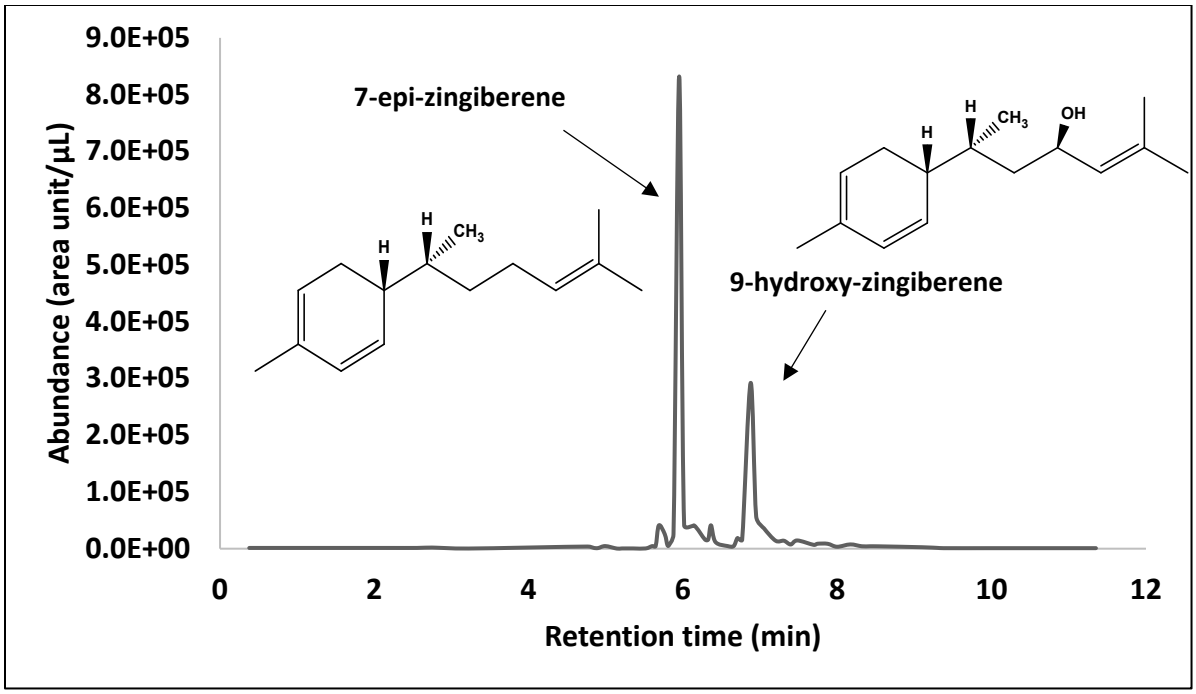

Figure 2-7 Chromatogram (GC-FID) of the *n*-hexane leaflet wash of the chemotype LA2329-A showing two major peaks at retention times of 6.00 and 6.91 min corresponding with 7-epi-zingiberene and 9-hydroxy-zingiberene, respectively.

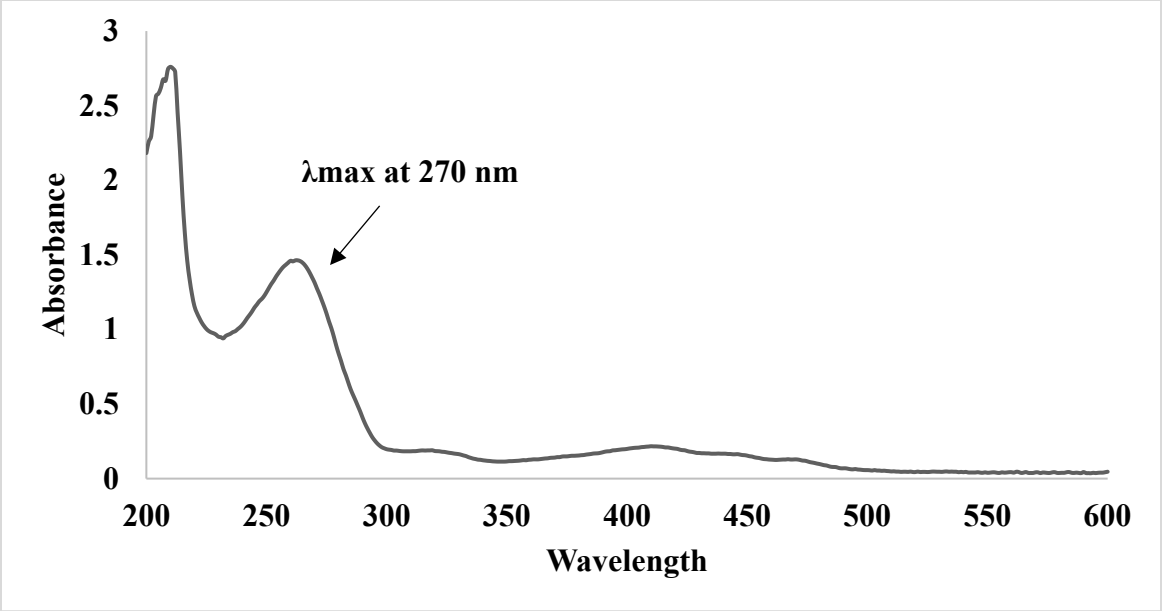

Figure 2-8 UV/visible scan (200-600 nm) of the leaflet wash obtained from chemotype LA2329-B illustrating 7 a λmax of 270 nm.

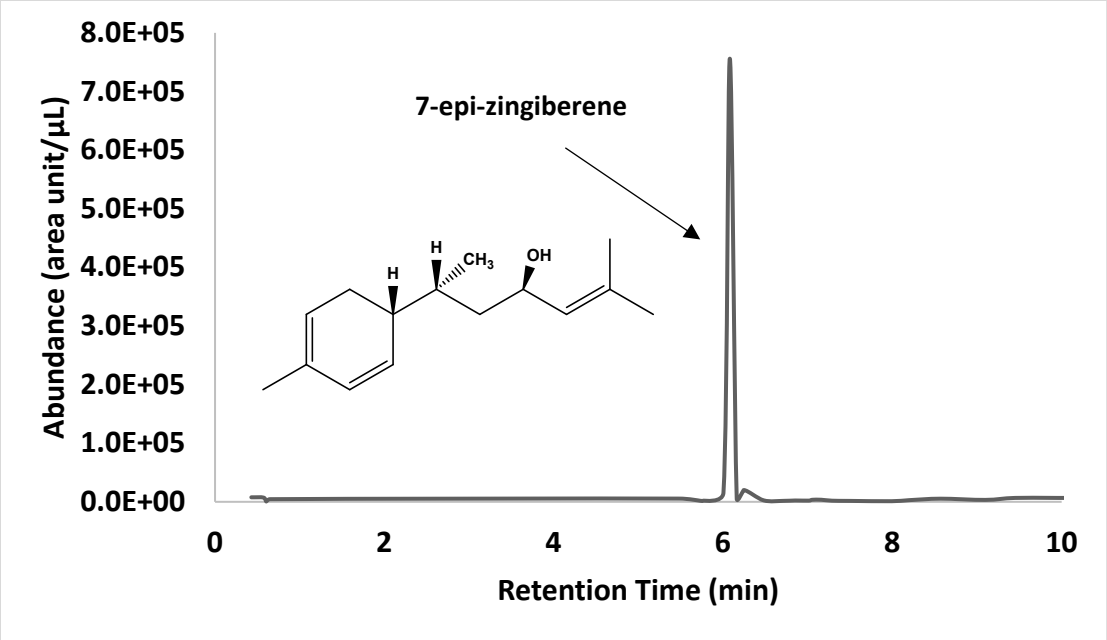

Figure 2-9 Chromatogram (GC-FID) of the *n*-hexane leaflet wash of the chemotype LA2329-B with one major peak at a retention time of 6.00 min corresponding to 7-epi--zingiberene.

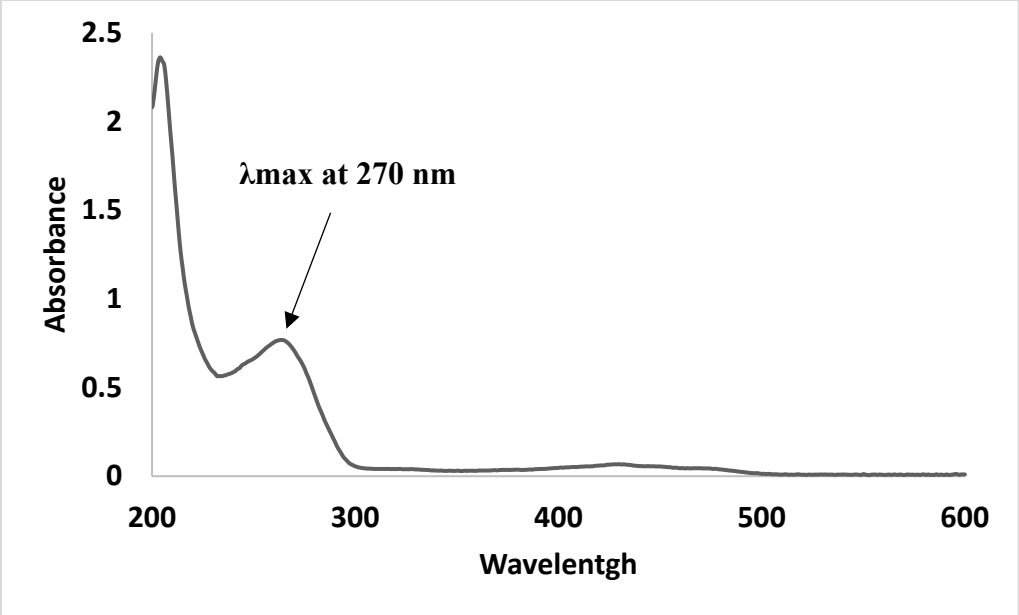

Figure 2-10 UV/visible scan (200-600 nm) of the leaflet wash from chemotype LA2329-A identifying absorbance peak at 270 nm, likely due to the presence of 7-epi-zingiberene and 9-hydroxy-zingiberene

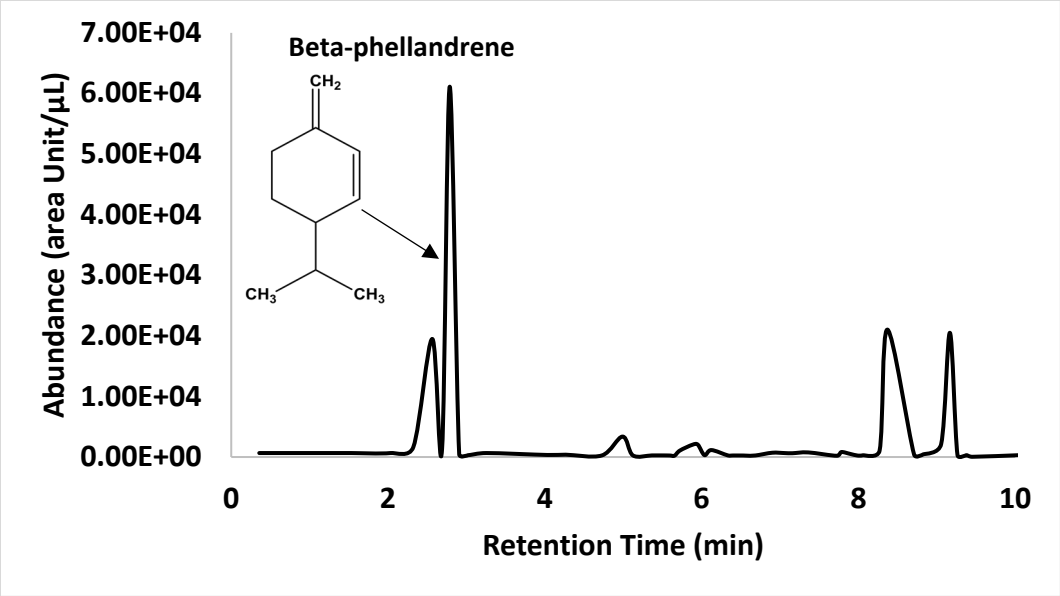

Figure 2-11 Chromatogram (GC-FID) of the *n*-hexane leaflet wash of recurrent tomato parent ('Zaofen 2') showing a major peak at 2.32 min which corresponds to β-phellandrene.

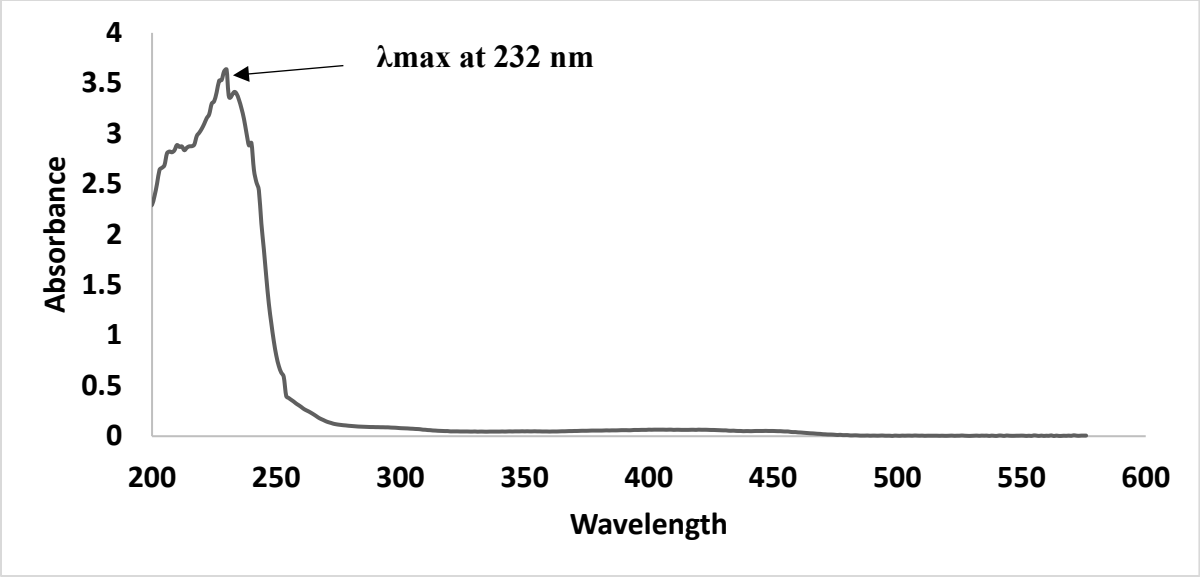

Figure 2-12 UV/visible scan (200-600 nm) of the crude n-hexane leaflet wash of recurrent parent ('Zaofen 2') illustrating a λmax of 232 nm.

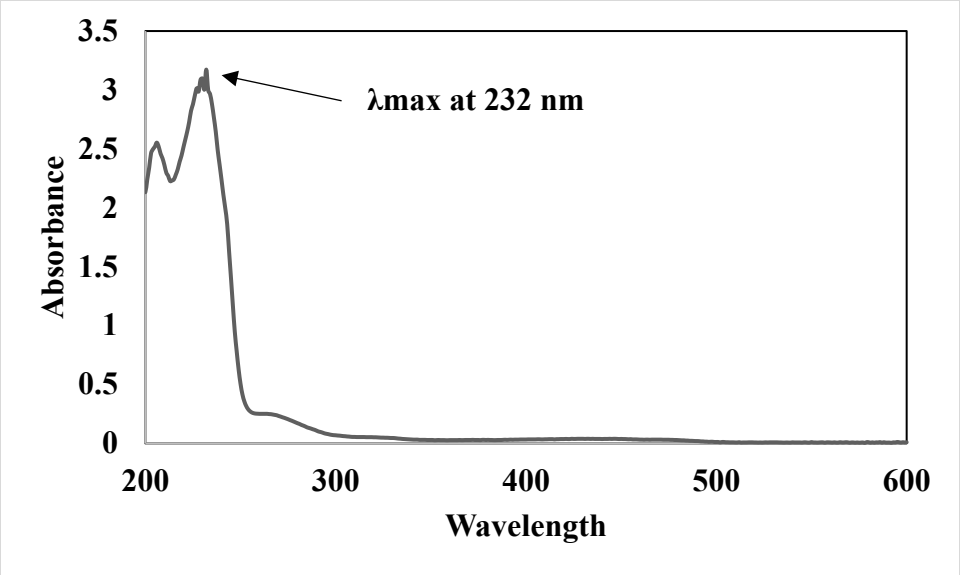

Figure 2-13 UV/visible scan (200-600 nm) of purified β-phellandrene. β-phellandrene had a λmax of 232 nm.

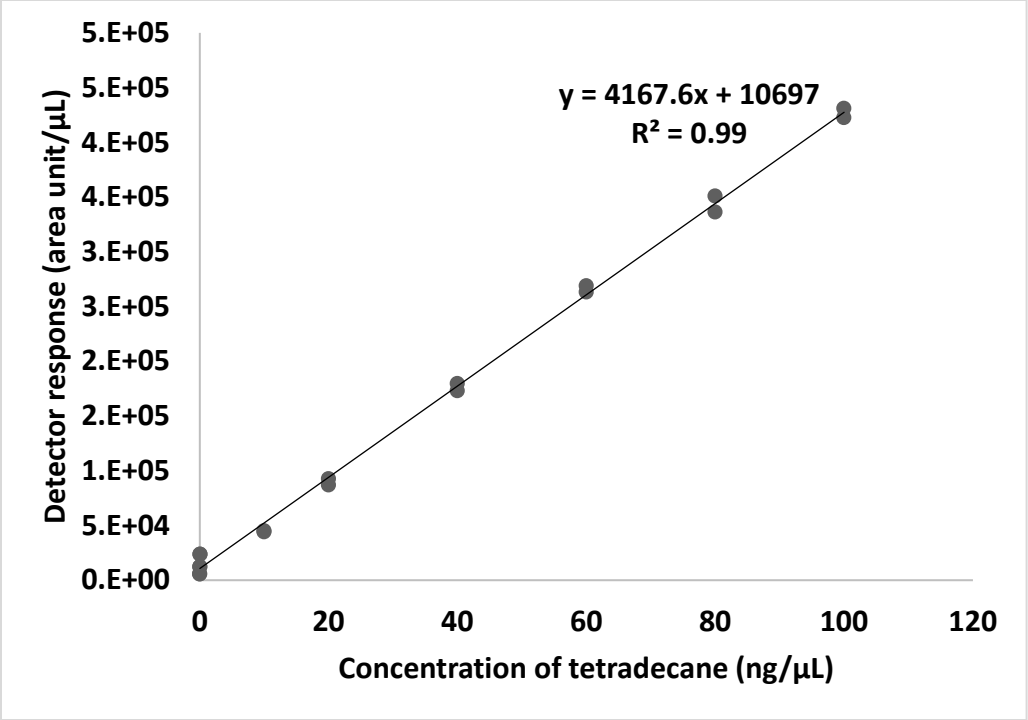

Figure 2-14 Standard curve between concentration of tetradecane (ng/µL) and GC-FID detector response (area unit/µL)

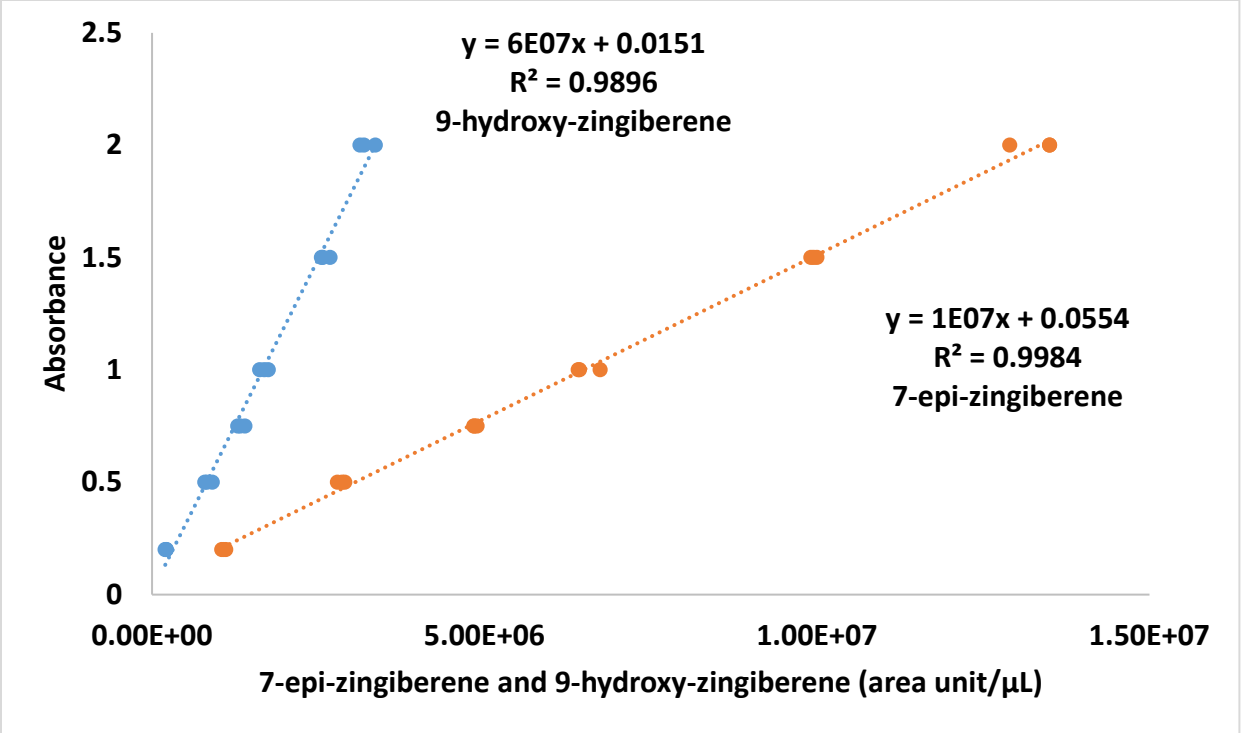

Figure 2-15 Association between abundance (area unit/µL) of purified 7-epi-zingiberene (orange trend line) and abundance of 9-hydroxy-zingiberene (blue trend line) with absorbance at 270 nm.

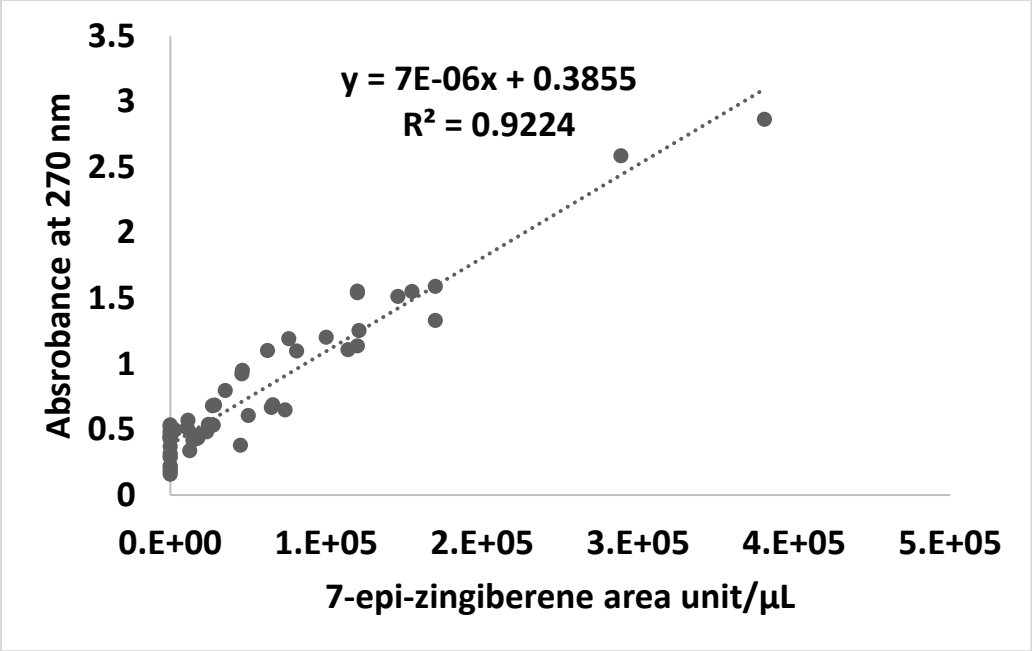

Figure 2-16 Association between 7-epi-zingiberene abundance (area unit/µL) measured by GC-FID and absorbance at 270 nm of *n*-hexane leaflet washes from BC4F2 interspecific hybrid plants, n=38

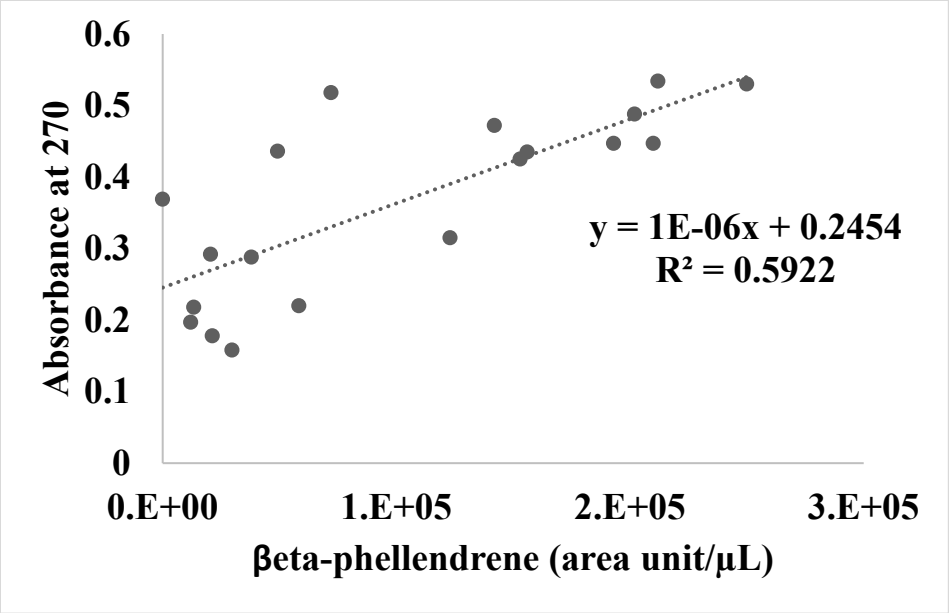

Figure 2-17 Association between abundance (area unit/µL) of β-phellandrene and absorbance at 270 nm wavelength of interspecific hybrid tomato BC4F2 subpopulation having no detectable zingiberene, n=19.

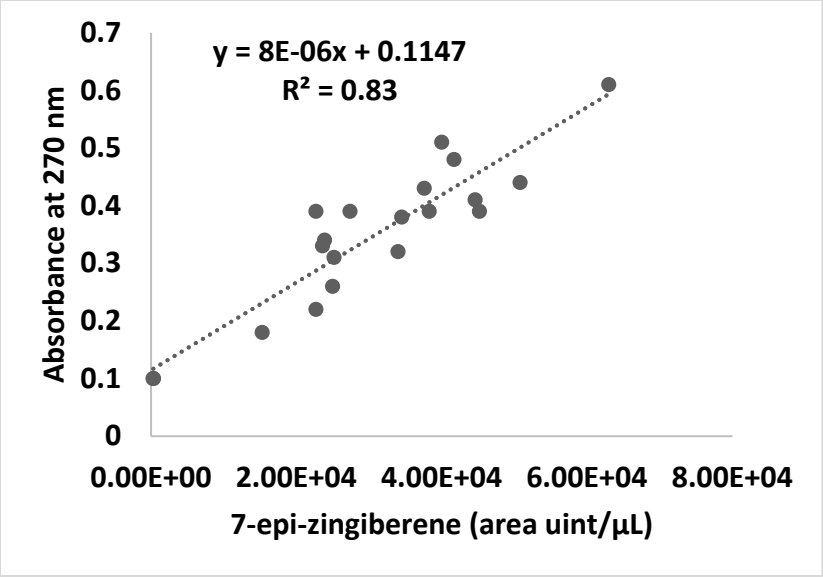

Figure 2-18 Association between 7-epi-zingiberene abundance (area unit/µL) of interspecific hybrid tomato $BC5F_1$ subpopulation and absorbance at 270, n=19. Plants in this subpopulation that had two major compounds, 7-epi-zingiberene and β-phellandrene.

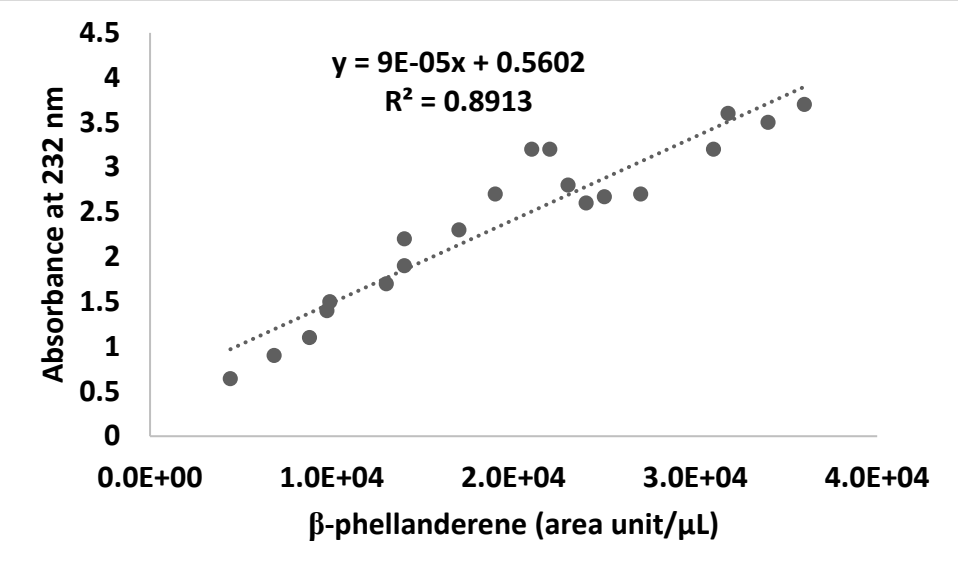

Figure 2-19 Association between β-phellandrene content (area unit/µL) and absorbance at 232 nm for an interspecific hybrid $BC5F_1$ subpopulation, n=21.

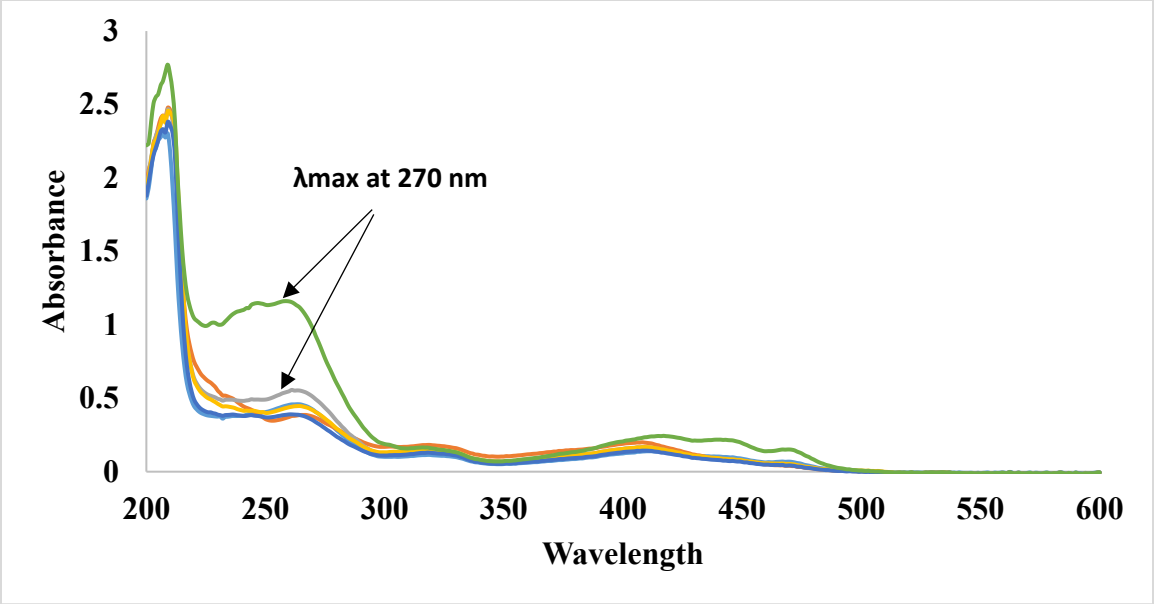

Figure 2-20 UV-visible scan (200-600 nm) of several BC3F5 genotypes. Besides 7-epi-zingiberene that λmax at 270, three other regions 230, 250 and 300 nm appeared to vary considerably among samples.

Table 2-1 Average 7-epi-zingiberene and β-phellandrene content (µg/cm² of foliage) for BC3F5, BC4F2 and $BC5F_1$ generations of interspecific hybrid subpopulations evaluated. Two sampling procedures were used for the BC3F5 population.

Generation	Compound µg/cm² of foliage	Mean (µg/cm²)	Range (µg/cm²) of foliage	
			low	high
BC3F5/ procedure B	7-epi-zingiberene	21±13.2	3.2	69.2
BC3F5/ procedure B	β-Phellandrene	0	0	0
BC3F5/ procedure A	7-epi-zingiberene	43±45	4.02	43.12
BC3F5/ procedure A	β-Phellandrene	0	0	0
BC4F2	7-epi-zingiberene	36±34	0	177
BC4F2	β-Phellandrene	50±40	0	115.51
$BC5F_1$	7-epi-zingiberene	14.27±7.18	0.14	29.19
$BC5F_1$	β-Phellandrene	9.06±4.37	2.04	16.67

Table 2-2 ANOVA table for the regression of absorbance at 270 nm on 7-epi-zingiberene concentration in 37 individuals from a BC4F2 interspecific hybrid population.

Source	DF	SS	MS	F	P
Regression	1	10.04	10.04	440.80	0.000
Error	35	0.80	0.02		
Total	36	10.83			

Table 2-3 ANOVA table for the regression of absorbance at 270 nm on β-phellandrene content (area unit/μL) for 19 interspecific BC4F2 hybrids that contained no zingiberene.

Source	DF	SS	MS	F	P
Regression	1	0.175	0.174	24.69	0.000
Error	17	0.120	0.007		
Total	18	0.301			

Table 2-4 Correlation matrix of β-phellandrene (area unit/µL) and absorbance at 270 nm for 19 interspecific BC4F2 hybrids

		β-phellandrene area unit/µL	Absorbance
β-phellandrene area unit/µL	Pearson's r	—	0.770 ***
	p-value	—	< .001
Absorbance	Pearson's r		—
	p-value		—

*Note. H_a is positive correlation. Note. * p < .05, ** p < .01, *** p < .001, one-tailed*

Table 2-5 Results of stepwise regression of 7-epi-zingiberene concentration on absorbance measured at four wavelengths (230, 250, 270 and 300 nm) for n-hexane leaf washes obtained by two sampling procedures (A and B) for 25 BC3F5 interspecific hybrid plants. In the models, the absorbance variable for each wavelength is abbreviated as abs plus the actual wavelength e.g. abs270 is absorbance at 270 nm, etc.

Model (No. of variables)	**Sample**	**Wavelength nm**	**Regression (R^2)**	**Equation**
1	A	270	0.92	7-epi-zingiberene = -9.48 + 41.22 (abs270)
	B		0.78	7-epi-zingiberene = -3.05 + 38.47 (abs270)
2	A	230 and 270	0.98	7-epi-zingiberene= 5.00 – 59.40 (abs230) + 77.63 (abs270)
	B		0.95	7-epi-zingiberene = 2.43 – 55.43 (abs230) + 72.45 (abs270)
3	A	230, 250 and 270	0.96	7-epi-zingiberene =2.30 – 45.41 (abs230) – 24.12 (abs250) + 92.38 (abs270)
	B		0.97	7-epi-zingiberene = 1.11 – 42.38 (abs230) – 2.25 (abs250) + 86.22 (abs270)
4	A	230, 250, 270, and 300	0.97	7-epi-zingiberene = 2.53 – 25.67 (abs230) – 42.93 (abs250) + 103.40 (abs270)-51.35 (abs300)
	B		0.97	7-epi-zingiberene =2.17 – 23.95 (abs230) – 40.07 (abs250) + 96.48 (abs270) – 47.92 (abs300)

Table 2-6 Correlations between β-phellandrene (area unit/μL) and absorbance at 232 nm for 19 interspecific $BC5F_1$ hybrid plants.

		β-phellandrene area unit/μL	Absorbance at 232 nm
β-phellandrene area unit/μL	Pearson's r	—	
	p-value	—	
Absorbance at 232 nm	Pearson's r	0.929 ***	—
	p-value	0.0002	—

Note. all tests one-tailed, for positive correlation. * p < .05, ** p < .01, *** p < .001, one-tailed

CHAPTER 3. THE ALCOHOL AND EPOXY ALCOHOL OF ZINGIBERENE, PRODUCED IN TRICHOMES OF WILD TOMATO, ARE MORE REPELLENT TO SPIDER MITES THAN ZINGIBERENE

3.1 ABSTRACT

Allelochemicals that are present in trichome secretions of wild tomato species play a major role in mediating interactions with arthropods, often conferring a high level of resistance via antibiosis and antixenosis. Many accessions of the wild tomato relative, *Solanum habrochaites* (*S.h*), possess high levels of resistance to arthropods. The monocyclic sesquiterpene hydrocarbon, 7-epi-zingiberene, is a major defensive component found in trichome secretions of certain accessions of *S.h.* We have used LA2329, an *S.h*. accession, as a donor in a breeding program designed to introgress zingiberene into cultivated tomato. However, the composition of trichome secretions in our population of LA2329 is segregating, with some individuals producing mainly 7-epi-zingiberene in their secretions while others producing two additional, unidentified compounds in their trichome secretions. To investigate if these other compounds may also contribute to arthropod resistance, trichome secretions were collected from plants of *S.h* LA2329 grown under greenhouse conditions and then major compounds were isolated by silica gel column chromatography and tested for their ability to repel two spotted-spider mite (TSSM), *Tetranychus urticae*. Isolation and identification of allelochemicals were aided by use of gas chromatography/mass spectroscopy. The results revealed the presence of three predominate chromatographic peaks: 7-epi-zingiberene, 9-hydroxy zingiberene and 9-hydroxy,10,11-epoxy-zingiberene. Results of testing isolated

compounds for repellency to TSSM using bridge bioassays revealed that the repellent activities of 9-hydroxy zingiberene and 9-hydroxy,10,11-epoxy-zingiberene were each significantly higher than that for 7-epi-zingiberene. These results support the idea that the degree of repellency may differ among plant allelochemicals and also emphasize the potential value of introgressing the presence of 9-hydroxy zingiberene and 9-hydroxy,10,11-epoxy-zingiberene into cultivated tomato to enhance its arthropod resistance.

Keywords: Breeding, Insect Resistance, 7-epi-zingiberene, 9-hydroxy-zingiberene, Tomato, Spider mites, Repellency, Antixenosis, Trichome

3.2 Introduction

The tomato, *Solanum lycopersicum,* is one of the most widely grown and important vegetables produced worldwide (Dorais, Ehret et al. 2008). Numerous factors can affect tomato productivity dramatically. One of these factors is arthropod infestation, resulting in direct damage and/or disease transmission (Nault and Speese III 2002). Most cultivated tomatoes are susceptible to diseases and pests, leading growers to apply pesticides and insecticides (Letourneau and Goldstein 2001) to control these problems. Breeding for resistance may provide an alternative to extensive pesticide use. Tomato has been greatly improved by introgression of disease resistance genes from wild relatives (Hajjar and Hodgkin 2007, Firdaus, van Heusden et al. 2012). There are wild tomato accessions that are highly resistant to a wide range of arthropod pests (Freitas, Maluf et

al. 2002), but introgression of arthropod resistance into commercially accepted tomato varieties remains an unfulfilled goal.

High levels of resistance to arthropods exist in several wild species of tomato and in many cases; resistance has been associated with trichomes and trichome secretions (Snyder, Guo et al. 1993, Schilmiller, Schauvinhold et al. 2009, Ekanayaka, Li et al. 2014). For example, acyl sugars secreted by trichomes on *S. pennellii, S. galapagense* and *S. pimpinellifolium* have been associated with resistance to an array of arthropods (Shapiro, Steffens et al. 1994, Liedl, Lawson et al. 1995, Mutschler, Doerge et al. 1996, Alba, Montserrat et al. 2009, de Resende, Maluf et al. 2009, Dias, Resende et al. 2013). Methyl ketones, primarily 2-tridecanone have been linked to arthropod antibiosis displayed by certain accessions of *S. habrochaites (S.h)* (Alba, Montserrat et al. 2009). For other accessions of *S.h*, resistance has been attributed to the presence of sesquiterpenes in their trichome secretions (Carter, Gianfagna et al. 1989, Weston, Johnson et al. 1989, Eigenbrode, Trumble et al. 1994, Chatzivasileiadis and Sabelis 1997).

It has been known for more than 25 years that trichomes on certain accessions of *S. h*. were associated with the production of sesquiterpenes and that there was structural diversity among sesquiterpenes produced (Guo, Weston et al. 1993). Additionally, oxygenated sesquiterpenoids, mainly carboxy acids of sesquiterpenes, have been documented in *S.h*. often as predominate components of trichome secretions (Coates, Denissen et al. 1988, Snyder, Guo et al. 1993). More recently, diversity and details of terpene production, especially those terpenes present in trichome secretions has been

elucidated in *S.h*. Much of the chemical diversity of trichome secretions in *S. h.* has been associated with sequence variation of sesquiterpene or monoterpene synthases associated with the TPS20 locus located on chromosome 8 of tomato. All of these enzymes utilize cisoid substrates, either neryl diphosphate for monoterpene production or 2z,6z-farnesyl diphosphate for sesquiterpene production (van der Hoeven, Monforte et al. 2000, Besser, Harper et al. 2009, Sallaud, Rontein et al. 2009, Schilmiller, Schauvinhold et al. 2009, Falara, Akhtar et al. 2011). There is an extensive collection of wild tomato germplasm curated by the Tomato Genetics Resource Center (TGRC), located in Davis, California. Over a period of many years, *S.h*. has been systematically collected over its natural geographic range. This extensive and valuable resource is maintained for researchers worldwide. Gonzales-Vigil, Hufnagel et al. (2012) have surveyed 79 accessions of *S.h*. from the collection at TGRC for diversity of trichome-associated terpene production. Trichome secretions on these accessions were predominated either by sesquiterpenes or by monoterpenes. 21 different terpenes were detected among these 79 accessions, and. Moreover, the accessions could be grouped by the predominate sesquiterpene present in the trichome secretions. For example, trichome secretions on more than ½ of the accessions were predominated by 7-epi-zingberene [1]. The sesquiterpene, gamma-elemene, was predominate in predominately on 19 accessions, beta caryophyllene was predominate in 9 accessions and trichome secretions in 8 of the 79 accessions were predominated by monoterpenes, either β-phellandrene or limonene. How this chemical diversity may relate to arthropod resistance has not been well characterized

Among accessions, there is an array of sesquiterpene hydrocarbons produced by trichomes on *S.h.* (Ekanayaka, Li et al. 2014). However, the presence of 7-epi-

zingiberene [1] has frequently been associated with pest resistance (Carter, Sacalis et al. 1989, Antonious and Kochhar 2003, Bleeker, Mirabella et al. 2012, Álvarez Gil 2015, de Oliveira, de Resende et al. 2018). Recently at the University of Kentucky, we have been attempting to introgress the presence of high levels of [1] and high type IV trichome density from *S.h.* LA2329 into cultivated tomato. We have been successful in the introgression of [1] from wild to cultivated tomato, and currently possess several advanced breeding lines (BC3F7) having yields and seed set similar to the recurrent parent with levels of [1] similar to that in the wild donor parent (Snyder, Dawood et al. 2018). As part of this breeding program, we have worked extensively with the *S.h.* donor line LA2329. Associated operations have included seed increase and monitoring production of [1]. Several years ago, we discovered that the composition of trichome secretions varied within our population of LA2329, with some plants having one major component and others having three major components as determined by gas chromatography. All plants produced [1], but some of the plants also produced two additional abundant compounds of unknown identity that were later eluting than [1] when separated by gas chromatography. We have maintained these two distinct chemotypes by selfing or sib-mating within chemotypes. Leaves of the two chemotypes appeared to be equally resistant to spider mites when bioassayed. However, after an unintended infestation of whiteflies *(Bemisia* spp.) in our greenhouse we observed that the plants having the three major compounds were less preferred than those with [1] alone. This difference in whitefly preference between chemotypes could have been related to quantitative or qualitative chemical differences in the chemotypes, or to other unmeasured differences.

Based on these observations, we were particularly interested in assessing the potential for qualitative differences of antixenotic activity of the three major compounds present in LA2329. Because the bridge bioassay has the ability to demonstrate differences in antixenotic activity or repellency to spider mites among closely related compounds (Snyder, Antonious et al. 2011), the goal of this research was to isolate and evaluate the spider mite repellency of the three major compounds present in trichome secretions of LA2329. We wanted to know whether these three compounds differed in their degree of repellency to spider mites.

3.3 Materials and Methods

3.3.1 Biological Materials

Seeds of *S.h.* accessions LA2329 and LA2167 were obtained from the Tomato Genetics Resource Center, Davis, CA and seeds of PI127826 were obtained from USDA-ARS, Geneva NY. After seedling establishment, plants were propagated by cuttings or from seed produced by selfing or sib-mating. Plants were grown in the greenhouse at the University of Kentucky, using Pro Mix BX (Premier Tech Horticulture, Quakertown, PA) as the growing medium. Plants were irrigated with a fertilizer solution containing Peter's Professional 5-11-26 (ICL SF USA & Canada, Dublin, OH) plus $CaNO_3$ (Viking Yara, Tampa, FL) to provide 180 ppm of N. Plants were grown during spring summer and fall, under natural daylength conditions. The average temperature and relative humidity of the greenhouse during this time period were 24°C, and 67% RH, respectively. Spider mites,

Tetranychus urticae (TSSM) were reared and handled using procedures as outlined by Snyder, Antonious et al. (2011).

3.3.2 Leaflet Wash Preparation for Determination of Qualitative and Quantitative Variation of Trichome Secretion Components

We investigated quantitative and qualitative variation of abundance of each major component of trichome sections on plants of the two chemotypes of LA2329 (one plant of each chemotype) and on LA2167 and PI127826 (one plant of each accession). To prepare leaflet washes the center 1/3 portion of each of three leaflets from the third leaf position of a plant was placed into a 20 ml vial and then 2.0 ml of *n*-hexane was added. The vials were vortexed for 30 seconds and then the extract was analyzed by GC-FID using procedures outlined below. The area of extracted leaf tissue was determined by using the open-source image analysis software ImageJ/Fiji (ImageJ 1.47v, National Institutes of Health, USA). Results were expressed as FID detector response (area units) per cm^2 of leaflet tissue. For this experiment, each plant was sampled three times.

3.3.3 Oleoresin Preparation for Bioassay and Chromatography

To prepare oleoresins of the two chemotypes of LA2329 for bioassays, leaflets, usually from the third or fourth leaf from the apex, were removed from plants and were then steeped in n-hexane for 30 minutes using approximately 1 ml of n-hexane per leaflet. Leaflets were then removed, and *n*-hexane was removed by use of a N_2 stream. Oleoresins were stored at 3°C and for bioassay were diluted in *n*-hexane.

To prepare extracts for subsequent open column chromatography, two kg of leaflets from the LA2329 chemotype that contained three major components (chemotype A) were

collected in a 10 L beaker. Leaflets were soaked in an excess of *n*-hexane. After stirring, leaflets were removed, and the extract was filtered (Whatman 934-AH). Subsequently over a period of several days the *n*-hexane was allowed to evaporate under a chemical hood. After that, additional *n*-hexane was eliminated from the oleoresin by use of a gentle stream of N_2. This extract was weighed and stored in a refrigerator at 3°C.

3.3.4 Gas Chromatography

A gas chromatograph, Hewlett Packard 5890 Series II, equipped with a flame ionization detector (GC-FID) and an RTX-5 column (5% diphenyl/95% dimethyl polysiloxane, 15 m, 0.53-mm ID, 0.5 µm, Restek Corporation, Bellefonte, PA) was operated as follows: injector 250ºC, detector 300ºC, oven initial temperature 50ºC for 1 min, then increasing at 20ºC/min to 260ºC. Helium was the carrier gas flowing at 14 ml/min. Tetradecane (Acros Organic®, New Jersey, USA) served as the external standard to verify retention time and detector response. Calculation of the concentration of components in trichome secretions was based on a detector response or on a tetradecane standard curve.

Gas chromatography-mass spectroscopy (GC-MS) was used to verify identities of [1] 7-epi-zingiberene, [2] 9-hydroxy zingiberene and [3] 9-hydroxy,10,11-epoxy-zingiberene in leaflet washes of LA2329. Identification was based on library spectra, published spectra (Anonymous 2017) as well as on spectra obtained from leaflet washes of the *S.h* accession LA2167, the source material used by Anonymous (2017) to identify [2] and [3]. The GC-MS was an Agilent model 6890N equipped an Agilent 5975 mass selective detector and 7673B injector. Oven temperature was programmed from 50 to 250°C at a rate of 20°C/min (1 min initial hold). Injector and detector temperatures were

set at 250 and 300 °C, respectively. A 30 m x 0.25 mm ID DB-5 capillary column (0.25 μm film thickness) was used. Helium was a carrier gas at 1.0 mL/min) AMDIS 32 [US National Institute for Standards and Technology, Gaithersburg, MD, USA] was used to extract and compare mass spectra.

3.3.5 Silica Gel Chromatography

To separate components of the oleoresin, open column chromatography was used. A glass column (20 X 0.8 cm) was filled with SiO_2 (230 –400 mesh, Natland International Corporation, NC, USA) in the usual fashion. 100 mg of oleoresin was loaded onto the column, which was then eluted with *n*-hexane: methyl-tert-butyl ether (MTBE). Ratio of *n*-hexane:MTBE ranged from 97:3 to 75:25. Two ml fractions were collected and elution was monitored by spotting one drop of each fraction onto a thin layer chromatography plate (Silica gel 60 A with fluorescent indicator, Whatman Int Ltd, England) followed by illumination with 254 nm ultraviolet light (UVGL-25, Mineralight Lamp, UVP Inc, CA, USA). Composition, concentration and purity of each uv-positive fraction were determined by GC-FID. For factions chosen for bioassay, solvent was evaporated by a stream of N_2 and the residue was dissolved in *n*-hexane.

3.3.6 Bridge Bioassay

The bridge bioassay was deployed as described by Snyder, Antonious et al. (2011) and Guo, Weston et al. (1993), which is a modest and easy technique for testing TSSM repellency of natural products. The bioassays were conducted on a lab bench. Temperatures averaged about 21C and varied ±2 degrees during days the bioassays were

conducted. To provide clarity for our results, a brief overview of the principal aspects of the bioassay is appropriate. The bioassay arenas (Figure 3-1) consisted of two small rectangles (1 X 0.75 cm each) of Whatman No. 1 filter papers held horizontally in a clamp. One rectangle was treated with a known concentration in *n*-hexane (20 µL/0.75 cm^2 of filter paper). The other filter paper rectangle was treated with *n*-hexane only as a control. After the *n*-hexane evaporated, a very small strip of filter paper (2 X 10 mm) was used as a bridge between the two rectangles of filter paper. Then an adult female spider mite was placed on the center of the bridge and mite movement was visually tracked. The filter paper over which the mite exited from the bridge was recorded and the mite was removed. Subsequently, a new (naive) spider mite was placed on the bridge. Two bridges constructed side-by-side permitted testing one concentration of a solution with 30 mites in less than 30 minutes. For these 30 mites, the sums of the exits over treatment and over control rectangles, was expressed as a ratio - the exit ratio. Methods for calculation of response index and more details on the bioassay are provided in prior publications (Snyder, Thacker et al. 2005, Snyder, Antonious et al. 2011).

The bridge bioassay was used to evaluate oleoresins from the two LA2329 chemotypes as well as to evaluate compounds purified by chromatography. In an experiment one concentration of oleoresin or compound was tested with 30 individual mites, so there were 30 replications for each concentration tested. The experiment to evaluate oleoresins was conducted three times, and the experiment that evaluated purified compounds was conducted twice.

In bridge bioassays quantitation of compounds was based on GC-FID and only included abundance of the major components present in the oleoresin or separated fraction. Concentrations of oleoresins evaluated in the bridge bioassay ranged from 0.018 to 9 μg/cm²; concentrations of fractions obtained from silica gel chromatography were tested in the range of 0.001 to 0.2 μg/cm².

3.3.7 Statistical Analysis

Statistical analyses were performed using the SAS Software package 9.4 (SAS Institute Inc., Cary, NC, USA). Quantities of trichome secretion components were analyzed by analysis of variance (ANOVA) and the means were compared using Duncan's multiple range test. Exit ratios for each concentration of each fraction evaluated in the bridge bioassay were tested for homogeneity between the two bridges in the arena by X^2. In all cases, exit ratios were homogeneous between two bridges. EC_{50} and EC_{90} values were then estimated by use of PROC PROBIT, (SAS Institute 9.4 version), as described by Snyder, Antonious et al. (2011). EC is an abbreviation for Effective Concentration, and an EC_{50} value is the predicted concentration at which 50% of the tested individuals respond to a stimulus; an EC_{90} value is the predicted concentration at which 90% of tested individuals respond.

3.4 Results

3.4.1 Composition and Abundance of Trichome Secretions on two chemotypes of LA2329 and on PI127826 and LA2167

Three major compounds, [1], [2], and [3], were present in *n*-hexane leaf washes LA2329, chemotype A (LA2329-A) (Figure 3-2). In contrast, there was only one major component in washes of LA2329, chemotype B (LA2329-B) (Figure 3.2). The presence of this qualitative difference in trichome secretion composition between the two chemotypes supports the idea that the synthesis of secondary metabolites is very different in the two chemotypes. Furthermore, the GC-FID tracings for trichome secretions from LA2167 and PI127826 demonstrated the presence of three compounds each having a retention time that was identical to one of the three components present in LA2329-A. This indicates that the secretion composition of PI127826 and LA2167 was more similar to that on LA2329-A than to that on LA2329-B. In terms of the quality of trichome secretions, the quality of the secretion from LA2329-A was nearly the same as the trichome secretion qualities on PI127826 and LA2167, and was very different from the quality of the secretion on LA2329-B.

When data for component abundances were analyzed by ANOVA, abundance of [1] ($F=6.15$, $P=0.029$), [2] ($F=61.87$, $P<0.0001$), and [3] ($F=42.25$, $P=0.0002$) varied significantly among plants (Figure 3.3).The abundance of [1] was highest in LA2329-A and PI127826, and lowest in LA2167. Concentration of [1] in LA2329-B was between these high and low concentrations. The abundance of [2] was highest in PI127826 and was not detected LA2329-B. Concentrations of [2] in LA2329-A and LA2167 were significantly less than those observed on PI127826 and greater than that observed on LA2329-B. Abundance of [3] differed significantly among all four plants with concentration highest on LA2167 and not detected in LA2329-B. Based on the presence of significant differences of concentrations of [1], [2], and [3] among these plants

supports the idea that metabolic differences among the four plants could lead to quantitative differences in their trichome secretions.

3.4.2 Repellent Activities of Oleoresins from LA2329 A and B Chemotypes

When oleoresins obtained from the two LA2329 chemotypes, A and B, were evaluated with the bridge bioassay, EC_{50} and EC_{90} values for the oleoresin from the B chemotype was considerably greater than that for the A chemotype (Table 3-1). In this assay, lower EC values indicate greater repellency so the EC value for the oleoresin having three major components from chemotype A (EC_{50}=0.00709 µg/cm²) indicated an approximately six-fold greater repellent activity than that for oleoresin from chemotype B containing a single component (EC_{50}=0.04791 µg/cm²).

3.4.3 Identification of 7-Epi-Zingiberene, 9-Hydroxy-Zingiberene, and 9-Hydroxy, 10,11-Epoxy-Zingiberene in LA2329 Chemotype A

Mass spectra of the three major compounds present in extracts of the A chemotype of LA2329 wild tomato accession showed a molecular ion (M+) at m/z 204 for [1]. For [2] the molecular ion (M+) was at m/z 220. And for [3], the molecular ion (M+) was located at m/z 236. These outcomes were consistent with a sesquiterpene hydrocarbon (C15H24), and sesquiterpenoids having empirical formulas of C15H24O and C15H24O2, respectively (Table 3-2 and Figure 3-4). Identical spectra (99% match) and retention times were obtained for each of the three major compounds present in leaf washes obtained from PI127826 and LA2167. Because of a complete match of retention times and spectra among the three accessions for the three compounds, we concluded that the identities of the three peaks present in LA2329-A were 7-epi-zingiberene [1], 9-hydroxy-zingiberene ([2]) and 9-hydroxy-10,11-epoxy-zingiberene ([3]).

3.4.4 Silica Gel Chromatography of LA2329 Chemotype A Oleoresin

The three compounds were well separated by silica gel chromatography. [1] was present in fractions 3 to 6 at purities, judged by GC-FID, ranging from 90 to 98% and concentrations ranging from 1 to 10 µg/mL. [2] was present in fractions 32 to 37 at purities ranging from 78 to 86% and concentrations ranging from 0.4 to 4.5 µg/mL and [3] was present in fractions 50 to 53 at purities ranging from 83 to 87% and concentrations ranging from 0.3 to 0.9 µg/ Fractions for bioassay were chosen based on the purity of the fraction. This minimized the influence of the presence of non-target chemicals on the bioassay results. Thus, fractions 4 and 5 for [1], fractions 33 and 34 for [2] and fractions 51 and 52 for [3] were chosen evaluation of repellency in the bridge bioassay.

Repellent activity of the isolated [1] as judged by EC_{50} and EC_{90} values obtained with the bridge bioassay was highest of the three compounds evaluated, indicating that [1] was least repellent of the three compounds tested (Table 3-3). EC_{50} and EC_{90} values for [2] and [3] were 5 to 10-fold less than that for [1], indicating that these two-oxygen-containing sesquiterpenoids were considerably more repellent than the hydrocarbon [1]. In other words, compared to [1], much smaller doses of [2] or [3] were required to repel spider mites, compared to [1]. The EC_{50} and EC_{90} estimates from probit analysis of data had narrow fiducial limits and non-significant X^2 values for lack of fit. Thus, the bioassays provided highly precise estimates of effective concentrations for the repellency of the tested compounds. Fiducial limits for the oxygen-containing sesquiterpenoids [2] and [3] did not overlap those for the hydrocarbon [1], supporting the conclusion that [1] was less repellent in the bridge bioassay than the two oxygen-containing

sesquiterpenoids, [2] and [3]. However, for these two sesquiterpenoids, fiducial limits did overlap, indicating that EC values and repellent activities of these two compounds, [2] and [3] were not differentiated by the bridge bioassay. In other words, the EC values and consequently the repellency of [2] and [3] were not separated statistically in this experiment.

3.5 Discussion

Wild tomato accessions such as those of *S.h.* are great potential sources of insect and disease resistance for tomato breeding (Vidavski 2007, Bleeker, Mirabella et al. 2012), partly because many of these accessions contain high levels of allelochemicals in their trichomes. One such allelochemical is 7-epi-zingiberene [1], which was present on both chemotypes of LA2329. The oleoresin from LA2329 chemotype B, which contained a single major component, [1] was repellent to spider mites in the bridge bioassay. However, the oleoresin from LA2329 chemotype A, having three major components, had considerably greater repellency than the oleoresin containing one major component. This observation supports the hypothesis that compounds other than [1] that were present in LA2329 chemotype A, namely compounds [2] and [3] are likely much more repellent to spider mites than [1].

We verified by GC-MS the identities of the major components present in LA2329 chemotype A. [1] was present as were [2] and [3]. Mass spectra and retention times completely matched those obtained for authentic compounds isolated from LA2167 (Anonymous 2017). [2] and [3] were also present in trichome secretions from the widely studied *S.h*. accession PI127826.

[1], [2] and [3] were successfully separated by silica gel chromatography. Quantities and purities of allelochemicals isolated by chromatography were sufficient to permit evaluation of spider mite repellency of each compound in bridge bioassays. The repellent activities of the two oxygenated components, [2] and [3], based on their EC_{50} and EC_{90}

values were 6 to 10 – fold more repellent than [1]. This result confirms our hypothesis that these two oxygenated sesquiterpenoids [2] and [3] are more repellent to spider mites than the sesquiterpene hydrocarbon, [1].

The results presented herein are in agreement with those of Guo, Weston et al. (1993). These authors concluded that the greater repellencies of trichome secretions on certain accessions of *S.h*. are likely due to the presence of compounds that are more polar than sesquiterpene hydrocarbons; [2] and [3] are each more polar than their parent sesquiterpene hydrocarbon, [1] and were more repellent in bridge bioassays than [1]. While we did not evaluate insect antibiotic properties of these molecules, it is possible that these oxygenated compounds may also have contributed to the strong antibiosis to beet army worm (*Spodoptera exigua)* of trichome secretions from *S.h*. LA2329 observed by Eigenbrode, Trumble et al. (1994). Furthermore, because we have reported that [2] and [3] are present in trichome secretions of *S.h*. PI127826, conclusions that the impute causes of resistance in this accession solely to the presence of the sesquiterpene hydrocarbon [1] need reconsideration (Maluf, Campos et al. 2001, de Azevedo, Faria et al. 2003).

In addition to spider mite repellency, oxygenated sesquiterpenoids present in trichome secretions may play a role in mediating interactions of *S.h.* with other arthropods. For example, sesquiterpenoid acids from the *S.h*. accession LA1777 reduce larval feeding and survival of *Heliocoverpa zea* and *Spodoptea exigua* (Frelichowski Jr and Juvik 2001) and surprisingly, stimulate oviposition of *H. zea* (Coates, Denissen et al. 1988). The occurrence of oxygenated sesquiterpenoid derivatives needs to be surveyed in this diverse

wild tomato species, as does their biochemical origins in the plant and their impact of behavior of significant arthropod pests such as the virus vector *Bemesia* spp. all need additional evaluation. Antixenosis, which seems to be present in many accessions of the *S.h.* species, should be emphasized (Guo, Weston et al. 1993, Snyder, Guo et al. 1993, Eigenbrode, Trumble et al. 1996, Snyder, Simmons et al. 1998).

It appears that not only are terpene profiles variable in *S.h,* the presence of oxygenated sesquiterpenoids in trichome secretions may also be variable. The trichome secretions on the *S.h.* accession LA1777 are predominated by the presence of alpha-santalenoic and endo- beta.-bergamotenoic acids (Coates, Denissen et al. 1988) and those on LA1363 are predominated by the presence of 2,3-dihydrofarnesoic acid (Guo, Weston et al. 1993). The biosynthetic origin of these oxygenated forms has not be delineated, but is likely the consequence of action by a cytochrome P450 on a parent sesquiterpene hydrocarbon, similar to the action the of the cytochrome P450 monooxygenase, CYP71AV1 in *Artemisia annua* that performs a three-step oxidation of the sesquiterpene hydrocarbon amorpha-4,11-diene to artemisinic acid (Ro, Paradise et al. 2006). The conversion of [1] into [2] and [2] into [3] in trichomes, as depicted in Figure 3-5, is reportedly also the result of action of a cytochrome P450 named zingiberene polyoxidase (Anonymous 2017). The apparent variation in the ratio of abundance of [1], [2] and [3] among LA2329-A, LA2167 and PI127826 (Figure 3-3) supports the idea that there may be considerable differences in the action of zingiberene polyoxidase among these three accessions.

Our research was aided considerably by the presence of two chemotypes in our population of *S.h*. LA2329. That we uncovered variation in trichome secretion composition within LA2329 is not surprising because it has been noted that there is more genetic variability within a single accession of self-incompatible wild tomato such as LA2329 than there is in all of cultivated tomato (Bai and Lindhout 2007). It is likely that a systematic search of the other wild tomato accessions would uncover chemical diversity in their trichome secretions. Also, it is likely that variation for presence of [2] and [3] in our population of LA2329 was directly connected to variation associated with the zingiberene polyoxidase locus.

Because of the apparent greater repellent activity of the oxygenated sesquiterpenoids, [2] and [3], plant breeders should consider introgression of these compounds into cultivated tomato, with a view toward additional evaluation of the role of these compounds in arthropod resistance of tomato. Hopefully, tomato plants that produce these more repellent compounds would demonstrate greater resistance to spider mites, and perhaps, to other arthropods.

Introgression of zingiberene oxidase may be a relatively straightforward breeding task. Likely a single enzyme, a cytochrome P450, is responsible for producing these two compounds from 7-epi-zingiberene (Anonymous 2017). Thus, introgression may be particularly direct when using a recurrent parent such as one of our advanced breeding lines that produce high concentrations of [1] in their trichomes. Introgression of the ability to produce the alcohol [2] and epoxide [3] may also lead to greater production of

total sesquiterpenoids on tomato, similar to the difference of sesquiterpenoid production between the LA2329-A and LA2329-B chemotypes (Figure 3-3).

The bridge or springboard bioassay has been demonstrated as sufficiently sensitive to detect differences in repellency of molecules having subtle structural differences, such as the presence or absence of a double bond (Snyder, Antonious et al. 2011). The results reported here support the conclusion that the bridge bioassay can be used to demonstrate differences of repellency between a sesquiterpene hydrocarbon, [1], and two oxygenated derivatives of zingiberene, [2] and [3]. However, this bioassay did not demonstrate any difference in repellent activity between the two oxygenated derivatives of [1].

Volatile substances present in plant glandular trichomes can prevent and/or reduce interactions with arthropod vectors of viral diseases (Aharoni, Jongsma et al. 2005), thereby reducing disease incidence. For example, the presence of exogenously applied [1] seems to repel the whitefly (*Bemisia tabaci*) from tomato plants. Because *B. tabaci* is a begomovirus vector (Rosen, Kanakala et al. 2015), Bleeker, Mirabella et al. (2012) suggested that introduction of the biosynthetic pathway for production of [1] would provide protection against virus transmission. Introduction of the ability of the plant to produce [2] and/or [3] could be even more beneficial if these oxygenated forms of zingiberene are more effective than [1] in repelling whiteflies, similar to our findings with TSSM.

Some wild tomato species are known as having very high levels of arthropod resistance (Rick 1984, Simmons and Gurr 2005, Barrantes, López-Casado et al. 2016). While the existence of these genetic resources is valuable, complete delineation of a resistant

phenotype for a highly resistant individual can be difficult. For example, evaluation by bioassay of causes of resistance in a plant having high levels of antixenosis could mask mechanisms of antibiosis. Likewise, presence of multiple active compounds such as in the A chemotype of LA2329, can considerably complicate delineation of causes of resistance. Although there are many wild sources of resistance to arthropods, the lack of released varieties having high levels of resistance is a good indication of difficulty of using these genetic resources.

Results of the research have implications not only for tomato breeders but also for chemical ecologists, and plant evolutionists. This research was prompted by the presence of two chemotypes within our locally maintained population of LA2329 and an observation that one chemotype appeared more resistant to whitefly than the other. According to the Tomato Genetics Resource Center (tgrc.ucdavis.edu) the *S.h*. accession LA2329 is allogamous-self-incompatible. However, our population of LA2329 was established from just a few plants, and because of this, as we have produced subsequent seed generations, we have also likely inbred some of the plants, which could have allowed expression of recessive genes. That we uncovered variation for composition of trichome secretions within an accession of *S.h*. is not surprising, given the genetic variability available in wild tomato accessions (Bai and Lindhout 2007). We believe that this is the first report of qualitative variation of trichome secretion composition within an accession of *S.h.* Furthermore, it is likely that the chemotypic variability observed in our population of LA2329 is associated with the variability in the expression or activity of the P450 oxidase responsible for production of oxygenated sesquiterpenoids [2] and [3] as described by Anonymous (2017). Furthermore, given the extensive genetic variability

that exists within wild tomato accessions, it is likely that components of trichome secretions may be variable within other accessions of *S.h*. The potential for variability of sesquiterpenoids in trichome sections of *S.h*. should attract research interest by chemical ecologists, plant evolutionists as well at those interested practical aspects of plant-arthropod interactions such as entomologists and plant breeders.

In conclusion, the preparative separation and purification of [1], [2] and [3] were successfully accomplished. After identification and purification, the three major allelochemical components were tested for TSSM repellency in the bridge bioassay. All allelochemical components repelled spider mites. Yet, the activities of [2] and [3] were both much higher than that for [1]. Future research should center on introgression of these sesquiterpenoids into cultivated tomato and evaluation of their antibiotic and antixenotic activities on arthropods other than TSSM. Future studies should also utilize these three allelochemical components to minimize pesticide use and guarantee long-term pest management.

3.6 Author Contributions

MHD conducted the experiments, performed the laboratory experiment by separating and purifying the allelochemical compounds, performed the bioassay tests and wrote the manuscript; and JCS analyzed the results, managed the data, contributed the experimental design and wrote the manuscript. Before submission, both authors read and approved the final manuscript.

3.7 Conflict of Interest Statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

3.8 Abbreviations

S.h, *Solanum habrochaites*; [1], 7-epi-zingiberene; [2], 9-hydroxy-zingiberene; [3], 9-hydroxy-10,11-epoxy-zingiberene; MTBE, methyl-tert-butyl ether; TSSM, two-spotted spider mite; GC-FID, Gas Chromatography - Flame Ionization Detector; GC-MS, Gas Chromatography Mass Spectroscopy.

3.9 Acknowledgements

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3.10 Data availability

The raw data supporting the conclusions of this manuscript will be made available by the authors, without undue reservation, to any qualified researcher

Table 3-1 EC50 and EC90 values (µg/cm² of filter paper) and their 95% fiducial limits, standard deviations and P-values of EC50 obtained from evaluation in the bridge bioassay of oleoresins collected from two chemotypes of *S habrochaites* LA2329. The A chemotype contained three major components, [1], [2] and [3], in its trichome secretions. The B chemotype had a single major component, [1].

Chemotype	EC_{50}	95% Fiducial Limits		EC_{90}	95% Fiducial Limits		Standard Deviation	P-Value
	(µg/cm²)	Lower	Upper	(µg/cm²)	Lower	Upper		
A (Three components)	0.00709	0.00513	0.00994	0.02561	0.02006	0.03567	0.0013	<.0001
B (One component)	0.04791	0.04975	0.05988	0.09716	0.08616	0.13398	0.0103	<.0001

Table 3-2 GC-FID and GC-MS retention times, molecular ion (M+) and characteristic mass fragments of the three allelochemical components extracted from wild tomato accessions Solanum habrochaites (LA2329, PI127826, and LA2167). Fragments are listed by m/z followed by relative abundance in parentheses.

Compound	Retention time (RT)		Molecular ions (M+), Fragments and (Relative Abundance)
	GC-FID	GC-MS	
[1] 7-epi-zingiberene	6.00	8.44	204 (M+), 93 (99), 91 (52), 77 (37), 69 (36), 41(32), 92 (21), 105 (19), 120 (15), 56 (13), 55 (13)
[2] 9-hydroxy-zingiberene	6.91	9.45	220 (M+), 105 (100), 95 (97), 93 (95), 91 (84), 132 (82), 119 (80), 77 (64), 120 (52), 85 (51) 41 (36)
[3] 9-hydroxy-10,11-epoxy-zingiberene	7.22	9.88	236 (M+), 119 (100), 93 (75), 91 (63), 105 (51), 77 (43), 85 (42), 120 (40), 92 (24), 43 (21), 55 (19)

Table 3-3 EC50 and EC90 values (µg/cm² of filter paper) and their 95% fiducial limits, standard deviations and P-values of three allelochemicals ([1], [2], and [3]) isolated by silica gel chromatography and tested in the bridge bioassay.

Allelochemical	EC_{50}	95% Fiducial Limits		EC_{90}	95% Fiducial Limits		Standard Deviation		P-Value EC_{50} and EC_{90}
	(µg/cm²)	Lower	Upper	(µg/cm²)	Lower	Upper	EC_{50}	EC_{90}	
[1] 7-epi-zingiberene	0.01655	0.01392	0.0198	0.0481	0.03785	0.06612	0.00030	0.00050	<.0001
[2] 9-hydroxy-zingiberene	0.00198	0.00165	0.00235	0.0073	0.0057	0.00103	0.00032	0.00014	<.0001
[3] 9-hydroxy,10,11-epoxy-zingiberene	0.00182	0.00167	0.00216	0.00448	0.00363	0.00602	0.00018	0.00001	<.0001

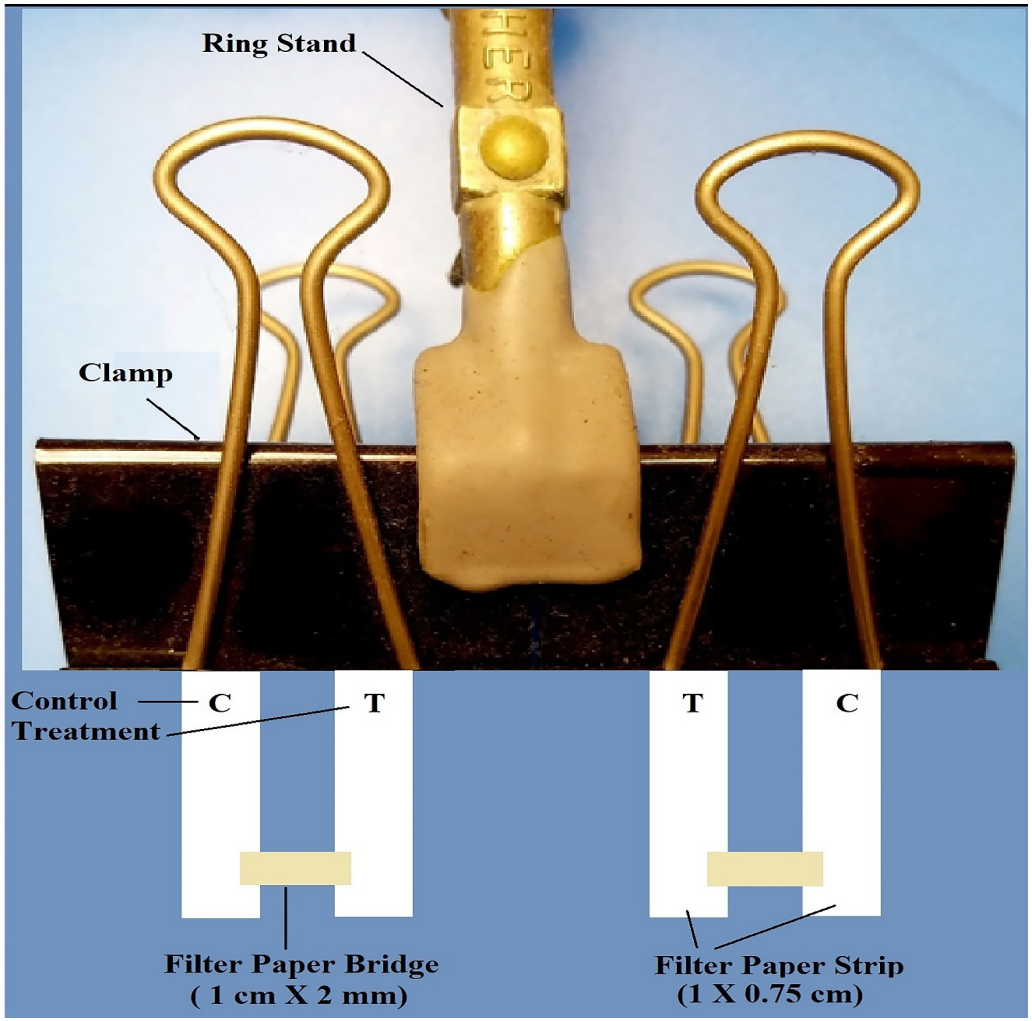

Figure 3-1 Bridge bioassay arenas used for repellency bioassays. Each consisted of two small rectangles (0.75 cm x 1 cm) of filter paper that were bridged by a very small strip of filter paper (1 cm x 2 mm). Filter paper rectangles labeled T were treated with a known concentration of test compound(s), while those labeled C were loaded with solvent (*n*-hexane) only.

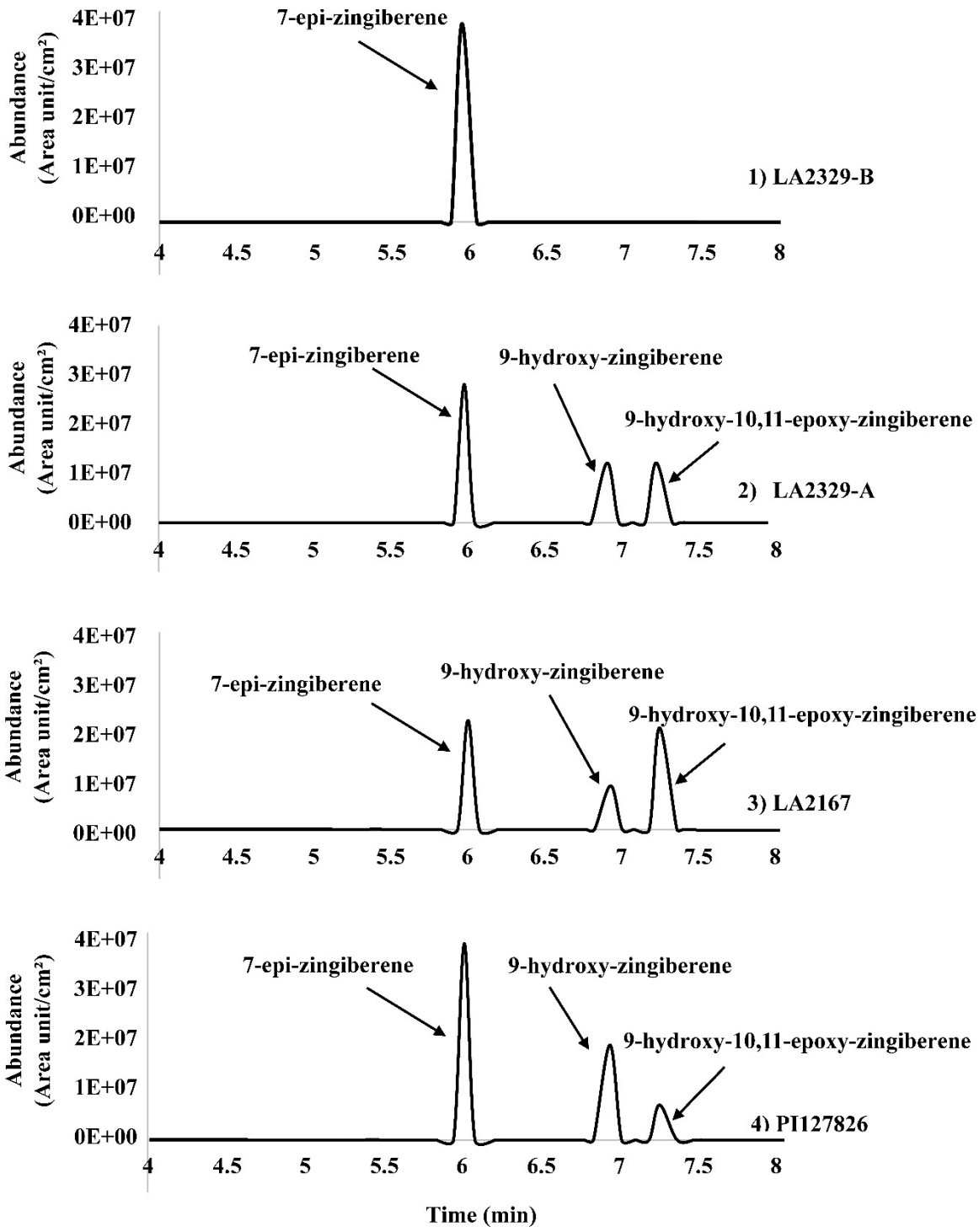

Figure 3-2 Chromatogram (GC-FID) of *n*-hexane leaflet wash from: (1) the LA2329-B chemotype showing a single major component at retention time of 6.00 min; (2) the LA2329-A chemotype showing three major components at retention times of 6.00, 6.91 and 7.23 min; (3) LA2167 showing three major components at retention times of 6.00, 6.91 and 7.23 min; (4) PI127826 showing three major components at retention times of 6.00, 6.91 and 7.23 min.

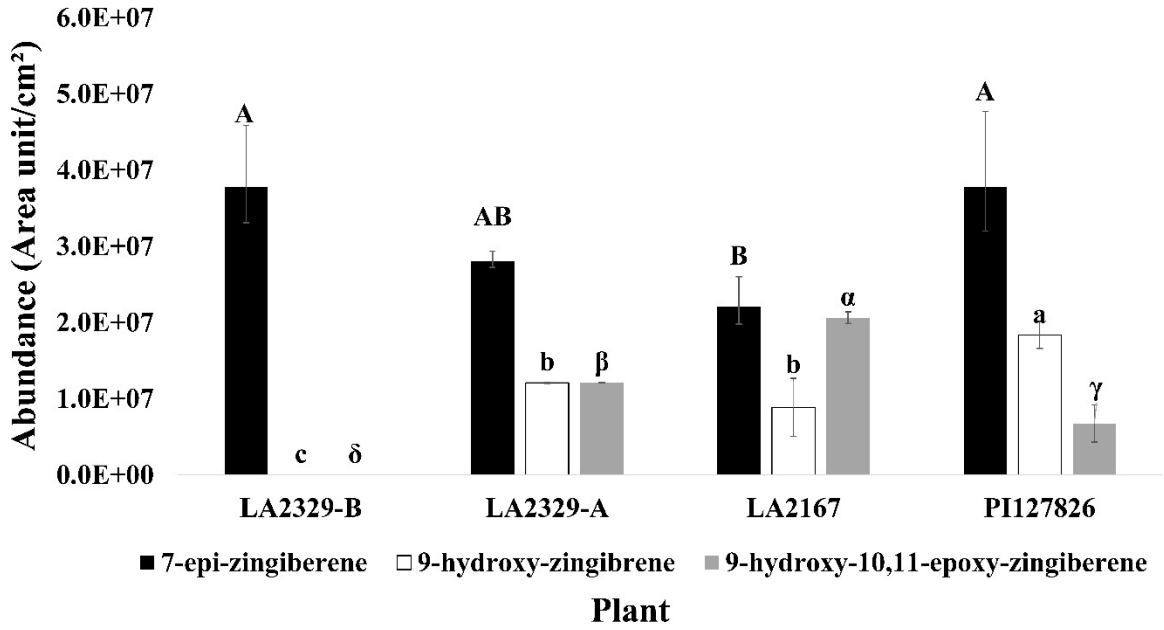

Figure 3-3 Abundance of 7-epi-zingiberene, 9-hydroxy-zingiberene, and 9-hydroxy,10-11-epoxy-zingiberene in four plants, two chemotypes of LA2329 (LA2329-A and LA2329-B), LA2167 and PI127826. Abundance of a compound labeled by the same letter(s) are not significantly different ($P < 0.05$).

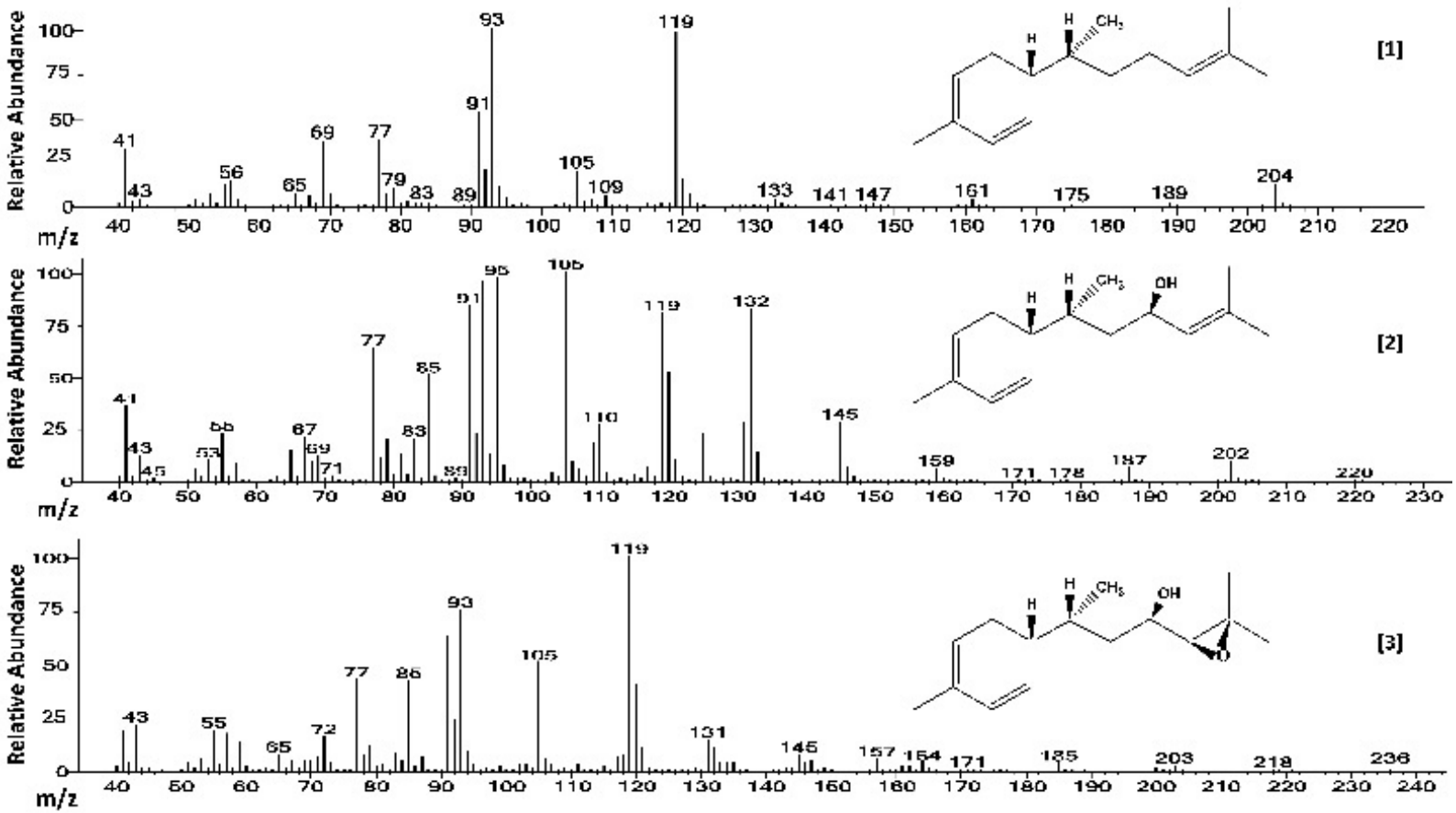

Figure 3-4 Electron ionization mass spectra of [1] 7-epi-zingiberene (C15H24), [2] 9-hydroxy-zingiberene (C15H24OH), and [3] of 9-hydroxy-10-11-epoxy-zingiberene (C15H24O2), present in trichome secretions of *S. habrochaites* accessions LA2329-A, LA2167 and PI127826. The names of three major components were obtained by comparing our results from GC/MS with those results published by Anonymous (2017) which was based on the analysis of trichome secretion components present on LA2167.

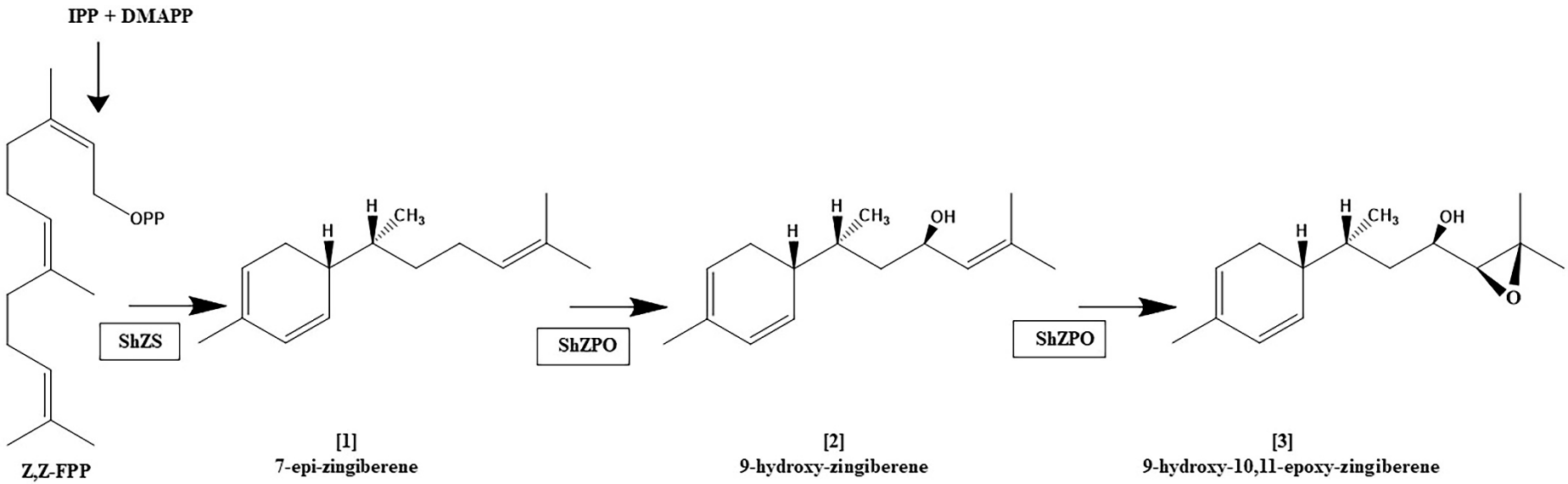

Figure 3-5 Proposed biosynthetic pathway for the zingiberene derivatives present in trichome secretion of S. *habrochaites* accession LA2167. zFPS: Z,Z-FPP synthase, ShZS: zingiberene synthase (also referred to herein as ShZIS), ShZPO, zingiberene polyoxidase. This figure is based on information in Anonymous (2017).

CHAPTER 4. YIELD, PHENOLIC, ASCORBIC ACID, SOLUBLE SOLIDS AND LYCOPENE OF FRUIT AND FOLIAR ZINGIBERENE CONTENT OF INTERSPECIFIC HYBRID TOMATOES

4.1 Abstract

Consumption of tomato (*Solanum lycopersicum)* products has been associated with improved human health. Tomato growers and breeders are looking for approaches to boost yields and the content of antioxidants and other nutrients. The purpose of this research was to evaluate yield, phenolic content, ascorbic lycopene and soluble solids of thirteen interspecific hybrid tomato lines (*S. lycopersicum* × *S. habrochaites*) of two families, D90 and F22, compared with two commercial F_1 hybrid lines ('Red Deuce' and 'BHN 589') grown in the open field. The interspecific hybrids had selected based on zingiberene content (µg/cm² of leaf) which is important in plant defense against an array of arthropods due to its repellence and toxicity. This study revealed that the yield of the F22 interspecific lines was 6.7 kg/plant, 7.5 kg/plant for the interspecific D90 lines and 11.4 kg/plant for commercial tomato F_1 hybrids. In interspecific hybrid families, average fruit weight was 110 g while in F_1 hybrids it was 290 g. The number of fruits per plant in the D90 family was the highest, 75 fruit/plant, whereas in the F22 family there was 58 fruit/plant compared to 40 fruit/plant for commercial hybrids. The content of foliage zingiberene in the F22 interspecific hybrid family was 45 µg/cm² and was 26 µg/cm² in the D90 family whereas there was no zingiberene in the F_1 cultivated tomatoes. The phenolic content in the interspecific hybrid families ranged from 325 to 427 µg/g fresh fruit whereas this compound was lower in F_1 hybrids. Ascorbic acid typically ranged between 483 and 498 µg/g fresh fruit in interspecific hybrids and was higher than that in F_1 hybrids

(337 µg /g). Lycopene content ranged from 31 to 40 µg/g and soluble solids ranged from 4 to 6.6%, whereas those compounds were lower in F_1 hybrids. These initial results show that it may be possible to breed tomatoes with high phenolic, ascorbic acid and zingiberene contents and maintain lycopene and yield at a reasonable level which could lead to improved human diets and host-plant resistance to arthropods.

Keywords: tomato, interspecific hybrids, zingiberene, yield, open field, phenolics

4.2 Introduction

Consumption of tomato (*Solanum lycopersicum)* products has been associated with improved human health (Viuda-Martos, Sanchez-Zapata et al. 2014, Perveen, Suleria et al. 2015). Tomato growers and breeders are looking for approaches to boost yields and the content of antioxidants and other nutrients.

Worldwide, tomato is the second most significant vegetable crop, next to potato. World production today amounts to around 200 million tons on 12 million acres (FAOSTAT 2017). In modern breeding programs genetic variation available in wild tomato relatives has often been the source of characteristics used to breed for enhanced yield, fruit quality, disease and insect resistance (Rick and Chetelat 1995). Yield is a genetically complex character and genetic selection for yield requires tremendous attention by the breeder. An increase in yield and quality of self-pollinated crops such as tomato is usually accomplished by choosing those genotypes that have the desired combination of phenotypic characters.

The sesquiterpene hydrocarbon, 7-epi-zingiberene, is a semi-volatile compound naturally synthesized by plants of *Solanum habrochaites*, a wild relative of cultivated tomato.

7-epi-zingiberene is one of the main anti-insect chemicals present in its leaf trichomes (Snyder, Guo et al. 1993, Antonious and Kochhar 2003, Antonious and Snyder 2006). Tomato breeders around the world are attempting to introgress high levels of 7-epi-zingiberene from wild tomatoes into cultivated types. Their intent in doing so is to improve insect resistance of tomato because 7-epi-zingiberene has been associated with resistance to arthropods such as spider mites, aphids, and whiteflies (Weston and Snyder 1990, Aragão, Dantas et al. 2000, Maluf, Campos et al. 2001, Freitas, Maluf et al. 2002, Gonçalves, Maluf et al. 2006, Bleeker, Diergaarde et al. 2011).

Because 7-epi-zingiberene is an oil, the tomato plant expends a great deal of energy to synthesize it, and because of this, there may be a negative association between fruit yield and production of 7-epi-zingiberene (de Azevedo, Faria et al. 2003). Also, yield in interspecific hybrids may be reduced due to genic incompatibilities, often referred to as Bateson-Dobzhansky-Muller interactions (Cutter 2012). This research is the first report of yields for interspecific hybrid tomatoes having high concentrations of 7-epi-zingiberene.

Tomato is a source of nutrients essential for human health (Borguini and Ferraz da Silva Torres 2009)\, because it is a great resource of ascorbic acid (vitamin C), carotenoids, lycopene and phenolics. For people with poor nutritional diets, tomatoes may be an important source of pro-vitamin A, carotenoids and vitamin C (Abushita, Hebshi et al. 1997, Agarwal and Rao 2000, Kaur, Savage et al. 2002). Carotenoids such as β-carotene and lycopene in the tomatoes may prevent oxidation of lipoproteins and vascular cells (Böhm 2018). Some epidemiological studies have shown that carotenoids and food rich in antioxidants can reduce cardiovascular risk (Mayne 1996). Consumption of tomato fruits is more associated with

cardiovascular protection (Böhm 2018). It is therefore wise to identify and consume food such as tomatoes as a source of cardiovascular protection, providing a combination of these antioxidants.

Total soluble-solids content (TSS) is one of the components of yield which shows an inverse relationship with fresh tomato yield (Pascale, Maggio et al. 2001). For example, water stress in the field results in substantial increases in TSS accompanied by a reduction in fruit yield. This is largely attributable to the limited physiological capacity of the plants to provide the raw materials needed for high fresh yield and TSS (Stevens 1978). Therefore, in breeding programs, it is necessary to take into account both the fresh yield and TSS. The parameter which is often used to characterize economic yield is the product of the two yield components which represents the soluble solids yield per plant or per unit area. An increase in TSS yield is of importance for fresh market tomatoes where taste is associated with TSS content. In processing-tomatoes, high yields and TSS values enable an efficient manufacturing of concentrates (Aoun, Lechiheb et al. 2013)

The purpose of this research was to obtain information on yield, phenolic content, ,ascorbic acid, lycopene and soluble solids and zingiberene content of interspecific hybrid tomato (thirteen BC3F7 lines) and two commercial F_1 hybrid lines '('Red Deuce' and 'BHN 589') grown in the open field. Also, to I wanted to learn how to adapt published methods used to measure nutrients for use in the lab and in a tomato breeding program.

4.3 Material and Methods

The experiment took place in 2019 at the Horticulture Research Farm, Lexington, KY (Figure 4-1). Each experimental plot consisted of four tomato plants spaced two feet apart within the row, and rows were set on seven-foot centers in raised beds with trickle irrigation and black plastic mulch. The statistical design was a randomized complete block design that included 13 interspecific hybrid breeding lines and two F_1 hybrid tomato cultivars in each of three blocks. The cultivars evaluated were 'BHN 589' and '''Red Deuce''. All 13 breeding lines were BC3F7 generation lines obtained from crossing between a wild tomato relative, S. *habrochaites* (LA2329) and 'Zaofen 2', a pink-fruited determinate variety released in 1962. The BC3F7 lines had been selected for high yield and for high zingiberene production and eight were chosen from the D90 family (lines SF89, SF91, SG23, SG73, SG83, SG87, SH68 and SH70) and five from the F22 family (lines SF37, SH13, SH17, SH18, and SH19). On 9 April, seeds were soaked in 50% sodium hypochlorite for 30 minutes and were then were directly sown into 72-cell flats containing Fort Light compost-based potting soil (Vermont Compost Co., Montpelier, VT). Transplanting occurred on 10 May. Transplant and field production cultural methods were followed in accordance with ID-36 (http://www2.ca.uky.edu/agcomm/pubs/id/id36/id36.pdf). Harvest began on 21 July 2019 and plants were harvested weekly for four weeks. Harvested tomatoes were weighed and counted.

4.3.1 Determination of 7-epi-zingiberene in Leaves of Plants

On 10 July 2019, the center 1/3 portion of one leaflet from the third or fourth leaf positions on each of the 4 plants in each replication in a plot was placed into a 20 ml vial and

then 2 mL of *n*-hexane containing 20 µL/L of *n*-tetradecane as internal standard was added. Vials were vortexed for 30 seconds. Subsequently the 7-epi-zingiberene content of the extract was determined by GC-FID (following the procedure outlined in chapter three.) Results were expressed as µg of 7-epi-zingiberene/cm² of leaflet. The area of extracted leaf tissue was determined by using the open-source image analysis software ImageJ/Fiji (ImageJ 1.47v, National Institutes of Health, USA).

4.3.2 Determination of Total Phenolics, Total Soluble Solids, Ascorbic Acid, and Lycopene

4.3.2.1 Sample preparation

For determination of phenolics, soluble solids and ascorbic acid, five fruit were randomly selected from each plot harvested on 27 July, 4 and 12 Aug, the last three harvests. Thus, there were 135 total samples (13 interspecific lines + 2 F_1 hybrid lines = 15 entries; 15 entries X 3 blocks X 3 replications = 90 samples). Fruits were weighed and gently washed with distilled water. Tomato fruits were chopped and then blended for one minute in an Oster® Blender. After that 50 g of this homogenate was filtered through Whatman No. 4 filter paper using a Buchner funnel and vacuum. The filtrate was collected and used for determination of phenolics, ascorbic acid and soluble solids. Samples for lycopene analysis were prepared in a similar fashion as those for analysis of phenolics, etc. except that these samples were obtained by choosing an additional five fruit from each plot harvested on 4 and 12 Aug harvests and five more fruit obtained from each plot on 22 Aug. Fruit were weighed, washed and homogenized as outlined above, but were not filtered. The homogenate was used for determination of lycopene. Zaofen-2 an open pollinated tomato cultivar, the recurrent

parent was inadvertently not grown as part of the trial. It was grown adjacent to the trial but was transplanted approximately one month (12 June) after the trial was planted. Fruit for analysis of ascorbic acid, lycopene and phenolic were harvested for analysis on 22 Aug .

4.3.2.2 Phenolic determination

Total phenolic content was estimated using the Folin–Ciocalteau Reagent (Sigma-Aldrich, St. Louis MO, USA) method as described by McGrath, Kaluza et al. (1982).phenolic of sample (filtrate), 0.5 ml ethanol (80%): (80 ethanol: water 20), 0.3 ml Folin–Ciocalteau reagent (FCR) and 5 ml ethanolamine (1%) were added in a glass tube to obtain 6.3 mL. After waiting for 20 minutes, all prepared samples were read spectrophotometrically at 750 nm. Results were expressed (µg/g) of fruit filtrate as chlorogenic acid (Sigma-Aldrich®, St. Louis MO, USA).

4.3.2.3 Ascorbic acid determination

Ascorbic acid was quantified using the potassium ferricyanide method as described by Hashmi (1973) and his method relies on reaction of ascorbic acid with potassium ferricyanide at pH 3.5. The ferricyanide ion is produced by the reaction and decomposed by the catalytic action of mercuric ions to produce ions which react with 1,10 phenanthroline to produce a red complex having an absorption maximum at 510 nm. This color reaction obeys Beer's Law between 0.5 and 5.5 µg/mL of ascorbic acid and is suitable for its determination. To prepare 0.4 M acetate buffer of 3.5 pH, 12.8 mL of 2M sodium acetate was mixed with 187.2 mL of 2N acetic acid to obtain 1 liter with deionized water. To prepare potassium ferricyanide solution, 26.3 mg of the reagent was dissolved to obtain 100 mL with deionized water. To prepare phenanthroline solution, 1.0812 g of the reagent was dissolved in the 0.4M acetate

buffer to obtain 500 mL. To prepare mercuric chloride solution, 1.354 grams of mercuric chloride was dissolved in 100 mL deionized water and from this solution, 1 mL was diluted with deionized water to prepare 100 mL of diluted solution. All chemicals that were used for ascorbic acid analysis (except L-ascorbic acid) were obtained from Fisher Scientific Company, Pittsburgh, Pa., USA. To prepare a standard ascorbic acid solution, 10 mg of ascorbic acid was dissolved in deionized water to obtain 100 mL. Then, 10 mL of this solution was diluted with deionized water to obtain 100 mL. Then the diluted solution contained 1 mg/100 mL of ascorbic acid. To determine ascorbic acid content of each sample of fruit filtrate, 1 ml potassium ferricyanide, 1 ml phenanthroline, 1 ml acetate buffer, 0.5 ml fruit filtrate 0.5 ml deionized H_2O and 1 ml mercuric chloride were added in a test tube to obtain 5 mL. Prepared samples were heated in a water bath at 100C for 15 minutes and then cooled and measured at 510 nm in spectrophotometer. Ascorbic acid content was determined as µg/g fruit filtrate using a standard curve of L-ascorbic acid (Sigma-Aldrich Ltd, St Louis, MO, USA).

4.3.2.4 Lycopene determination

Lycopene was measured using the method as described by Rao, Waseem et al. (1998). Lycopene from tomato homogenate was extracted with *n*-hexane: methanol: acetone (2:1:1), including 2.5% butylated hydroxy toluene (BHT), (Sigma-Aldrich Ltd, St Louis, MO, USA) to eliminate oxidation. 0.5 gram of homogenate was weighed and placed into a scintillation vial (20 mL) containing 10 mL of *n*-hexane: methanol: acetone (2:1:1). The vials were shaken for 30 seconds using a vortex mixer and then were allowed to stand for 5 minutes until all fruit tissues were converted into a white layer below the orange-colored upper phase. Then, 2 ml of the upper phase was taken to the spectrophotometer. The optical density of the upper

phase was assessed spectrophotometrically at 502 nm against an *n*-hexane: methanol: acetone blank. Lycopene was calculated using the extinction coefficient (E1%) of 3150.6 (Rao, Waseem et al. 1998). Results are reported in parts per million (ppm) and converted to µg/g of fresh weight.

4.3.2.5 Total soluble solids determination

Total soluble solids (TSS) was measured using a digital refractometer (VEE GEE MDX-101, Wilmington, NC) with accuracy of ±0.2° Brix at room temperature (25℃), by placing 1 drop of the filtrated of each prepared sample on the prism. Before reuse, the prism was rinsed with deionized water and dried by blotting paper. Before taking any sample, each juice vial was shaken for 5 seconds. The results were expressed as a percentage.

4.4 Statistical Analysis

All data were analyzed using the GLM procedure of SAS version 9.4 statistical software (version 9.4; SAS Institute, Cary, NC). Means were compared using the Duncan's multiple range test at 5% level of probability or LSMeans. In order to evaluate the relationship between yield and 7-epi-zingiberene content in the interspecific hybrids, the Pearson correlation coefficient was determined for these two variables.

4.5 Results and Discussion

Analysis of variance (Table 4.1) revealed significant differences among lines for yield ($P<0.05$). Total fruit weight on average, was 11.39 kg per plant for the cultivars compared with 6.88 kg per plant for the interspecific hybrid lines, 6.7 kg for F22 and 7.5 for D90

families (Table 4-2). Fruit from the interspecific hybrid lines was much smaller, 111 grams/fruit, compared to the very large fruit produced on the hybrid cultivars, 310 grams/fruit (Tables 4-1 and 4-3). Fruit number per plant was significantly higher in interspecific lines compared to the 'Red Deuce' and 'BHN 589' ($P<0.05$). For the interspecific hybrid lines within the D90 and F22 families, the highest number of fruits was for line SF89, which produced 80 fruit/plant, while the lowest number was for SF37 which produced 46 fruit/plant. The cultivar 'Red Deuce' produced 34 fruit/plant and 'BHN 589' produced 45 fruit/plant (Tables 4-1 and 4-4).

Certain interspecific hybrid lines in the D90 and F22 families such as SH13, SH17 and SH18 produced very high levels of 7-epi-zingiberene (Tables 4-1 and 4-5) However, only a few significant difference among lines were observed (PP>0.05), (Table 4-1). The F22 family had a higher level 7-epi-zingiberene content than that in D90 family whereas there was no 7-epi-zingiberene detected in the tomato cultivars (Tables 4-1 and 4-5). I also investigated the relationship between 7-epi-zingiberene content and yield. There was a significant negative association, $r = -0.75$ and PP=0.001 between average plant yield and 7-epi-zingiberene content when the F_1 hybrid lines were included in the correlation analysis, indicating that as 7-epi-zingiberene content increased, yield tended to decline. However, when the F_1 hybrid lines were eliminated from this analysis, the degree of correlation was considerably reduced, $r= -0.28$, $P=0.17$. These results indicated that production of high levels of 7-epi-zingiberene may be mildly associated with a yield penalty.

On average, phenolic content in interspecific BC3F7 lines was higher than that in the F_1 hybrids ('BHN 589' and 'Red Deuce') (Table 4-6)), but, there was no difference in

phenolic content between recurrent parent Zoafen-2 and its progenies, the individuals in the D90 and F22 families. High phenolic content was recorded (400 µg/g of fresh weight) in interspecific hybrid lines such as SF37 and SG87 (Table-4-66). These results agreed with our previous work on phenolics in interspecific hybrid families (Dawood, Snyder et al. 2018) and also agreed with (Kavitha, Shivashankara et al. 2014).These results are close to those reported by Kavitha, Shivashankara et al. (2014) who studied phenolics in wild tomato species, interspecific hybrids and cultivated tomatoes. These authors found that phenolic content was much greater in wild species and interspecific hybrids than that in cultivated tomatoes. However, because the phenolic content of our interspecific hybrids was similar to that of our recurrent parent this study provides little evidence that improved phenolic content of the interspecific hybrids traced to the wild parent. Thus, without additional information, the interspecific BC3F7 lines may be no better than the recurrent parent as sources of improved phenolic content.

Ascorbic acid was somewhat higher in BC3F7 lines compared to 'Red Deuce' F_1 hybrid and the recurrent parent 'Zaofen 2' (Table 4.7). In some interspecific lines such as the SG89 line ascorbic acid content reached a high level (560 µg/g). Also, there was a significant difference between the two F_1 hybrid cultivars. The recurrent parent ('Zaofen 2') had the lowest ascorbic acid content 256 µg/cm². There are several possibilities why some interspecific hybrid lines had ascorbic acid levels greater than that in the F_1 hybrids and recurrent parent, 'Zaofen 2'. One explanation could be that these interspecific hybrid families had less ascorbate oxidase activity that decreased the ascorbic acid levels in fruits during repining (Yahia, Contreras-Padilla et al. 2001).

The tomato cultivar, ‘Zaofen 2’, had very low levels of ascorbic acid (Table 4.7) One possibility is that this open pollinated variety does not have that enables it to produce high levels of ascorbic acid. Another alternative is that because this line was not grown as part of the yield trial, the results may not be directly comparable. That said, lines from interspecific hybridizations may be an excellent source to obtain plants having high levels of ascorbic acid. In the future it would be good to repeat this study and also validate why interspecific hybrids have more ascorbic acid content than the ‘Red Deuce’ F_1 hybrid and the recurrent parent ‘Zaofen 2’. Ascorbic acid contents in this experiment was comparable or superior to those reported by Di Matteo, Sacco et al. (2010) and Ntinas, Kadoglidou et al. (2019).

Lycopene content varied among interspecific BC3F7 lines and the recurrent parent, ‘Zaofen 2’ (Tables 4.1 and 4.8). The highest content was in SF91, SH68 and SG83, all from the D90 family. There was a significant difference of lycopene level found among a few of the interspecific BC3F7 lines and the recurrent parent ‘Zaofen 2’. F_1 hybrids (‘Red Deuce’ and ‘BHN 589’) had the lowest lycopene levels Table 4-88). Because the interspecific BC3F7 populations were obtained by crossing between a wild tomato relative and an open pollinated tomato variety, ‘Zaofen 2’ released in 1962, there may be certain alleles present in the wild species that increased lycopene (Tanksley, Grandillo et al. 1996, Bernacchi, Beck-Bunn et al. 1998) which have been transferred into our breeding population. The results suggest that the best sources of lycopene were found in the interspecific BC3F7 lines. These lines, in particular SF91, SH68 and SG83 are potential parents that could be used in a breeding program designed to improve lycopene content. These results are somewhat lower than those reported by Kavitha, Shivashankara et al. (2014) who found lycopene content of cultivated varieties ranged from 58 to 129 µg/g fresh weight. My results for lycopene concentration were

in the same range as those reported by Saad, Ibrahim et al. (2016). Consequently, there appears to be variability of lycopene concentration in the interspecific hybrids evaluated herein, but its actual concentration may inaccurate. Additional work is needed especially with regard to accuracy of methods used for lycopene determination. Additional work is needed especially with regard to accuracy of methods used for lycopene determination.

Tables 4-1 and 4-10 demonstrate that the highest level of soluble solids was 6.6% in interspecific line SH13 (family F22). Both F_1 hybrids slower soluble solids, ranging between 5.3-5.6 % However, there was no difference between soluble solids of the interspecific hybrid and that of 'Zaofen 2' indicating that soluble solids were not significantly better in the interspecific hybrids, compared to the recurrent parent. Thus, based on this information it is unlikely that any of the interspecific hybrid lines have a breeding value better than the recurrent parent for improvement of soluble solids.

This experiment provides a snapshot of field performance of selected inbred BC3F7 lines. Several lines were successfully identified as having high yield similar to their recurrent parent and 7-epi-zingiberene production similar that in the wild donor parent. Higher phenolics, ascorbic acid, and TSS were found in some interspecific hybrid lines than that in the F_1 hybrids and 'Zaofen 2'. These initial results indicate that it may be possible to breed tomatoes with an improved phenolic, ascorbic acid, lycopene content, and TSS with yield similar to the recurrent parent and foliar 7-epi-zingiberene content similar to the wild donor parent. This may also lead to improvement in yield production, nutrition value and plant pest resistance. The wide variation for yield, 7-epi-zingiberene, phenols, ascorbic acid, lycopene and TSS detected in the interspecific BC3F7 lines (D90 and F22) from a wide genetic

background provides an opportunity for using some of these lines as parents in future breeding programs. This research also was a huge step towards further study to verify and improve these parameters.

4.6 Conclusion

The current study revealed that total yield per plant was higher in cultivars than that in interspecific BC3F7 lines. Fruit number per plant in interspecific BC3F7 lines was higher than that in F_1 hybrids and recurrent parent. Yield per plant was not significantly correlated with 7-epi-zingiberene content. However, because zingiberene is an oil that contains a lot of energy, the relationship between yield and its production needs to be continually monitored. Despite the breeding and selection challenges that can occur in interspecific BC3F7 development, it is possible to successfully transfer traits from wild species into cultivated types with yield similar to their recurrent parent and 7-epi-zingiberene production similar that in the wild donor parent. In some interspecific BC3F7 lines had a higher level of ascorbic acid, lycopene and TSS than that in F_1-bybrids and ’Zaofen’. These initial results show that it may be possible to breed tomatoes using some of the methods outlined here as selection tools. However, additional validation of these laboratory procedures is required.

Figure 4-1 Interspecific hybrids lines (BC3F7), Solanum habrochaites , F_1 hybrids (‘BHN588’ and ‘Red Deuce’) and cultivated tomato (’Zaofen’) grown in raised black plastic mulch at Field Horticulture Research Farm, Fayette County, Kentucky, USA, Lexington, KY, 2019.

Table 4-1 ANOVA table for-epi-zingiberene area µg/cm² of foliage, average yield (kg/plant), average fruit weight (g/fruit), average fruit number/plant, phenolics (µg/g), ascorbic acid (µg/g), lycopene(µg/g), and soluble solids (%) for the 13 BC3F7 lines selected for high zingiberene, recurrent parent (Zaofen 2) and for two cultivated tomato F_1 hybrid families.

Variable	Dependent Variable	Sum of Squares	df	Mean Square	F-test	P-value
Family	7-epi-zingiberene concentration (µg/cm²)	5316	2	2658	39	<.0000***
Line		8171	14	584	0.23	0.994^{ns}
Family	Average yield (kg/plant)	97.31	2	49	55	<.0000***
Line		1121	14	8	11	<.0000***
Family	Average fruit weight (g/fruit)	0.81	2	0.40	321	<.0000***
Line		0.84	14	0.060	116	<.0000***
Family	Average fruit number/plant	4557	2	2279	23.5	<.0000***
Line		6220	14	444	5.5	<.0000***
Family	Phenolics (µg/g)	64382	3	21461	4.915	0.003**
Line		104882	15	6992	1.568	0.092^{ns}
Family	Ascorbic acid (µg/g)	541211	3	44242	8.1	<.0000***
Line		104882	15	44242	1.89	0.03**
Family	Lycopene (µg/g)	1024	3	341.2	4.28	0.006***
Line		2428	15	162	2.12	0.013**
Family	Soluble solids (%)	8.56	3	2.85	5.20	0.002***
Line		15.87	15	1.058	2.12	0.024**

, * statistical significance at $p \leq 0.05$ and 0.01, respectively. ns implies statistically no significant.

Table 4-2 Average fruit yield (kg/plant) for 13 interspecific hybrid BC3F7 lines selected for high zingiberene and for two cultivated tomato F_1 hybrids.

Line	Family	Mean	Grouping			
'BHN 589'	F_1 Hybrid	12.1	A			
'Red Deuce'	F_1 Hybrid	10.6		B		
SF91	D90	8.4			C	
SF89	D90	8.3			C	
SG73	D90	7.7			C	D
SG23	D90	7.4			C	D E
SH70	D90	7.3			C	D E
SH17	F22	7.1			C	D E
SG83	D90	7.0			C	D E
SH18	F22	7.0			C	D E
SH68	D90	6.9				D E
SH19	F22	6.9				D E
SH13	F22	6.8				D E
SG87	D90	6.6				D E
SF37	F22	6.0				E

Means with the same letter are not significantly different for P>0.05(Duncan's test).

Table 4-3 Average fruit weight (g/fruit) for 13 interspecific hybrid BC3F7 lines selected for high zingiberene and for two cultivated tomato F_1 hybrids.

Line	Family	Mean	Grouping				
'Red Deuce'	F_1 Hybrid	318.3	A				
'BHN 589'	F_1 Hybrid	257.0		B			
SF37	F22	128.7			C		
SF89	D90	121.7			C	D	
SH17	F22	115.3			C	D	E
SG83	D90	115.3			C	D	E
SH19	F22	114.7			C	D	E
SH18	F22	112.3			C	D	E F
SH13	F22	112.3			C	D	E F
SH68	D90	108.0				D	E F
SG23	D90	106.0				D	E F
SF91	D90	104.0					E F
SG73	D90	103.3					E F
SG87	D90	99.3					E F
SH70	D90	97.0					F

Means with the same letter are not significantly different for P>0.05(Duncan's test).

Table 4-4 Average fruit number/plant for 13 interspecific BC3F7 tomato lines selected for high zingiberene and for two cultivated tomato F_1 hybrids.

Line	Family	Mean	Grouping				
SF91	D90	80	A				
SH70	D90	76	A	B			
SG73	D90	75	A	B	C		
SG23	D90	70	A	B	C		
SF89	D90	69	A	B	C		
SG87	D90	67	A	B	C		
SH68	D90	64		B	C		
SH18	F22	62		B	C		
SH17	F22	62		B	C	D	
SH13	F22	61		B	C	D	
SG83	D90	61			C	D	
SH19	F22	60			C	D	
'BHN 589'	F_1 Hybrid	47				D	E
SF37	F22	47				D	E
'Red Deuce'	F_1 Hybrid	34					E

Means with the same letter are not significantly different for P>0.05(Duncan's test).

Table 4-5 Average fruit 7-epi-zingiberene content µg/cm² of foliage for 13 interspecific BC3F7 tomato lines selected for high zingiberene and for two cultivated tomato F_1 hybrids.

Line	Family	Mean	Grouping
SH13	F22	49.0	A
SH17	F22	43.8	A B
SH18	F22	41.9	C
SH68	D90	32.5	D
SG87	D90	31.3	E
SG73	D90	30.9	EE
SH70	D90	28.5	FF
SG23	D90	25.1	GG
SH19	F22	22.3	HH
SF89	D90	20.6	II
SF91	D90	20.1	JJ
SF37	F22	19.0	JJ
SG83	D90	18.6	LL
'Red Deuce'	F_1 Hybrid	0.0	
'BHN 589'	F_1 Hybrid	0.0	

Means with the same letter are not significantly different for P>0.05(Duncan's test).

Table 4-6 Average phenolic content µg/g for 13 interspecific hybrid tomato lines selected for high zingiberene, recurrent parent ('Zaofen 2') and for two cultivated tomato F_1 hybrids.

Line	Family	Mean	Grouping		
SF37	F22	405.2	A		
SG87	D90	399.2	A		
SG83	D90	395.4	A		
SH18	F22	393.0	A		
SH19	F22	387.2	A	B	
SH17	F22	380.6	A	B	
SG73	D90	371.5	A	B	C
SH68	D90	371.5	A	B	C
SF91	D90	368.1	A	B	C
SG23	D90	367.4	A	B	C
Zaofen 2	R. Parent	358.2	A	B	C
SF89	D90	358.1	A	B	C
SH70	D90	358.0	A	B	C
SH13	F22	335.8		B	C
'BHN 589'	F_1 Hybrid	319.7			C
'Red Deuce'	F_1 Hybrid	313.1			C

Means with the same letter are not significantly different for P>0.05(Duncan's test).

Table 4-7 Average ascorbic acid content µg/g for 13 interspecific hybrid tomato lines selected for high zingiberene, recurrent parent (’Zaofen 2’) and for two cultivated tomato F_1 hybrids

Line	Family	Mean	Grouping			
SG87	D90	560	A			
SG83	D90	511	A	B		
SH18	F22	508	A	B		
SG23	D90	508	A	B		
SH13	F22	501	A	B	C	
SF37	F22	500	A	B	C	
SH17	F22	497	A	B	C	
SH19	F22	487	A	B	C	
SH68	D90	484	A	B	C	
SF89	D90	467	A	B	C	
SG73	F22	464	A	B	C	
SF91	D90	451	A	B	C	
SH70	D90	439		B	C	D
‘BHN 589’	F_1 Hybrid	415			C	D
’Red Deuce’	F_1 Hybrid	336				D
Zaofen 2	R. Parent	275				D

Means with the same letter are not significantly different for P>0.05(Duncan’s test).

Table 4-8 Average lycopene content µg/g for 13 interspecific hybrid tomato lines selected for high zingiberene, recurrent parent ('Zaofen 2') and for two cultivated tomato F_1 hybrids.

Line	Family	Mean	Grouping					
SF91	D90	40.6	A					
SH68	D90	40.3	A	B				
SG83	D90	38.2	A	B	C			
SH17	F22	36.8	A	B	C	D		
SF89	D90	35.3	A	B	C	D	E	
SH18	F22	33.5	A	B	C	D	E	F
SH70	D90	32.9		B	C	D	E	F
SH19	F22	32.3			C	D	E	F
SG73	D90	31.8			C	D	E	F
SG23	D90	31.7			C	D	E	F
SF37	F22	31.5			C	D	E	F
SH13	F22	30.1				D	E	F
SG87	D90	29.2					E	F
Zaofen 2	R. Parent	28.8					E	F
'BHN 589'	F_1 Hybrid	28.5					E	F
'Red Deuce'	F_1 Hybrid	27.7						F

Means with the same letter are not significantly different for P>0.05(Duncan's test).

Table 4-6 Average soluble solid % for 13 interspecific hybrid tomato lines selected for high zingiberene, recurrent parent ('Zaofen 2') and for two cultivated tomato F_1 hybrids.

Line	Family	Mean	Grouping			
SH13	F22	6.6	A			
SG83	D90	6.5	A	B		
SG87	D90	6.4	A	B		
SF37	F22	6.4	A	B		
SH17	F22	6.4	A	B		
SH19	F22	6.3	A	B		
SH68	D90	6.3	A	B	C	
SG23	D90	6.2	A	B	C	
SH18	F22	6.1	A	B	C	D
SF91	D90	6.1	A	B	C	D
SG73	D90	6.0	A	B	C	D
ZH2	R. Parent	5.8		B	C	D
SH70	D90	5.8		B	C	D
'BHN 589'	F_1 Hybrid	5.8		B	C	D
SF89	D90	5.6			C	D
'Red Deuce'	F_1 Hybrid	5.5				D

Means with the same letter are not significantly different for P>0.05(Duncan's test).

CHAPTER 5. CONCLUSIONS AND FUTURE EXPECTATIONS

One goal of this research was to evaluate the potential for using a spectrophotometric method to enable accurate selection for 7-epi-zingiberene content in breeding lines derived from an interspecific cross and in wild accessions, such as LA2329 that had multiple allelochemicals such as 7-epi-zingiberene and 9-hydroxy-zingiberene. Another intent was also to examine the accuracy of Maluf, Campos et al. (2001) procedure to quantify 7-epi-zingiberene content in lines involving interspecific hybridizations. The results revealed that plants derived from interspecific crosses may have high levels of 7-epi-zingiberene as demonstrated with high absorbance values. However, plants having no 7-epi-zingiberene had low, but non-zero absorbance values indicating the presence of interfering compounds. β-phellandrene and other unidentified compounds likely contributed to absorbance values, but their contribution was minimal, and this interference is more pronounced at low 7-epi-zingiberene concentrations. Regression analysis indicated a strong linear association between 7-epi-zingiberene content and UV-absorbance value at 270 nm. In general, by increasing 7-epi-zingiberene content, the UV absorption reading increased (R^2=0.922). Other compounds such as 9-hydroxy-zingiberene in the wild tomato accessions LA2329, was present in trichome secretions and caused interference in determining 7-epi-zingiberene concentrations. 7-epi-zingiberene content can be estimated by using UV-spectrophotometer if plants did not contain oxygenated forms of 7-epi-zingiberene in their leaf tissue. Without interfering compounds, this technique could be a cost-effective and faster technique, compared to gas chromatography, for selecting plants in a breeding program designed to transfer 7-epi-zingiberene from wild to cultivated tomato with a view toward improving arthropod resistance

of tomato. Using the UV-visible scanning technique along with GC-FID would be the best for determination of multiple compounds in the foliage secretions.

The second goal was to investigate if compounds beside 7-epi-zingiberene may contribute to arthropod resistance. Isolation and identification of three major allelochemicals, 7-epi-zingiberene, 9-hydroxy-zingiberene and 9-hydroxy-10,11-epoxy-zingiberene, were undertaken by use of open column and gas chromatography/mass spectroscopy. The results revealed the presence of three predominate chromatographic peaks: 7-epi-zingiberene, 9-hydroxy zingiberene and 9-hydroxy,10,11-epoxy-zingiberene. Results of testing isolated compounds for repellency to TSSM using bridge bioassays revealed that the repellent activities of 9-hydroxy zingiberene and 9-hydroxy,10,11-epoxy-zingiberene were each significantly higher than that for 7-epi-zingiberene. These results support the idea that the degree of repellency may differ among plant allelochemicals and also emphasized the potential value of introgressing the presence of 9-hydroxy zingiberene and 9-hydroxy,10,11-epoxy-zingiberene into cultivated tomato to enhance its arthropod resistance.

The last goal was to evaluate yield, phenolic content, lycopene and soluble solids of thirteen interspecific BC3F7 lines of two families, D90 and F22, and two commercial F_1 hybrid lines '('Red Deuce' and 'BHN 589') grown in the open field. This study revealed that the yield of lines of the F22 interspecific lines were 6.7 kg/plant, 7.5 kg/plant for the interspecific D90 lines and 11.4 kg/plant for commercial tomato F_1 hybrids. In interspecific BC3F7 families, average fruit weight was 110 g while in F_1 hybrids it was 290 g. The number of fruits per plant in the D90 family was the highest, 75 fruit/plant, whereas in the F22 family there was 58 fruit/plant compared to 40 fruit/plant for commercial hybrids. The content of

foliage zingiberene in the F22 interspecific hybrid family was 45 μg/cm² and was 26 μg/cm² in the D90 family whereas there was no zingiberene in the F_1 cultivated tomatoes. The phenolic content in some of interspecific hybrid lines was high, as high as 427 μg/g fresh fruit. Ascorbic acid content of the interspecific hybrids was very similar to the ascorbic acid content of the F_1 hybrid cultivated tomatoes. Lycopene content ranged from 31 to 40 μg/g and soluble solids ranged from 4 to 6.6%, whereas those compounds were generally lower in F_1 hybrids. These initial results show that it may be possible to breed tomatoes with a higher phenolic, lycopene and zingiberene contents and maintain ascorbic acid concentrations, TSSTSS and yield at a reasonable levels, which could lead to improved human diets and host-plant resistance to arthropods. Additional work is needed to verify these results and find plants within the earlier generations of the interspecific population that may be capable of producing fruit with higher phenolic, ascorbic acid, lycopene, and TSS than those reported for BC3F7 hybrids. These initial results suggest that it may be possible to elevate these components ought to breed, leading to better nutritive value and/or plant disease resistance.

REFERENCES

Abushita, A. A., E. A. Hebshi, H. G. Daood and P. A. Biacs. 1997. Determination of antioxidant vitamins in tomatoes. Food Chemistry 60(2): 207-212.

Achinewhu, S. 1983. Ascorbic acid content of some Nigerian local fruits and vegetables. Plant Foods for Human Nutrition 33(4): 261-266.

Agarwal, S. and A. V. Rao. 2000. Tomato lycopene and its role in human health and chronic diseases. Cmaj 163(6): 739-744.

Aharoni, A., M. A. Jongsma and H. J. Bouwmeester. 2005. Volatile science? Metabolic engineering of terpenoids in plants. Trends in Plant Science 10(12): 594-602.

Alasalvar, C., J. M. Grigor, D. Zhang, P. C. Quantick and F. Shahidi. 2001. Comparison of volatiles, phenolics, sugars, antioxidant vitamins, and sensory quality of different colored carrot varieties. Journal of Agricultural and Food Chemistry 49(3): 1410-1416.

Alba, J. M., M. Montserrat and R. Fernández-Muñoz. 2009. Resistance to the two-spotted spider mite (*Tetranychus urticae*) by acylsucroses of wild tomato (*Solanum pimpinellifolium*) trichomes studied in a recombinant inbred line population. Experimental and Applied Acarology 47(1): 35-47.

Álvarez Gil, M. 2015. Resistencia a insectos en tomate (*Solanum* spp.). Cultivos Tropicales 36(2): 100-110.

Anonymous (2017). Gene And Protein For The Synthesis Of Oxidized Zingiberene Derivatives. EP, LEIBNIZ-INSTITUT FÜR PFLANZENBIOCHEMIE.

Ansari, M. S., M. A. Moraiet and S. Ahmad (2014). Insecticides: impact on the environment and human health. Environmental Deterioration and Human Health, Springer**:** 99-123.

Antonious, G. F. and T. S. Kochhar. 2003. Zingiberene and curcumene in wild tomato. Journal of Environmental Science and Health, Part B 38(4): 489-500.

Antonious, G. F. and J. C. Snyder. 2006. Natural products: repellency and toxicity of wild tomato leaf extracts to the two-spotted spider mite, *Tetranychus urticae* Koch. Journal of Environmental Science and Health Part B 41(1): 43-55.

Aoun, A. B., B. Lechiheb, L. Benyahya and A. Ferchichi. 2013. Evaluation of fruit quality traits of traditional varieties of tomato (*Solanum lycopersicum*) grown in Tunisia. African Journal of Food Science 7(10): 350-354.

Aragão, C. A., B. F. Dantas and F. R. G. Benites. 2000. Foliar trichome in tomato with contrasting levels of alelochemical 2-tridecanone. Scientia Agricola 57(4): 813-816.

Aubin, B. S. (2019). Genetic Analysis of Glandular Trichome Development and Metabolism in Cultivated Tomato, Michigan State University.

Bahorun, T., A. Luximon‐Ramma, A. Crozier and O. I. Aruoma. 2004. Total phenol, flavonoid, proanthocyanidin and vitamin C levels and antioxidant activities of Mauritian vegetables. Journal of the Science of Food and Agriculture 84(12): 1553-1561.

Bai, Y. and P. Lindhout. 2007. Domestication and breeding of tomatoes: what have we gained and what can we gain in the future? Annals of Botany 100(5): 1085-1094.

Balasundram, N., K. Sundram and S. Samman. 2006. Phenolic compounds in plants and agri-industrial by-products: Antioxidant activity, occurrence, and potential uses. Food Chemistry 99(1): 191-203.

Barrantes, W., G. López-Casado, S. García-Martínez, A. Alonso, F. Rubio, J. J. Ruiz, R. Fernández-Muñoz, A. Granell and A. J. Monforte. 2016. Exploring new alleles involved in tomato fruit quality in an introgression line library of *Solanum pimpinellifolium*. Frontiers in Plant Science 7: 1172.

Bauchet, G. and M. Causse. 2012. Genetic diversity in tomato (*Solanum lycopersicum*) and its wild relatives. Genetic Diversity in Plants 133: 162.

Bernacchi, D., T. Beck-Bunn, Y. Eshed, J. Lopez, V. Petiard, J. Uhlig, D. Zamir and S. Tanksley. 1998. Advanced backcross QTL analysis in tomato. I. Identification of QTLs for traits of agronomic importance from *Lycopersicon hirsutum*. Theoretical and Applied Genetics 97(3): 381-397.

Besser, K., A. Harper, N. Welsby, I. Schauvinhold, S. Slocombe, Y. Li, R. A. Dixon and P. Broun. 2009. Divergent regulation of terpenoid metabolism in the trichomes of wild and cultivated tomato species. Plant Physiology 149(1): 499-514.

Bhagwat, S., D. B. Haytowitz and J. M. Holden (2014, Last Update Date). "USDA database for the flavonoid content of selected foods, Release 3.1." Series USDA database for the flavonoid content of selected foods, Release 3.1 Edition. Retrieved Access Date, Access 2014, from https://www.ars.usda.gov/northeast-area/beltsville-md-bhnrc/beltsville-human-nutrition-

research-center/nutrient-data-laboratory/docs/usda-database-for-the-flavonoid-content-of-selected-foods-release-31-december-2013/.

Bhatt, A., Y. Naidoo and A. Nicholas. 2010. The foliar trichomes of Plectranthus laxiflorus Benth [Lamiaceae]: an important medicinal plant. New Zealand Journal of Botany 48(2): 55-61.

Bhattarai, K., F. J. Louws, J. D. Williamson and D. R. Panthee. 2016. Diversity analysis of tomato genotypes based on morphological traits with commercial breeding significance for fresh market production in eastern USA. Australian Journal of Crop Science 10(8): 1098.

Blázquez, M. A. 2014. Role of natural essential oils in sustainable agriculture and food preservation. Journal of Scientific Research and Reports: 1843-1860.

Bleeker, P. M., P. J. Diergaarde, K. Ament, S. Schütz, B. Johne, J. Dijkink, H. Hiemstra, R. de Gelder, M. T. de Both and M. W. Sabelis. 2011. Tomato-produced 7-epizingiberene and R-curcumene act as repellents to whiteflies. Phytochemistry 72(1): 68-73.

Bleeker, P. M., R. Mirabella, P. J. Diergaarde, A. VanDoorn, A. Tissier, M. R. Kant, M. Prins, M. de Vos, M. A. Haring and R. C. Schuurink. 2012. Improved herbivore resistance in cultivated tomato with the sesquiterpene biosynthetic pathway from a wild relative. Proceedings of the National Academy of Sciences 109(49): 20124-20129.

Bohlmann, J., G. Meyer-Gauen and R. Croteau. 1998. Plant terpenoid synthases: molecular biology and phylogenetic analysis. Proceedings of the National Academy of Sciences 95(8): 4126-4133.

Böhm, V. (2018). Lycopene, Tomatoes, and Cardiovascular Diseases. Lycopene and Tomatoes in Human Nutrition and Health, CRC Press**:** 51-68.

Bolland, H. R., J. Gutierrez and C. H. Flechtmann (1998). World catalogue of the spider mite family (Acari: Tetranychidae), Brill.

Borguini, R. G. and E. A. Ferraz da Silva Torres. 2009. Tomatoes and tomato products as dietary sources of antioxidants. Food Reviews International 25(4): 313-325.

Bravo, L. 1998. Polyphenols: chemistry, dietary sources, metabolism, and nutritional significance. Nutrition Reviews 56(11): 317-333.

Brødsgaard, H. F. and R. Albajes (1999). Insect and mite pests. Integrated pest and disease management in greenhouse crops, Springer**:** 48-60.

Buta, J. and D. Spaulding. 1997. Endogenous levels of phenolics in tomato fruit during growth and maturation. Journal of Plant Growth Regulation 16(1): 43-46.

Butu, M., M. Butnariu, S. Rodino and A. Butu. 2014. STUDY OF ZINGIBERENE FROM LYCOPERSICON ESCULENTUM FRUIT BY MASS SPECTOMETRY. Digest Journal of Nanomaterials & Biostructures (DJNB) 9(3).

Carter, C. D., T. J. Gianfagna and J. N. Sacalis. 1989. Sesquiterpenes in glandular trichomes of a wild tomato species and toxicity to the Colorado potato beetle. Journal of Agricultural and Food Chemistry 37(5): 1425-1428.

Carter, C. D., J. N. Sacalis and T. J. Gianfagna. 1989. Zingiberene and resistance to Colorado potato beetle in *Lycopersicon hirsutum* f. *hirsutum*. Journal of Agricultural and Food Chemistry 37(1): 206-210.

Chatzivasileiadis, E. A., J. J. Boon and M. W. Sabelis. 1999. Accumulation and turnover of 2-tridecanone in *Tetranychus urticae* and its consequences for resistance of wild and cultivated tomatoes. Experimental and Applied Acarology 23(12): 1011-1021.

Chatzivasileiadis, E. A. and M. W. Sabelis. 1997. Toxicity of methyl ketones from tomato trichomes to *Tetranychus urticae* Koch. Experimental & Applied Acarology 21(6-7): 473-484.

Chatzivasileiou, A. O. (2019). Engineering a novel pathway for isoprenoid synthesis, Massachusetts Institute of Technology.

Cheynier, V. 2005. Polyphenols in foods are more complex than often thought. The American Journal of Clinical Nutrition 81(1): 223S-229S.

Choi, W.-S., B.-S. Park, S.-K. Ku and S.-E. Lee. 2002. Repellent activities of essential oils and monoterpenes against Culex pipiens pallens. Journal of the American Mosquito Control Association 18(4): 348-351.

Clifford, M. (1985). Chapter 5, Chlorogenic acids. In “Coffee. Vol. 1: Chemistry,” ed. by Clarke, RJ and Macrae, R, Elsevier Applied Science, London.

Coates, R. M., J. F. Denissen, J. A. Juvik and B. A. Babka. 1988. Identification of α-santalenoic and endo β-bergamotenoic acids as moth oviposition stimulants from wild tomato leaves. The Journal of Organic Chemistry 53(10): 2186-2192.

Cutter, A. D. 2012. The polymorphic prelude to Bateson–Dobzhansky–Muller incompatibilities. Trends in Ecology & Evolution 27(4): 209-218.

da Silveira Vasconcelos, M., E. F. Mota, N. F. Gomes-Rochette, D. C. S. Nunes-Pinheiro, S. M. Nabavi and D. F. de Melo (2019). Ginger (*Zingiber officinale Roscoe*). Nonvitamin and Nonmineral Nutritional Supplements, Elsevier**:** 235-239.

Dawood, H. D., C. J. Snyder and G. F. Antonious. Phenolic Content of Fruit and Leaves of Interspecific Hybrid Tomatoes. Fruit and Vegetable PR-: 34-36.

Dawood, M. H. and J. C. Snyder. 2020. The alcohol and epoxy alcohol of zingiberene, produced in trichomes of wild tomato, are more repellent to spider mites than zingiberene. Frontiers in Plant Science 11: 35.

Dawood, M. H., J. C. Snyder and G. F. Antonious. 2018. Phenolic Content of Fruit and Leaves of Interspecific Hybrid Tomatoes. Fruit and Vegetable: 34.

de Azevedo, S. M., M. V. Faria, W. R. Maluf, A. C. B. De Oliveira and J. A. de Freitas. 2003. Zingiberene-mediated resistance to the South American tomato pinworm derived from Lycopersicon hirsutum var. hirsutum. Euphytica 134(3): 347-351.

de Freitas, J., W. Maluf, M. d. G. Cardoso and A. de Oliveira. 2000. Selection of tomato plant resistant to arthropod pests mediated by zingiberene. Acta Scientiarum 22(4): 919-923.

de Oliveira, J. R., J. T. de Resende, W. R. Maluf, T. Lucini, R. B. de Lima Filho, I. P. de Lima and C. Nardi. 2018. Trichomes and allelochemicals in tomato genotypes have antagonistic effects upon behavior and biology of Tetranychus urticae. Frontiers in Plant Science 9: 1132.

de Resende, J. T., W. R. Maluf, M. d. G. Cardoso, L. D. Gonçalves, M. V. Faria and I. R. do Nascimento. 2009. Resistance of tomato genotypes to the silverleaf whitefly mediated by acylsugars. Horticultura Brasileira 27(3): 345-348.

de Sena Fernandes, M. E., F. L. Fernandes, N. O. Silva, D. J. H. da Silva, F. G. Pinto and R. S. Ramos. 2014. Selection of tomato hybrids with zingiberene concentration for breeding programs to pest resistance. Journal of Agricultural Science 6(6): 148.

de Souza, L. M., P. C. T. Melo, R. R. Luders and A. M. Melo. 2012. Correlations between yield and fruit quality characteristics of fresh market tomatoes. Horticultura Brasileira 30(4): 627-631.

Di Matteo, A., A. Sacco, M. Anacleria, M. Pezzotti, M. Delledonne, A. Ferrarini, L. Frusciante and A. Barone. 2010. The ascorbic acid content of tomato fruits is associated with the expression of genes involved in pectin degradation. BMC Plant Biology 10(1): 163.

Dias, D., J. Resende, M. Faria, L. Camargo, R. Chagas and I. Lima. 2013. Selection of processing tomato genotypes with high acyl sugar content that are resistant to the tomato pinworm. Genetics and Molecular Research 12: 381-389.

Dias, D., J. Resende, M. Faria, L. Camargo, R. Chagas and I. Lima. 2013. Selection of processing tomato genotypes with high acyl sugar content that are resistant to the tomato pinworm. Genetics and Molecular Research 12(1): 381-389.

Dorais, M., D. L. Ehret and A. P. Papadopoulos. 2008. Tomato (*Solanum lycopersicum*) health components: from the seed to the consumer. Phytochemistry Reviews 7(2): 231-250.

Duffey, S. and M. Isman. 1981. Inhibition of insect larval growth by phenolics in glandular trichomes of tomato leaves. Experientia 37(6): 574-576.

Eigenbrode, S. D., J. T. Trumble, J. G. Millar and K. K. White. 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*. Journal of Agricultural and Food Chemistry 42(3): 807-810.

Eigenbrode, S. D., J. T. Trumble and K. K. White. 1996. Trichome exudates and resistance to beet armyworm (Lepidoptera: Noctuidae) in *Lycopersicon hirsutum* f. typicum Accessions. Environmental Entomology 25(1): 90-95.

Ekanayaka, E. P., C. Li and A. D. Jones. 2014. Sesquiterpenoid glycosides from glandular trichomes of the wild tomato relative *Solanum habrochaites*. Phytochemistry 98: 223-231.

Falara, V., T. A. Akhtar, T. T. Nguyen, E. A. Spyropoulou, P. M. Bleeker, I. Schauvinhold, Y. Matsuba, M. E. Bonini, A. L. Schilmiller and R. L. Last. 2011. The tomato terpene synthase gene family. Plant physiology 157(2): 770-789.

FAOSTAT (2017). Food and Agriculture Organization of the United Nations (FAO), United Nations Publications.

Firdaus, S., A. W. van Heusden, N. Hidayati, E. D. J. Supena, R. G. Visser and B. Vosman. 2012. Resistance to *Bemisia tabaci* in tomato wild relatives. Euphytica 187(1): 31-45.

Fleuriet, A. and J.-J. Macheix. 1985. Tissue compartmentation of phenylpropanoid metabolism in tomatoes during growth and maturation. Phytochemistry 24(5): 929-932.

Fleuriet, A. and J. Macheix. 1976. Effets des conditions anaérobies sur les composés phénoliques de fruits de Tomates «Cerise». Physiologie Végétale 14: 407-414.

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

Freitas, J. A., W. R. Maluf, M. das Graças Cardoso, L. A. A. Gomes and E. Bearzotti. 2002. Euphytica 127(2): 275-287.

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

Frelichowski Jr, J. E. and J. A. Juvik. 2001. Sesquiterpene carboxylic acids from a wild tomato species affect larval feeding behavior and survival of *Helicoverpa zea* and *Spodoptera exigua* (Lepidoptera: Noctuidae). Journal of Economic Entomology 94(5): 1249-1259.

Fufa, H., P. Baenziger, B. Beecher, I. Dweikat, R. Graybosch and K. M. Eskridge. 2005. Comparison of phenotypic and molecular marker-based classifications of hard red winter wheat cultivars. Euphytica 145(1-2): 133-146.

Garcia-Salas, P., A. Morales-Soto, A. Segura-Carretero and A. Fernández-Gutiérrez. 2010. Phenolic-compound-extraction systems for fruit and vegetable samples. Molecules 15(12): 8813-8826.

Gerson, U. and P. G. Weintraub. 2007. Mites for the control of pests in protected cultivation. Pest Management Science: formerly Pesticide Science 63(7): 658-676.

Goddard, M. and R. Matthews. 1979. Contribution of fruits and vegetables to human nutrition. HortScience.

Gonçalves, L. D., W. R. Maluf, M. d. G. Cardoso, J. T. V. d. Resende, E. M. d. Castro, N. M. Santos, I. R. d. Nascimento and M. V. Faria. 2006. Relação entre zingibereno, tricomas foliares e repelência de tomateiros a Tetranychus evansi. Pesquisa Agropecuária Brasileira 41(2): 267-273.

Gonzales‐Vigil, E., D. E. Hufnagel, J. Kim, R. L. Last and C. S. Barry. 2012. Evolution of TPS20‐related terpene synthases influences chemical diversity in the glandular trichomes of the wild tomato relative *Solanum habrochaites*. The Plant Journal 71(6): 921-935.

Guo, Z., P. A. Weston and J. C. Snyder. 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.

Gutensohn, M., D. A. Nagegowda and N. Dudareva (2012). Involvement of compartmentalization in monoterpene and sesquiterpene biosynthesis in plants. Isoprenoid Synthesis in Plants and Microorganisms, Springer**:** 155-169.

Hajjar, R. and T. Hodgkin. 2007. The use of wild relatives in crop improvement: a survey of developments over the last 20 years. Euphytica 156(1-2): 1-13.

Hashmi, M. U. H. (1973). Spectrophotometric determination with potassium ferricyanide. Assay of Vitamins in Pharmaceutical Preparations London, UK, John Wiley and Sons. **57**.

Hessey, N. and W. Parr. 1963. The effect of glasshouse red spider mite on the yield of cucumber. Journal of Horticultural Science 38: 255-263.

Hu, S.-X., J.-G. Yu and E. Zeng. 2010. A theoretical study on UV-spectroscopy, electronic structure and reactivity properties of sesquiterpenes. Atmospheric Chemistry and Physics Discussions 10(10): 24325-24343.

Ilic, Z. and L. Milenkovic (2012). The influence of photo-selective shade nets on quality of tomatoes grown under plastic tunnels and field conditions. Proceedings of an International Conference on BioScience: Biotechnology and Biodiversity, Step in the Future, Novi Sad Serbia.

Isman, M. B. and S. S. Duffey. 1982. Toxicity of tomato phenolic compounds to the fruitworm, *Heliothis zea*. Entomologia Experimentalis et Applicata 31(4): 370-376.

Kaur, R., G. Savage and P. Dutta. 2002. Antioxidant vitamins in four commercially grown tomato. Proceedings of the Nutrition Society 27: 69.

Kavitha, P., K. S. Shivashankara, V. K. Rao, A. T. Sadashiva, K. V. Ravishankar and G. J. Sathish. 2014. Genotypic variability for antioxidant and quality parameters among tomato cultivars, hybrids, cherry tomatoes and wild species. Journal of the Science of Food and Agriculture 94(5): 993-999.

Khatun, S., U. Cakilcioglu and N. C. Chatterjee. 2011. Pharmacognostic value of leaf anatomy and trichome morphology for identification of forskolin in a novel medicinal plant Coleus forskohlii. Biological Diversity and Conservation 4(2): 165-171.

Klein, B. and A. Perry. 1982. Ascorbic acid and vitamin A activity in selected vegetables from different geographical areas of the United States. Journal of Food Science 47(3): 941-945.

Klingler, H., S. Frosch and E. Wagner. 1991. In vitro effects of monoterpenes on chloroplast membranes. Photosynthesis Research 28(3): 109-118.

Kokosa, J. M., A. Przyjazny and M. Jeannot (2009). Solvent Microextraction: Theory and Practice, John wiley & sons.

Lange, B. M., R. Rios-Estepa and G. W. Turner (2012). Production of terpenes and terpenoids in glandular trichome-bearing plants, Google Patents.

Lange, W. H. and L. Bronson. 1981. Insect pests of tomatoes. Annual Review of Entomology 26(1): 345-371.

Lattanzio, V., V. M. Lattanzio and A. Cardinali. 2006. Role of phenolics in the resistance mechanisms of plants against fungal pathogens and insects. Phytochemistry: Advances in Research 661(2): 23-67.

Le Gall, G., M. S. DuPont, F. A. Mellon, A. L. Davis, G. J. Collins, M. E. Verhoeyen and I. J. Colquhoun. 2003. Characterization and content of flavonoid glycosides in genetically modified tomato (*Lycopersicon esculentum*) fruits. Journal of Agricultural and Food Chemistry 51(9): 2438-2446.

Letourneau, D. and B. Goldstein. 2001. Pest damage and arthropod community structure in organic vs. conventional tomato production in California. Journal of Applied Ecology 38(3): 557-570.

Liedl, B. E., D. M. Lawson, K. K. White, J. A. Shapiro, D. E. Cohen, W. G. Carson, J. T. Trumble and M. A. Mutschler. 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.

Lim, Y. Y., T. T. Lim and J. J. Tee. 2007. Antioxidant properties of several tropical fruits: A comparative study. Food Chemistry 103(3): 1003-1008.

Lima, I., J. Resende, J. Oliveira, M. Faria, N. Resende and R. Lima-Filho. 2015. Indirect selection of industrial tomato genotypes rich in zingiberene and resistant to *Tuta absoluta* Meyrick. Genetics and Molecular Research 14(4): 15081-15089.

Lima, I. P., J. T. Resende, J. R. Oliveira, M. V. Faria, D. M. Dias and N. C. Resende. 2016. Selection of tomato genotypes for processing with high zingiberene content, resistant to pests. Horticultura Brasileira 34(3): 387-391.

Lin, S. Y., J. T. Trumble and J. Kumamoto. 1987. Activity of volatile compounds in glandular trichomes of *Lycopersicon* species against two insect herbivores. Journal of Chemical Ecology 13(4): 837-850.

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

Luczynski, A., M. Isman, D. Raworth and C. Chan. 1990. Chemical and morphological factors of resistance against the twospotted spider mite in beach strawberry. Journal of Economic Entomology 83(2): 564-569.

Macheix, J., A. Fleuriet and J. Billot. 1990. Phenolic compounds in fruit processing. Fruit Phenolics 1: 295-358.

Maluf, W. R., G. A. Campos and M. das Graças Cardoso. 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, W. R., V. de Fátima Silva, M. das Graças Cardoso, L. A. A. Gomes, Á. C. G. Neto, G. M. Maciel and D. A. C. Nízio. 2010. Resistance to the South American tomato pinworm *Tuta absoluta* in high acylsugar and/or high zingiberene tomato genotypes. Euphytica 176(1): 113-123.

Marsic, N. K., L. Gasperlin, V. Abram, M. Budic and R. Vidrih. 2011. Quality parameters and total phenolic content in tomato fruits regarding cultivar and microclimatic conditions. Turkish Journal of Agriculture and Forestry 35(2): 185-194.

Martínez‐Valverde, I., M. J. Periago, G. Provan and A. Chesson. 2002. Phenolic compounds, lycopene and antioxidant activity in commercial varieties of tomato (*Lycopersicum esculentum*). Journal of the Science of Food and Agriculture 82(3): 323-330.

Mayne, S. T. 1996. Beta-carotene, carotenoids, and disease prevention in humans. The FASEB Journal 10(7): 690-701.

McGrath, R. M., W. Z. Kaluza, K. H. Daiber, W. B. Van der Riet and C. W. Glennie. 1982. Polyphenols of sorghum grain, their changes during malting and their inhibitory nature. Journal of Agricultural and Food Chemistry 30(3): 450-456.

Melis, A., F. K. Davies, H.-C. C. Wintz and A. Zurbriggen (2018). Production of β-phellandrene using genetically engineered cyanobacteria, Google Patents.

Miller, J. and S. Tanksley. 1990. RFLP analysis of phylogenetic relationships and genetic variation in the genus *lycopersicon*. Theoretical and Applied Genetics 80(4): 437-448.

Muniappan, R. (2012). Arthropod pests of horticultural crops in tropical Asia, CABI.

Mutschler, M., R. Doerge, S. Liu, J. Kuai, B. Liedl and J. Shapiro. 1996. QTL analysis of pest resistance in the wild tomato *Lycopersicon pennellii*: QTLs controlling acylsugar level and composition. Theoretical and Applied Genetics 92(6): 709-718.

Naczk, M. and F. Shahidi. 2006. Phenolics in cereals, fruits and vegetables: Occurrence, extraction and analysis. Journal of Pharmaceutical and Biomedical Analysis 41(5): 1523-1542.

Nakazato, T. and E. A. Housworth. 2011. Spatial genetics of wild tomato species reveals roles of the Andean geography on demographic history. American Journal of Botany 98(1): 88-98.

Nault, B. A. and J. Speese III. 2002. Major insect pests and economics of fresh-market tomato in eastern Virginia. Crop Protection 21(5): 359-366.

Neiva, I. P., A. A. d. Silva, J. F. Resende, R. d. C. Carvalho, A. M. S. d. Oliveira and W. R. Maluf. 2019. Tomato genotype resistance to whitefly mediated by allelochemicals and Mi gene. Chilean Journal of Agricultural Research 79(1): 124-130.

Ntinas, G. K., K. Kadoglidou, N. Tsivelika, K. Krommydas, A. Kalivas, P. Ralli and M. Irakli. 2019. Performance and Hydroponic Tomato Crop Quality Characteristics in a Novel Greenhouse Using Dye-Sensitized Solar Cell Technology for Covering Material. Horticulturae 5(2): 42.

Østerlie, M. and J. Lerfall. 2005. Lycopene from tomato products added minced meat: Effect on storage quality and colour. Food Research International 38(8-9): 925-929.

Pascale, S. D., A. Maggio, V. Fogliano, P. Ambrosino and A. Ritieni. 2001. Irrigation with saline water improves carotenoids content and antioxidant activity of tomato. The Journal of Horticultural Science and Biotechnology 76(4): 447-453.

Peralta, I. E., D. M. Spooner and S. Knapp. 2008. Taxonomy of wild tomatoes and their relatives (*Solanum* sect. Lycopersicoides, sect. *Juglandifolia*, sect. *Lycopersicon*; *Solanaceae*). Systematic Botany Monographs 84.

Perveen, R., H. A. R. Suleria, F. M. Anjum, M. S. Butt, I. Pasha and S. Ahmad. 2015. Tomato (*Solanum lycopersicum*) carotenoids and lycopenes chemistry; metabolism, absorption,

nutrition, and allied health claims—A comprehensive review. Critical Reviews in Food Science and Nutrition 55(7): 919-929.

Pichersky, E. 2020. The complete functional characterisation of the terpene synthase family in tomato. New Phytologist: 1.

Pingali, P. (1995). Impact of pesticides on farmer health and the rice environment: an overview of results from a multidisciplinary study in the Philippines. Impact of Pesticides on Farmer Health and The Rice Environment, Springer**:** 3-21.

Popp, J., K. Pető and J. Nagy. 2013. Pesticide productivity and food security. A review. Agronomy for Sustainable Development 33(1): 243-255.

Pretty, J. 2008. Agricultural sustainability: concepts, principles and evidence. Philosophical Transactions of the Royal Society B: Biological Sciences 363(1491): 447-465.

Racke, K. D. 2013. Insecticides in Environmental Matrices. Organophosphates Chemistry, Fate, and Effects: Chemistry, Fate, and Effects: 47.

Raffo, A., G. La Malfa, V. Fogliano, G. Maiani and G. Quaglia. 2006. Seasonal variations in antioxidant components of cherry tomatoes (*Lycopersicon esculentum* cv. Naomi F1). Journal of Food Composition and Analysis 19(1): 11-19.

Rao, A., Z. Waseem and S. Agarwal. 1998. Lycopene content of tomatoes and tomato products and their contribution to dietary lycopene. Food Research International 31(10): 737-741.

Reis, S. L., A. G. Mantello, J. M. Macedo, E. A. Gelfuso, C. P. Da Silva, A. L. Fachin, A. M. Cardoso and R. O. Beleboni. 2016. Typical monoterpenes as insecticides and repellents against stored grain pests. Molecules 21(3): 258.

Resende, J., A. da Silva, A. Gabriel, A. Zeist, R. Favaro, D. Nascimento, R. Zeist and C. Camargo. 2018. Resistance to *Helicoverpa armigera* mediated by zingiberene and glandular trichomes in tomatoes for industrial processing. Genetics and Molecular Research 17(2).

Rick, C. and R. Chetelat (1995). Utilization of related wild species for tomato improvement. I International Symposium on Solanacea for Fresh Market 412.

Rick, C. M. 1984. Germplasm resources in the wild tomato species. Acta Horticulturae 190: 39-47.

Rivas, N. and B. Luh. 1968. Polyphenolic compounds in canned tomato pastes. Journal of Food Science 33(4): 358-363.

Rivero, R. M., J. M. Ruiz and L. Romero. 2003. Can grafting in tomato plants strengthen resistance to thermal stress? Journal of the Science of Food and Agriculture 83(13): 1315-1319.

Ro, D.-K., E. M. Paradise, M. Ouellet, K. J. Fisher, K. L. Newman, J. M. Ndungu, K. A. Ho, R. A. Eachus, T. S. Ham and J. Kirby. 2006. Production of the antimalarial drug precursor artemisinic acid in engineered yeast. Nature 440(7086): 940-943.

Rosen, R., S. Kanakala, A. Kliot, B. C. Pakkianathan, B. A. Farich, N. Santana-Magal, M. Elimelech, S. Kontsedalov, G. Lebedev and M. Cilia. 2015. Persistent, circulative transmission of begomoviruses by whitefly vectors. Current Opinion in Virology 15: 1-8.

Rubatzky, V. E. and M. Yamaguchi (2012). World vegetables: principles, production, and nutritive values. World Population, Land Area, and Food Situation UK, Springer Science & Business Media.

Saad, A. M., A. Ibrahim and N. El-Bialee. 2016. Internal quality assessment of tomato fruits using image color analysis. Agricultural Engineering International: CIGR Journal 18(1): 339-352.

Sallaud, C., D. Rontein, S. Onillon, F. Jabès, P. Duffé, C. Giacalone, S. Thoraval, C. Escoffier, G. Herbette and N. Leonhardt. 2009. A novel pathway for sesquiterpene biosynthesis from Z, Z-farnesyl pyrophosphate in the wild tomato *Solanum habrochaites*. The Plant Cell 21(1): 301-317.

Salunkhe, D., S. Jadhav and M. Yu. 1974. Quality and nutritional composition of tomato fruit as influenced by certain biochemical and physiological changes. Qualitas Plantarum 24(1-2): 85-113.

Sances, F., J. Wyman and I. Ting. 1979. Morphological responses of strawberry leaves to infestations of twospotted spider mite. Journal of Economic Entomology 72(5): 710-713.

Schilmiller, A. L., I. Schauvinhold, M. Larson, R. Xu, A. L. Charbonneau, A. Schmidt, C. Wilkerson, R. L. Last and E. Pichersky. 2009. Monoterpenes in the glandular trichomes of tomato are synthesized from a neryl diphosphate precursor rather than geranyl diphosphate. Proceedings of the National Academy of Sciences 106(26): 10865-10870.

Scott, J. (2005). Fresh market tomato breeding in the USA. XV Meeting of the EUCARPIA Tomato Working Group 789.

Shapiro, J. A., J. C. Steffens and M. A. Mutschler. 1994. Acylsugars of the wild tomato *Lycopersicon pennellii* in relation to geographic distribution of the species. Biochemical Systematics and Ecology 22(6): 545-561.

Simmons, A. T. and G. M. Gurr. 2005. Trichomes of *Lycopersicon* species and their hybrids: effects on pests and natural enemies. Agricultural and Forest Entomology 7(4): 265-276.

Smirnoff, N. 1996. Botanical briefing: the function and metabolism of ascorbic acid in plants. Annals of Botany 78(6): 661-669.

Snyder, C. J., M. H. S. A. Dawood and A. S. AL-Bayati (2018). "Introgression of zingiberene and type IV trichome density from *Solanum habrochaites* LA2329 into *S. lycopersicum* – Progress Report " 47th Tomato Breeders Roundtable. Retrieved June 2, 2019, from http://tgc.ifas.ufl.edu/TBRT%202018/TBRT_Program_Book_Final.pdf.

Snyder, J. C., G. F. Antonious and R. Thacker. 2011. A sensitive bioassay for spider mite (*Tetranychus urticae*) repellency: a double bond makes a difference. Experimental and Applied Acarology 55(3): 215-224.

Snyder, J. C., Z. Guo, R. Thacker, J. P. Goodman and J. S. Pyrek. 1993. 2,3-Dihydrofarnesoic acid, a unique terpene from trichomes of *Lycopersicon* hirsutum, repels spider mites. Journal of Chemical Ecology 19(12): 2981-2997.

Snyder, J. C., Z. Guo, R. Thacker, J. P. Goodman and J. S. Pyrek. 1993. 2, 3-Dihydrofarnesoic acid, a unique terpene from trichomes of *Lycopersicon hirsutum*, repels spider mites. Journal of Chemical Ecology 19(12): 2981-2997.

Snyder, J. C., A. M. Simmons and R. R. Thacker. 1998. Attractancy and ovipositional response of adult *Bemisia argentifolii* (Homoptera: Aleyrodidae) to type IV trichome density on leaves of *Lycopersicon hirsutum* grown in three day-length regimes. Journal of Entomological Science 33(3): 270-281.

Snyder, J. C., R. R. Thacker and X. Zhang. 2005. Genetic transfer of a twospotted spider mite (Acari: *Tetranychidae*) repellent in tomato hybrids. Journal of Economic Entomology 98(5): 1710-1716.

Stevens, M. A. 1978. Genetic potential for overcoming physiological limitations on adaptability, yield, and quality in the tomato. Hort-Science 13: 673-678.

Tanksley, S., S. Grandillo, T. Fulton, D. Zamir, Y. Eshed, V. Petiard, J. Lopez and T. Beck-Bunn. 1996. Advanced backcross QTL analysis in a cross between an elite processing line of tomato and its wild relative *L. pimpinellifolium*. Theoretical and Applied Genetics 92(2): 213-224.

Toor, R. K. and G. P. Savage. 2005. Antioxidant activity in different fractions of tomatoes. Food research international 38(5): 487-494.

Topwal, M. and D. K. Singh. 2018. A Review on Genetic Diversity among Tomato (*Solanum lycopersicon* (MLL.) Wettsd.) Genotypes. Int. J. Pure App. Biosci 6(1): 368-375.

Tulipani, S., M. M. Huelamo, M. R. Ribalta, R. Estruch, E. E. Ferrer, C. Andres-Lacueva, M. Illan and R. M. Lamuela-Raventós. 2012. Oil matrix effects on plasma exposure and urinary excretion of phenolic compounds from tomato sauces: Evidence from a human pilot study. Food Chemistry 130(3): 581-590.

Tuttle, D. M. and E. W. Baker. 1968. Spider mites of southwestern United States and a revision of the family Tetranychidae.

Valadares, R. N., R. A. Melo, I. V. Sarinho, N. S. Oliveira, F. A. Rocha, J. W. Silva and D. Menezes. 2018. Genetic diversity in accessions of melon belonging to momordica group. Horticultura Brasileira 36(2): 253-258.

van der Hoeven, R. S., A. J. Monforte, D. Breeden, S. D. Tanksley and J. C. Steffens. 2000. Genetic control and evolution of sesquiterpene biosynthesis in *Lycopersicon esculentum* and *L. hirsutum*. The Plant Cell 12(11): 2283-2294.

Van der Rest, B., S. Danoun, A.-M. Boudet and S. F. Rochange. 2006. Down-regulation of cinnamoyl-CoA reductase in tomato (*Solanum lycopersicum* L.) induces dramatic changes in soluble phenolic pools. Journal of Experimental Botany 57(6): 1399-1411.

Vidavski, F. S. (2007). Exploitation of resistance genes found in wild tomato species to produce resistant cultivars; pile up of resistant genes. Tomato Yellow Leaf Curl Virus Disease. H. Czosnek. Dordrecht, Springer**:** 363-372.

Viuda-Martos, M., E. Sanchez-Zapata, E. Sayas-Barberá, E. Sendra, J. Pérez-Álvarez and J. Fernández-López. 2014. Tomato and tomato byproducts. Human health benefits of lycopene and its application to meat products: a review. Critical Reviews in Food Science and Nutrition 54(8): 1032-1049.

Walker, J. 1962. Phenolic acids in ‚cloud' and normal tomato fruit wall tissue. Journal of the Science of Food and Agriculture 13(7): 363-367.

Walter, J. 1967. Hereditary resistance to disease in tomato. Annual Review of Phytopathology 5(1): 131-160.

Weaver, R. D., D. J. Evans and A. Luloff. 1992. Pesticide use in tomato production: Consumer concerns and willingness - to - pay. Agribusiness 8(2): 131-142.

Weston, P., D. Johnson, H. Burton and J. Snyder. 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, P. A. and J. C. Snyder. 1990. Thumbtack Bioassay: A Quick Method for Measuring Plant Resistance to Twospotted Spider Mites (Acari: Tetranychidae). Journal of Economic Entomology 83(2): 500-504.

Wu, M.-a. and R. Burrell. 1958. Flavonoid pigments of the tomato (*Lycopersicum esculentum* Mill.). Archives of Biochemistry and Biophysics 74(1): 114-118.

Yahia, E. M., M. Contreras-Padilla and G. Gonzalez-Aguilar. 2001. Ascorbic acid content in relation to ascorbic acid oxidase activity and polyamine content in tomato and bell pepper fruits during development, maturation and senescence. LWT-Food Science and Technology 34(7): 452-457.

Zeist, A. R., A. A. da Silva, J. T. V. de Resende, W. R. Maluf, A. Gabriel, D. S. Zanin and E. P. Guerra (2018). Tomato Breeding for Insect-Pest Resistance. Recent Advances in Tomato Breeding and Production, IntechOpen.

Zhang, W., H. J. McAuslane and D. J. Schuster. 2004. Repellency of ginger oil to *Bemisia argentifolii* (Homoptera: Aleyrodidae) on tomato. Journal of Economic Entomology 97(4): 1310-1318.

VITA

Name:

Mohammad Hasan Salman Ali Dawood

Education:

M.Sc. in Science of Horticulture, University of Ege, College of Agriculture, Izmir, TURKEY 2007.

BC.C in Agricultural Economics, University of Mosul, College of Agriculture and Forests, Mosul, IRAQ 2002.

Awards:

1- Tomato Genotype and the Role of Trichome Secretions on Arthropod Resistance "In recognition for excellence for the presentation. University of Kentucky, (2016).
2- Scholarship from Republic of Iraq Ministry of Higher Education and Scientific Research to obtain PhD degree "2014-2019".

Positions Held:

Graduate Research Assistant, University of Kufa, College of Agriculture and Landscape, Horticulture Science, 2009-2013.

Instructor at University of Kufa, College of Agriculture and Landscape (2009-2013).

Presentations and Workshops of Research:

1- Dawood, M.H.S.A. 2016. Tomato Genotype and the Role of Trichome Secretions on Arthropod Resistance. University of Kentucky, College of Agriculture, Food and Environment, Student Mini-Symposium. Lexington, Kentucky.
2- Dawood, M.H.S.A. 2017. Can UV Absorbance be Used for Quantitation of Zingiberene in Interspecific Tomato Populations? University of Kentucky, College of Agriculture, Food and Environment, Student Mini-Symposium. Lexington, Kentucky.
3- Dawood, M.H.S.A. and Snyder, C.J. 2018. A New Sesquiterpene Alcohol from Wild Tomato Repelled the Two-Spotted Spider Mite, Tetranychus Urticae. Ohio Agricultural Research and Development Center, Wooster, Ohio.
4- Dawood, M.H.S.A. and Snyder, C.J. 2016. Vegetable Tour Horticulture Research Farm Twilight Tour. Tomato Breeding for Mite Resistance. University of Kentucky, College of Agriculture, Food and Environment, Horticultural Research Farm, Lexington, Kentucky.

5- Dawood, M.H.S.A. 2017. Maricopa Field-Based High Throughput Phenotyping Workshop. College of Agriculture, University of Arizona Maricopa Agricultural Center, Maricopa, Arizona.
6- Dawood, M.H.S.A. 2018. Spider Mite Repellency of 7-Epi-Zingiberene, 9-Hydroxy-Zingiberene, and 9-Hydroxy, 10,11-Epoxy-Zingiberene Isolated from Wild Tomato. University of Kentucky, College of Agriculture, Food and Environment, Student Mini-Symposium. Lexington, Kentucky.

Publications and Abstract (During PhD study):

1- Diao, W., J. C. Snyder, S. Wang, J. Liu, B. Pan, G. Guo, W. Ge and M. H. S. A. Dawood (2018). "Genome-Wide Analyses of the NAC Transcription Factor Gene Family in Pepper (Capsicum annuum L.): Chromosome Location, Phylogeny, Structure, Expression Patterns, Cis-Elements in the Promoter, and Interaction Network." *International journal of molecular sciences* 19(4): 1028. https://www.ncbi.nlm.nih.gov/pubmed/29596349
2- Dawood, M.H.S.A. and Snyder, C.J. (2017). A New Sesquiterpene Alcohol from Wild Tomato Repelled the Two-Spotted Spider Mite, Tetranychus Urticae. " Tomato Breeders Roundtable", Ohio State (47) http://tgc.ifas.ufl.edu/TBRT%202018/TBRT_Program_Book_Final.pdf
3- Snyder, C.J., AL-Bayati, A.S and Dawood, M.H.S.A. (2017). Introgression of Zingiberene and Type IV Trichome Density from Solanum. Habrochaites LA2329 into S. lycopersicum – Progress Report "Tomato Breeders Roundtable", Ohio State (47). http://tgc.ifas.ufl.edu/TBRT%202018/TBRT_Program_Book_Final.pdf
4- Mohammad H Dawood, George F. Antonious and John C. Snyder (2018). Phenolic Content of Fruit and Leaves of Interspecific Hybrid Tomatoes. "Fruit and Vegetable Crops Research Report" (PR-757). http://www2.ca.uky.edu/agcomm/pubs/PR/PR757/PR757.pdf
5- Antonious, G., Turley, E., & Dawood, M. (2019). Ascorbic Acid, Sugars, Phenols, and Nitrates Concentrations in Tomato Grown in Animal Manure Amended Soil. *Agriculture*, 9(5), 94. http://www.mdpi.com/2077-0472/9/5/94.
6- Dawood, M. H. and J. C. Snyder. (2019). Results of selection for high yield and zingiberene content of interspecific hybrid tomatoes grown in the open field. Fruit and Vegetable PR-762: 26-28. http://www2.ca.uky.edu/agcomm/pubs/PR/PR762/PR762.pdf#page=28
7- Dawood, M. H. and J.C. Snyder. (2019). Yield, Phenolic, Ascorbic Acid, Soluble Solids and Lycopene of Fruit and Foliar Zingiberene Content of Interspecific Hybrid Tomatoes "Tomato Breeders Roundtable", University of Florida (48).

8- Dawood, M. H., & Snyder, J. C. (2020). The Alcohol and Epoxy Alcohol of Zingiberene, Produced in Trichomes of Wild Tomato, are More Repellent to

Spider Mites than Zingiberene. Frontiers in Plant Science, 11, 35. https://www.frontiersin.org/articles/10.3389/fpls.2020.00035/full