POTENTIAL INSECT DETERRENCE IN A TRI-SPECIES COTTON HYBRID

A Thesis

by

RAVEN S. ALLISON

Submitted to the Office of Graduate and Professional Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Chair of Committee,	David L. Kerns
Co-Chair of Committee,	Charles P. Suh
Committee Member,	Steven Hague
Head of Department,	Phillip Kaufman

May 2021

Major Subject: Entomology

ABSTRACT

Currently, insecticide applications and *Bt* cotton are the primary means for managing insect pests in cotton, but many of these pests have developed resistance to pesticides. Because of resistance, host plant resistance may be a viable solution to prevent significant yield losses. Tri-species cotton hybrids consisting of either *Gossypium hirsutum* L., *G. arboreum*, and *G. armouranium*, or *G. hirsutum*, *G. arboreum*, and *G. turneri* have been reported to contain β-caryophyllene derivatives (12-hydroxy-β-caryophyllene and hydroxy-β-caryophyllene acetate) and demonstrated resistance to nematodes, drought, and heat. Yet, there is a lack of evidence whether these hybrids affect cotton insect pests.

A series of field, greenhouse and laboratory experiments were conducted to assess the impact a tri-species cotton hybrid expressing β-caryophyllene, or its derivatives, β-caryophyllene acetate and alcohol, and β-caryophyllene alcohol, have on tobacco thrips, *Frankliniella fusca* (Hinds), western flower thrips, *Frankliniella occidentalis* (Pergande), cotton aphid, *Aphis gossypii* Glover, fall armyworm, *Spodoptera frugiperda* (J.E. Smith), and bollworm, *Helicoverpa zea* (Boddie). Gas chromatography-mass spectrometry (GC/MS) was initially used to confirm the presence of β-caryophyllene and its derivatives in individual tri-species cotton hybrid plants.

The impact of the tri-species cotton hybrid plants on preventing thrips injury in field trials was inconclusive. At the 1-2 true leaf stage, the tri-species hybrid plants expressing β-caryophyllene and its derivatives (c. acetate and alcohol and c. alcohol) did not differ from *G. hirsutum* or *G. hirsutum* seed treated with the insecticide imidacloprid (IST). At the 2-3 and 3-4 true leaf stages, differences among treatments in the frequency distribution of thrips injury ratings was more evident, and although slight, injury tended to be lower for the tri-species hybrid

than for *G. hirsutum* and *G. hirsutum* (IST), particularly for the two β-caryophyllene derivatives in 2020. Although inconclusive in field trials, in a greenhouse choice test, the tri-species hybrid cotton had less colonization and fewer alate cotton aphids than *G. hirsutum*. Furthermore, in a cotton aphid reproduction study, intrinsic rate of increase, and finite rate of increase was lower for the tri-species hybrid plants expressing β-caryophyllene or its derivative c. alcohol relative to *G. hirsutum*.

When fed leaf tissue, the tri-species cotton hybrid did not affect cotton bollworm larvae development, relative to *G. hirsutum*. However, fall armyworm larvae feeding upon the leaves of the tri-species cotton hybrid expressing c. alcohol, were smaller and did not mature as quickly as larvae feeding on *G. hirsutum* or the tri-species cotton hybrid expressing only β-caryophyllene, or the c. acetate and alcohol derivative. Results suggest that the tri-species cotton hybrid, especially those capable of expressing c. alcohol, negatively impacts, albeit minor, several insect pests of cotton.

DEDICATION

This thesis is dedicated to Jesus Christ.

ACKNOWLEDGEMENTS

I would like to thank my major advisors Drs. David Kerns and Charles Suh for believing in me and providing the opportunity to earn a Master of Science degree. Their guidance and knowledge of row crop entomology provided confidence in my potential and goals for my thesis. Furthermore, I acknowledge them for challenging me and encouraging me to not give up. I would also like to thank the member of my committee, Dr. Steven Hague, for his guidance and continuous advice for my thesis research.

There are many people who have supported me while I attended Texas A&M University. Dr. Alois Bell, from USDA-ARS, provided his extensive knowledge and supplied me with tri-species cotton hybrids. I would like to thank Dr. Jose Perez for his guidance and support throughout this research. Thank you for teaching me how to perform plant chemical extractions and operate the GC/MS. Special thanks to Dr. Fei Yang for supplying the cotton bollworm and fall armyworm populations for this research, and Dr. Sara Duke for assisting with the experimental designs, statistical analyses, and guidance using JMP.

I would like to thank the entomology faculty and staff members for their kindness, encouragement, and understanding during my studies. I would like to acknowledge my colleagues from the TAMU row crop entomology lab, as well as, the summer workers for their assistance in my research work and for their friendship. I would like to present my gratefulness to my family and friends for their constant support, inspiration, and love during my journey.

Thank you to my mentors who have contributed greatly throughout the course of this research. I would like to give a big thanks to Dr. Katherine Parys and Dr. Jeffrey Gore from whom I have learned so much from shadowing the both of you. My joy and passion for

entomology began with Dr. Katherine Parys and the push and drive began with Dr. Jeffrey Gore. I am truly thankful and because of all of you, I persevered!

CONTRIBUTORS AND FUNDING SOURCES

This work was supervised by a thesis committee consisting of Dr. David Kerns of the Department of Entomology from Texas A&M University, Dr. Charles Suh with the Insect Control and Cotton Disease Research Unit at the USDA-ARS in College Station, Texas, and Dr. Steven Hague of the Department of Soil and Crop Science at Texas A&M University.

The experimental designs and data analyses was assisted by Dr. Sara Dukes at USDA-ARS in College Station, Texas.

All other work conducted for this thesis was completed by Raven Allison independently.

The tri-species cotton hybrids used throughout this research were supplied by Dr. Alois Bell at USDA-ARS in College Station, Texas. The cotton bollworm and fall armyworm populations used for this research were supplied by Dr. Fei Yang of the Department of Entomology at Texas A&M University.

Graduate research was supported by Dr. Dionel Avilés ’53 and Dr. James Johnson ’67 Fellowship, Lechner Excellence Fellowship, and Assistantship from Texas A&M University. I would like to acknowledge USDA-ARS in College Station, Texas for their support as well.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER I

INTRODUCTION

Upland cotton, *Gossypium hirsutum* L., is grown throughout much of the world with at least 80% of its textile fiber produced in underdeveloped countries (Deguine et al. 2008). Advances in cotton cultivation and production practices has offered substantial economic growth; however, several factors have limited growth of the cotton industry. One factor includes yield limiting infestations by arthropod pests. Economically important cotton pests are generally considered a threat to fruiting structures such as squares, flowers, and developing bolls, but also include arthropods that consume leaf tissue resulting in defoliation, or sucking pests that remove photosynthates, stress the plant, or produce exudates that contaminate the lint (Deguine et al. 2008).

Crop protection is required for safeguarding against major cotton pests to prevent unacceptable yield loss (Oerke 2004). Before the adoption of transgenic crops, synthetic pesticides were applied extensively to manage insect pests (Naranjo et al. 2008). Approximately 22.5% of total insecticides used worldwide are associated with cotton (Naranjo et al. 2008). Unfortunately, widespread pesticide use has resulted in reduced production and profitability. One aspect that has affected the profitability of cotton is the cost of pesticide applications. Another is the development of resistance to pesticides by many insect pests, which has resulted in the need for additional insecticide applications and associated expense, or lack of ability to manage the pest. Additionally, there is great concern regarding the harmful effects pesticides may have on human health and the environment (Sun et al. 2020). As a result, it is often necessary to develop alternative pest management strategies. Integrated pest management (IPM) is a strategy used

globally in agricultural systems to combat infestations of agricultural pests for a sustainable production in the interest of producers and the environment (Naranjo 2011, Luttrell et al. 2015). IPM programs combine a variety of management tactics such as biological control, plant resistance, and cultural management techniques (Oerke 2004). For example, transgenic crops, particularly those expressing the *Bacillus thuringiensis* (*Bt*) toxins, have been successfully used to manage lepidopoteran pests, and has resulted in a reduction in broad-spectrum insecticide use, and thus the preservation of natural enemies and subsequent reduction in secondary pest outbreaks.

Major Cotton Pests: Thrips, Cotton Aphid, Fall Armyworm, and Cotton Bollworm

Thrips (Thysanoptera: Thripidae)

Cotton seedlings in the southern U.S. are susceptible to injury from several species of thrips, such as tobacco thrips (*Frankiniella fusca* Hinds), flower thrips (*F. tritci* Fitch), western flower thrips (*F. occidentalis* Pergande), onion thrips (*Thrips tabaci* Lindeman), and soybean thrips (*Neohydatothrips variabilis* Beach) (Akbar et al. 2019). Thrips are slender, cigar shaped insects approximately 0.17 cm in length, can reproduce parthenogenetically and insert their eggs into the abaxial side of leaf tissue (Layton and Reed 2002, Reed et al. 2006, Vyvavhare et al. 2018). Developmental stages of thrips consist of egg, two larval forms, a non-feeding prepupal stage, and an adult stage (Allen et al. 2018). Both adults and nymphs rasp plant cells causing damage to leaf tissues and the apical meristem. Adults are usually winged and primarily dispersed by wind, but short, directed flight can allow for colonization of different hosts, such as weeds and crops (Allen et al. 2018).

Thrips infestation in cotton occurs after one or more generations are completed on alternate hosts in the spring (Allen et al. 2018). Thrips belonging to the *Frankliniella* genus are

the most significant pests of cotton production in southeastern and mid-southern regions of the U.S. (Toews et al. 2010). Infestation in cotton can range from minor to severe and if left unmanaged, can often lead to plant death, stunting, loss of apical dominance, and delayed maturity (Kaur et al. 2018). Cotton plants at the seedling stage through the 4 true leaf stage, typically consisting of about 4-5 weeks of cotton development, are most susceptible; however injury varies among species in cotton producing regions (Kaur et al. 2018).

Thrips can cause severe injury and economic loss in cotton across much of the U.S. Cotton Belt (Toews et al. 2010). Recently, a study reported that thrips populations can result in up to 50% yield reduction in cotton (Akbar et al. 2019). However, the effect of thrips injury on cotton yields varies by year and location (Cook et al. 2011, Arnold et al. 2012). In 2014, thrips severely damaged 7 million acres, resulting in a loss of ~150,000 bales in the southern U.S. (Kaur et al. 2018).

IPM strategies for thrips management in cotton include biological control, delayed planting to avoid pest issues, promoting cover crop residue, prophylactic insecticide seed treatments, and post-emergence foliar insecticides. When used alone, most of these tactics are not completely effective. Further, accurate identification of *Frankliniella* spp. influences management or control strategies as not all species respond to the same management practices (Reed et al. 2006). For instance, prophylactic at-planting treatments (in-furrow/liquid insecticides or seed treatments) suppressed populations before injury, but these applications can quickly lead to insecticide resistance in some areas (Cook et al. 2011). Currently, neonicotinoids are a popular class of insecticides that are used to control thrips. Two of the seven chemicals belonging to this class, imidacloprid and thiamethoxam, are primarily used for thrips control in cotton (Darnell-Crumpton et al. 2018). Since there has been documented neonicotinoid

resistance in thrips, it is critical to explore alternative control strategies for thrips management in cotton to complement or reduce insecticide applications (Zhao et al. 1995, Huseth et al. 2016).

Cotton Aphid (Hemiptera: Aphididae)

Aphids have piercing-sucking mouthparts and ingest phloem sap, robbing the plant of photosynthates. Aphids feed on a great many species of plants, including many cultivated crops and ornamentals, and many species of aphids are known to alternate between one or more closely related plants (Goff and Tissot 1932, Isely 1946, Blackman and Eastop 1984, Ebert and Cartwright 1997). Alates (winged forms) are the primary aphid types that disperse between hosts, while apterous (wingless forms) adults are generally the most common form encountered in large numbers in cotton. Immature apterous aphids resemble wingless adults. Additionally, aphids may act as vectors of a number of plant pathogenic viruses (Elmer and Brawner 1975, Allen et al. 2018).

The cotton aphid, *Aphis gossypii* Glover, is a pest of cotton and many other crops and ornamentals worldwide. Cotton aphids feed on cotton sporadically throughout the growing season, and their coloration varies from light yellow to dark green to black (Cattaneo and Kerns 2008, Gore et al. 2013). Cotton aphids are typically found on the underside of leaves, on stems, terminals, fruit bracts, squares and flowers (Cattaneo and Kerns 2008). Allen et al. (2018) described cotton aphids as the most severe aphid species in cotton.

Cotton aphid infestation timing varies regionally and may begin at plant emergence, flowering, or boll development. Large cotton aphid infestations may result in the downward curling of younger leaves, the yellowing and shedding of older leaves, the abscission of squares and small bolls, and the pre-mature opening of bolls. Furthermore, the excretion of honeydew from aphids on open bolls results in decreased fiber quality and yield (Cattaneo and Kerns 2008).

In the southern U.S., farmers greatly depend on insecticides as a major control strategy for aphids (Gore et al. 2013). Neonicotinoids are a popular class of chemicals (acetamiprid, thiamethoxam, and imidacloprid) used to suppress sucking pests, including the cotton aphid. However, Bass et al. (2015) reported neonicotinoid resistance in cotton aphid populations. In addition to chemical control, entomopathogenic fungi, predatory wasps and other natural enemies are important in naturally suppressing or controlling aphid populations. Unfortunately, insecticide applications intended for other insect pests often negatively affect predatory arthropods that feed upon aphids resulting in an increase in aphid populations following insecticide application (Allen et al. 2018). Preservation of beneficial insects is highly desirable, driving a need to find alternative control strategies that are effective against cotton aphids while preserving natural enemies.

Fall Armyworm (Lepidoptera: Noctuidae)

Fall armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae), is a native pest to tropical and subtropical America. Fall armyworm oviposit clusters of eggs (ranging from 10-500) in the lower two-thirds of cotton plant canopy on the underside of the leaf (Hardke et al. 2015). Fall armyworm larvae complete six instars before pupating, but species identification can be difficult for early instars. A distinguishable feature of all fall armyworm larvae is the noticeable inverted “Y” on their heads. In addition, larvae have four circular spots positioned dorsally on the last abdominal segment. Fall armyworm larval feeding depends on its instar. After hatching, neonates typically display gregarious behavior and feed on the leaf adjacent to hatching site (Hardke et al. 2015). Older instars (>2nd instars) migrate throughout the plant canopy and to neighboring plants to feed on fruiting structures (Ali et al. 1989, 1990).

In the southern U.S., the fall armyworm is an economically important pest of many commercial crops, including cotton (Hardke et al. 2015). In cotton, larvae primarily feed on reproductive structures (squares, bolls, and flowers) and infestations can be unpredictable and difficult to detect (Barros et al. 2010, Hardke et al. 2015). This sporadic pest migrates annually from subtropical and tropical regions and infests crops throughout the U.S. Cotton Belt (Hardke et al. 2015). The insect does not diapause and cannot survive severe cold, thus the fall armyworm only overwinters in southern Florida and southern Texas in the U.S. (Luginbill 1928).

Effective control of fall armyworm larva is difficult because of its preference for plant structures in the lower area of the canopy, where penetration of foliar insecticides is greatly reduced (Reed and Smith 2001). Furthermore, mature caterpillars can develop a higher tolerance to insecticides (Yu 1983, Mink and Luttrell 1989). Fall armyworm infestations can result in yield losses ranging from 30% in controlled populations to 90% in uncontrolled populations (de Sousa Ramalho et al. 2011). In addition to insecticide resistance, this highly polyphagous pest is resistant to certain Cry proteins in transgenic *Bt* cotton in the U.S. and other countries, threatening the viability of *Bt* technology in cotton and other crops attacked by the fall armyworm (Yang et al. 2016, Chen et al. 2019).

Cotton Bollworm (Lepidoptera: Noctuidae)

Cotton bollworm, *Helicoverpa zea* (Boddie), is the most polyphagous and economically important pest of several agronomic crops in the southern U.S., such as corn (*Zea mays* L.), sorghum (*Sorghum bicolor* L.), and soybean *(Glycine max* L.) (Luttrell and Jackson 2012). Cotton bollworms are also serious pests of commercial cotton, attacking terminal buds and fruiting structures (squares, flowers, and bolls) (Domingo and Damo 1996). Oviposition by the female moth generally occurs on cotton during blooming and fruiting stages (Javaid et al. 2005).

Eggs are deposited as single pearl-like eggs above or below the leaf in the upper canopy. However, oviposition can also occur in the lower canopy of the plant (Vyavhare et al. 2018), especially when high winds occur. The neonates typically emerge from eggs between three to five days, depending on environmental conditions. Cotton bollworms undergo five or six larval development instars, and mature larvae express cannibalistic behavior. Cotton bollworm adults can disperse readily leading to annual migrations from the southern U.S. up to Canada (Capinera 2000). Cotton bollworms have the ability to overwinter as a pupa for approximately two to three months underneath the soil and emerge when environmental conditions are optimal (Dicke 1939, Hardwick 1965, Reisig et al. 2019). The number of generations per year range from four to six, depending on the cotton production region (Pan et al. 2015).

The cotton bollworm is difficult to target across regional agricultural landscapes, leading to significant yield losses (up to 50%) (Dhawan et al. 1998, Luttrell and Jackson 2012). Over the last three decades, major insect control technologies have been developed to manage lepidopteran pests such as the cotton bollworm (Luttrell 2015). However, resistance to insecticide classes, such as organophosphate and pyrethroids, well as high insecticide costs have made effective and economic pest control difficult (Gore et al. 2001, Hamadin and Chambers 2001, Jacobson et al. 2009). This led to widespread use of *Bt* cotton, which has been one of the most successful methods to manage lepidopteran pests and reduce insecticide applications, but cotton bollworms have developed resistance to certain *Bt* proteins in cotton, including Cry1Ac and Cry2a (Tabashnik et al. 2013, Tabashnik 2015).

The Significance of Host Plant Resistance

Cotton plants are under continuous insect pressure at all stages of production (Leghari et al. 2001). Uncontrollable infestations of various insect pests result in the reduction of yield and

quality of seed cotton (Amer et al. 1999). Given the inherent difficulties in effective management of economic important pests of cotton with conventional chemical control strategies and *Bt* technology in crops, it is important to explore alternative methods of control, such as host plant resistance (HPR) (Hardke et al. 2015).

Host plant resistance has become a useful resource for the discovery of control measures for herbivorous pests. Host plant resistance is centered around the idea of exploring the genetic makeup of plant species in order to exploit favorable mechanisms to suppress pest populations. The development of resistance in plants encompasses an in-depth study of the relationship between the plant and its pests, with the ultimate goal of providing economic benefits in the field (Leghari et al. 2001). The implementation of HPR strategies has been added into integrated pest management systems in response to pest control failures (Kennedy 2008). Several mechanisms of HPR, such as genetic markers or characters (i.e. high gossypol and hairy leaf), play an important role managing arthropod pests (Leghari et al. 2001).

Essential oils in plants, composed of various secondary plant metabolites, are by-products of plant metabolism and are used for protection against numerous insects, mites, and pathogens (Park and Tak 2016). Essential oils can be released as volatile compounds that deter plant-feeding insect pests, or aid as chemical communication between different plants or animals (i.e. allelochemical) (Langenheim 1994). These chemicals may act as antifeedant agents or manipulate oviposition behavior (Akhtar et al. 2012). Pak and Tak (2016) reported that essential oils have been used to manage arthropod pests along with other non-chemical strategies, such as biological and cultural control methods. Ultimately, these strategies aim to reduce pest populations and the amount of synthetic pesticides applied in agroecosystems. Currently, the use

of secondary plant metabolites as a natural self-defense against pests has proven to provide effective control for some plant species (Miresmailli and Isman 2014).

Tri-Species Cotton Hybrid

Gossypium, a genus of Malvaceae family, has forty-five diploid species (2n = 2x =26) and five allotetraploid species (2n = 4x = 52) (Wendel and Albert 1992, Seelanan et al. 1997, Wendel and Cronn 2003). Upland cotton, *G. hirsutum* L. is one of the five allotetraploid cottons that dominates the cotton industry accounting for more than 95% of the cotton production world-wide (Gillham 1995, Chen et al. 2018). *G. hirsutum* in its undomesticated state is predominantly found in Mesoamerica and the Caribbean (Brubaker et al. 1993). In its domesticated state, it is the most productive *Gossypium* crop species. Due to the economic importance of cotton and its limited genetic base, plant breeders have used wild cotton species as a means of increasing useful allelic diversity (Chaundhary et al. 2008).

William et al. (1997) identified β-caryophyllene and its derivatives as the most abundant volatile sesquiterepenes from essential oils in wild cottons, *G. armouranium, G. harknessii and G. turneri*. β-caryophyllene derivatives, 12-hydroxy-β-caryophyllene and 12-hydroxy-β-caryophyllene acetate are unique in that these compounds are only found in these three wild cotton species (Williams et al. 1997). Based on anecdotal observations, the derivatives are believed to reduce insect injury and populations in these wild cotton species. USDA-ARS scientists identified the genetic basis for the expression of these β-caryophyllene derivatives leading to the incorporation of these genes into tri-species cotton hybrids: *G. hirsutum, G. armouranium,* and *G. arboreum* or *G. hirsutum, G. arboreum,* and *G. turneri*. The ultimate goal of the development of these tri-species hybrids is to develop commercially available cotton varieties utilizing traits unique to the tri-species cotton hybrid that impart benefits such as

resistance to insect pests. These traits may be integrated into commercial cotton varieties through traditional plant breeding techniques, or through gene editing techniques such as CRISPR.

Objectives

The objectives of this research were to 1) confirm β-caryophyllene derivatives are expressed in tri-species cotton hybrids using a gas chromatography-mass spectrometry (GC/MS). 2) Assess the response of several economically important cotton insect pests to a tri-species cotton hybrid expressing the β-caryophyllene derivatives (c. acetate and alcohol and c. alcohol) to determine whether these derivatives provide some level of insect deterrence.

CHAPTER II

INFLUENCE OF BETA-CARYOPHYLLENE DERIVATIVES IN A TRI-SPECIES COTTON ON THRIPS INJURY

Introduction

Thrips (Thysanoptera: Thripidae) are a major pest of cotton (*Gossypium hirsutum* L.) in the U.S. Thrips are most injurious to cotton from seedling emergence through the 4 true leaf stage (Kaur et al. 2018). Both adults and nymphs rasp plant cells causing leaf tissue and apical meristem damage (Kaur et al. 2018). Cook et al. (2011) indicated, across all U.S. cotton acreage, that thrips cause yield loss ranging from 0.12% - 0.88%. However, when concentrating on incidences where thrips are severe, unmanaged thrips may result in yield reductions as high as 58% (Herbert et al. 2007). Agronomic impacts of thrips damage range from stunted growth, delayed development, reduction in lint quality, reduced flowering, lower boll set, loss of apical dominance, and plant death (Cook et al. 2011).

Species of thrips that are commonly identified on cotton in the U.S. include tobacco thrips, *Frankliniella fusca* (Hinds); flower thrips, *Frankliniella tritici* (Fitch); onion thrips, *Thrips tabaci* (Lindeman); western flower thrips, *Frankliniella occidentalis* (Pergande); and soybean thrips, *Neohydatothrips variabilis* (Beach) (Leigh et al.1996, Albeldano et al. 2008). Thrips belonging to the genus *Frankliniella* have been documented as the most economically important pests of cotton seedlings throughout the South and Mid-South (Reed et al. 2006).

Currently, insecticide applications are the primary means of managing thrips in cotton. Prophylactic insecticide seed treatments such as acephate, imidacloprid, or thiamethoxam, and foliar insecticide applications have been recommended to control infestations when there are

more than 2-3 thrips per plant (Toews et al. 2010, Kerns et al. 2009, Parker et al. 2009, Bacheler and Reisig 2010). However, resistance of thrips to insecticide seed treatments and foliar sprays has been reported across much of the cotton production regions (Huseth et al. 2016, Stewart et al. 2010). Because thrips have developed resistance against insecticides, an alternative approach to suppress populations and minimize crop damage is critically needed (D'Ambrosio et al. 2020).

Among alternative strategies to insecticides, host plant resistance may be a viable solution. Differences in susceptibility to thrips injury among cotton varieties has been demonstrated, although the mechanism for resistance is not known (Kerns et al. 2017). Plants produce a variety of defensive compounds, such as essential oils, which are volatile, natural, complex compounds characterized by distinct odors (Bakkali et al. 2008). Essential oils in crops can provide plant protection by reducing insect pest appetite or preference (Park and Tak 2016). β-caryophyllene is a naturally occurring sesquiterpene found in essential oils extracted from many plant species, such as cloves (*Syzygium aromaticum*), rosemary (*Salvia rosmarinus*), and oregano (*Origanum vulgare).* Essential oils containing β-caryophyllene have been reported as having repellant properties against important insect pests, such as mosquitoes (Sun et al. 2020). Upland cotton (*Gossypium hirsutum* L.) produces this unique compound in its pigment glands. The aroma of a cotton field is composed primarily of β-caryophyllene and this compound can adversely affect undesirable insects, by acting as a feeding deterrent or attractant to natural enemies of insect pests of cotton (Flint et al. 1979, Opitz et al. 2008).

In addition to β-caryophyllene, its derivatives, 12-hydroxy-β-caryophyllene (c. alcohol) and 12-hydroxy-β-caryophyllene acetate (c. acetate and alcohol) have been hypothesized to reduce injury from insects (C. Suh, personal communication, 2017). William et al. (1997) detected the β-caryophyllene derivatives, c. acetate and alcohol and c. alcohol in three species of

non-cultivated cotton, *G. armourianum, G. harknessii,* and *G. turneri*. These β-caryophyllene derivatives appear to be unique to these three wild cotton species. Subsequently, the genes responsible for the production of these derivatives were identified and a traditional backcross breeding technique was used to develop a tri-species cotton, which includes *G. hirsutum, G. armouranium,* and *G. arboreum* parent lines. The tri-species hybrid expresses β-caryophyllene, as well as c. acetate and alcohol and c. alcohol depending on segregation. Furthermore, the tri-species hybrid has been observed to be more heat, drought, and disease resistant, but its susceptibility to cotton pests is unknown (C. Suh, personal communication, 2017). The objective of this study was to collect and identify thrips species infesting the tri-species hybrid cotton and evaluate if thrips feeding injury is reduced on the hybrid relative to *G. hirsutum* under field conditions.

Materials and Methods

Cotton Source

Two cotton germplasm lines, a tri-species hybrid and 'Tamcot 73' (*G. hirsutum*), were used in this field experiment. The tri-species hybrid was developed at USDA Southern Plains Agricultural Research Center in College Station, Texas. Tamcot 73 (*G. hirsutum*) was developed in the Cotton Improvement Laboratory at Texas A&M University in College Station, Texas (Smith et al. 2011).

Field Study

To evaluate the tri-species hybrid's impact on thrips colonization and injury, field experiments were conducted at the Texas A&M University Field Laboratory in Snook, Texas, in 2019 and 2020. The field-planting included the tri-species hybrid, *G. hirsutum* treated with imidacloprid at 0.375 mg-a.i./seed (Gaucho® 600F, [Bayer CropScience LP, Research Triangle

Park, NC]) henceforth referred to as *G. hirsutum* (IST), and non-treated *G. hirsutum* control plants. All seeds were treated with fungicide azoxystrobin at 0.10-3.75 fl oz/100 lbs seed (Dynasty®, [Syngenta Crop Protection, Greensboro, NC]). Plots were arranged in a randomized complete block design with four replicates. The plots dimensions were 4.06 m wide × 12.19 m in length. Each seed was hand planted 15 cm apart resulting in approximately 80 plants per row. No pesticides were applied to the plants throughout the study. The field was manually cultivated and irrigated as necessary. Although the tri-species hybrid plants express β-caryophyllene, they may or may not express c. acetate and alcohol and c. alcohol depending on genetic segregation. Thus, within the tri-species hybrid plots, the treatment varied among the plants, consisting of plants expressing either β-caryophyllene, c. acetate and alcohol, or c. alcohol.

Expression of the β-caryophyllene derivatives is not evident until about the 5 true leaf stage (Bell and Stipanovic 1977). Because thrips primarily occur and damage cotton before the 5 true leaf stage, I was not able to confirm which plants expressed the various β-caryophyllene derivatives until after thrips injury assessments were made. Thus, each plant among the tri-species hybrid plants was individually labelled for data collection. Once I was able to determine which plant expressed either β-caryophyllene, c. acetate and alcohol, or c. alcohol, I was able to match each thrips injury rating to the appropriate plant treatment.

The presence of β-caryophyllene derivatives in tri-species hybrid plants was elucidated by using gas chromatography mass spectrometry (GC/MS). Each plant was sampled by collecting the terminal leaf at the 5 true leaf stage and storing it in a labeled 2 ml microcentrifuge tube. Samples were immediately placed in a cooler for transport to the laboratory. The samples were subsequently stored in a -20°C freezer until extraction.

Leaf Extraction and Analysis

The β-caryophyllene derivatives of interest were extracted by pulverizing the frozen leaf material using a tube pestle. Dichloromethane (900 µl) was added to the leaf material and the mixture was vortexed for approximately 20 seconds. The mixtures were then sonicated for thirty minutes, followed by a ten-minute centrifugation phase. After centrifugation, a 200 µl pipette was used to remove extracts from tubes. The resulting extracts were then placed into a second set of labeled tubes and concentrated in the fume hood. The leaf material was extracted a second time in a similar manner to ensure maximum extraction of the desired metabolites. Both extracts were pooled and the solution was concentrated for two hours under a fume hood. The concentrated extracts were transferred into labeled GC vials with volume reducing inserts and submitted for GC/MS analysis.

GC/MS analyses were performed using a Shimadzu GCMS-QP2010 Ultra (Shimadzu Scientific Instruments, Columbia, MD) equipped with a Zebron ZB-WAX plus (30 m length × 0.25 mm I.D. × 0.25 µm film thickness; (Phenomenex, Torrence CA, USA), and methods described by Perez et al. (2019). The carrier gas was helium at a flow rate of 1.2 ml min^{-1}. The temperature of the injection port was 220°C and a 1µL sample was injected in a split-less mode. The column temperature program consisted of an initial temperature of 100 °C, held for 5 min followed by a 10 °C/min ramp to 220 °C and held for 10 min. The program was ended with a 40°C/min ramp to an ending temperature of 250 °C. The mass spectrometry conditions were: electron impact ionization (EI); interface temperature of 250 °C; and ion source temperature of 200 °C. Based on the GC/MS results, each tri-species hybrid plant was identified as expressing either β-caryophyllene, c. acetate and alcohol, or c. alcohol.

Thrips Sampling

In 2019 and 2020, thrips injury was assessed on three different sampling dates during each year at the 1-2, 2-3, and 3-4 true leaf stages using an injury rating scale of 1-5 (Cook et al. 2020) (Figure 1), 1 = no thrips damage, 2 = slight damage, 3 = moderate injury, 4 = severe injury, and 5 = severe stunting, terminal bud necrosis, or plant death. The plants sampled and scored included all of the tri-species hybrid cotton, according to the expression of β-caryophyllene and its derivatives, and thirty plants were sampled from the middle rows (rows 2 and 3) of *G. hirsutum* (IST) and *G. hirsutum* treatment groups. Additionally, forty plants from *G. hirsutum* and *G. hirsutum* (IST) plots were destructively sampled at the cotyledon stage to identify thrips composition. Plants were placed into 946 ml plastic jars filled with 70% ethanol. Jar samples were returned to the laboratory where they were filtered using methods described by Burris et al. (1989). I, with assistance from Xanthe Shirley, USDA-APHIS, counted and identified each adult thrips based on criteria outlined by Reed et al. (2006).

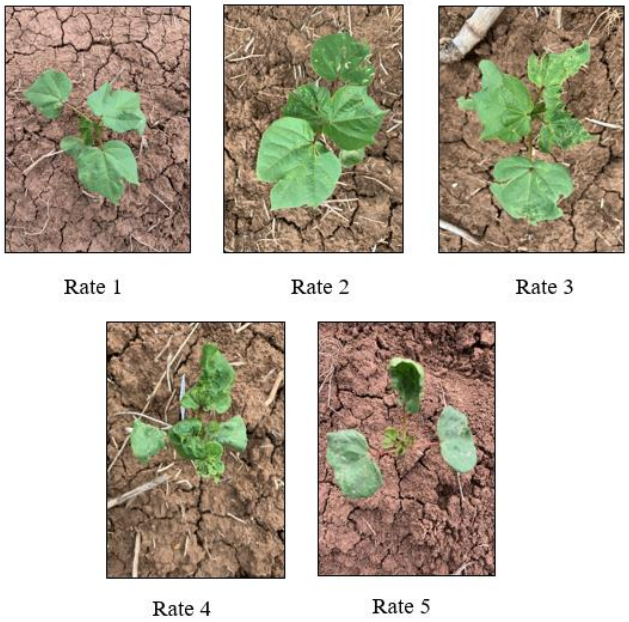

Figure 1. Visual ratings of thrips injury to tri-species cotton. Rating description: 1-no damage, 2-slight damage, 3-moderate injury, 4-severe injury, and 5-severe stunting or plant death.

Statistical Analysis

Injury ratings (response variable) were compared across treatments, *G. hirsutum*, *G. hirsutum* (IST), β-caryophyllene, c. acetate and alcohol, and c. alcohol using categorical frequency analysis for the dates sampled as a repeated measure since individual plant were measured repeatedly. Pearson's Chi-square test ($P \leq 0.05$) was used to determine differences among treatments by date (JMP Pro software, version 15.1.0; SAS Institute Inc., Cary, NC).

Results

A total of 63 (71.6 %) tobacco thrips and 25 (28.4 %) western flower thrips were identified from 40 *G. hirsutum* and *G. hirsutum* (IST) plants in 2019. In 2020, 35 (71.4 %) tobacco thrips and 14 (28.6 %) western flower thrips were identified from forty plants. Across both years, the total number of thrips decreased from 88 to 49 but their general distribution did

not change. A slight decrease in tobacco thrips and a slight increase in western flower thrips was observed from 2019 to 2020.

In 2019, there were no differences in thrips injury among treatments at the 1-2 true leaf stage (Table 1). At the 2-3 true leaf stage, *G. hirsutum* had significantly greater injury than the other treatments (Tables 2). The *G. hirsutum* (IST), β-caryophyllene, c. acetate and alcohol, and c. alcohol treatments sustained similar thrips injury (Table 2). Injury was high across all treatments, with 27.3% of plants scoring ratings ≥3 (Table 3). There were no differences among treatments at the 3-4 true leaf stage (Table 3).

Cotton injury at the 1-2 true leaf stage in 2020 did not differ among treatments (Table 4). However, there were significant differences in injury rating frequencies at the 2-3 and 3-4 true leaf stages (Tables 5 and 6). At the 2-3 true leaf stage, thrips injury was moderate with frequency injury ratings ≥3, averaging 30.7%, 29.6%, 30.1%, 28.1% and 26.1% on *G. hirsutum, G. hirsutum* (IST), β-caryophyllene, c. acetate and alcohol, and c. alcohol, respectively (Table 5). The greatest frequency difference at the 2-3 true leaf stage occurred within the 2 and 3 scaled injury ratings with *G. hirsutum, G. hirsutum* (IST), β-caryophyllene, c. acetate and alcohol, and c. alcohol, averaging 40%, 31.1%, 27.1%, 31.4%, and 30.9%, respectively. The greatest difference in injury frequency among treatments occurred within the 3-injury rating category during the 3-4 true leaf stage. On average, 71.1% of *G. hirsutum* plants possessed moderate injury, which was 20% greater than, that observed for *G. hirsutum* (IST) and 31.5% higher than the lowest value, which was c. alcohol at 39.6%. However, when pooling moderate to severe injury (injury ratings ≥3) differences among treatments were relatively small, averaging 30.73%, 26.63%, 30.10%, 28.23% and 26.07% for *G. hirsutum, G. hirsutum* (IST), β-caryophyllene, c. acetate and alcohol, and c. alcohol, respectively (Table 6).

Table 1. Frequency distribution of thrips injury ratings on 1-2 true leaf stage cotton plants, Snook, TX, 2019.

	Injury rating (1-5)[1]					
Treatment	1	2	3	4	5	≥3[2]
G. hirsutum	0.8	55.0	40.0	3.3	0.8	44.1
G. hirsutum (IST)	4.2	45.8	41.7	8.3	0.0	50.0
β-caryophyllene	5.3	46.9	43.5	4.3	0.0	47.8
c. acetate and alcohol	4.4	43.2	46.1	6.3	0.0	52.4
c. alcohol	5.6	36.6	51.4	6.3	0.0	57.7

P = 0.1686. Differences among treatments based on categorical frequency analysis and Pearson's Chi-square test ($P \leq 0.05$).

[1]1 = no damage, 2 = slight damage, 3 = moderate injury, 4 = severe injury, and 5 = severe stunting, terminal bud necrosis, or plant death (Cook et al. 2020).

[2]Mean injury of 3 or higher; injury that may result in yield loss (Akbar et al. 2018).

Table 2. Frequency distribution of thrips injury ratings on 2-3 true leaf stage cotton plants, Snook, TX, 2019.

	Injury (1-5)[1]					
Treatment	1	2	3	4	5	≥3[2]
G. hirsutum	0.0	18.3	60.8	7.5	13.3	81.6
G. hirsutum (IST)	1.7	22.5	62.5	6.7	6.7	75.9
β-caryophyllene	1.4	25.6	60.4	7.2	5.3	72.9
c. acetate and alcohol	0.0	29.1	63.6	4.9	2.4	70.9
c. alcohol	0.0	21.8	69.7	3.5	4.9	78.1

P = 0.0105. Differences among treatments based on categorical frequency analysis and Pearson's Chi-square test ($P \leq 0.05$).

[1]1 = no damage, 2 = slight damage, 3 = moderate injury, 4 = severe injury, and 5 = severe stunting, terminal bud necrosis, or plant death (Cook et al. 2020).

[2]Mean injury of 3 or higher; injury that may result in yield loss (Akbar et al. 2018).

Table 3. Frequency distribution of thrips injury ratings on 3-4 true leaf stage cotton plants, Snook, TX, 2019.

	Injury (1-5)[1]					
Treatment	1	2	3	4	5	≥3[2]
G. hirsutum	0.0	9.2	48.3	18.3	24.2	90.8
G. hirsutum (IST)	0.0	19.2	48.3	13.3	19.2	80.8
β-caryophyllene	0.0	23.7	48.3	11.6	16.4	76.3
c. acetate and alcohol	0.5	19.9	51.5	14.6	13.6	79.7
c. alcohol	0.0	18.3	52.8	11.3	17.6	81.7

P = 0.1955. Differences among treatments based on categorical frequency analysis and Pearson's Chi-square test ($P \leq 0.05$).

[1]1 = no damage, 2 = slight damage, 3 = moderate injury, 4 = severe injury, and 5 = severe stunting, terminal bud necrosis, or plant death (Cook et al. 2020).

[2]Mean injury of 3 or higher; injury that may result in yield loss (Akbar et al. 2018).

Table 4. Frequency distribution of thrips injury ratings on 1-2 true leaf stage cotton plants, Snook, TX, 2020.

	Injury (1-5)[1]					
Treatment	1	2	3	4	5	≥3[2]
G. hirsutum	0.0	18.3	60.8	7.5	13.3	81.6
G. hirsutum (IST)	1.7	22.5	62.5	6.7	6.7	75.9
β-caryophyllene	1.4	25.6	60.4	7.2	5.3	72.9
c. acetate and alcohol	0.0	29.1	63.6	4.9	2.4	70.9
c. alcohol	0.0	21.8	69.7	3.5	4.9	78.1

P = 0.07834. Differences among treatments based on categorical frequency analysis and Pearson's Chi-square test ($P \leq 0.05$).

[1]1 = no damage, 2 = slight damage, 3 = moderate injury, 4 = severe injury, and 5 = severe stunting, terminal bud necrosis, or plant death (Cook et al. 2020).

[2]Mean injury of 3 or higher; injury that may result in yield loss (Akbar et al. 2018).

Table 5. Frequency distribution of thrips injury ratings on 2-3 true leaf stage cotton plants, Snook, TX, 2020.

	Injury (1-5)[1]					
Treatment	1	2	3	4	5	≥3[2]
G. hirsutum	0.0	7.8	72.2	20.0	0.0	92.2
G. hirsutum (IST)	0.0	11.1	51.1	37.8	0.0	88.9
β-caryophyllene	0.0	9.8	44.4	45.9	0.0	90.3
c. acetate and alcohol	0.0	15.4	47.4	35.8	1.2	84.4
c. alcohol	0.0	21.6	40.2	38.2	0.0	78.4

P = 0.0003. Differences among treatments based on categorical frequency analysis and Pearson's Chi-square test ($P \leq 0.05$).
[1]1 = no damage, 2 = slight damage, 3 = moderate injury, 4 = severe injury, and 5 = severe stunting, terminal bud necrosis, or plant death (Cook et al. 2020).
[2]Mean injury of 3 or higher; injury that may result in yield loss (Akbar et al. 2018).

Table 6. Frequency distribution of thrips injury ratings on 3-4 true leaf stage cotton plants, Snook, TX, 2020.

	Injury (1-5)[1]					
Treatment	1	2	3	4	5	≥3[2]
G. hirsutum	0.0	7.8	71.1	21.1	0.0	92.2
G. hirsutum (IST)	0.0	11.1	51.1	37.8	0.0	88.9
β-caryophyllene	0.0	9.8	47.4	42.9	0.0	90.3
c. acetate and alcohol	0.0	15.4	46.2	37.3	1.2	84.7
c. alcohol	0.0	21.8	39.6	38.6	0.0	78.2

P = 0.0009. Differences among treatments based on categorical frequency analysis and Pearson's Chi-square test ($P \leq 0.05$).
[1]1 = no damage, 2 = slight damage, 3 = moderate injury, 4 = severe injury, and 5 = severe stunting, terminal bud necrosis, or plant death (Cook et al. 2020).
[2]Mean injury of 3 or higher; injury that may result in yield loss (Akbar et al. 2018).

Discussion

Given that β-caryophyllene is found in all members of the genus *Gossypium*, and that the derivatives of β-caryophyllene have been demonstrated to adversely affect the growth and development of some bacteria and fungi (Huang et al. 2012, Sun et al. 2018), it was critical to explore if these compound could possibly prevent thrips injury. β-caryophyllene has been shown to neither attract nor repel the western flower thrips, *Frankliniella occidentalis* (Koschier et al.

2002), but its impact on *F. fusca*, or the impact of the β-caryophyllene derivatives c. acetate and alcohol and c. alcohol have not been evaluated. β-caryophyllene has been documented to serve as a volatile defensive compound against herbivores (Langenheim 1994). Langenheim (1994) describes this compound to have adverse effects on the growth and survival of insects feeding on cotton.

Relative to *G. hirsutum* and *G. hirsutum* (IST), β-caryophyllene and its derivatives (c. acetate and alcohol and c. alcohol) in our tri-species cotton hybrid had minimal impact on preventing thrips injury. Thrips injury at the 1-2 true leaf stage was indistinguishable among treatments during both 2019 and 2020. β-caryophyllene and its derivatives are expressed in the internal glands of cotton leaves throughout the genus *Gossypium*. The density of the glands can vary among species, and will be less dense in underdeveloped apical tissue (Stipanovic et al. 1977). Thus, at the 1-2 true leaf stage there may not have been sufficient internal glands producing β-caryophyllene and its derivatives to deter thrips colonization and/or feeding. However, thrips injury on the positive control, *G. hirsutum* (IST), which should be protected from thrips feeding injury by the imidiacloprid seed treatment, was not appreciably lower than that observed on the negative control, *G. hirsutum*. Thus, the lack of differences at the 1-2 true leaf stage was most likely due to low thrips populations. By the 2-3 true leaf stage, imidacloprid seed treatment usually ceases to offer acceptable thrips control (Cook et al. 2011). In our study, differences among treatments in the frequency distribution of thrips injury ratings were more evident at the 2-3 and 3-4 true leaf stages, and although slight, injury tended to be lower for the tri-species hybrid than for *G. hirsutum* and *G. hirsutum* (IST), particularly for the two β-caryophyllene derivatives in 2020. Thrips injury severity distribution was greater in 2020 than 2019, so the reason more profound differences were not observed under lower injury potential in

2019 remains uncertain, but may have been due to cooler growing conditions in 2020. Although, the current study suggests that the β-caryophyllene derivatives, c. acetate and alcohol and c. alcohol, found in the tri-species hybrid will only minimally protect the plant from thrips feeding injury, it might still be a viable protection strategy for other insects and/or when used with other plant protection strategies.

CHAPTER III

THE EFFECT OF BETA-CARYOPHYLLENE DERIVATIVES IN A TRI-SPECIES COTTON HYBRID ON COTTON APHID INFESTATION AND POPULATION GOWTH POTENTIAL

Introduction

The cotton aphid, *Aphis gossypii* Glover, is an occasional pest of cotton, *Gossypium hirsutum* L., but can be a major threat to cotton production worldwide (Gore et al. 2013, Chen et al. 2018). Several aphid species are known to infest cotton in the U.S., including cowpea aphid, *Aphis craccivora* Koch; the bean aphid, *Aphis fabae* Scopoli; the corn root aphid, *Anuraphis maidiradicis* Forbes; the potato aphid, *Macrosiphum euphorbiae* (Thomas); the green peach aphid, *Myzus persicae* (Sulzer); the rice root aphid, *Rhopalosiphum rufiadominale* (Sasaki); and the bean root aphid; *Smynthurodes betae* Westwood; yet, the cotton aphid is the most common species to infest U.S. cotton (Goff and Tissot 1932, Leigh et al. 1996, Stoetzel et al. 1996). Generally, cotton aphid populations increase during the seedling and pre-bloom stages, but are often most abundant at peak bloom (Slosser et al. 1989, Kerns and Gaylor 1993b). Cotton aphid can cause economic loss in cotton by acting as a stress factor from feeding upon the plant's phloem sap (Kerns et al. 2015). The excretion of honeydew from aphids on open bolls results in decreased fiber quality and yield (Cattaneo and Kerns 2008). Cotton aphids may also affect cotton by vectoring cotton leafroll dwarf virus (Avelar et al. 2019).

Fuchs and Minzenmayer (1995) and Layton et al. (1996) reported that more than fifty aphids per leaf caused yield reductions between 167 and 244 kg of lint per hectare. An economic threshold developed for the southwestern U.S. reported that the mean economic threshold across varied control cost, market price, and yield potential were 110 ± 48, 70 ± 31, 45 ± 19, and 29 ±

13 aphids per leaf at lead times of 1, 3, 5, and 7 d, respectively (Kerns et al. 2015). Population densities tend to increase due to insecticide resistance and reduction of natural enemies as a consequence of pesticide applications for other pests (Kerns and Gaylor 1993a, b). Furthermore, it has been confirmed that some insecticides may stimulate aphid reproduction (Dunnam and Clark 1941, Slosser et al. 1989, Kerns and Gaylor 1993a, b, Kidd et al. 1996, Kidd and Rummel 1997). Because of the difficulties achieving long-term sustainable control of cotton aphid, alternative management strategies are needed.

Host plant resistance (HPR) has become an effective approach by offering natural defense to crops to maintain sustainability (Kennedy 2008), while preserving natural enemy populations. Plants produce defensive compounds, such as essential oils, which are composed of volatile sesquiterpenes characterized by distinct odors (Bakkali et al. 2008). β-caryophyllene is a sesquiterpene found in essential oils produced by a variety plants, including upland cotton. These essential oils are produced in the pigment glands of cotton. β-caryophyllene has been reported to act as a feeding deterrent for insect pests or attractant for their natural enemies (Flint et al. 1979, Opitz et al. 2008). β-caryophyllene derivatives, 12-hydroxycaryophyllene (caryophyllene alcohol) and 12-hydroxycaryophyllene acetate (caryophyllene acetate), or the combination of the two, are natural defensive compounds found in wild cotton species *G. armouranium*, *G. harknessii*, and *G. turneri*. These wild cottons emit volatiles composed of caryophyllene derivatives that are speculated to reduce injury from a wide group of insects (William et al. 1997, C. Suh, personal communication, 2017). Tri-species cotton hybrids (*G. hirsutum, G. arboreum,* and *G. armouranium*) and (*G. hirsutum*, *G. arboreum,* and *G. turneri*) were developed using traditional breeding techniques after identifying the genes responsible for the production of these caryophyllene derivatives. The tri-species cotton hybrids were developed to not only tolerate

harsh field conditions and provide some resistance to nematodes and diseases, but to also affect insect pests.

The objective of this study was to evaluate cotton aphid population density, plant selection, and population growth potential on a tri-species hybrids expressing β-caryophyllene derivatives under field, greenhouse, and laboratory conditions.

Materials and Methods

Cotton Source

Two cotton germplasm lines, tri-species cotton and 'Tamcot 73' (*G. hirsutum*), were used in field, laboratory, and greenhouse experiments. The tri-species cotton hybrid was developed at USDA Southern Plains Agricultural Research Center in College Station, Texas. Cotton variety Tamcot 73 (*G. hirsutum*) was developed in the Cotton Improvement Laboratory at Texas A&M University in College Station, Texas (Smith et al. 2011).

β-Caryophyllene Derivative Characterization

The presence of β-caryophyllene derivatives in tri-species hybrid plants was elucidated by using gas chromatography mass spectrometry (GC/MS). Each plant was sampled by collecting the terminal leaf at the 5 true leaf stage and storing it in a labeled 2 ml microcentrifuge tube. Samples were immediately placed in a cooler for transport to the laboratory. The samples were subsequently stored in a -20°C freezer until extraction. Based on the GC/MS results, each tri-species hybrid plant was identified as expressing either β-caryophyllene, c. acetate and alcohol, or c. alcohol, and labelled accordingly in the field, laboratory, and greenhouse experiments.

Field Study

To evaluate insect deterrence, field experiments were conducted at the Texas A&M University Field Laboratory in Snook, Texas, in 2019 and 2020. The field-planting consisted of the tri-species hybrid, *G. hirsutum* treated with imidacloprid at 0.375 mg-a.i./seed (Gaucho® 600F, [Bayer CropScience LP, Research Triangle Park, NC]) (henceforth referred to as *G. hirsutum* (IST)), and non-treated *G. hirsutum* control plants. All seeds were treated with fungicide azoxystrobin at 0.10-3.75 fl oz/100 lbs seed (Dynasty®, [Syngenta Crop Protection, Greensboro, NC]). Plots were arranged in a randomized complete block design with four replicates. The plots dimensions were 4.06 m wide × 12.19 m in length. Each seed was hand planted 15 cm apart resulting in approximately 80 plants per row. No pesticides were applied to the plants throughout the study. The field was manually cultivated and irrigated as needed. Although the tri-species hybrid plants express β-caryophyllene, they may or may not express c. acetate and alcohol and c. alcohol, depending on genetic segregation. Thus, within the tri-species hybrid plots, the treatment varied among the plants, consisting of plants expressing β-caryophyllene, c. acetate and alcohol and c. alcohol. Typically, expression of the β-caryophyllene derivatives is not evident until about the 5 true leaf stage (Bell and Stipanovic 1977). As described in chapter II, I was not able to confirm which plants expressed the various β-caryophyllene derivatives until after aphid plant infestation assessments were made. Thus, each plant among the tri-species hybrid plants was individually labelled for data collection. Once I was able to determine which plant expressed either β-caryophyllene, c. acetate and alcohol, or c. alcohol, I was able to match each aphid count to the appropriate plant treatment.

Thirty plants were sampled from the middle rows (rows 2 and 3) of *G. hirsutum* (IST) and *G. hirsutum* treatment groups. All plants were sampled within the tri-species hybrid treatment groups. Aphids were sampled on June 6th, June 11th, June 16th, and June 21st in 2019 and on May 27th, June 1st, June 6th, and June 11th in 2020. Aphid populations were evaluated within each plot by estimating the number present on a single first-expanded leaf in the upper plant canopy at the first sample date. These leaves were marked and subsequent counts were taken from the same leaf.

A mixed model analysis was used to analyze the mean aphid population density per leaf for each treatment in JMP (JMP Pro14 software, version 14.1.0 SAS Institute Inc., Cary, NC). Treatments, *G. hirsutum*, *G. hirsutum* (IST), β-caryophyllene, c. acetate and alcohol, and c. alcohol, date, and treatment by date were set as fixed effects and replication [treatment] were set as the random effect in a randomized complete block design of four replications. Treatments were separated by date using Tukey-Kramer HSD, $P \leq 0.05$. Using these averages, cumulative aphid days (CAD) was calculated using Brewer et al. (2017) formula, $\Sigma[(x_i + x_{i-1})/2] \times (t_i - t_{i-1})$, where $(x_i + x_{i-1})/2$ is the aphid density x between progressive sampling periods i, and $(t_i - t_{i-1})$ is the number of days t between sampling periods.

Choice Test Study

A single apterous cotton aphid adult was collected from an established laboratory colony reared on pesticide free *G. hirsutum* grown in a USDA-ARS greenhouse. This colony was isolated and maintained on *G. hirsutum* in a growth chamber at 26 ± 1˚C and a photoperiod of 13:11 (L:D) h. Treatments were arranged in a completely randomized design and replicated ten times. Treatments consisted of *G. hirsutum* and the tri-species hybrid expressing either β-caryophyllene, c. acetate and alcohol, and c. alcohol. Two seeds were planted in 2-gallon nursery

pots (Horticulture Source, Vancouver, Washington) filled with standard potting soil and watered as necessary. Fifteen plants were planted for each treatment. Once the plants reached the 1-2 true leaf stage, one of the plants was removed leaving a healthy plant. Each treatment plant was placed in a Bug Dorm (MegaView Science Co., Taichung, Taiwan) with each of the four treatment plants randomly placed in each corner of the cage and oriented such that the plant did not contact the treatment plants. The caged plants were maintained in the greenhouse at 22.2˚± 3°C with a photoperiod of 12:12 h (L:D). At the 8-9 true leaf stage, a cotton aphid-infested *G. hirsutum* plant from the growth chamber was placed in the center of the Bug Dorm, with approximately equal distance to each of the four treatment plants. All but ~100 aphids were removed from each infested plant.

Cotton aphids were allowed to disperse from the centered infested plant onto the treatment plants, and populations were allowed to develop for seven days. After which, the number of cotton aphids on the first fully expanded and 7th-node leaves were counted on each of the treatment plants.

The numbers of alate, apterous, and total aphids were analyzed separately using JMP (JMP Pro14 software, version 14.1.0 SAS Institute Inc., Cary, NC). A mixed model ANOVA was used to analyze all data. *G. hirsutum*, β-caryophyllene, c. acetate and alcohol, and c. alcohol were set as the fixed effects and trials set as random effects in each model. Treatments were separated using Tukey-Kramer HSD, $P \leq 0.05$.

Life Table Study

A life table study of the cotton aphid, based on methods described by Neupane et al. (2019), was conducted to determine aphid population growth potential and aphid longevity. Cotton aphids originating from a single apterous adult, as previously described, were utilized for this study. The life table study was conducted in a growth chamber maintained at 26 ± 1˚C and a photoperiod of 13:11 (L:D) h. Individual treatments (*G. hirsutum*, β-caryophyllene, c. acetate and alcohol, and c. alcohol) were assigned eight plant replicates in a completely randomized design. Two seeds from each treatment were planted into 15-cm diameter plastic pots filled with standard potting soil and watered as needed. At the 1-2 true leaf stage, one of the plants was removed leaving a single healthy plant per pot. Clip cages were constructed using the methods described in Neupane et al. (2019), to confine cotton aphids on the abaxial side of individual leaves. At the 4 true leaf stage, 2-3 apterous adults were randomly selected from a laboratory colony and carefully positioned on the first fully expanded leaf in the terminal of individual plants using a fine paint brush. Upon reproduction, all aphids but one newborn nymph, were removed from the plant. After nymphs reached adulthood, their offspring were counted and removed daily. The monitoring and recording of aphid pre-reproductive period and fecundity continued until the death of each respective adults.

Aphid life table parameters (pre-reproductive period, intrinsic rate of increase, doubling time, finite rate of increase, and longevity) were calculated according to parameters and formulas described by Neupane et al. (2019). Pre-reproductive period is the time, in days, required to reach reproductive maturity. Intrinsic rate of increase is described as the rate of increase per individual. Finite rate of increase is the rate of increase for each individual per unit of time, and doubling time is the amount of time necessary for a population to double in size. Lastly,

longevity is the time in days from birth until death. The intrinsic rate (r_m) for aphids on individual treatments was calculated using the formula $r_m = (\log (R_0))/d$, where R_0 is the total number of nymphs produced by each female adult within its lifetime and d is defined as the pre-reproductive period (Birch 1948). Formulas for calculating finite rate of increase ($\lambda F = e^{rm}$) and doubling time ($DT = \ln (2)/r_m$) were also derived from Birch (1948).

All life table statistic data were analyzed using a one-way analysis of variance (ANOVA) with *G. hirsutum*, β-caryophyllene, c. acetate and alcohol, and c. alcohol set as the treatments in a completely randomized design with eight replications per treatment(JMP Pro14 software, version 14.1.0 SAS Institute Inc., Cary, NC). Means were separated using Tukey-Kramer HSD, $P \leq 0.05$.

Results

Field Study

In 2019, numbers of aphids were not significantly different among treatments, but non-treated *G. hirsutum* plants had numerically more aphids than *G. hirsutum* (IST), β-caryophllyene, c. acetate and alcohol, and c. alcohol plants across all four sampling dates, 6-21 June (Table 7). In 2020, there were no differences in aphid infestation among the treatments until the last sampling date, 11 June, when the imidacloprid-treated *G. hirsutum* had fewer aphids than *G. hirsutum*, but did not differ from any of the tri-species hybrid β-caryophyllene derivatives (Table 8). Based on total cumulative aphid days, there were no significant differences detected among treatments (df = 4, $F = 2.19$, $P > 0.12$) and treatment by date interaction in 2019 (df = 3, $F = 0.93$, $P > 0.52$) (Figure 2). However, significant differences were detected between treatments (df = 4, $F = 8.96$, $P = 0.01$) and treatment by date (df = 3, $F = 6.11$, $P < 0.02$) in 2020 (Figure 3). On 1 June 2020 the *G. hirsutum* (IST), c. acetate and alcohol, and c. alcohol had fewer

cumulative aphid days than *G. hirsutum.* But *G. hirsutum* and *G. hirsutum* (IST) were the only two treatments to differ from each other on 6 and 11 June. β-caryophyllene, c. acetate and alcohol, and c. alcohol treatments never differed from each other across all collection dates during either year.

Choice Test Study

Aphid infestation was significantly higher on *G. hirsutum* (98.44) relative to β-caryophllyene (34.58 aphids per leaf), c. acetate and alcohol (63.56 aphids per leaf), and c. alcohol (28.35 aphids per leaf) treatments (Figure 4). However, β-caryophllyene and c. alcohol treatments had significantly fewer aphids than c. acetate and alcohol treatment. Similarly, the mean numbers of alates were most abundant on *G. hirsutum* (5.64) compared to β-caryophllyene (1.80), c. acetate and alcohol (3.83), and c. alcohol (1.59) treatments (Figure 5). There were significantly more apterous aphids feeding upon control plants (92.80) than on the tri-species hybrids (Figure 6). Aphid population development was significantly reduced on β-caryophllyene (32.77) and c. alcohol (26.76) treatments than c. acetate and alcohol (59.72).

Life Table Study

The average intrinsic rate of increase of cotton aphid was higher on *G. hirusutum* relative to tri-species hybrid treatments (Figure 7). However, the intrinsic rate of increase of cotton aphid was significantly lower when exposed to tri-species hybrids, β-caryophyllene and c. alcohol. C. alcohol had an adverse effect on the average aphid population doubling time compared to *G. hirsutum* (Figure 8). The aphid population doubling time was not significantly different among tri-species cotton hybrids. The average finite rate of increase of cotton aphid was significantly lower on β-caryophyllene and c. alcohol than on *G. hirsutum* (Figure 9). The cotton aphid life

span and generation time were not significantly influenced by β-caryophllyene and its derivatives (Figure 10 and 11).

Table 7. Mean aphids per first-expanded terminal leaf by date, Snook, TX, 2019.

	Date			
Treatment	June 6th	June 11th	June 16th	June 21st
G. hirsutum	18.54 ± 3.60 a	9.65 ± 5.12 a	12.00 ± 7.09 a	15.48 ± 7.34 a
G. hirsutum (IST)	6.71 ± 1.21 a	3.71 ± 1.54 a	4.25 ± 1.81 a	9.48 ± 5.07 a
β-caryophyllene	2.70 ± 0.92 a	4.46 ± 2.44 a	5.55 ± 2.62 a	6.54 ± 3.29 a
c. acetate and alcohol	4.37 ± 1.13 a	3.95 ± 2.37 a	6.89 ± 3.93 a	6.77 ± 3.37 a
c. alcohol	3.85 ± 2.36 a	2.38 ± 1.52 a	6.25 ± 3.85 a	5.96 ± 3.82 a

Means followed by the same letter are not significantly different. Means were separated using a two-way ANOVA (Tukey Kramer HSD, $P \leq 0.05$).

Table 8. Mean aphids per first-expanded terminal leaf by date, Snook, TX, 2020.

	Date			
Treatment	May 27th	June 1st	June 6th	June 11th
G. hirsutum	3.51 ± 1.71 a	9.79 ± 4.84 a	7.07 ± 2.20 a	16.00 ± 5.39 a
G. hirsutum (IST)	0.08 ± 0.08 a	0.39 ± 0.39 a	1.06 ± 0.63 a	0.72 ± 0.72 b
β-caryophyllene	5.09 ± 2.20 a	1.81 ± 0.12 a	7.36 ± 3.59 a	3.38 ± 1.87 ab
c. acetate and alcohol	1.83 ± 0.09 a	1.83 ± 0.03 a	6.25 ± 3.07 a	5.41 ± 1.79 ab
c. alcohol	1.78 ± 0.09 a	1.80 ± 0.05 a	5.11 ± 1.44 a	4.55 ± 2.80 ab

Means followed by the same letter are not significantly different. Means were separated using a two-way ANOVA (Tukey Kramer HSD, $P \leq 0.05$).

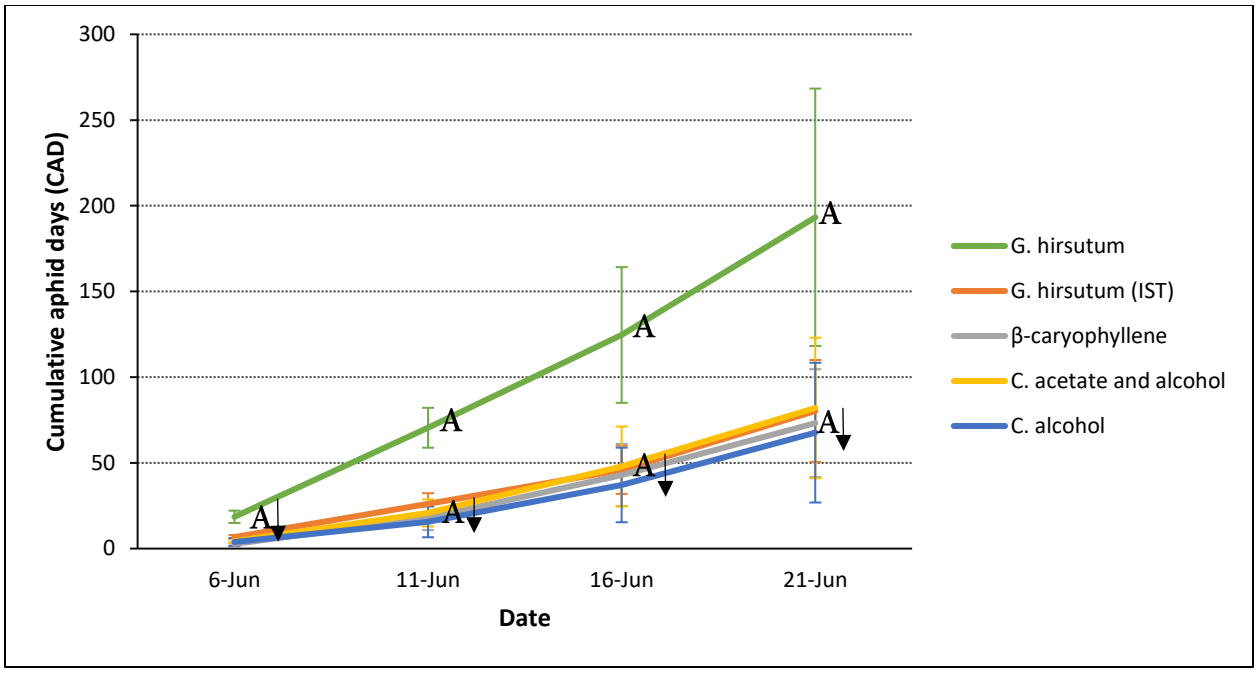

Figure 2. Cumulative aphid days by date during 2019. Means followed by the same letter are not significantly different based on a two-way ANOVA (Tukey Kramer HSD, $P \leq 0.05$).

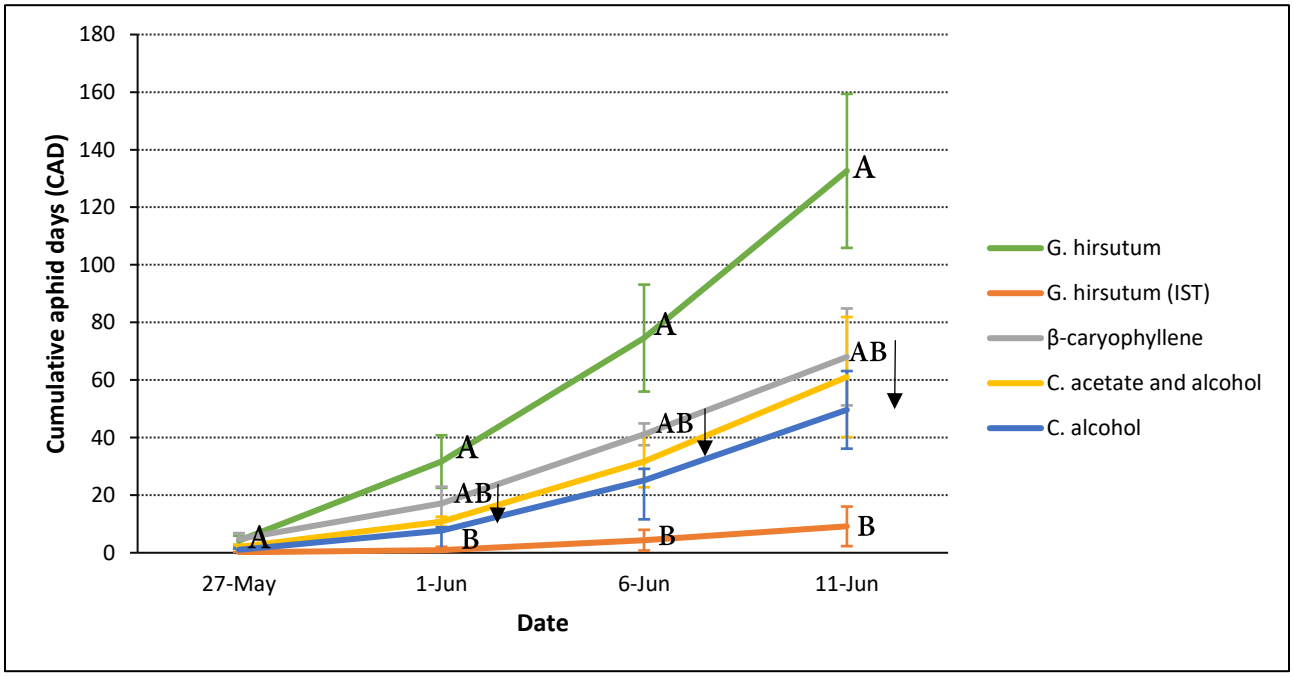

Figure 3. Cumulative aphid days by date during 2020. Means followed by the same letter are not significantly different based on a two-way ANOVA (Tukey Kramer HSD, $P \leq 0.05$).

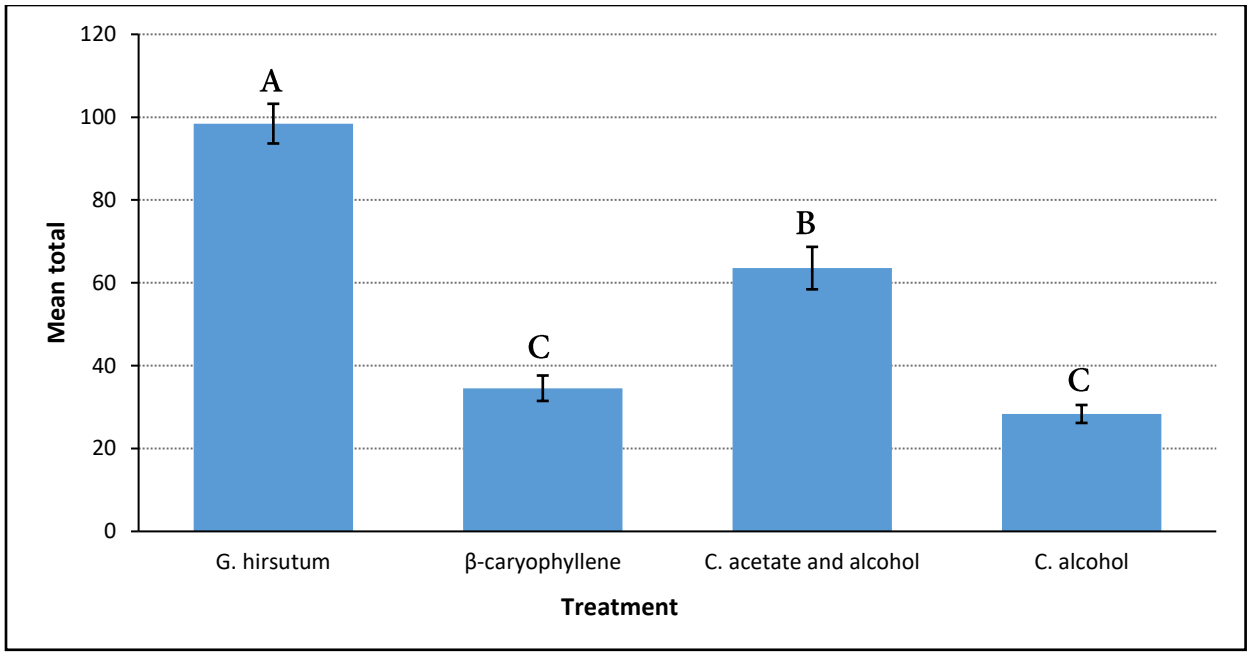

Figure 4. Mean total cotton aphids on individual treatments. Means followed by the same letter are not significantly different based on a one-way ANOVA (Tukey Kramer HSD, $P \leq 0.05$).

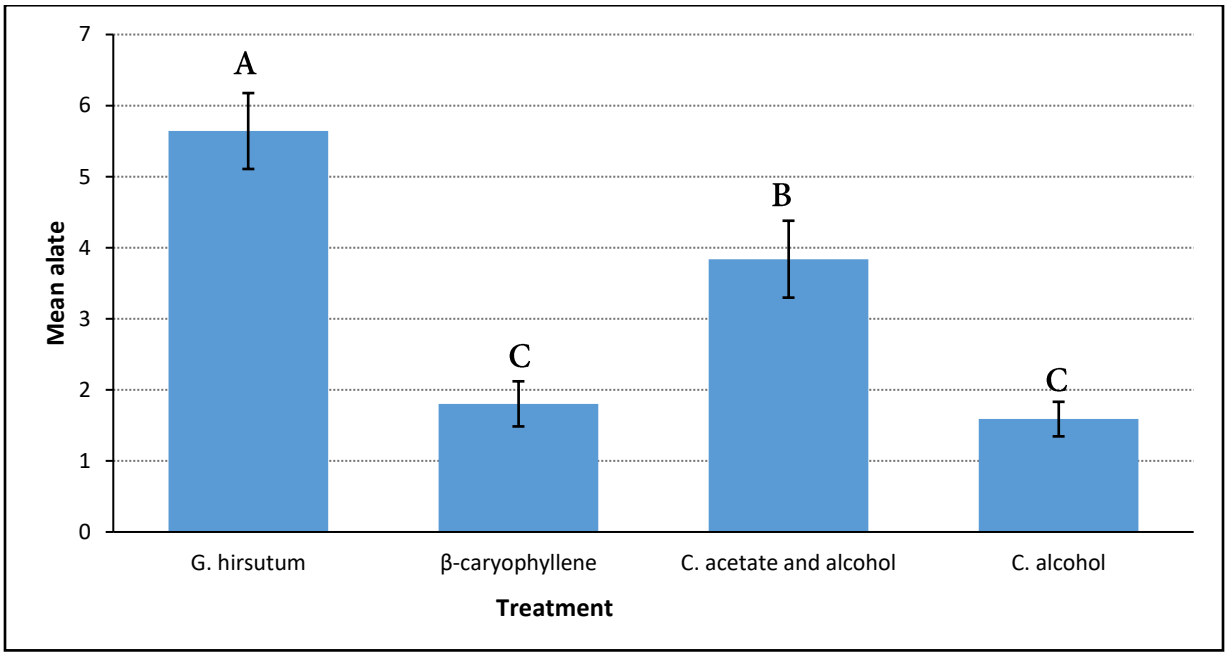

Figure 5. Mean alate cotton aphids on individual treatments. Means followed by the same letter are not significantly different based on a one-way ANOVA (Tukey Kramer HSD, $P \leq 0.05$).

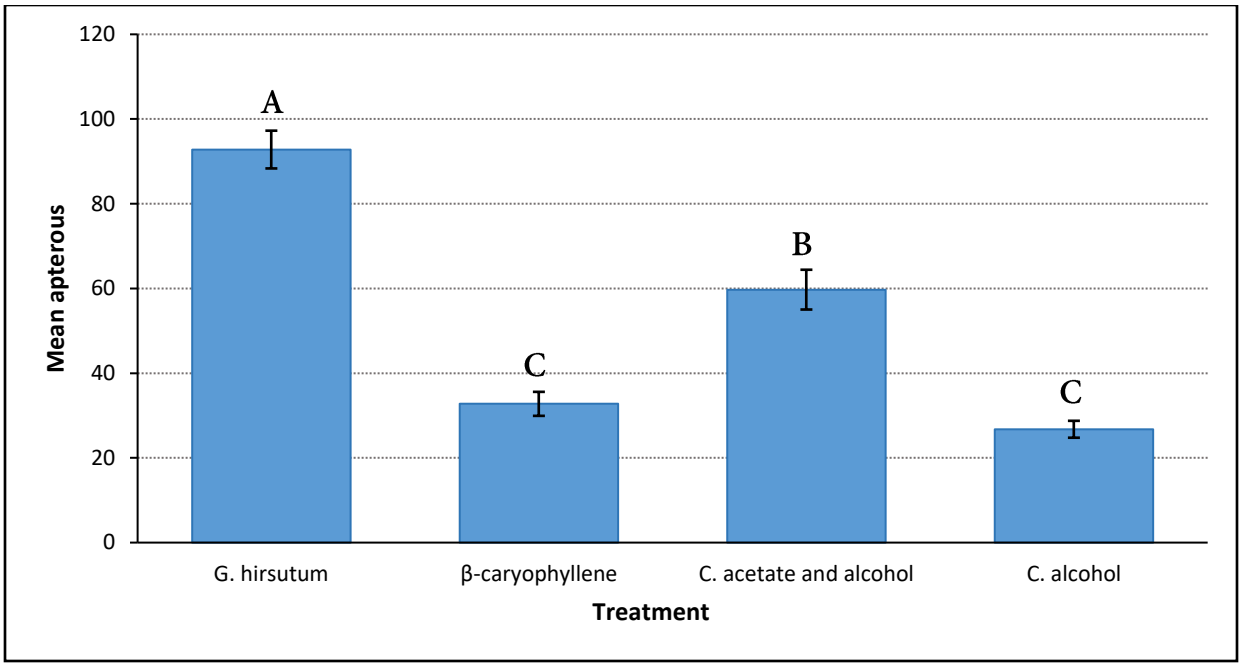

Figure 6. Mean apterous cotton aphids on individual treatments. Means followed by the same letter are not significantly different based on a one-way ANOVA (Tukey Kramer HSD, $P \leq 0.05$).

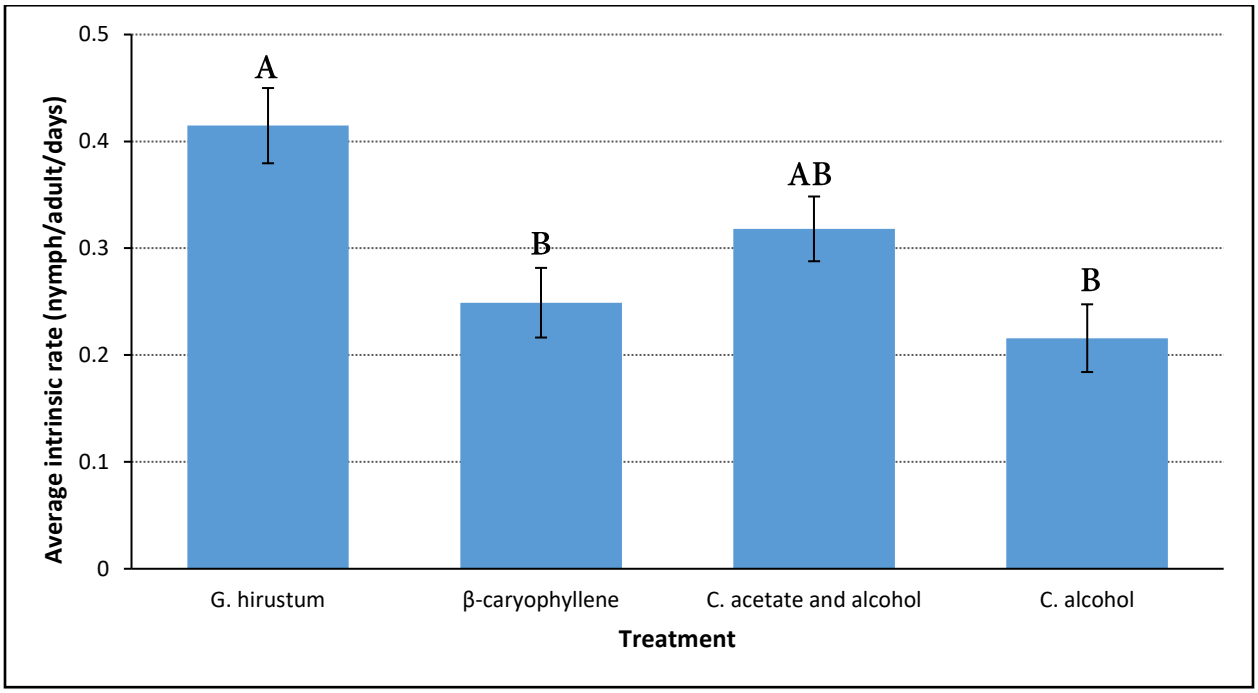

Figure 7. Average intrinsic rate of cotton aphids expressed as nymphs per adult per day on individual treatments. Means followed by the same letter are not significantly different based on a one-way ANOVA (Tukey Kramer HSD, $P \leq 0.05$).

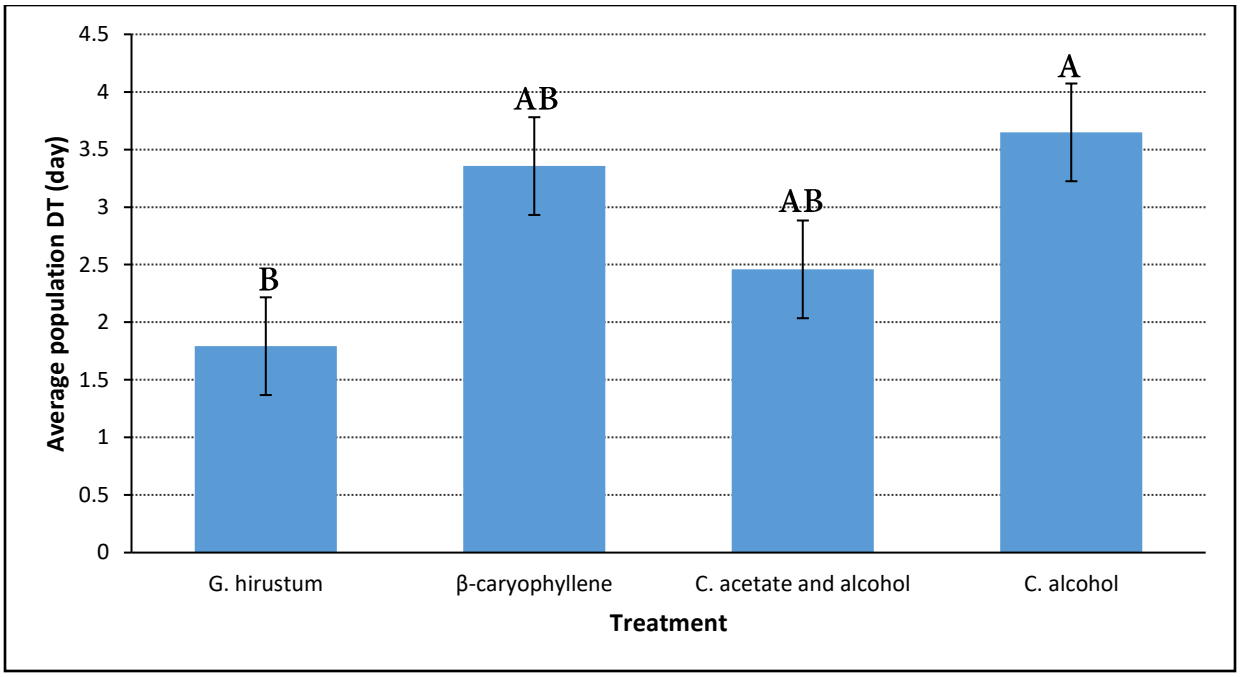

Figure 8. Average population doubling time of cotton aphids on individual treatments. Means followed by the same letter are not significantly different based on a one-way ANOVA (Tukey Kramer HSD, $P \leq 0.05$).

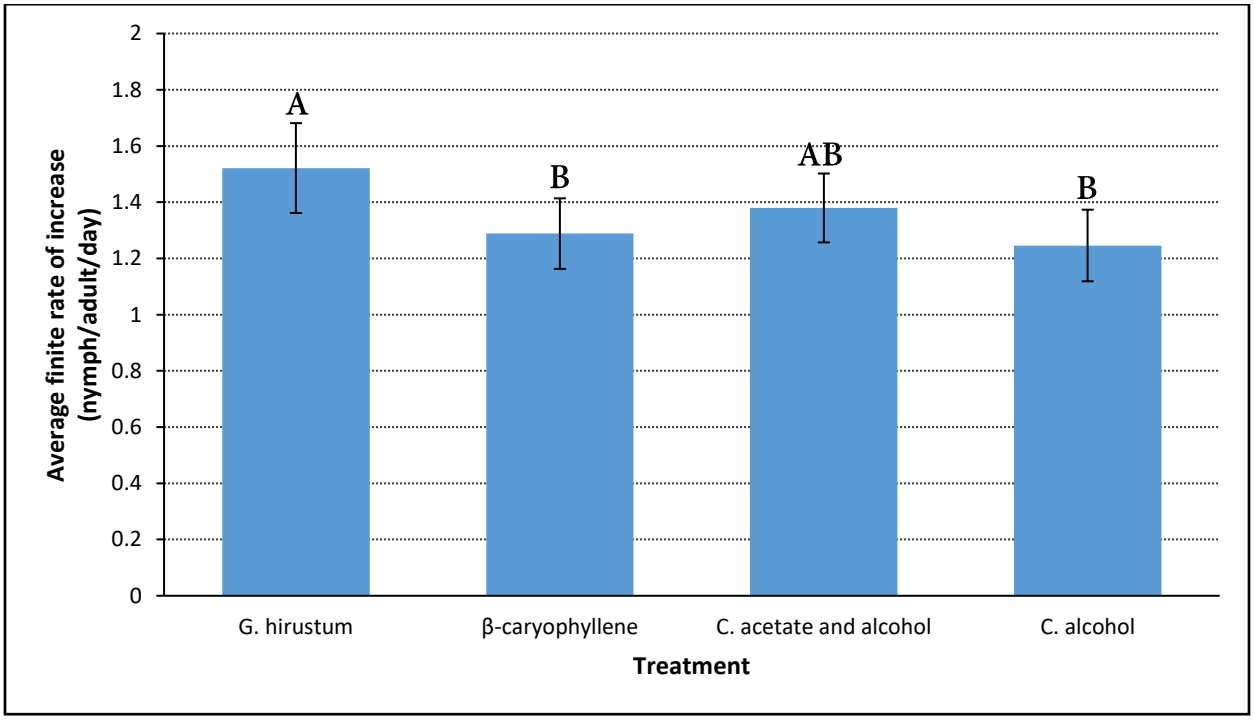

Figure 9. Average finite rate of increase of cotton aphids expressed as nymphs per adult per day on individual treatments. Means followed by the same letter are not significantly different based on a one-way ANOVA (Tukey Kramer HSD, $P \leq 0.05$).

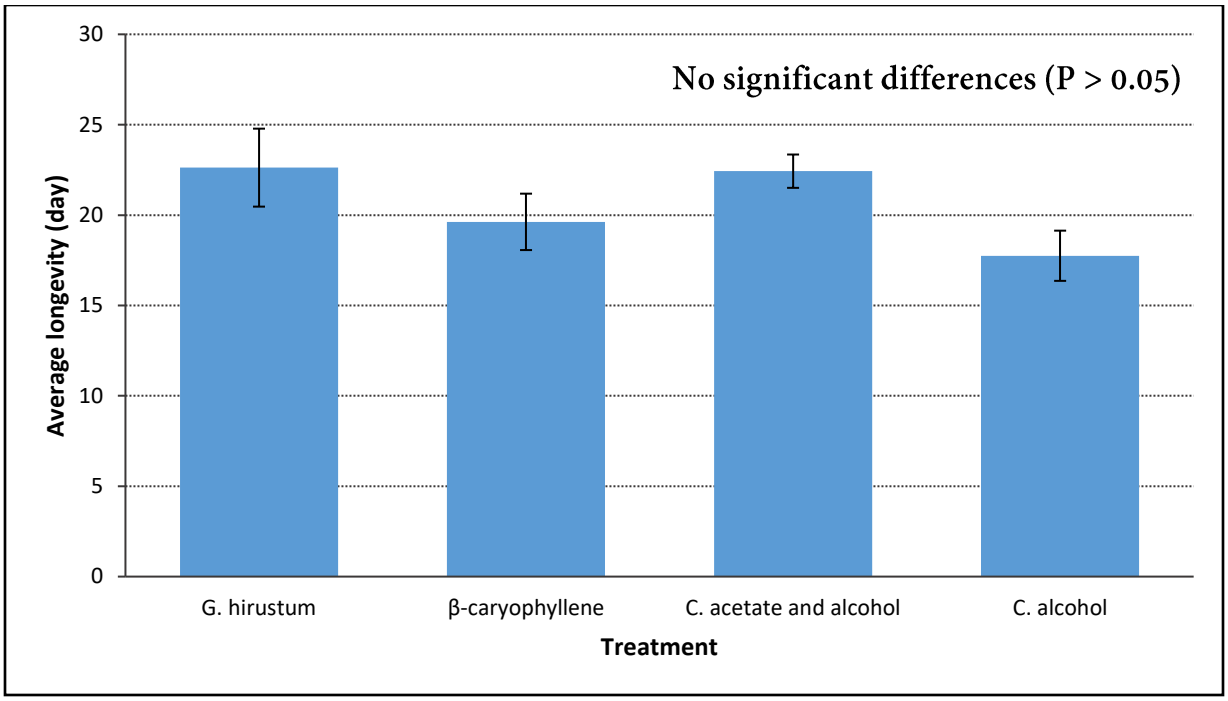

Figure 10. Average longevity of cotton aphids on individual treatments.

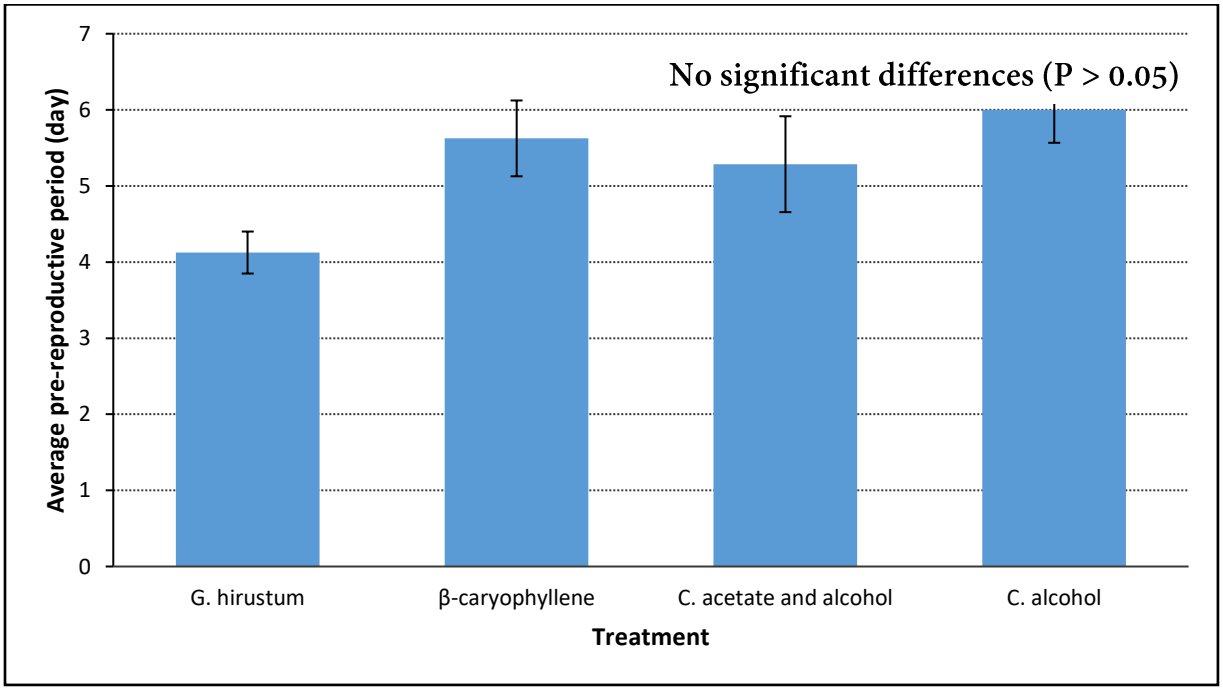

Figure 11. Average pre-reproductive period of cotton aphids on individual treatments.

Discussion

β-caryophllyene derivatives expressed in a tri-species cotton hybrid did not significantly affect cotton aphid infestations in field experiments relative to *G. hirsutum* and *G. hirsutum* (IST) during 2019 or 2020. However, the tri-species cotton hybrid, similar to *G. hirsutum* (IST) treated with an insecticide seed treatment, had fewer aphids than *G. hirsutum* (non-seed treated control). In a recent study, Zhang et al. (2020) confirmed that β-caryophllyene in cotton had an adverse effect on aphid population development and was an attractant to its parasitoid, *Aphidius gifuensis*. Our results suggested that treatments, c. acetate and alcohol and c. alcohol may have a similar effect on aphid population development and could be the reason for fewer aphids on plants.

In choice test study, tri-species hybrids containing β-caryophllyene and its derivatives negatively affected aphid population development compared to *G. hirsutum*. β-caryophllyene and c. alcohol differed from c. acetate and alcohol treatment by having fewer aphid infestations. Similary, the average intrinsic rate of increase of the cotton aphid was significantly less on β-caryophllyene and c. alcohol treatments. The aphid population doubling time was significantly longer when exposed to c. alcohol compared to *G. hirsutum*. β-caryophllyene and c. alcohol treatments had an adverse effect on the average finite rate of increase of cotton aphids. However, the tri-species cotton hybrid did not significantly affect the cotton aphid life span and generation time.

Field studies conducted in 2019 and 2020 suggest a subtle negative impact of the tri-species hybrid on cotton aphid population infestation relative to *G. hirsutum*; similar to what is observed when *G. hirsutum* seed is treated with imidacloprid. However, there was no obvious effect on aphid infestation among the tri-species β-caryophllyene or its derivatives. Thus, the

reduction in aphid infestation on the tri-species hybrid plant appears to be due to some other factor associated with the tri-species hybrid. The field data is supported by the choice test and the life table studies. In the choice test, the tri-species hybrid generally had fewer aphids colonizing and developing on the plants than *G. hirsutum*. Similarly, intrinsic rate of increase, finite rate of increase, and doubling time of aphids tended to be negatively affected by the tri-species hybrid relative to *G. hirsutum*. However, responses on the tri-species, β-caryophyllene acetate and alcohol derivative plants were inconsistent. The reason for this result is uncertain, but might be related to the quantity of derivatives expressed in each plant.

The impact of the tri-species hybrid on cotton aphid plant infestation and population development was not dramatic. Yet, even slight negative effects can be important for cotton aphid management in an IPM system. Where aphid infestation and population growth is suppressed, natural enemies may be better able to manage the infestation and prevent the need for curative insecticide (Oerke 2004). Furthermore, β-caryophyllene released during the feeding of insect pest on non-floral tissues serves as an attractant to the pests' natural enemies (Köllner et al. 2008, Rasmann et al. 2005).

β-caryophyllene is produced in the pigment glands of upland cotton (*Gossypium hirsutum* L.) and has been reported to reduce the fitness and survivability of insects feeding on cotton (Langenheim 1994). It is plausible that β-caryophyllene derivatives (c. acetate and alcohol and c. alcohol) could also contribute to the efficacy of insect control. These unique derivatives are only found in certain wild cotton species. There are no reports, to date, of the efficacy of these caryophyllene derivatives for insect pest control in cotton. As such the data obtained in this study may catalyze further studies to elucidate the biological activities of these compounds in pest management.

CHAPTER IV

THE EFFECT OF BETA-CARYOPHYLLENE DERIVATIVES IN A TRI-SPECIES COTTON HYBRID ON MID TO LATE-SEASON PESTS (FALL ARMYWORM AND COTTON BOLLWORM) SURVIVAL AND DEVELOPMENT

Introduction

Growers routinely rely on insecticide applications in order to control infestations of insect pests (Gore et al. 2001). Growing environmental, health and economic concerns, as well as insecticide resistance, have resulted from repetitive insecticide applications. As such, transgenic crops became a widely adopted substitute for managing some insect pests (Bravo et al. 2011, Roubos et al. 2014, Kranthi et al. 2002). Transgenic crops, such as *Bt* cotton (*Gossypium hirsutum* L.), express *Bacillus thuringiensis* (*Bt*) proteins that have proven to be highly effective in managing many lepidopteran insect pests. After ingestion, the *Bt* proteins disrupts the insect's midgut tissues. The disruptions causes the gut contents to leak into the insect's hemocoel resulting in death (Raymond et al. 2010). Since 1996, this technology has been highly successful for managing the cotton bollworm, *Helicoverpa zea* (Boddie) and fall armyworm, *Spodoptera fruigperda* (J.E. Smith) in cotton (Bravo et al. 2011, Hardke et al. 2015). The widespread adoption of *Bt* cotton has resulted in a reduction of insecticide applications, and ultimately, provided human health, environmental and economic benefits (Frisvold et al. 2006).

Despite the success of *Bt* cotton for control of lepidopteran pests, some target pests have evolved resistance to the *Bt* proteins (Carriére et al. 2010, Chen et al. 2018, Palumbi 2001, Onstad and Guse 2008). Insect resistance to *Bt* cotton has made effective and economical control of bollworm difficult throughout most cotton producing regions in the U.S. (Gore et al. 2001).

Ineffective control of fall armyworm and cotton bollworm demonstrates the importance of discovering alternative methods to enhance currently utilized pest management techniques (Hardke et al. 2015).

Host plant resistance (HPR) may be an effective approach to help manage difficult to control cotton pests and reduce insect resistance selection pressure. HPR is an important component of integrated pest management (IPM) that relies on plants with favorable characteristics (i.e. plant resistance to pests) (Sharma et al. 2001, Leghari et al. 2001). For centuries, plants have been known to possess essential oils that are composed of defensive secondary metabolites (Waiss et al. 1977). Essential oils in crops can provide plant protection by reducing insect pest performance or preference (Park and Tak 2016). β-caryophyllene is an essential oil found in many plant species, such as cloves (*Syzygium aromaticum*), rosemary (*Salvia rosmarinus*), and oregano (*Origanum vulgare),* and has a repellant effect on important insect pests, such as mosquitoes (Sun et al. 2020). Upland cotton (*Gossypium hirsutum* L.) produces β-caryophyllene in its pigment glands. The aroma of a cotton field is composed primarily of β-caryophyllene (Flint et al. 1979). Furthermore, β-caryophyllene has been reported to act as a feeding deterrent for insect pests or attractant for their natural enemies (Opitz et al. 2008).

β-caryophyllene derivatives, 12-hydroxycaryophyllene (caryophyllene alcohol) and 12-hydroxycaryophyllene acetate (caryophyllene acetate), or the combination of the two have been speculated to reduce injury from a wide group of insects (C. Suh, personal communication, 2017). William et al. (1997) detected the β-caryophyllene derivatives, c. acetate and alcohol and c. alcohol, in three species of non-cultivated cotton, *G. armourianum, G. harknessii,* and *G. turneri*. The genes regulating the production of these derivatives have been identified and

traditional backcross breeding techniques have been used to develop a tri-species cotton hybrid, which includes *G. hirsutum, G. arboreum*, and *G. turneri* (C. Suh, personal communication, 2017). This tri-species hybrid expresses β-caryophyllene, and can also express its derivatives, c. acetate and alcohol and c. alcohol, depending on genetic segregation. Additionally, the tri-species hybrid has been observed to be more heat, drought, and disease resistant, but its impact on cotton pests are unknown (C. Suh, personal communication, 2017).

The objectives of this experiment were to 1) confirm the presence of β-caryophyllene derivatives in a tri-species cotton hybrid using gas chromatography mass spectrometry (GC/MS) analysis and 2) determine the effect of tri-species hybrid expressing β-caryophyllene derivatives on fall armyworm and cotton bollworm larval mortality, growth, and development.

Materials and Methods

Cotton Source

Two cotton germplasm lines, a tri-species hybrid and 'Tamcot 73' (*G. hirsutum*), were used in the laboratory experiment. The tri-species hybrid was developed at USDA Southern Plains Agricultural Research Center in College Station, Texas. Tamcot 73 (*G. hirsutum*) was developed in the Cotton Improvement Laboratory at Texas A&M University in College Station, Texas (Smith et al. 2011). Two seeds were planted in 2-gallon nursery pots (Horticulture Source, Vancouver, Washington) filled with standard potting soil. Fifteen plants were planted for each treatment. Once the plants reached the 1-2 true leaf stage, each pot was thinned to a single plant. Plants were maintained in a greenhouse and watered as needed.

Although tri-species hybrid plants express β-caryophyllene, they may or may not express c. acetate and alcohol and c. alcohol depending on genetic segregation. Thus, within the tri-

species hybrid group, the treatment varied among the plants, consisting of plants expressing β-caryophyllene, c. acetate and alcohol, and c. alcohol.

Chemical Analysis

Expression of the β-caryophyllene derivatives is not evident until about the 5 true leaf stage (Bell and Stipanovic 1977). The presence of β-caryophyllene derivatives in tri-species hybrid plant was elucidated by using gas chromatography mass spectrometry (GC/MS). Each plant was sampled by collecting the terminal leaf at the 5 true leaf stage and storing it in a labeled 2-ml microcentrifuge tube. Samples were immediately placed in a cooler with ice for transport to the laboratory. The samples were subsequently stored in a -20°C freezer until ready for extraction.

The β-caryophyllene derivatives of interest were extracted by pulverizing the frozen leaf material using a tube pestle. Dichloromethane (900µl) was added to the leaf material and vortexed for approximately 20 seconds. The mixtures were sonicated for thirty minutes, followed by a ten-minute centrifugation phase. After centrifugation, a 200µl pipette was used to remove extracts from tubes. The resulting extracts were then placed into a second set of labeled tubes and concentrated in the fume hood. The concentrated extracts were transferred into labeled GC vials fitted with volume reducing inserts and submitted for GC/MS analysis.

GC/MS analysis was conducted on a Shimadzu GCMS-QP2010 Ultra (Shimadzu Scientific Instruments, Columbia, MD) equipped with a Zebron ZB-WAX plus (30 m length × 0.25 mm I.D. × 0.25 µm film thickness) (Phenomenex, Torrence CA, USA) utilizing the methods described by Perez et al. (2019). Helium was used as a carrier gas with a flow rate of 1.2 ml/min. The temperature of the injection port was 220°C and a 1µL sample was injected in a split-less mode. The column temperature program consisted of an initial temperature of 100°C, held for 5

min followed by a 10 °C/min ramp to 220°C and held for 10 min. The program ended with a 40°C/min ramp to an ending temperature of 250°C. The mass spectrometry conditions were: electron impact ionization (EI); interface temperature of 250 °C; and ion source temperature of 200°C.

Based on the GC/MS results, each tri-species hybrid plant was identified as expressing either β-caryophyllene, c. acetate and alcohol, or c. alcohol and labelled accordingly in the greenhouse.

No Choice-Bioassay

Fall armyworm larvae were established from a colony collected from non-*Bt* corn in Snook, TX. Cotton bollworm larvae were established from eggs obtained from Benzon Research in Carlisle, PA. Survivorship and development of fall armyworm and cotton bollworm larvae were evaluated on leaf tissue of *G. hirsutum*, and the tri-species cotton hybrid expressing either β-caryophyllene, c. acetate and alcohol, or c. alcohol. At the 8 true leaf stage, one fully-expanded leave was excised from each of nodes 4-7. Each leaf was placed adaxial side up into a sterile Petri dish (100 x15 mm) lined with moistened filter paper (9-cm diam; Fisherbrand, Pittsburgh, PA). Five fall armyworm or cotton bollworm neonates (<24 h old) were placed on the surface of each leaf. Petri dishes were sealed with a lid and secured with weights. Petri dishes were placed in a growth chamber maintained at 27 ± 1°C, ~50% RH, and a photoperiod of 14:10 (L:D) h. Filter paper was saturated with water daily, and leaves were replaced every two days. Each treatment combination was replicated four times, totaling 80 larvae assessed for each treatment combination. Larval mortality, weight, and instar development were assessed seven days after infestation. A correction for natural mortality was calculated using Abbott's formula based on mortality from *G. hirsutum* (Abbott 1925). The average weight was calculated by weighing all

surviving fall armyworm or cotton bollworm larvae and dividing the weight by the number of live insects.

Statistical Analysis

Mortality, larval instar development, and average weight data were analyzed using JMP (JMP Pro14 software, version 14.1.0 SAS Institute Inc., Cary, NC). A mixed model ANOVA was used to analyze all data. *G. hirsutum*, β-caryophyllene, c. acetate and alcohol, and c. alcohol were set as the fixed effects and trials set as random effects. Treatment differences were separated using Tukey-Kramer HSD, $P \leq 0.05$.

Results

There were no differences in fall armyworm mortality among treatments , but signifcant differences were observed for larval weight and mean instar (Table 9). The mean instar after 7 days for fall armyworm was greater on *G. hirsutum* than on the tri-species hybrid expressing β-caryophyllene or c. alcohol, but did not differ from the tri-species hybrids expressing c. acetate and alcohol (Table 9). Neither mean instar, larval weight, nor mortality of the cotton bollworm differed among treatments, but mortality was numerically higher on the tri-species hybrid plants expressing c. alcohol. (Table 10).

Table 9. Mortality, growth, and development of fall armyworm larvae feeding on the leaves of *G. hirsutum* and a tri-species cotton hybrid expressing β-caryophyllene or its derivatives.

	7 Days after infestation		
Treatment	Mean percentage mortality (± SEM) [1]	Mean instar (± SEM)	Mean weight (mg) (± SEM)
G. hirsutum	4.24 ± 2.07 a	2.26 ± 0.04 a	3.88 ± 0.22 a
β-caryophyllene	6.85 ± 2.51 a	2.13 ± 0.03 b	3.53 ± 0.21 a
c. acetate and alcohol	5.50 ± 2.48 a	2.17 ± 0.03 ab	3.35 ± 0.16 a
c. alcohol	9.06 ± 2.97 a	2.05 ± 0.03 b	2.35 ± 0.14 b

Means in a column followed by the same letter are not significantly different based on a one-way ANOVA (Tukey Kramer HSD, $P \leq 0.05$).

[1]A correction for natural mortality was calculated using Abbott's formula based on mortality from *G. hirsutum* (Abbott 1925).

Table 10. Mortality, growth, and development of cotton bollworm larvae feeding on the leaves of *G. hirsutum* and a tri-species cotton hybrid expressing β-caryophyllene or its derivatives.

	7 Days after infestation		
Treatment	Mean percentage mortality (± SEM)[1]	Mean instar (± SEM)	Mean weight (mg) (± SEM)
G. hirsutum	5.50 ± 2.08 a	2.17 ± 0.05 a	2.25 ± 0.14 a
β-caryophyllene	5.26 ± 2.52 a	2.11 ± 0.03 a	1.83 ± 0.13 a
c. acetate and alcohol	8.31 ± 2.19 a	2.23 ± 0.08 a	2.04 ± 0.22 a
c. alcohol	10.71 ± 3.47 a	2.08 ± 0.04 a	2.20 ± 0.14 a

Means in a column followed by the same letter are not significantly different based on a one-way ANOVA (Tukey Kramer HSD, $P \leq 0.05$).

[1]A correction for natural mortality was calculated using Abbott's formula based on mortality from *G. hirsutum* (Abbott 1925).

Discussion

The over-reliance on insecticides to manage insect pests has been a serious problem in crop production. As a result, there is great concern with the development of insect resistance, and the harmful effects of pesticides on human health and the environment (Sun et al. 2020). To address these concerns, the use of natural plant defensive compounds have been explored as alternative to insecticides (Sun et al. 2020). Essential oils have been reported to protect plants by insecticidal, repellent, and antimicrobial activities (Sun et al. 2020). Exposure to these natural plant compounds could offer crop protection by inducing sub-lethal effects, such as growth inhibition, oviposition deterrence, and antifeedant activity (Akhtar and Isman 2013, Akhtar et al. 2012).

In the current study, the growth and survivability of the fall armyworm and cotton bollworm were evaluated on leaves expressing β-caryophyllene derivatives in a tri-species cotton hybrid, relative to *G. hirsutum*. Although no significant effect on instar development, weight, and mortality was observed on cotton bollworm, some impact on fall armyworm was evident. The fall armyworm instar development was significantly reduced on β-caryophyllene and its derivative c. alcohol. Additionally, fall armyworm larvae also exhibited reduced weight when they fed upon tri-species hybrid cotton expressing c. alcohol. This effect was most likely due to an antifeedant response. The results reported in the current study are similar to that reported for caryophyllene and caryophyllene oxide on *H. virescens* (Gunasena et al. 1988). However, we did not observe this effect on *H. zea*. Furthermore, Gunasena et al. (1988) also reported that *H. virescens* exhibited lower pupae weight upon feeding on β-caryophyllene, but that time to pupation was only slightly increased. Gunasena et al. (1988) concluded that there was a synergistic interaction between β-caryophyllene and gossypol, where low concentrations of both

resulted in a slight positive growth effect. They also reported that caryophyllene oxide acted synergistically with gossypol, increasing the growth stimulating effects, but in contrast, β-caryophyllene with higher levels of gossypol resulted in a reduction in larval growth. Our results suggest that β-caryophyllene and its c. alcohol derivative, may offer some plant protection against fall armyworm by negatively influencing the insects' fitness (Akhtar et al. 2012, Zalkow et al. 1979). Slower growing and less fit larvae may have increased probability of being preyed upon by natural enemies and cause less crop injury. In turn, more time is available for curative remedial action in which insects may be more susceptible to insecticides. Thus, slight negative impacts on pest development may have important implication in IPM.

CHAPTER V

CONCLUSIONS

Producers greatly depend on *Bt* cotton and insecticides for effective management of multiple insect pests in cotton. However, widespread use of these control tactics has revealed limitations, including field-evolved resistance across many pest species. Because of this, alternative management strategies, such as host-plant resistance (HPR) using native traits, can help to suppress pest populations and merits further investigation within the cotton production system. William et al. (1997) identified β-caryophyllene and its derivatives (12-hydroxy-β-caryophyllene and 12-hydroxy-β-caryophyllene acetate) as the most abundant volatile sesquiterepenes from essential oils in wild cottons, *G. armouranium G. harknessii, and G. turneri*. These unique derivatives are only expressed in these wild cotton species. Tri-species cotton hybrids (*G. hirsutum, G. arboreum,* and *G. armouranium*) and (*G. hirsutum, G. arboreum,* and *G. turneri*) were developed by USDA-ARS using traditional breeding techniques. These natural defensive compounds have been observed anecdotally to control infestations of insect pests on wild cotton plants but their effect in a tri-species hybrid on cotton pest species remains unknown (C. Suh, personal communication, 2017). To address this knowledge gap, a tri-species cotton hybrid expressing β-caryophyllene derivatives was evaluated in field, laboratory, and/or greenhouse experiments for its host plant resistance potential against tobacco thrips, *Frankliniella fusca* (Hinds), western flower thrips, *Frankliniella occidentalis* (Pergande), cotton aphid, *Aphis gossypii* Glover, fall armyworm, *Spodoptera frugiperda* (J.E. Smith), and bollworm, *Helicoverpa zea* (Boddie).

At the 2-3 and 3-4 true leaf stages differences in thrips injury ratings among the tri-species hybrids expressing β-caryophyllene and its derivatives, relative to *G. hirsutum* and *G. hirsutum* treated with the seed insecticide imidacloprid (IST), was evident but slight. Injury tended to be lower for the tri-species hybrid than for *G. hirsutum* and *G. hirsutum* (IST), particularly for the two β-caryophyllene derivatives in 2020. Thrips injury severity distribution was greater in 2020 than 2019, so the reason more profound differences were not observed under lower injury potential in 2019 remains uncertain. Although, the current study suggests that the β-caryophyllene derivatives, c. acetate and alcohol and c. alcohol, found in the tri-species hybrid will only minimally protect the plant from thrips feeding injury, it may still be a viable protection strategy when used with other plant protection strategies.

Tri-species cotton hybrid plants expressing β-caryophyllene derivatives did not appear to affect initial cotton aphid infestation in field experiments relative to *G. hirsutum* and *G. hirsutum* (IST) during both 2019 and 2020. Additionally, there were no significant differences among treatments in cumulative aphid days in 2019. However, in 2020 the *G. hirsutum* (IST) treatment had fewer cumulative aphid days than *G. hirsutum*, but did not differ from any of the tri-species hybrid treatments, suggesting that the tri-species hybrid may have a slight negative effect on cotton aphid population growth similar to the level provided by the seed treatment.

In contrast to field observations, the number of alate aphids settling on tri-species cotton plants, regardless of β-caryophyllene expression, was lower than for *G. hirsutum* in a controlled greenhouse choice experiment. Additionally, the resulting number of apterous aphids, as well as total aphids, was lower on the tri-species hybrid. Among the tri-species hybrid plants expressing β-caryophyllene or its derivatives, the plants expressing β-caryophyllene alone, or the c. alcohol derivative had significantly fewer alates, apterous and total aphids than plants expressing the β-

caryophyllene acetate and alcohol derivative. The reason for this difference among the tri-species hybrid treatments is not known but might be related to the quantities of these compounds expressed in pigment glands.

In a controlled growth chamber study investigating the effect of the tri-species hybrid on cotton aphid life table statistics relative to *G. hirsutum*, the results were similar to those observed in the greenhouse choice experiment. Cotton aphid intrinsic rate of increase, and finite rate of increase was lower for the tri-species hybrid plants expressing β-caryophyllene or its derivative c. alcohol relative to *G. hirsutum*, but did not differ from tri-species plants expressing c. acetate and alcohol. The cotton aphid population doubling time was significantly longer on tri-species hybrid plants expressing c. alcohol than for *G. hirsutum* but did not differ from the other tri-species hybrid treatments. However, the cotton aphid life span and generation time was not significantly influenced by β-caryophyllene and its derivatives. Thus, the tri-species hybrid appears to be influencing aphid population development.

Fall armyworm and cotton bollworm development and survival were determined on fully expanded leaves expressing β-caryophyllene derivatives in tri-species cotton hybrid in controlled feeding bioassays under laboratory conditions. Results indicate the tri-species cotton negatively affected fall armyworm development, but had no impact on development of the cotton bollworm. Fall armyworm mass gain was significantly reduced on plants expressing c. alcohol and instar development was slowed on plants expressing β-caryophyllene and c. alcohol treatments. β-caryophyllene has been documented to exhibit either repellant or antifeedant effects on insect pests (Sun et al. 2019). Since c. alcohol adversely affected fall armyworm growth and development, this compound may offer some control of fall armyworm.

Overall, U.S. grown cotton cultivars do not incorporate HPR as a mechanism to aid in control of significant insect pest species. Host plant resistance is a major component of highly successful Integrated Pest Management programs. If a single HPR trait could be identified that impacts multiple pest species, and complements *Bt* technology in that it is constantly present, the potential impact on pest management and cotton production could greatly enhance sustainability. Development of a tri-species cotton hybrid that expresses β-caryophyllene derivatives that negatively impact insect pests could be a useful HPR source for cotton breeders. In the current set of studies, my findings suggest that this tri-species hybrid had slight or inconclusive negative effects on thrips, no impact on bollworm survival and development, but did exhibit some impact on cotton aphid population development and fall armyworm larvae development.

REFERENCES

Abbott, W. S. 1925. A method of computing the effectiveness of an insecticide. J. Econ. Entomol. 18(2): 265-267.

Akbar, W., A. Gowda, J. E. Ahrens, J. W. Stelzer, R. S. Brown, S. L Bollman, ... and S. D. Stewart. 2019. First transgenic trait for control of plant bugs and thrips in cotton. Pest Manag. Sci. 75(3): 867-877.

Akhtar, Y. and M. B. Isman. 2013. Plant natural products for pest management: the magic of mixtures. *In* Advanced technologies for managing insect pests. pp. 231-247. Springer, Dordrecht.

Akhtar, Y., E. Pages, A. Stevens, R. O. D Bradbury, C. A. da Camara, and M. B. Isman. 2012. Effect of chemical complexity of essential oils on feeding deterrence in larvae of the cabbage looper. Physiol. Entomol. 37(1): 81-91.

Albeldano, W. A., J. E. Slosser, and M. N. Parajulee. 2008. Identification of thrips species on cotton on the Texas Rolling Plains. Southwest Entomol. 33: 43-51.

Ali, A., R. G. Luttrell, H. N. Pitre, and F. M. Davis. 1989. Distribution of fall armyworm (Lepidoptera: Noctuidae) egg masses on cotton. Environ. Entomol. 18: 881-885.

Ali, A., R. G. Luttrell, and H. N. Pitre. 1990. Feeding sites and distribution of fall armyworm (Lepidoptera: Noctuidae) larvae on cotton. Environ. Entomol. 19: 1060-1067.

Allen, K. C., R. G. Luttrell, T. W. Sappington, L. S. Hesler, and S. K. Papiernik. 2018. Frequency and abundance of selected early-season insect pests of cotton. J. Integr. Pest Manag. 9(1): 20.

Amer, M., S. A. S. Hussain, L. Khan, M. Khattak, and G. S. Shah. 1999. The comparative efficacy of insecticides for the control of the insect pest complex of cotton (*Gossypium hirsutum* L.). Pak. J. Biol. Sci.

Arnold, M. D., J. K. Dever, M. N. Parajulee, S. C. Carroll, and H. D. Flippin. 2012. Simple and effective method for evaluating cotton seedlings for resistance to thrips in a greenhouse, and a thrips species composition on the Texas High Plains. Southwestern Entomol. 37(3): 305-313.

Avelar, S., D. W. Schrimsher, K. Lawrence and J. K. Brown. 2019. First report of cotton leafroll dwarf virus associated with cotton blue disease symptoms in Alabama. Plant Dis. 103(3): 592-592.

Bacheler, J. S. and D. D. Reisig. 2010. Insect control in cotton. *In* 2010 North Carolina agricultural chemicals manual. College of agriculture and life sciences, North Carolina State University. (pp. 83-88).

Bakkali, F., S. Averbeck, D. Averbeck, and M. Idaomar. 2008. Biological effects of essential oils–a review. Food Chem Toxicol. 46(2): 446-475.

Barros, E. M., J. B. Torres, J. R. Ruberson, and M. D. Oliveira. 2010. Development of *Spodoptera frugiperda* on different hosts and damage to reproductive structures in cotton. Entomol. Exp. Appl. 137(3): 237-245.

Bass C., I. Denholm, M. S. Williason, and R. Nauen. 2015. The global status of insect resistance to neonicotinoid insecticides. Pestic. Biochem. Physiol. 121: 78-87.

Bell, A. A. and R. D. Stipanovic. 1977. The chemical composition, biological activity, and genetics of pigment glands in cotton. *In* Proceedings of the Beltwide Cotton Conference, *eds.* Dugger C.P. and Richter D.A. National Cotton Council of America, New Orleans, LA, pp. 244-258.

Birch, L. 1948. The intrinsic rate of natural increase of an insect population. J. anim. Ecol. 17(1): 15-26.

Blackman, R. L. and V. F. Eastop. 1984. Aphid on the world's crops; an identification and information guide. John Wiley and Sons, Chichester, UK.

Bravo, A., S. Likitvivatanavong, S. S. Gill, and M. Soberón. 2011. *Bacillus thuringiensis*: a story of a successful bioinsecticide. Insect Biochem. Mol. Biol. 41(7): 423-431.

Brewer, M. J., J. W. Gordy, D. L. Kerns, J. B. Woolley, W. L. Rooney, and R. D. Bowling. 2017. Sugarcane aphid population growth, plant injury, and natural enemies on selected grain sorghum hybrids in Texas and Louisiana. J. Econ. Entomol. 110(5): 2109-2118.

Brubaker, C. L., J. A. Koontz, and J. F. Wendel. 1993. Bidirectional cytoplasmic and nuclear introgression in the new world cottons, *Gossypium barbadense* and *G. hirsutum* (Malvaceae). Am. J. Bot. 80: 1203-1208.

Burris E., K. J. Ratchford, A. M. Pavloff, D. J. Boquet, B. R. Williams, and R. L. Rogers. 1989. Thrips on seedling cotton: related problems and control. Louisiana agricultural experiment station bulletin 811. LSU AgCenter, Baton Rouge, LA.

Capinera, J. L. 2000. Corn earworm, *Helicoverpa* (= *Heliothis*) *zea* (Boddie) (Lepidoptera: Noctuidae). Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida. EENY-145 (IN302).

Carrière, Y., D.W. Crowder, and B.E. Tabashnik. 2010. Evolutionary ecology of insect adaptation to Bt crops. Evol. Appl. 3(5-6):561-573.

Cattaneo, M. and D. L. Kerns. 2008. Managing cotton insects in the Lower Rio Grande Valley-2008. Texas FARMER Collection.

Chaudhary, B., R. Hovav, R. Rapp, N. Verma, J. A. Udall, and J. F. Wendel. 2008. Global analysis of gene expression in cotton fibers from wild and domesticated *Gossypium barbadense*. Evol. Dev. 10(5): 567-582.

Chen, T., R. Zeng, W. Guo, X. Hou, Y. Lan, and L. Zhang. 2018. Detection of stress in cotton (*Gossypium hirsutum* L.) caused by aphids using leaf level hyperspectral measurements. Sensors. 18(9): 2798.

Chen, X., G. P. Head, P. Price, D. L. Kerns, M. E. Rice, F. Huang, ... and F. Yang. 2019. Fitness costs of Vip3A resistance in *Spodoptera frugiperda* on different hosts. Pest Manag. Sci. 75(4): 1074-1080.

Chen, Y., S. Feng, T. Zhao, and B. Zhou. 2018. Overcoming obstacles to interspecific hybridization between *Gossypium hirsutum* and *G. turneri*. Euphytica. 214(2): 35.

Cook, D., A. Herbert, D. S. Akin, and J. Reed. 2011. Biology, crop injury, and management of thrips (Thysanoptera: Thripidae) infesting cotton seedlings in the United States. J. Integr. Pest Manag. 2(2): B1-B9.

Cook, D. R., S. D. Stewart, J. Gore, G. M. Lorenz, A. L. Catchot, D. L. Kerns, S. Brown, G. Studebaker, N. Bateman, W. Crow, B. Thrash, and K. C. Allen 2020. Managing thrips in mid-south cotton. *In* Proceedings of the Beltwide Cotton Conference, *eds.* Boyd S, Huffman M, Krogman L, and Sarkissian A. National Cotton Council of America, Austin, TX, pp. 666-674.

D'Ambrosio, D. A., G. G. Kennedy, and A. S. Huseth. 2020. *Frankliniella fusca* and *Frankliniella occidentalis* response to thrips-active Cry51Aa2. 834_16 Bt cotton with and without neonicotinoid seed treatment. Crop Prot. 129(105042).

Darnell-Crumpton, C., A. L. Catchot, D. R. Cook, J. Gore, D. M. Dodds, S. C. Morsello, and F. R. Musser. 2018. Neonicotinoid insecticide resistance in tobacco thrips (Thysanoptera: Thripidae) of Mississippi. J. Econ. Entomol. 111(6): 2824-2830.

Deguine, J. P., P. Ferron, and D. Russell. 2008. Sustainable pest management for cotton production. A review. Agron. Sustain. Dev. 28(1): 113-137.

Dicke, F. F. 1939. Seasonal abundance of the corn earworm. J. Agric. Res. 59: 237–257.

Dhawan, A. K., A. J. Sindhu, and G. S. Simwat. 1998. Monitoring the seasonal abundance of cotton bollworms with pheromone traps. Indian J. Ecol. 23: 129-131.

Domingo, E. and M. Damo. 1996. Yield loss assessment due to insect pests. Crop Protection Department Annual Accomplishment Report for CY 1995-1996. Cotton Research and Development Institute, Batac, Ilocos Norte, Philippines.

Dunnam, E. W. and J. C. Clark. 1941. Cotton aphid multiplication following treatment with calcium arsenate. J. Econ. Entomol. 34: 587-588.

Ebert, T. A. and B. Cartwright. 1997. Biology and ecology of *Aphis gossypii* Glover (Homoptera: Aphididae). Southwest. Entomol. 22: 116–153.

Elmer, H. S. and O. L. Brawner. Control of brown soft scale in central valley. Citrograph 1975, 60: 402–403.

Flint, H. M., S. S. Salter, and S. Walters. 1979. Caryophyllene: an attractant for the green lacewing. Environ. Entomol. 8(6): 1123-1125.

Frisvold, G. B.,J. M. Reeves, and R. Tronstad. 2006. *Bt* cotton adoption in the United States and China: international trade and welfare effects.

Fuchs, T. W. and R. Minzenmayer. 1995. Effect of *Aphis gossypii* on cotton development and yield in west Texas. Southwest. Entomol. 20: 341-349.

Gillham, F. E. 1995. Cotton production prospects for the next decade (Vol. 23). World Bank Publications.

Goff, C. C. and A. N. Tissot. 1932. The melon aphid, *Aphis gossypii* Glover. Florida Ag. Exp. Sta. Bull. 252: 1-23.

Gore, J., B. R. Leonard, and J. J. Adamczyk. 2001. Bollworm (Lepidoptera: Noctuidae) survival on 'Bollgard' and 'Bollgard II' cotton flower bud and flower components. J. Econ. Entomol. 94(6): 1445-1451.

Gore, J., D. Cook, A. Catchot, B. R. Leonard, S. D. Stewart, G. Lorenz, and D. Kerns. 2013. Cotton aphid (Heteroptera: Aphididae) susceptibility to commercial and experimental insecticides in the southern United States. J. Econ. Entomol. 106(3): 1430-1439.

Gunasena, G. H., S. B. Vinson, H. J. Williams, and R. D. Stipanovic. 1988. Effects of caryophyllene, caryophyllene oxide, and their interaction with gossypol on the growth and development of *Heliothis virescens* (F.) (Lepidoptera: Noctuidae). J. Econ. Entomol. 81(1): 93-97.

Hamadin, E. I. and H. W. Chambers. 2001. Susceptibility and mechanisms underlying the relative tolerance to five organophosphorus insecticides in tobacco budworms and corn earworms. Pestic. Biochem.Physiol. 69: 35–47.

Hardke, J. T., R. E. Jackson, B. R. Leonard, and J. H. Temple. 2015. Fall armyworm (Lepidoptera: Noctuidae) development, survivorship, and damage on cotton plants

expressing insecticidal plant-incorporated protectants. J. Econ. Entomol. 108(3): 1086-1093.

Hardwick, D. F. 1965. The corn earworm complex. Mem. Entomol. Soc. Canada. 40: 1–247.

Herbert D. A., J. Bacheler, S. Malone, D. Mott. 2007. Thrips control options in Virginia/North Carolina: overviews, insights and updates, pp. 1649–1653. Boyd S Huffman M Richter D Robertson B (eds.), Proceedings 2007 Beltwide Cotton Conferences. National Cotton Council, Memphis, TN.

Huang, M., A. M. Sanchez-Moreiras, C. Abel, R. Sohrabi, S. Lee, J. Gershenzon, and D. Tholl. 2012. The major volatile organic compound emitted from *Arabidopsis thaliana* flowers, the sesquiterpene (E)-β-caryophyllene, is a defense against a bacterial pathogen. New Phytol. 193(4): 997-1008.

Huseth, A. S., T.M. Chappell, K. Langdon, S. C. Morsello, S. Martin, J. K. Greene, A. Herbert, A. L. Jacobson, F.P. Reay-Jones, T. Reed, et al. 2016. *Frankliniella fusca* resistance to neonicotinoid insecticides: an emerging challenge for cotton pest management in the eastern United States. Pest Manag. Sci. 72: 1934-1945.

Kranthi, K. R., D.R. Jadhav, S. Kranthi, R.R. Wanjari, S. S. Ali, and D. A. Russell. 2002. Insecticide resistance in five major insect pests of cotton in India. Crop Prot. 21(6): 449-460.

Isely, D. 1946. The cotton aphid. Ark. Agr. Exp. Sts. Bull. 462:1–29.

Jacobson, A., R. Foster, C. Krupke, W. Hutchison, B. Pittendrigh, and R. Weinzierl. 2009. Resistance to pyrethroid insecticides in *Helicoverpa zea* (Lepidoptera: Noctuidae) in Indiana and Illinois. J. Econ. Entomol. 102(6): 2289-2295.

Javaid, I., J. Joshi, R. B. Dadson, F. M. Hashem, A. L. Allen. 2005. The potential of *Bt* corn as a trap crop for the control of corn earworm, *Helicoverpa zea* Boddie, in soybean. J. Sustain. Agric. 26: 115-121.

Kaur, B., V. Kuraparthy, J. Bacheler, H. Fang, and D. T. Bowman. 2018. Screening germplasm and quantification of components contributing to thrips resistance in cotton. J. Economic Entomol. 111(5): 2426-2434.

Kennedy, G. G. 2008. Integration of insect-resistant genetically modified crops within IPM programs. In integration of insect-resistant genetically modified crops within IPM programs. pp.1-26. Springer, Dordrecht.

Kerns C., J. Greene, H. Wang, J. Smoak, D. Robinson, A. Greene, F. Reay-Jones, P. Roberts, M. Toews, A. Jacobson, and D. Reisig. 2017. Effects of planting date and variety on thrips populations and injury to cotton in the southeastern United States. 2017 Beltwide cotton conferences proceedings. pp. 704-709.

Kerns, D. L., C. G. Sansone, K. T. Siders, and B. A. Baugh. 2009. Suggested insecticides for managing cotton insects in the High Plains, Rolling Plains, and Trans Pecos areas of Texas 2009. Texas AgriLife Extension Service, Texas A&M.

Kerns, D. L., and M. J. Gaylor. 1993a. Biotic control of cotton aphids (Homoptera: Aphididae) in cotton influenced by two insecticides. J. Econ. Entomol. 86: 1824–1834.

Kerns, D. L., and M.J. Gaylor. 1993b. Induction of cotton aphid outbreaks by insecticides in cotton. Crop Prot. 12: 387–393.

Kerns, D. L., J. A. Yates, and B. A. Baugh. 2015. Economic threshold for cotton aphid (Hemiptera: Aphididae) on cotton in the southwestern United States. J. Econ. Entomol. 108: 1795-1803.

Kidd, P. W. and D. R. Rummel. 1997. Effect of insect predators and a pyrethroid insecticide on cotton aphid, *Aphis gossypii* Glover, population density. Southwestern Entomol. 22: 381-393.

Kidd, P. W., D. R. Rummel and H. G. Thorvilson, 1996. Effects of cyhalothrin on field populations of the cotton aphid, *Aphis gossypii* Glover, in the Texas High Plains. Southwestern Entomol. 21: 293-301.

Köllner T. G., M. Held, C. Lenk, I. Hiltpold, T. C. J. Turlings, J. Gershenzon, J. Degenhardt. 2008. A maize (E)-b-caryophyllene synthase implicated in indirect defense responses against herbivores is not expressed in most American maize varieties. Plant cell. 20:482-494.

Koschier, E. H., K. A. Sedy, and J. Novak. 2002. Influence of plant volatiles on feeding damage caused by the onion thrips *Thrips tabaci*. Crop Prot. 21(5):419-425.

Langenheim, J. H. 1994. Higher plant terpenoids: a phytocentric overview of their ecological roles. J. Chem. Ecol. 10:1223-1280.

Layton, B. and J. T. Reed. 2002. Biology and control of thrips on seedling cotton in Mississippi. Mississippi State University Extension Service, Mississippi State, MS. Publication 2302: 1-10.

Layton, M. B., H. R. Smith, and G. Andrews. 1996. Cotton aphid infestations in Mississippi: efficacy of selected insecticides and impact on yield. In: P. Dugger and D. Richter (eds), Proceedings, Beltwide Cotton Conference. National Cotton Council, Memphis TN, pp. 892-893.

Leigh, T. F., S. H. Roach, and T. F. Watson. 1996. Biology and ecology of important insect and mite pests of cotton. In E. King, J. Phillips and R. Coleman (eds.), Cotton insect and mites: characterization and management. The cotton foundation, Memphis, TN. (pp. 17-86).

Leghari, M. A., Kalroo, A. M., and Leghari, A. B. 2001. Studies on host plant resistance to evaluate the tolerance/susceptibility against cotton pests. Pak. J. Biol. Sci.

Luginbill, P. 1928. The fall armyworm. U.S. Dept. Agric. Tech. Bull. 34:1–91.

Luttrell, R. G. and R. E. Jackson. 2012. *Helicoverpa zea* and *Bt* cotton in the United States. *GM* crops and food. 3(3): 213-227.

Luttrell, R. G., T. G. Teague, and M. J. Brewer. 2015. Cotton insect pest management. Cotton. 509-546.

Mink J. S. and R. G. Luttrell. 1989. Mortality of fall armyworm, *Spodoptera frugiperda* (Lepidoptera: Noctuidae) eggs, larvae and adults exposed to several insecticides on cotton. J. Entomol. Sci. 24: 563-571.

Miresmailli, S. and M. B. Isman 2014: Botanical insecticides inspired by plant-herbivore chemical interactions. Trends Plant Sci. 19: 29-35.

Naranjo, S. E. 2011. Impacts of *Bt* transgenic cotton on integrated pest management. J. Agric. Food Chem. 59(11): 5842-5851.

Naranjo, S. E., J. R. Ruberson, H. C. Sharma, L. Wilson, and K. Wu. 2008. The present and future role of insect-resistant genetically modified cotton in IPM. Integration of insect-resistant genetically modified crops within IPM programs. Springer, Dordrecht. 159-194.

Neupane, S. B., D. L. Kerns, and A. Szczepaniec. 2019. The impact of sorghum growth stage and resistance on life history of sugarcane aphids (Hemiptera: Aphididae). J. Econ. Entomol.

Oerke, E. C. and H. W. Dehne. 2004. Safeguarding production—losses in major crops and the role of crop protection. Crop Prot. 23(4): 275-285.

Onstad, D. W. and C. A. Guse. 2008. Concepts and complexities of population genetics. In D.W. Onstad, ed. Insect Resistance Management. Biology. Economics and Predictions. pp. 69–88. Academic Press, London.

Opitz, S., G. Kunert, and J. Gershenzon. 2008. Increased terpenoid accumulation in cotton (*Gossypium hirsutum*) foliage is a general wound response. J. Chem. Ecol. 34(4): 508-522.

Pan, Z., D. Onstad, P. Crain, A. Crespo, W. Hutchison, D. Buntin, P. Porter, A. Catchot, D. Cook, C. Pilcher, and L. Flexner. 2015. Evolution of resistance by *Helicoverpa zea* (Lepidoptera: Noctuidae) infesting insecticidal crops in the southern United States. J. Econ. Entomol. 109(2): 821-831.

Palumbi, S. R. 2001. Humans as the world's greatest evolutionary force. Sci. 293: 1786–1790.

Park, Y. L., and J. H. Tak. 2016. Essential oils for arthropod pest management in agricultural production systems. In essential oils in food preservation, flavor and safety. Academic Press. pp. 61-70.

Parker, R. D., M. J. Jungman, S. P. Biles, and D. L. Kerns. 2009. Suggested insecticides for managing cotton insects in the Southern, Eastern, and Blackland areas of Texas 2009. Texas AgriLife Extension Service, Texas A&M.

Perez, J. L., C.P.-C. Suh, and A. A. Bell. 2019. Chemotypic variation of caryophyllene derivatives in cotton germplasm lines. In S. Boyd, M. Huffman, L. Krogman, A. Sarkissian [eds] 2019 Beltwide Cotton Conferences Proceedings. (pp. 467-471).

Rasmann S., T. G. Köllner, J. Degenhardt, I. Hiltpold, S. Toepfer, U. Kuhlmann, J. Gershenzon, and T.C.J. Turlings. 2005. Recruitment of entomopathogenic nematodes by insect damaged maize roots. Nature. 434: 732-737.

Raymond, B., P. R. Johnston, C. Nielsen-LeRoux, D. Lereclus, and N. Crickmore. 2010. *Bacillus thuringiensis*: an impotent pathogen? Trends Microbiol. 18, 189e194.

Reed, J. T., C. Allen, R. Bagwell, D. Cook, E. Burris, B. Freeman, R. Leonard, and G. Lentz. 2006. A key to the thrips on seedling cotton in the Midsouthern United States. MAFES.

Reed, J. T. and D. B. Smith. 2001. Droplet size and spray volume effects on insecticide deposit and mortality of heliothine (Lepidoptera: Noctuidae) larvae in cotton. J. Econ. Entomol. 94: 640-647.

Reisig, D., D. Kerns, J. Gore, and F. Musser. 2019. Managing pyrethroid-and *Bt*-resistant bollworm in southern US cotton. Crops and Soils. 52(1): 30-35.

Roubos, C. R., C. Rodriguez-Saona, and R. Isaacs. 2014. Mitigating the effects of insecticides on arthropod biological control at field and landscape scales. Biological control. 75: 28-38.

de Sousa Ramalho, F., T. L. Azeredo, A. R. B de Nascimento, F. S. Fernandes, J. L. Nascimento Júnior, J.B Malaquias, C.A.D. da Silva, and J.C. Zanuncio. 2011. Feeding of fall armyworm, *Spodoptera frugiperda*, on *Bt* transgenic cotton and its isoline. Entomol. Exp. Appl. 139(3): 207-214.

SAS Institute. 2020. JMP Software, Version 15.1.0. SAS Inst. Inc., Cary, NC.

Seelanan, T., A. Schnabel, and J.F. Wendel. 1997. Congruence and consensus in the cotton tribe (Malvaceae). Syst. Bot. 22:259-290.

Sharma, H. C., K. K. Sharma, N. Seetharama, and R. Ortiz. 2001. Genetic transformation of crop plants: risks and opportunities for the rural poor. Curr. Sci. 80(12): 1495-1508.

Slosser J. E., W. E. Pinchak, and D. R. Rummel. 1989. A review of known and potential factors affecting the population dynamics of the cotton aphid. Southwest. Entomol. 14: 302-313.

Smith, C. W., S. Hague, and D. Jones. 2011. Registration of 'Tamcot 73'upland cotton cultivar. J. Plant Regist. 5(3): 273-278.

Stewart, S. D., R. Patrick, and A. McClure. 2010. 2010 Cotton insect control recommendations. In 2010 insect control recommendations for field crops, cotton, soybeans, field corn, sorghum, wheat, and pasture. PB 1768, University of Tennessee Extension. (pp. 3-16).

Stipanovic, R. D., A. A. Bell, and M. J. Lukefahr. 1977. Natural insecticides from cotton (*Gossypium*).

Stoetzel, M. B., G. L. Miller, P. J. O'Brien, and J. B. Graves. 1996. Aphids (Homoptera: Aphididae) colonizing cotton in the United States. Fl. Entomol. 79: 193-205.

Suh, C. P. personal communication, January 1 2017.

Sun, J. S., Y. Feng, Y. Wang, J. Li, K. Zou, H. Liu, Y. Hu, Y. Xue, L. Yang, S. S. Du, and Y. Wu. 2020. α-pinene, caryophyllene and β-myrcene from *Peucedanum terebinthaceum* essential oil: insecticidal and repellent effects on three stored-product insects. Re Nat. Prod. 14(3): 189.

Sun, W. M., Y. N. Ma, Y. J. Yin, C. J. Chen, F. R. Xu, X. Dong, and Y. X. Cheng. 2018. Effects of essential oils from Zingiberaceae plants on root-rot disease of *Panax notoginseng*. Molecules. 23(5): 1021.

Tabashnik, B. E. 2015. ABCs of insect resistance to *Bt*. PLoS Genet. 11(11): e1005646.

Tabashnik B. E., T. Brévault, and Y. Carrière. 2013. Insect resistance to *Bt* crops: lessons from the first billion acres. Nat Biotechnol.31: 510–521.

Toews, M. D., R. S. Tubbs, D. Q. Wann, and D. Sullivan. 2010. Thrips (Thysanoptera: Thripidae) mitigation in seedling cotton using strip tillage and winter cover crops. Pest Manag. Sci. 66(10): 1089-1095.

Vyvavhare, S. S., D. Kerns, C. Allen, R. Bowling, M. Brewer, and M. Parajulee. 2018. Managing cotton insects in Texas. College Station, TX: The Texas A&M University System.

Waiss Jr, A. C., B. G. Chan, and C. A. Elliger. 1977. Host plant resistance to insects.

Wendel, J. F. and V. A. Albert. 1992. Phylogenetics of the cotton genus *Gossypium* - character-state weighted parsimony analysis of chloroplastDNA restriction site data and its systematic and biogeographic implications. Syst. Bot. 17: 115–143.

Wendel, J. F. and R. C. Cronn. 2003. Polyploidy and the evolutionary history of cotton. Adv. Agron. 78: 139-186.

Williams, H. J., G. Moyna, S. B. Vinson, A. I. Scott, A. A. Bell, and R. D. Stipanovic. 1997. β-Caryophyllene derivatives from the wild cottons. Nat. Prod. Lett. 11(1): 25-30.

Yang, F., D. L. Kerns, S. Brown, R. Kurtz, T. Dennehy, B. Braxton, ... and F. Huang. 2016. Performance and cross-crop resistance of Cry1F-maize selected *Spodoptera frugiperda* on transgenic *Bt* cotton: implications for resistance management. Scientific reports. (6): 1-7.

Yu, S. J. 1983. Age variation in insecticide susceptibility and detoxification capacity of fall armyworm (Lepidoptera: Noctuidae) larvae. J. Econ. Entomol. 76: 219-222.

Zalkow, L. H., M. M. Gordon, and N. Lanir. 1979. Antifeedants from rayless goldenrod and oil of pennyroyal: toxic effects for the fall armyworm. J. Econ. Entomol. 72(6): 812-815.

Zhang, L., G. Lu, X. Huang, H. Guo, X. Su, L. Han, ... and H. Cheng. 2020. Overexpression of the caryophyllene synthase gene GhTPS1 in cotton negatively affects multiple pests while attracting parasitoids. Pest Manag. Sci. 76(5): 1722-1730.

Zhao, G., W. Liu, and J. M. Brown.1995. Insecticidal resistance in field and laboratory strains of western flower thrips (Thysanoptera: Thripidae). J. Econ. Entomol. 88: 1164-1170.