EVALUATION OF CULTURAL TACTICS, INSECTICIDES, AND PEANUT GENOTYPES FOR THRIPS AND SPOTTED WILT DISEASE MANAGEMENT IN PEANUT

by

PIN-CHU LAI

(Under the Direction of Rajagopalbabu Srinivasan)

ABSTRACT

Selected management tactics, including genotype selection, insecticides, and cultural practices, against tobacco thrips and *Tomato spotted wilt virus* (TSWV) in peanut were evaluated. Insecticide efficacy of thrips control and the impact of TSWV field resistant peanut genotypes on thrips-mediated TSWV transmission and thrips fitness were further investigated. The first objective was to integrate tactics for improved spotted wilt disease management in peanut, including selected insecticides, TSWV resistant cultivars, and cultural practices, namely row patterns and tillage systems. The second objective assessed the effectiveness of insecticides on tobacco thrips mortality and reducing feeding damage over time; the putative resistance status in tobacco thrips from field populations was also evaluated. The third objective focused on the effects of newly released TSWV field resistant genotypes as well as diploid wild species relative of cultivated peanut on thrips transmission and thrips biological fitness.

INDEX WORDS: Spotted wilt of peanut, insecticide efficacy, TSWV resistant genotypes

EVALUATION OF CULTURAL TACTICS, INSECTICIDES, AND PEANUT GENOTYPES FOR THRIPS AND SPOTTED WILT DISEASE MANAGEMENT IN PEANUT

by

PIN-CHU LAI

B.S., National Taiwan University, Taiwan, 2013

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

MASTER OF SCIENCE

ATHENS, GEORGIA

2015

EVALUATION OF CULTURAL TACTICS, INSECTICIDES, AND PEANUT GENOTYPES FOR THRIPS AND SPOTTED WILT DISEASE MANAGEMENT IN PEANUT

by

PIN-CHU LAI

Major Professor:	Rajagopalbabu Srinivasan
Committee:	Mark Abney
	Albert Culbreath

Electronic Version Approved:

Suzanne Barbour
Dean of the Graduate School
The University of Georgia
December 2015

To my parents.

ACKNOWLEDGEMENTS

I would like to express my deepest gratitude to my major advisor, Dr. Rajagopalbabu Srinivasan for his excellent mentoring and guidance throughout my Masters study. I am so thankful for having the opportunity to join your lab and learn how to conduct research under your supervision. My sincere appreciation goes to my committee members, Dr. Mark Abney and Dr. Albert Culbreath for contributing precious time, expertise, and positive feedbacks to help with my research all the way through. I would like to thank Dr. Shyam Tallury for providing peanut seeds for my experiments as well as all the funding sourses, USDA-NIFA, USDA- SR-IPM, National Peanut Board, and Georgia Peanut Commission, for supporting me conducting the research. I am also very grateful to Mr. Stan Diffie, Mr. Simmy McKeown, and Ms. Sheran Thompson, who worked so hard and provided me with endless help and encourangement. Without all your support and assistance during my research, I could not have done this great job. It is always so pleasant and enjoyable to work with all of you. To Mr. Billy Mills and Mr. Clint Powell, thank you both for the great assistance with the field work. To Ms. Carol Ireland and Ms. Jenny Granberry, thank you for all your support and assistance specifically with all the paper works in our department. I would like to extend my sincere gratitude to my colleagues, Ms. Anita Shrestha and Ms. Wendy Marchant for teaching me all the professional research and experimental skills as well as giving me constant cheer and support. I am so blessed to have you both to working with. I am also thankful to Dr. Babu's student workers: Nan Bostick, Julia Kitchens, Paige Still for assisting and helping me with large amount of work in the field, greenhouse, and lab. To my friend, Ms. Kathleen Marasigan, thank you for providing me with

precious assistance and support all the time. I would like to express countless thanks to my roommate and best friend, Ms. Jean Liu for always being there to help with anything you can. Last but not least, to my parents and brother, thank you all for the endless love and support. Thank you for giving me all the best and leading me to where I am today.

TABLE OF CONTENTS

Page

LIST OF TABLES

Page

LIST OF FIGURES

Page

CHPATER 1

INTRODUCTION

Peanut (*Arachis hypogaea* L.), also known as groundnut, is an important economic crop worldwide. Peanut contains high protein content, unsaturated fatty acids, and lots of micronutrients that are beneficial to human health (Francisco & Resurreccion, 2008). The United States is the third largest peanut producer in the world, while China and India rank first and second, respectively (Boriss & Kreith, 2013). The total value of peanut production in the United States in 2014 was 1.1billion US dollars (NASS, 2015). In the United States, peanut production is centered in the South. Georgia produces more than 45% of the United States peanut crop every year (Boriss & Kreith, 2013).

In the southeastern United States, spotted wilt disease caused by *Tomato spotted wilt virus* (TSWV) (family *Bunyaviridae*, genus *Tospovirus*) has been one of the biggest constraints in peanut production. Spotted wilt disease severely reduces yields and quality of peanut. Losses due to spotted wilt disease in peanut dramatically increased in the 1980s (Culbreath & Srinivasan, 2011). In 1997, an estimated value of $40 million in losses was reported in Georgia alone (Bertrand, 1998). More recently, reduction of spotted wilt incidence has been achieved due to considerable research efforts devoted to spotted wilt management (Brown et al., 2005, Culbreath & Srinivasan, 2011). The symptoms of spotted wilt disease in peanut include concentric ring-spots, various mosaic patterns and chlorosis on leaflets; stunting of all above-ground plant parts; small or reduced pegs, pods and kernels; discoloration and cracking of the seed coats; and necrosis of roots (Culbreath et al., 2003).

Thrips (order Thysanoptera), as pests of peanut, not only feed on foliage and blooms but also serve as vectors of TSWV. The tobacco thrips, *Frankliniella fusca* (Hinds) and the Western flower thrips, *Frankliniella occidentalis* (Pergande) are two major vector species of TSWV in peanut in the southeastern United States (Culbreath et al., 2003). *Frankliniella fusca* is a better colonizer of peanut seedlings than *F. occidentalis* in the early season when peanut plants are most vulnerable to virus infection. Thus, it is considered that *F. fusca* is the predominant vector of TSWV in peanut (Culbreath et al., 2003, Lowry et al., 1992).

Since the identification of *Tomato spotted wilt virus* (TSWV) in 1930 (Samuel et al., 1930), TSWV has been a severe plant pathogen causing diseases in many important crops, not only in peanut but also in tomato, pepper, and tobacco (Culbreath et al., 2003, Pappu, 2008, Pappu et al., 2009). TSWV is exclusively transmitted by several species of thrips in a persistent and propagative manner (Lewis, 1997, Whitfield et al., 2005). In nature, viruliferous thrips and TSWV infected inoculum sources are responsible for transmission of TSWV. Those appear to be the significant factor of TSWV epidemics in various cropping systems (Culbreath et al., 2003, Whitfield et al., 2005). TSWV transmission by thrips vector is a stage-specific event (Bragard et al., 2013). To become a competent TSWV vector, thrips can only acquire TSWV in the first or early second instar stages. Following ingestion by thrips larvae, TSWV replicates and infects thrips cells and reaches salivary glands. Infected thrips transmit TSWV in the late larval stage and throughout adulthood (Moritz et al., 2004, Whitfield et al., 2005). Spotted wilt disease is one of the most detrimental plant diseases in the world that causes severe yield losses. TSWV and most of the vector thrips species have wide and over lapping host ranges; this makes TSWV epidemics hard to predict and manage (Pappu et al., 2009).

There is no single management tactic that can provide sufficient spotted wilt disease control in peanut. An integrated management program has been developed with TSWV-resistant cultivars, insecticides, and a number of cultural practices (Brown et al., 2005). Cultivar selection is considered the most important factor in TSWV and spotted wilt management in the Southeast (Culbreath et al., 2003). Peanut cultivars with field resistance to TSWV have been bred and released since the first field resistance cultivar discovered in the mid-1980s (Black, 1991). TSWV and spotted wilt incidence have been greatly suppressed in resistant cultivars compared with susceptible cultivars. However, cultivars with moderate levels of field resistance may still suffer severe spotted wilt damage during an intensive TSWV outbreak (Culbreath et al., 2003). Therefore, it is always necessary to incorporate insecticides and cultural tactics with resistant cultivars in order to provide sufficient control (Culbreath et al., 2013). Second- and third-generation resistant cultivars with higher TSWV-resistance have been released in recent years (Branch, 2007, Branch, 2013, Branch & Culbreath, 2011, Holbrook et al., 2008). The performance of newly released cultivars and the efficacy when used in different integrated management programs need to be demonstrated.

Development of peanut genotypes with higher level of resistance to TSWV has been a major objective in peanut breeding programs in the Southeast. Researchers are also looking for different TSWV resistant sources. Wild species relatives have been investigated and in some cases used for commercial crop breeding programs (Rao et al., 2003). Cultivated peanut, *Arachis hypogaea* L., is a tetraploid (2n=4x=40) species. Low genetic diversity makes peanut very vulnerable to plant pathogens (Ratnaparkhe et al., 2011). Diploid wild species (2n=2x=20) in the genus *Arachis* are considered the closest relatives of cultivated peanut. *Arachis diogoi*, a diploid wild species, has been suggested to possess resistance to TSWV as well as thrips (Lyerly et al.,

2002). Recently, *A. diogoi* has been used in peanut breeding lines. In the early stage of incorporating resistant genes from wild species, more research investigating the compatibility and performance of hybrid genotypes is desirable.

In the TSW pathosystem, controlling vectors has always been an important component in disease management that could reduce thrips feeding damage and potentially suppress spotted wilt incidence. Insecticides are used to control thrips populations in peanuts. Even so, most of the insecticides are ineffective at suppressing spotted wilt incidence in peanut because they fail to prevent TSWV inoculation by viruliferous adult thrips (Chamberlin et al., 1993). The reason for this failure is unknown. It is suggested that most of the commonly used insecticides have limited efficacy against viruliferous adult thrips emigrating from adjacent fields (Chamberlin et al., 1993). Thrips can transmit virus in 5 minutes, and transmission may occur before insecitcide active ingredients take action (Culbreath et al., 2003, Wijkamp, 1995). Furthermore, the effectiveness of the insecticides through time has been questionable. It is concerned that insecticides do not last long enough to suppress thrips population and prevent thrips inoculation. Phorate, an organophosphorus insecticide (OP), has been reported to suppress spotted wilt incidence (Wiatrak et al., 2000). However, the underlying mechanism of reduction of spotted wilt incidence by phorate is unclear. Also, the highly toxic property of organophosphorus insecticide has been associated with negative environmental impacts. Several neonicotinoid insecticides without broad-spectrum non-target effects are relatively safer than widely used carbamates and Ops, and they have been labeled for use in peanut. Previous studies using neonicotinoid insecticides to control thrips and spotted wilt in peanut have provided inconsistent results. Imidacloprid application in peanut resulted in higher spotted wilt incidence compared to other insecticides and an untreated control (Todd et al., 1994). Preliminary studies also indicate

that thiamethoxam is not as effective at suppressing thrips damage as phorate and imidacloprid. On the other hand, neonicitinoid insecticide resistance in thrips has been speculated in peanut. Resistance to thiamethoxam in thrips has been reported in cotton. Factors affecting the efficacy of insecticides for spotted wilt disease management in peanut need to be clarified.

Along with the use of field-resistant cultivars and insecticides, a number of cultural practices have been manipulated for spotted wilt management. Alteration of planting dates, row patterns, seedling rates, and tillage systems are several important factors that are commonly considered and included in integrated management of spotted wilt disease (Brown et al., 2005, Culbreath et al., 2003). Selecting different planting dates can affect spotted wilt incidence due to the dynamics of thrips populations in the field (Todd et al., 1995). Changing row patterns or seeding rates will affect plant populations in a plot that will subsequently influence spotted wilt incidence (Brown et al., 2005). Tillage system determines the surrounding vegetation of the cropping system that will affect thrips populations on crops (Johnson et al., 2001). As in many pathosystems, several concerns that could affect efficacy of management tactics such as loss of host resistance in plants, evolution of highly virulent strains, and development of insecticide resistance remain. As new management options become available, and/or when some options are no longer effective, the management package has to be refined.

In this study, three objectives have been set focusing on spotted wilt management in peanut. The first objective was to evaluate the efficacy of selected insecticides and several cultural practices along with TSWV-resistant cultivars against thrips and spotted wilt disease in peanut. Second, we investigated the residue levels of selected insecticides in peanut plants over time and their efficacy against thrips population and thrips damage. Direct toxicity of insecticides, to assess thrips susceptibility to selected insecticides, was evaluated through feeding assays on

thrips. The third objective was to investigate the effects of several newer TSWV-resistant genotypes on TSWV transmission by thrips and on thrips fitness.

Literature cited

Bertrand, P.F. (1998) 1997 Georgia Plant Disease Loss Estimates. Univ. Georgia Coop. Ext. , Pub. Path. 98-007.

Black, M. (1991) Effects of spotted wilt on selected peanut varieties. *Proc. Am. Peanut Res. Ed. Soc.* , **23**, 52.

Boriss, H. and Kreith, M. (2013) Peanut profile. AgMRC.

Bragard, C., Caciagli, P., Lemaire, O., Lopez-Moya, J.J., Macfarlane, S., Peters, D., Susi, P. and Torrance, L. (2013) Status and prospects of plant virus control through interference with vector transmission. *Annu Rev Phytopathol*, **51**, 177-201.

Branch, W.D. (2007) Registration of 'Georgia-06G' peanut. *Journal of Plant Registrations*, **1**, 120-120.

Branch, W.D. (2013) Registration of 'Georgia-12Y' peanut. *Journal of Plant Registrations*, **7**, 151-153.

Branch, W.D. and Culbreath, A.K. (2011) Registration of 'Georgia-10T' peanut. *Journal of Plant Registrations*, **5**, 279-281.

Brown, S.L., Culbreath, A.K., Todd, J.W., Gorbet, D.W., Baldwin, J.A. and Beasley, J.P., Jr. (2005) Development of a method of risk assessment to facilitate integrated management of spotted wilt of peanut. *Plant Disease*, **89**, 348-356.

Chamberlin, J.R., Todd, J.W., Culbreath, A.K., Johnson, W.C., Iii and Demski, J.W. (1993) Post-harvest management of tobacco thrips (Thysanoptera: Thripidae) overwintering in peanut fields. *Journal of Entomological Science*, **28**, 433-446.

Culbreath, A.K. and Srinivasan, R. (2011) Epidemiology of spotted wilt disease of peanut caused by Tomato spotted wilt virus in the southeastern U.S. *Virus Research*, **159**, 101-109.

Culbreath, A.K., Todd, J.W. and Brown, S.L. (2003) Epidemiology and management of tomato spotted wilt in peanut. *Annual review of phytopathology,* **41**, 53-75.

Culbreath, A.K., Tubbs, R.S., Tillman, B.L., Beasley, J.J.P., Branch, W.D., Holbrook, C.C., Smith, A.R. and Smith, N.B. (2013) Effects of seeding rate and cultivar on tomato spotted wilt of peanut. *Crop Protection,* **53**, 118-124.

Francisco, M.L.D.L. and Resurreccion, A.V.A. (2008) Functional Components in Peanuts. *Critical Reviews in Food Science & Nutrition,* **48**, 715-746.

Holbrook, C.C., Timper, P., Culbreath, A.K. and Kvien, C.K. (2008) Registration of 'Tifguard' peanut. *Journal of Plant Registrations,* **2**, 92-94.

Johnson, W.C., Iii, Brenneman, T.B., Baker, S.H., Johnson, A.W., Sumner, D.R. and Mullinix, B.G., Jr. (2001) Tillage and pest management considerations in a peanut-cotton rotation in the Southeastern Coastal Plain. *Agronomy Journal,* **93**, 570-576.

Lewis, T. (1997) Pest thrips in perspective. *Thrips as crop pests.* (ed. T. Lewiss), Wallingford, Oxon, UK ; New York, NY, USA : CAB International, c1997.

Lowry, V.K., Smith, J.W. and Mitchell, F.L. (1992) Life-Fertility Tables for Frankliniella fusca (Hinds) and F. occidentals (Pergande) (Thysanoptera: Thripidae) on Peanut. *Annals of the Entomological Society of America,* **85**, 744-754.

Lyerly, J.H., Hoffman, K., Moyer, J.W. and Stalker, H.T. (2002) Evaluation of Arachis species for resistance to tomato spotted wilt virus. *Peanut science,* **29**, 79-84.

Moritz, G., Kumm, S. and Mound, L. (2004) Tospovirus transmission depends on thrips ontogeny. *Virus Research,* **100**, 143-149.

Nass (2015) National Statistics for Peanuts.

Pappu, H.R. (2008) Tomato Spotted Wilt Virus. *Encyclopedia of Virology (Third Edition).* (ed.

B.W.J.M.H.V.V. Regenmortels), pp. 133-138. Academic Press, Oxford.

Pappu, H.R., Jones, R.a.C. and Jain, R.K. (2009) Global status of tospovirus epidemics in diverse cropping systems: Successes achieved and challenges ahead. *Virus Research,* **141**, 219-236.

Rao, N.K., Reddy, L.J. and Bramel, P.J. (2003) Potential of wild species for genetic enhancement of some semi-arid food crops. *GENETIC RESOURCES AND CROP EVOLUTION*, **50**, 707-721.

Ratnaparkhe, M.B., Wang, X., Li, J., Compton, R.O., Rainville, L.K., Lemke, C., Kim, C., Tang, H. and Paterson, A.H. (2011) Comparative analysis of peanut NBS-LRR gene clusters suggests evolutionary innovation among duplicated domains and erosion of gene microsynteny. p. 164. John Wiley & Sons.

Samuel, G., Bald, J.G. and Pittman, H.A. (1930) Investigations on " Spotted Wilt " of Tomatoes. *Bulletin of the Council for Scientific and Industrial Research, Australia.* pp. 64-64. Melbourne.

Todd, J.W., Culbreath, A.K., Chamberlin, J.R., Beshear, R.J. and Mullinix, B.G. (1995) Colonization and population dynamics of thrips in peanuts in the southern United States. *Thrips biology and management: proceedings of the 1993 International Conference on Thysanoptera.* eds. B.L. Parker, M. Skinner & T. Lewiss), pp. 453-460. Plenum Publishing Co. Ltd, London; USA.

Todd, J.W., Culbreath, A.K., Rogers, D. and Demski, J.W. (1994) Contraindications of insecticide use relative to vector control and spotted wilt disease progress in peanut. *Proc. Am. Peanut Res. Ed. Soc.*, **26**, 42 (Abstr.).

Whitfield, A.E., Ullman, D.E. and German, T.L. (2005) Tospovirus-thrips interactions. *Annu Rev*

Phytopathol, **43**, 459-89.

Wiatrak, P.J., Wright, D.L., Marois, J.J., Grzes, S., Koziara, W. and Pudelko, J.A. (2000) Conservation tillage and thimet effects on tomato spotted wilt virus in three peanut cultivars. *Proceedings - Soil and Crop Science Society of Florida*, **59**, 109-111.

Wijkamp, I. (1995) Virus-vector relationships in the transmission of tospoviruses. *Agric.* p. 143. Univ. Wageningen, The Netherlands.

CHAPTER 2

LITERATURE REVIEW

Peanut: a worldwide important crop

The commercially grown peanut is a tetraploid (2n=4x=40) species *Arachis hypogaea* L., which belongs to the family Leguminosae. Peanut, also known as groundnut, is an annual plant adapted to various agro-climatic zones around the world (Carley, 1983). It blooms above ground, and then the flower withers and bends down after it is self-pollinated. The ovary develops underground and eventually becomes the fruit or the pod (Stalker & Pattee, 1995). Cultivated peanut was domesticated at least 3500 years ago in South America, and spread out to Europe, Africa, Asia, and North America by explorers and traders (Burow et al., 2009, Carley, 1983). Being brought by African slaves to the United States, peanuts were regarded as food for poor and grown for feeding livestock at first. After the civil war, peanuts became a regional food in the southern United States. Along with the green revolution in the early 1900's, peanuts are grown extensively in the United States today (APC, 2014, Carley, 1983).

The United States is the third largest peanut producer in the world, preceded only by China and India (Boriss & Kreith, 2013). The total value of peanut production in the U.S. in 2014 was 1.1billion US dollars (NASS 2015). Peanut production is mainly located in mid-atlantic, southeastern, and mid-south regions of the United States. Georgia is the number one producer with more than 45 percent of the U.S. peanut crop every year (Boriss & Kreith, 2013, Carley, 1983). Four types of peanuts, the runner, Virginia, Spanish and Valencia, are produced in different regions for diverse use in the U.S. Runner type peanuts are mostly planted in the

Southeast and constitute about 80 percent of the planted acreage in the U.S. Forty five percent of peanuts produced in the U.S. are used for peanut butter, followed by 30 percent for gourmet snacks, and 25 percent for candy and confections (APC, 2014, Boriss & Kreith, 2013).

Peanut seeds provide a rich source of protein (~25%), fat (~50%), vitamins and minerals (Carley, 1983). Beside food products, peanut is an important oilseed crop. Peanut is naturally cholesterol-free with about 80% unsaturated fat. Consumption of tree nuts and peanuts reduces the risk of heart disease incidence (Kris-Etherton et al., 2008). Not only because of unsaturated fatty acid, peanut also contains other micronutrients that are beneficial to human health, such as α-tocopherol, ferulic acid, resveratrol, and other phenolic compounds (King et al., 2008, Kris-Etherton et al., 2008). Recently, peanut has been recognized as functional food that categorized in a heart-healthy diet (Francisco & Resurreccion, 2008). High protein composition makes peanut an important animal feed worldwide (Carley, 1983). Peanut protein serves as an important replacement for animal proteins, especially in some developing countries where meat is not readily available for most of the population (Adjou et al., 2012). Peanut is becoming more and more popular for snacks, plant oil, and even main food around the world.

Spotted Wilt Disease of Peanut

Peanut production in the United States is constrained by numerous pests and diseases. In the early 1900's, some fungal diseases diseases such as white mold were noticed as well as several insect pests and nematodes (Carley, 1983). Spotted wilt disease caused by *Tomato spotted wilt virus* (TSWV) has been one of the most detrimental factors in the southeastern United States since late 1980s (Culbreath & Srinivasan, 2011).

In the United States, spotted wilt disease of peanut was first reported in Texas in 1971 (Halliwell & Philley, 1974). In the late 1980s, significant losses to spotted wilt in peanut and TSWV epidemics were reported in other peanut producing states such as Alabama and Georgia (Black et al., 1987, Hagan et al., 1990, Todd et al., 1995). Peanut yields can be greatly reduced due to the stunting or death of entire plants and reduced pod production. In 1997, estimated yield losses to spotted wilt disease were 12% of the entire crop, representing value of 40 million USD in Georgia alone (Bertrand, 1998). Based on data from 1996 to 2006, the average loss to TSWV in peanut was estimated to be $12.3 million in Georgia (Riley et al., 2011). Losses in the peanut crop due to spotted wilt disease fluctuate over time. Loss estimates have decreased in recent years, likely due to the development and improvement of TSWV management programs. Nevertheless, the Georgia plant disease loss estimate report indicated that spotted wilt disease of peanut is still a major constraint (Kemerait, 2013).

History, Host Range, and Symptoms of *Tomato spotted wilt virus*

The disease known as spotted wilt was first described on tomato (*Lycopersicon esculentum* Miller) in Australia in 1915 (Brittlebank, 1924). A few years later, the involvement of onion thrips (*Thrips tabaci* Lindeman) in transmitting the disease-causing agent was reported (Pittman, 1927). A virus, which was named Tomato spotted wilt virus (TSWV), was identified to be the causative agent of spotted wilt disease in 1930 (Samuel et al., 1930).

TSWV has a wide host range. TSWV is reported to infect at least 1090 plants species in over 85 families including monocots and dicots (Culbreath et al., 2003, Parrella et al., 2003, Tsompana & Moyer, 2008). Many of those plants are hosts for both the vectors and the virus that contribute to spotted wilt epidemics in crop plants (Groves et al., 2001, Groves et al., 2002,

Srinivasan et al., 2014). A number of weeds and important economic crops are hosts of TSWV such as tomato, peanut, pepper, potato, and tobacco (Culbreath et al., 2003, German et al., 1992, Pappu, 2008, Riley et al., 2011). TSWV is one of the plant viruses with the largest host range, and more and more plant species have been found to be the host of TSWV (Gognalons et al., 1998, Krstic´ et al., 2008). The worldwide geographic distribution of TSWV in temperate regions is associated with its thrips vector (Tsompana & Moyer, 2008).

The symptoms of spotted wilt disease vary greatly depending on host plant species, cultivar, plant age, virus strain, and environmental conditions (German et al., 1992, Riley et al., 2011). The typical symptoms of spotted wilt induced by TSWV include ring spots, speckling, mottling, chlorosis, necrotic lesions on leaflets, and yellowing, stunting, wilting in plants (Riley et al., 2011). Subsequently, it usually results in severe yield losses. An estimate of worldwide annual losses to TSWV was over $1 billion in 1998 (Prins & Goldbach, 1998, Scholthof et al., 2011, Ullman et al., 1997). In Georgia alone, there were $326 million in losses from TSWV epidemics on peanut, tobacco, tomato, and pepper from 1996 to 2006 (Riley et al., 2011).

Spotted wilt disease of peanut was first reported in Brazil in the mid-1900s (Costa, 1941). Reports addressing spotted wilt or similar disease in peanut were also made from South Africa (Klesser, 1967), Australia (Helms et al., 1961), India, and the United States (Halliwell & Philley, 1974) in the following decades. On peanuts, TSWV infection induces symptoms of spotted wilt such as concentric ring-spots, various mosaic patterns and chlorosis on leaflets; stunting of all above-ground plant parts; small or reduced pegs, pods and kernels; discoloration and cracking of the seed coats; and necrosis of roots (Culbreath & Srinivasan, 2011, Culbreath et al., 2003). Asymptomatic infections of TSWV occur as frequently as symptomatic infections (Culbreath et al., 1992). Peanut plants showing chlorosis, yellowing or wilting symptoms without typical

TSWV aboveground symptoms were shown to be highly associated with TSWV infection (Culbreath et al., 1991, Culbreath et al., 2003).

Taxonomy, Structure and Genome Organization of *Tomato spotted wilt virus*

Tomato spotted wilt virus (TSWV) belongs to the genus *Tospovirus* in the family *Bunyaviridae*. The family *Bunyaviridae* contains more than 300 virus species recognized as pathogens transmitted by arthropods, but the genus *Tospovirus* is the only plant-infecting group (Nagata & Peters, 2001, Pappu, 2008, Whitfield et al., 2005). Except for infecting plant hosts, tospoviruses share many morphological and molecular characteristics typical of other members in the family *Bunyaviridae* (Haan et al., 1989, Tsompana & Moyer, 2008). Some of the other virus species in this family are important arthropod-transmitted pathogens which cause a suite of human and animal diseases such as *Hantavirus, Heartland virus* (HRTV), and *Bunyamwera virus* (Bosco-Lauth et al., 2015, Charbonnel et al., 2014, Nagata & Peters, 2001, Odhiambo et al., 2014). TSWV is considered the type species of the genus *Tospovirus*. The *Tospovirus* genus was categorized as a monotypic virus group with a single virus (TSWV) until the report of *Impatiens necrotic spot virus* (INSV) in 1991 (Laviña & Batlle, 1994, Ruter & Gitaitis, 1993). Initially, *Peanut bud necrosis virus* (PBNV) was considered a strain of TSWV, but now PBNV is classified as a distinct virus in the *Tospovirus* genus (Reddy et al., 1992). There are currently more than 20 species in the genus *Tospovirus* that cause severe disease; they are classified primarily based on serological properties and the amino acid sequence identity of the viral structural proteins (Avila et al., 1993, Bragard et al., 2013).

TSWV virus particles are spherical, 80-120nm diameter with a host-derived membrane. Two viral glycoproteins (Gn/Gc) are integral components of the membrane, which form 5-10nm long

surface projections (German et al., 1992, Tsompana & Moyer, 2008, Whitfield et al., 2005). The core of the virion is composed of ribonucleoproteins (RNPs) and a few copies of the RNA-dependent RNA polymerase (RdRp); RNPs are a complex of the single-stranded RNA encapsidated by the nucleoprotein (N) (Tsompana & Moyer, 2008, Whitfield et al., 2005).

TSWV has a tripartite negative-stranded RNA genome, which is a distinctive characteristic of the family *Bunyaviridae*. The genome consists of three single-stranded RNAs, namely S (2.9kb), M (4.8kb), and L (8.9kb) RNA. The RNAs have a panhandle structure created by base pairing of the termini with the inverted complementary sequences of each strand; the eight-nucleotide sequence (5' AGAGCAAU 3') is strictly conserved among all tospoviruses(Tsompana & Moyer, 2008, Whitfield et al., 2005). The panhandle conformation is suggested to serve as a promoter for replication (Tsompana & Moyer, 2008). All the proteins of TSWV are expressed by translation of subgenomic messenger RNA species (mRNA) (German et al., 1992).

L RNA is completely negative (complementary) sense that encodes the L protein. L protein has been identified as the putative RNA-dependent RNA polymerase (RdRp) based on sequence motifs characteristic of polymerase, functional analyses and sequence homology with all segmented negative-stranded RNA viruses (Haan et al., 1991, Tsompana & Moyer, 2008). The viral RdRp in TSWV and, by analogy to other viruses in the Bunyaviridae, is a multifunctional protein associated with replication that has activities of NTPase, polymerase, nuclease, and helicase (Pappu, 2008, Whitfield et al., 2005).

M RNA is ambisense with the positive (viral) sense coding for a nonstructural protein (NSm) and the negative (complementary) sense coding for the two viral membrane glycoproteins, Gn and Gc (Kormelink et al., 1992). There is a potential transcription termination hairpin loop in the intergenic region between the two genes in the M RNA; the stable hairpin structure remains in

the subgenomic mRNA products (German et al., 1992). The NSm serves as a viral movement protein supported by the profile of early expression after infection and tubule formation; NSm facilitates virus cell-to-cell movement through plamodesmata of plant hosts (Kormelink et al., 1992, Storms et al., 1998). The glycoproteins (Gn/Gc) are suggested to associate with virus assembly and vector transmission. Both Gn and Gc are anchored in the viral membrane with cytoplasmic tails, which are expected to interact with RNPs and play a vital role in virion packaging (Whitfield et al., 2005). In addition to virus assembly, it is indicated that glycoproteins are involved in virus acquisition by thrips vectors and probably serve as viral binding and fusion proteins during virus entry(Whitfield et al., 2005). TSWV virus particles lacking envelop and glycoproteins were not thrips transmissible (Nagata et al., 2000).

S RNA is also an ambisense RNA segment. The nonstructural protein (NSs) is encoded in positive (viral) sense, while the nucleoprotein (N) is encoded in viral complementary (negative) sense of S RNA (Haan et al., 1990). The NSs has been shown to function in suppression of RNA silencing, a defense system against virus infection in plants (Takeda et al., 2002). The nucleoprotein serves as a structural protein attached to ssRNAs to form RNPs with putative regulatory activities; it is also suggested the nucleoprotein plays a role in regulation of the initiation of transcription and replication of TSWV (Pappu, 2008, Whitfield et al., 2005).

Thrips as pests and vectors of TSWV: morphology, biology, and ecology

Plant-infected tospoviruses are exclusively transmitted by several species of thrips in nature (Bragard et al., 2013, Whitfield et al., 2005). Thrips is the common name of insects in the order Thysanoptera(Lewis, 1997). Thrips is one of the important crop pests worldwide. Among all known thrips species, most of the serious crop pests and pathogen vectors are in the suborder

Terebrantia, especially in the family Thripidae (Mound, 1997, Riley et al., 2011). Adult thrips are usually tiny, slender insects only 1-2 millimeters long. Most long-winged adults have two pairs of band-like wings all with a fringe of long hairs, which is the most distinct characteristic in Thysanoptera. However, some species can have reduced wing form adults along with the long-winged form in the population (Lewis, 1973). For example, tobacco thrips, *Frankliniella fusca*, have been documented to have two wing morphs in a population, namely macropterous (winged) and brachypterous thrips. Macropterous thrips have fully functioned wings leading to higher dispersal abilities, while the wings of brachypterous thrips are greatly reduced and nonfunctional. The roles of two wing morphs thrips in TSWV transmission and epidemics have been investigated. Wells et al. (2002) found no difference in the transmission abilities between macropterous and brachypterous thrips in a laboratory study; however, the proportion the two wing morphs in the field population changed over time in a year. Brachypterous forms are predominant in the fall and winter, whereas macropterous forms dominate during the spring and summer. The number of macropterous thrips collected in fields that tested positive for TSWV was more than the number of brachypterous thrips. It was concluded that macropterous thrips are more likely to colonize and subsequently transmit TSWV to newly emerged crops, while brachypterous tobacco thrips help harbor TSWV over the winter (Wells et al., 2002).

The body of adult thrips is dorsoventrally flattened and color varies from yellow to brown, black (Moritz, 1997). The asymmetric, piercing-sucking mouthparts of thrips cause a lot of damage to plant tissues. While feeding, the mouth cone punctures the leaf epidermis and ingests the cytoplasm from epidermal and /or mesophyll cells (Kirk, 1997, Ullman et al., 1992a). Many thrips species are polyphagous with a wide host range, either feed on foliage, pollen, flower, or fruit of plants (Lewis, 1973).Whole virus particles were observed in thrips guts with intact plant

organelles by using the electron microscope (Takeda et al., 2002), which indicated thrips acquire virus through feeding. Two feeding modes have been observed in *F. occidentalis*. Probe with short duration is the mode that occurs more often; thrips salivate and empty the contents of plant cells just under the epidermal surface. Probes with longer duration which consist of a quick salivation followed by a long period of ingestion are seldom made (Hunter et al., 1994, Kindt et al., 2003, Kirk, 1997). TSWV infection is known to affect thrips vectors' feeding behaviors. Stafford et al. (2011) indicated that male *F. occidentalis*, one of the competent vectors, infected with TSWV had more feeding damage, which is up to threefold frequency than uninfected males; and the higher frequency of feedings subsequently increased the probability of TSWV inoculation.

Thrips can injure host plants by direct feeding; more importantly, thrips indirectly threaten host plants by transmitting viruses in some cases (Kirk, 1997). For example, *Frankliniella fusca* (Hinds) is known as a serious pest of seedling peanuts in the U.S.; they feed on terminals of peanut plants, which causes distorted leaflets and stunted plants (Riley et al., 2011, Young et al., 1972). Additionally, *F. fusca* was confirmed to act as the vector of TSWV in peanut and is responsible for secondary spread of spotted wilt disease within peanuts (Lowry et al., 1992, Sakimura, 1962).

Most of the thrips species use arrhenotoky reproductive strategy. It is one of the sexual reproductive strategies, which is also known as haplodiploid sex-determination. After copulation, females using arrhenotoky strategy lay fertilized and unfertilized eggs. Eggs with diploid number of chromosomes, which are fertilized, will become females; while haploid, unfertilized eggs will develop as males. A few thrips species reproduce asexually and have only female progeny, which is known as thelytokous parthenogenesis (Chatzivassiliou et al., 2002, Moritz, 1997).

Different reproductive strategies in thrips populations may lead to variation in vector ability and further influence viral epidemics (Chatzivassiliou et al., 2002).

The tobacco thrips, *Frankliniella fusca* (Hinds) and the western flower thrips, *Frankliniella occidentalis* (Pergande) are two of the nine known vectors of TSWV (Riley et al., 2011), and both species appear in most peanut-producing areas of the United States (Culbreath et al., 2003). *F. fusca* is the predominant thrips species on cultivated peanut plants (>80%) whereas *F. occidentalis* is predominant in vegetation around cultivated fields (Lowry et al., 1992). Moreover, *F. occidentalis* primarily feed on the floral parts of the plant. *F. fusca* are flower and foliage feeders and better colonizers in the early season when peanut seedlings are vulnerable to virus infection (Riley et al., 2011, Todd et al., 1995). Based on the feeding site and reproductive ability on peanuts, *F. fusca* is considered the primary competent vector of TSWV in peanuts in the southern United States (Bragard et al., 2013, Culbreath et al., 2003, Lowry et al., 1992).

The life cycle of thrips varies from one species to another (Riley et al., 2011). The development and life cycle of *F. fusca* have been investigated and documented since it is endemic to peanut fields in the southern United States (Lowry et al., 1992). In terebrantian species, female adults insert bean like or kidney-shaped eggs into peanut leaf, flower, or fruit tissue of the host plant (Moritz, 1997). *F. fusca* oviposit in the leaf tissue of peanut plants. At 30°C, eggs will hatch in six days, and enter the first instar stage. First instar larvae molt into second instar larvae in ~24 h. The second instar stage lasts for about 2.5 days, and the larvae enter the prepupaal stage (Lowry et al., 1992). The first and second instars are feeding stages; they are unskeletized and pale or light yellow in color, and they resemble a miniature version of adults (Moritz, 1997). The prepupae and the following pupae stage are quiescent (mostly immobile) and non-feeding stages; they have faded or colorless, unsclerotized cuticle with setae

and well-developed wing pads (Lewis, 1973, Moritz, 1997). The prepupae stage lasts for 1 day and pupae stage is 1.4 days long in average (Lowry et al., 1992). The duration of development in one generation of *F. fusca* is around 12 days, from eggs to adults. Fecundity of *F. fusca* at 30°C is 24 eggs per female, and female adults can live for 9 days. *F. fusca* females tend to oviposit more eggs in the early adulthood. Thrips development is known to be temperature-dependent, which means the developmental time decreases as temperature increases. *F. fusca* has the highest reproductive capacity and developmental rate at 30°C (Lowry et al., 1992).

Most of the plant disease vector species share important ecological characteristics such as extreme polyphagy and a broad host range of plants that can support successful reproduction (Mound, 1997). Many thysanopteran species are highly polyphagous. The western flower thrips attacks over 60 plant families that include several crop plants (Childers, 1997, Yudin et al., 1986); it is a cosmopolitan pest was spread through international trade of ornamental plants in the late 1900s (Perrings et al., 2005). The tobacco thrips has been a domestic species reported in all states in the U.S. until its recent discovery in Japan (Nakao et al., 2011). It is especially abundant in the southeastern U.S. (Diffie et al., 2008, Riley et al., 2011). *F. fusca* are documented to infest many weeds and economically important crops in the southeast, such as peanut, tomato, tobacco, cotton, and onion (Gitaitis, 2014, Groves et al., 2003, Salguero Navas et al., 1991, Srinivasan et al., 2014, Todd et al., 1995).

TSWV transmission and epidemics

Epidemics of thrips-transmitted plant pathogens such as TSWV require the interaction of three biological entities: the thrips vectors, TSWV pathogen, and plants serving as hosts for both the virus and their vectors (German et al., 1992, Ullman et al., 1997). In nature, mobile viruliferous

thrips and source of TSWV inoculum are responsible for transmission of TSWV, (Culbreath & Srinivasan, 2011, Culbreath et al., 2003, Pappu, 2008, Ullman et al., 1997, Whitfield et al., 2005). Weed hosts susceptible to TSWV can be important sources of inoculum when their growing seasons overlap with economic crops (Pappu, 2008). TSWV can be mechanically inoculated into host plants under experimental conditions, but in nature he virus is exclusively transmitted by thrips (Mandal et al., 2001, Shrestha et al., 2015). Mechanical transmission by physical contact of plants does not appear to be important in nature (Culbreath et al., 2003, Whitfield et al., 2005). There is no evidence of seed transmission of TSWV in peanut, even though TSWV can be found in the pod shell and testae of seed from infected peanut plants (Culbreath et al., 2003, Pappu et al., 1999).

TSWV is transmitted by several species of thrips in a persistent, and propagative manner (Bragard et al., 2013, German et al., 1992, Pappu, 2008, Whitfield et al., 2005). The relationship between thrips vectors and tospoviruses is very specific as many insect vector and plant virus interactions (German et al., 1992, Whitfield et al., 2005). TSWV will distribute, replicate and can be passed transstadially in the body of thrips vectors (Ullman et al., 1992a, Whitfield et al., 2005, Wijkamp & Peters, 1993). TSWV, as well as other members of tospovirus, establish a unique and specific relationship with their thrips vectors that infected or viruliferous adult thrips can only acquire virus during their larval stages (German et al., 1992). To become a TSWV transmitter, the larvae have to feed on an infected host plant that serves as an inoculum source to acquire the virus. First and sometimes second instar larvae are able to acquire virus, and the acquisition rates decrease as larvae age (Pappu, 2008, Tsompana & Moyer, 2008, van de Wetering et al., 1996). After acquisition, the virus replicates in the thrips body throughout all developmental stages. The emerging adults are capable of transmitting the virus to non-infected

plants in their saliva during feeding (Pappu, 2008, Tsompana & Moyer, 2008). TSWV infection and TSWV replication occur in both larval and adult stages; however, acquisition by early instar larvae is a prerequisite for TSWV transmission (van de Wetering et al., 1996, Whitfield et al., 2005). TSWV infection has been observed in adult thrips in the midgut and surrounding muscle cells after an acquisition access period, but not in ligament-like tissue or salivary glands; it is suggested that the basal lamina serves as potential barrier to virus movement out of the midgut of thrips (Ullman et al., 1992b, Whitfield et al., 2005). Transovarial transmission of tospoviruses has not been demonstrated, thus virus can only be maintained in thrips for one generation (Pappu, 2008). Many plants can be infested by thrips and infected by tospovirus; however, if plants cannot support thrips development, the epidemic of TSWV will cease when plants serve as dead end hosts (Pappu, 2008, Whitfield et al., 2005).

After acquisition by thrips vectors via feeding, TSWV viral particles travel from the alimentary canal through the foregut and arrive at the midgut where they bind and enter insect cells (Assis Filho et al., 2002, Ullman et al., 1992b). The fact that only nine out of over 5000 described thrips species are known to transmit TSWV indicates the specificity of TSWV- thrips interaction (Pappu, 2008, Riley et al., 2011). The specificity may be due to the specific binding of TSWV glycoproteins (Gn/Gc) with thrips midgut receptors, which may be absent in non-vector species (Pappu, 2008). TSWV without functional glycoproteins are not transmittable by thrips(Nagata et al., 2000). It is suggested that TSWV Gn protein plays an important role in recognition and binding vector gut cells, while Gc protein serves as a fusion protein and facilitate the entry into the vector gut (Pappu, 2008, Whitfield et al., 2005). A putative thrips receptor was found in *F. occidentalis*; a 50-kDa protein was identified in larval gut cells, but not in adult guts (Bandla et al., 1998).

TSWV virus particles cross over the membrane of microvilli aligning in thrips gut lumen followed by the infection of visceral muscle cells surrounding the midgut (Nagata et al., 1999). Virus must travel to the primary salivary glands of the thrips vector to be transmitted (Assis Filho et al., 2005, Kritzman et al., 2002). Two possible mechanisms of virus entry into salivary glands have been proposed and supported (Whitfield et al., 2005). The most compelling hypothesis is based on thrips ontogeny. The distance between internal organs changes during thrips development (Moritz et al., 2004). A study done by Moritz et al. on *F. occidentalis* showed that the primary salivary glands, midgut, and visceral muscle cells are compressed into an area of thorax where they are in direct contact in the first instar stage through the early second instar stage. It is proposed that virus move from midgut to the primary salivary glands while the primary salivary glands directly contact with membranes of the visceral muscle cells (Moritz et al., 2004). This hypothesis is consistent with the phenomenon that only adults that acquired virus in larval stages can transmit the virus (Whitfield et al., 2005). Another hypothesis suggested that virus moves from midgut to the primary salivary glands through structures connecting those two organs. The tubular salivary glands and thin-ligament-like structures are the two known structures connecting midgut and the primary salivary glands based on thrips anatomy (Nagata et al., 1999, Ullman et al., 1992b). Several studies supported the assumption that the ligament-like structure may serve as a conduit for virus movement by providing evidence of TSWV infection of this structure in vector species, but not in non-vector thrips species. Infection of ligament-like structure occurred prior to the infection of salivary glands (Assis Filho et al., 2002, Nagata et al., 2000, Nagata et al., 1999). However, there is no infection or virus replication in the tubular salivary glands (Assis Filho et al., 2002). A less plausible hypothesis, without any direct evidence, has been addressed. Refers to other persistently transmitted viruses,

it is suggested that TSWV enters and circulates in the hemocoel and eventually infects the primary salivary glands (Tsompana & Moyer, 2008, Whitfield et al., 2005). After reaching the salivary glands of thrips, TSWV is able to be transported via saliva to host plants during thrips feeding (Kirk, 1997).

TSWV and Spotted Wilt Disease Management

TSWV causes significant economic losses in several cropping systems annually, mainly due to suppression of plant growth, reduction of yields and quality. Spotted wilt disease pathosystem, which includes thrips vectors, TSWV, and host plants, is very complex due to the overlapping host ranges of both TSWV and thrips vectors(Pappu, 2008). Vector control is usually inadequate to suppress TSWV epidemics due to high fecundity of thrips, insecticide resistance development, and extensive external sources of inoculum (Tsompana & Moyer, 2008). An integrated management approach must be adopted since there is no single tactic that provides effective control by itself (Pappu, 2008). To improve spotted wilt disease management in peanut in the Southeast, an interdisciplinary research program has been employed. Critical factors or tools contributing to manage spotted wilt epidemics have been identified. Combinations of multiple management practices have proven to provide better control of TSWV than applying only one or two tactics at a time (Culbreath et al., 2003, Todd et al., 2000). Integrated management of spotted wilt disease in peanut primarily combines cultivar selection, chemical control, and several cultural practices.

Cultivar selection

Among all the tactics in spotted wilt management in peanut, cultivar selection is the most important factor in the southeastern United States (Culbreath et al., 2003). Peanut cultivar

"Southern Runner" was first observed to possess moderate field resistance to TSWV in the mid-1980s in Texas (Black, 1991). A TSWV epidemiology study in Georgia provided consistent results that Southern Runner had lower TSWV incidence rate than Florunner, a major cultivar used at that time (Culbreath et al., 1992). Ever since the observation of TSWV field resistance in peanut genotypes, intensive cultivar and breeding line screening has been conducted. Numerous runner-type cultivars with field resistance to TSWV were released after resistant sources were identified. Georgia Browne (Culbreath et al., 1994), Georgia Green (Culbreath et al., 1996), and Tamrun 96 (Smith et al., 1998) are examples of first generation TSWV resistant cultivars. The performance of field resistant cultivars has been consistent in comparison with susceptible cultivars; however, cultivars with moderate levels of field resistance may still suffer severe damage during TSWV outbreaks (Culbreath et al., 2003). In regard to reduction of spotted wilt incidence in resistant cultivars, test results in the field trials through thrips-mediated transmission and from greenhouse experiments by mechanical inoculation were not consistent in some cases (Mandal et al., 2002, Pereira et al., 1995). Differential responses of peanut plants to varying levels of virus amount in thrips vectors and virus inoculum sources may occur and lead to the inconsistent performance of resistant cultivars (Culbreath et al., 2003). A transmission study on several newly released field resistant genotypes suggested that the resistant trait in TSWV-resistant cultivars is tolerance instead of true resistance (Shrestha et al., 2013). Complete virus resistance has not been found, and the underlying mechanism of the field resistance /tolerance in peanut breeding lines has not been clarified. Even so, field resistant cultivars are still an indispensable component in integrated spotted wilt management programs. Georgia Green was a standard cultivar that was widely planted in the Southeast soon after it was released until 2010 (Culbreath et al., 2000, Culbreath & Srinivasan, 2011). Second generation TSWV-resistant

cultivars with higher level of field resistance than previously released were released later. One of the second-generation cultivars, Georgia-06G, has replaced Georgia Green as the predominant cultivar grown in the Southeast since 2010 (Beasley J. P., 2011, Monfort, 2015). The high yielding property and sufficient field resistance to TSWV made Georgia-06G (Branch, 2007) the most widely used cultivar. By using second-generation cultivars as parental species in breeding lines, third-generation resistant cultivars were developed and released. Examples Georgia-10T (Branch & Culbreath, 2011) and Georgia-12Y (Branch, 2013). It is believed that the TSWV resistance level is even higher in third-generation cultivars; however, their actual performance needs to be verified. The development of new cultivars with greater levels of resistance to TSWV is the most potential and desirable way to improve management of spotted wilt in peanut that could reduce the dependence on chemicals and other cultural tactics; also the integrated management program can be more flexible. On the other hand, researchers are also searching for new sources of TSWV resistance. A possible alternative source of TSWV resistance is the wild species of *Arachis*. In fact, those relatives of cultivated peanut possess high levels of resistance to pets and disease (Stalker & Pattee, 1995). Several *Arachis* species have been identified as highly resistant to virus, such as *A. diogoi* and *A. correntina*, and they have been used as parents in crossing programs to incorporate TSWV resistance genes in to *A. hypogaea* (Lyerly et al., 2002).

Chemical control

Insecticides are used to manage thrips, which are the exclusive vectors of TSWV (Culbreath et al., 2003). Nonetheless, insecticide applications have been ineffective in suppressing spotted wilt in peanut due to the failure to prevent plant inoculation by viruliferous adult thrips (Chamberlin et al., 1993). Transmission of tospovirus occurs fast; inoculation could be completed in as short

as 5 minutes to 30 minutes before vectors are killed by insecticides (Culbreath et al., 2003, German et al., 1992). Insecticides applied in winter or spring, which are usually not the growing seasons, were also used to control overwintering thrips in fallow fields or on volunteer peanut plants; however, controlling early populations of thrips has not resulted in consistent reduction of spotted wilt in the subsequent peanut crop (Todd et al., 1996). Aldicarb, a carbamate insecticide, and phorate, an organophosphate insecticide, have been the standard in-furrow insecticides applied in peanut in the Southeast (Baldwin, 2001, Culbreath et al., 2003, Herbert et al., 2007). A Study by Todd et al. (1996) indicated that the application of phorate typically offers no better control than other insecticides in reducing thrips populations; thus the mechanism responsible for suppression of spotted wilt by phorate remains unclear. Phorate can be phytotoxic on peanuts and often causes marginal chlorosis and necrosis on peanut foliage. It is suggested that the underlying mechanism of phorate is related to defense response of young host plants or inhibition of virus replication or movement (Gallo-Megher et al., 2001, Jain et al., 2015). Other types of compounds such as plant defense activator or botanical insecticide have been evaluated for spotted wilt management, but results of those chemical compounds on peanut were not consistent. Almost none of them is effective and economically feasible (Culbreath et al., 2003). Fungicide seed treatment is a standard practice on peanut in the United States (Culbreath et al., 2003); a combination of fungicide seed treatments has been reported to increase plant populations and greater plant stands resulted in reduced spotted wilt incidence (Brenneman & Walcott, 2001). Use of herbicides combined with insecticides has shown both positive and negative effects on spotted wilt incidence in peanut in different scenarios (Culbreath et al., 2003). There is a limited choice of chemicals for spotted wilt management in peanut. The use of phorate itself is not sufficient to prevent yield losses due to spotted wilt. In addition, the highly

toxic and broad-spectrum properties of commonly used carbamate and organophosphate insecticides have been concerning in recent years with rising environmental awareness (Singh et al., 2010, Williams, 1997). Due to the broad-spectrum and highly toxic properties, aldicarb will be completely phased out in 2018 (AgroNews, 2010). Phorate, on the other hand, is also highly toxic to a broad-spectrum of non-target species that environmental risks of phorate are concerned (Singh et al., 2010, Williams, 1997). Alternative insecticides with less environmental impact are needed to integrate with other management tactics. Neonicotinoid insecticides with less non-target effects are now available for use in peanut, yet the outcomes of applying those insecticides were not consistent in regard to spotted wilt control (Culbreath et al., 2003).

Development of insecticide resistance is also a crucial concern when selection pressure from a sinlge insecticide mode of action is high for an extended period of time. *F. fusca* resistant to thiamethoxam has been reported in cotton in Georgia (Johnson, 2014). Therefore, it is critical to apply insecticide effectively and efficiently. To avoid insecticide resistance development, rotation of insecticide application with multiple mode of actions (MOAs) is recommended, and insecticides with new MOAs are always desirable in insecticide resistance management as well as sustainable disease management.

Planting date

In peanut production area, planting date has been reported to be an important factor in epidemics of TSWV (Culbreath et al., 2010, Culbreath et al., 2003, Hurt et al., 2005, Nuti et al., 2014, Tillman et al., 2007). Dynamics of thrips populations in peanut plants and non-crop plants are putative factors affecting peanut crops planted in different dates (Culbreath et al., 2003). In the southeast, larger populations of TSWV vector *F. fusca* were found on April-planted peanuts, while peanut planted in May had fewer numbers of thrips (Todd et al., 1995). Studies indicated

that later planting in May or even June has provided significant suppression of spotted wilt incidence compared with planting in April (Hurt et al., 2005, Nuti et al., 2014, Tillman et al., 2007). In fact, later planting dates have been adopted widely in peanut producing areas.Some environmental factors differ by time and might play crucial roles in spotted wilt incidence. Lower temperature (25-30°C) promotes TSWV systemic infection of peanut plants by mechanical inoculation compared with inoculation under higher temperature (30-37°C) (Mandal et al., 2002). In Georgia, a shift of peanut planting dates from late April to early or middle of May occurred after emergence of spotted wilt disease (Culbreath et al., 2003). However, with other factors affecting timing of planting such as weather and equipment limitation, sometimes growers prefer to start planting peanuts earlier than the optimum planting dates for spotted wilt management. Researchers have been evaluating the effectiveness of different combinations of management tactics such as using higher-level TSWV resistant cultivars to reduce the risk of severe TSWV outbreak mediated by high thrips population in early planting (Culbreath et al., 2010).

Plant population

Manipulation of crop population is another viable tool for suppressing spotted wilt in peanut. Higher plant populations are likely to reduce the percentage of infected plants, even though the number of infected plants does not decrease at all (Brown et al., 2005, Culbreath et al., 2003). Several studies indicated that higher seedling rates or plant stands have resulted in lower spotted wilt incidence and higher yields (Branch et al., 2003, Tubbs et al., 2011, Wehtje et al., 1994). Field survey data have also indicated that when the seedling rate was below 13 plants per meter of row, the spotted wilt incidence will severely increase (Brown et al., 2005).

Row pattern

Selection of row pattern can also influence the incidence of spotted wilt disease and yield for peanut (Tubbs et al., 2011). Single row pattern is default in peanut planting in the Southeast that peanuts are often planted on beds 1.8m wide with two single rows spaced about 91cm apart. The planting of twin rows, which are spaced 18-24 cm apart at the same seeding rate per acre as single row, has been largely adopted in the Southeast (Brown et al., 2005). Studies have reported significant reduction in spotted wilt incidence and higher yields when row pattern was altered from single to twin rows (Culbreath et al., 2008, Tillman et al., 2006). Healthy plants in twin rows are assumed to be able to compensate for stunted plants due to TSWV. The reason for spotted wilt incidence reduction remains unknown, but may involve visual interference with host plant recognition of thrips vectors (Culbreath et al., 2003).

Tillage system

Conventional tillage system has been compared with conservation tillage system in peanut production for disease management. Conservation tillage is a method of soil cultivation that leaves residues on the soil surface. Studies indicated that use of minimum tillage resulted in lower incidence of spotted wilt (Baldwin, 2001, Chamberlin et al., 1993, Johnson et al., 2001), lower thrips populations (Brown et al., 1996), and less thrips feeding damage (Minton et al., 1991). However, extra plant residues appear in the field would possibly increase the occurrence of secondary pests. Conventional tillage system is usually labor-intensive and time-consuming. Therefore, conservation tillage can reduce the cost of crop production and potentially suppress the incidence of spotted wilt disease in peanut (Culbreath et al., 2003).

Weed control

Weeds such as non-crop plants or volunteer peanuts nearby crops may serve as reservoirs for TSWV and thrips vectors. Thrips populations on weeds can be important and affect spotted wilt

epidemics (Culbreath et al., 2003, Groves et al., 2002). A study done by Srinivasan et al. (2014) indicated that some winter weeds differentially supported thrips reproduction and development; moreover, they were effectiveTSWV reservoirs.

Spotted wilt risk index

To promote the adoption of integrated management program by peanut growers, a spotted wilt risk index has been developed in Georgia, Florida, and Alabama. The index gives a score based on cultivar selection, planting date, plant population, in-furrow insecticide, disease history, row pattern, tillage system, and herbicide usage in a particular field. It allows growers to assess the relative risk of spotted wilt and identify the best combination of disease-suppressive factors for them to apply (Brown et al., 2005, Culbreath et al., 2003).

Other control strategies

A transgenic approach has been used to develop peanut lines with resistance to TSWV by incorporating TSWV coat protein gene. However, TSWV field resistant levels in transgenic peanut lines have been inconsistent (Li et al., 1997, Magbanua et al., 2000). An entomoparasitic nematode, *Thripinema fuscum*, has been studied and considered as a biological control agent for spotted wilt management; the ability of *Thripinema fuscum* to infect TSWV vector *F. fusca* and further interfere with its feeding behavior and TSWV transmission has been reported (Funderburk et al., 2002, Sims et al., 2009). The potential for utilizing this agent to aid in spotted wilt disease management has not yet been fully demonstrated. RNA interference (RNAi) tool has been evaluated and studied as a thrips control agent (Badillo-Vargas et al., 2015). Although it is still in the early stage, RNAi has the potential to improve disease management in the near future.

Conclusion

Although the outcome of integrated spotted wilt management programs has been mostly positive and encouraging by far in terms of minimizing losses to spotted wilt disease, spotted wilt disease continues to seriously threaten peanut production. Investigating factors contributing to epidemics of spotted wilt and developing strategies for disease management are two major tasks for researchers. Due to the complexity of the spotted wilt disease pathosystem, developing TSWV-resistant cultivars with higher resistance levels is one of the most important priorities. Use of chemicals and adoption of cultural practices need careful consideration in order to reach goals in all aspects such as reducing costs and negative environmental impacts, and increasing crop yields and quality. While peanut is produced in varying environments with lots of changing factors, such as development of insecticide resistance, and the evolution of virulent isolates of the virus, there is no ultimate integrated disease management program that could last forever. The efficacy of management programs with newer peanut cultivars, insecticides, and cultural practices should be evaluated and monitored over time.

Scope of investigation

In this study, we focused on the management of spotted wilt disease in peanuts. Emphasis was put on TSWV-resistant peanut genotypes, certain newer insecticides, and several cultural tactics. There were three research objectives in this investigation.

The first objective was to evaluate the efficacy of newly released TSWV-resistant cultivars, alternative insecticides, and selected cultural practices in spotted wilt management. We provide information on the performance of newer peanut cultivars and alternative insecticides under field conditions along with different, row patterns and tillage types. Interactions between those

different options were also investigated. Transmission studies involving different peanut cultivars and insecticides were further conducted in the greenhouse.

The second objective was to assess the effectiveness of selected insecticides for controlling thrips. Our goal was to investigate the efficacy of insecticides by means of testing their direct toxicity to thrips and the effectiveness as systemic insecticide over time. The direct toxicity to thrips and the median lethal concentration (LC50) of three selected insecticides were determined. The residual status of active ingredients and the effectiveness over time were tested and analyzed. Several thrips populations from the field were included for monitoring insecticide resistance development.

In the third objective, we evaluated peanut genotypes with increased TSWV-resistance for a number of transmission parameters, including infection percentages, virus copy numbers and thrips acquisition percentages. We aimed to validate the level of TSWV-resistance in genotypes using thrips mediated transmission assays. The impact of TSWV susceptibility of peanut genotypes on thrips fitness was further investigated. Transmission and biological fitness experiments were conducted on two sets of genotypes, including four runner-type peanut cultivars and one Virginia-type cultivar. A wild species peanut relative and the hybrid were also evaluated.

Literature cited

Adjou, E.S., Dahouenon-Ahoussi, E. and Soumanou, M.M. (2012) Investigations on the mycoflora and processing effects on the nutritional quality of peanut (Arachis hypogea L. var. TS 32-1). *Journal of Microbiology, Biotechnology and Food Sciences,* **2**, 1025-1039.

Agronews (2010) Bayer CropScience to close a plant as Temik phasing out.

Apc (2014) The Peanut Industry. American Peanut Council.

Assis Filho, F.M.D., Naidu, R.A., Deom, C.M. and Sherwood, J.L. (2002) Dynamics of Tomato spotted wilt virus replication in the alimentary canal of two thrips species. *Phytopathology,* **92**, 729-733.

Assis Filho, F.M.D., Stavisky, J., Reitz, S.R., Deom, C.M. and Sherwood, J.L. (2005) Midgut infection by tomato spotted wilt virus and vector incompetence of Frankliniella tritici. *Journal of Applied Entomology,* **129**, 548-550.

Avila, A.C.D., Haan, P.D., Kormelink, R., Resende, R.D.O., Goldbach, R.W. and Peters, D. (1993) Classification of tospoviruses based on phylogeny of nucleoprotein gene sequences. *Journal of General Virology,* **74**, 153-159.

Badillo-Vargas, I.E., Rotenberg, D., Schneweis, B.A. and Whitfield, A.E. (2015) RNA interference tools for the western flower thrips, Frankliniella occidentalis. *Journal of Insect Physiology,* **76**, 36-46.

Baldwin, J.A. (2001) A REGIONAL STUDY TO EVALUATE TILLAGE, ROW PATTERNS, IN-FURROW INSECTICIDE, AND PLANTING DATE ON THE YIELD, GRADE, AND TOMATO SPOTTED WILT VIRUS INCIDENCE OF THE GEORGIA GREEN PEANUT CULTIVAR.

Bandla, M.D., Campbell, L.R., Ullman, D.E. and Sherwood, J.L. (1998) Interaction of tomato

spotted wilt tospovirus (TSWV) glycoproteins with a thrips midgut protein, a potential cellular receptor for TSWV. *Phytopathology,* **88**, 98-104.

Beasley J. P., J. (2011) 2011 Peanut update. *Cultivar Options for 2011* Univ. Georgia Coop. Ext. .

Bertrand, P.F. (1998) 1997 Georgia Plant Disease Loss Estimates. Univ. Georgia Coop. Ext. , Pub. Path. 98-007.

Black, M. (1991) Effects of spotted wilt on selected peanut varieties. *Proc. Am. Peanut Res. Ed. Soc. ,* **23**, 52.

Black, M., Stewart, J., Kearney, N., Gasch, C. and Lummus, P. (1987) Tomato Spotted Wilt Virus in Texas Peanuts. *Texas Agric. Exp. Stn. Ext. Ser. Rep.*, 15.

Boriss, H. and Kreith, M. (2013) Peanut profile. AgMRC.

Bosco-Lauth, A.M., Panella, N.A., Root, J.J., Gidlewski, T., Lash, R.R., Harmon, J.R., Burkhalter, K.L., Godsey, M.S., Savage, H.M., Nicholson, W.L., Komar, N. and Brault, A.C. (2015) Serological investigation of heartland virus (Bunyaviridae: Phlebovirus) exposure in wild and domestic animals adjacent to human case sites in Missouri 2012-2013. *American Journal of Tropical Medicine and Hygiene,* **92**, 1163-1167.

Bragard, C., Caciagli, P., Lemaire, O., Lopez-Moya, J.J., Macfarlane, S., Peters, D., Susi, P. and Torrance, L. (2013) Status and prospects of plant virus control through interference with vector transmission. *Annu Rev Phytopathol,* **51**, 177-201.

Branch, W.D. (2007) Registration of 'Georgia-06G' peanut. *Journal of Plant Registrations,* **1**, 120-120.

Branch, W.D. (2013) Registration of 'Georgia-12Y' peanut. *Journal of Plant Registrations,* **7**, 151-153.

Branch, W.D., Baldwin, J.A. and Culbreath, A.K. (2003) Genotype x Seeding Rate Interaction

among TSWV-Resistant, Runner-Type Peanut Cultivars. *PEANUT SCIENCE,* **30**, 108-111.

Branch, W.D. and Culbreath, A.K. (2011) Registration of 'Georgia-10T' peanut. *Journal of Plant Registrations,* **5**, 279-281.

Brenneman, T.B. and Walcott, R. (2001) Defining the relationship between plant stand, tomato spotted wilt, and pod yield from peanut seed treatment trials. *Proc. Am. Peanut Res. Ed. Soc. ,* **33**, 21 (Abstr.).

Brittlebank, C.C. (1924) Tomato diseases. *Journal of the Department of Agriculture of Victoria, Australia,* **22**, 433-434.

Brown, S.L., Culbreath, A.K., Todd, J.W., Gorbet, D.W., Baldwin, J.A. and Beasley, J.P., Jr. (2005) Development of a method of risk assessment to facilitate integrated management of spotted wilt of peanut. *Plant Disease,* **89**, 348-356.

Brown, S.L., Todd, J.W. and Culbreath, A.K. (1996) Effect of selected cultural practices on incidence of tomato spotted wilt virus and populations of thrips vectors in peanuts. *Acta Horticulturae,* 491-498.

Burow, M.D., Simpson, C.E., Faries, M.W., Starr, J.L. and Paterson, A.H. (2009) Molecular biogeographic study of recently described B- and A-genome Arachis species, also providing new insights into the origins of cultivated peanut. *Genome,* **52**, 107-119.

Carley, D.H.S.T.H.M.K.H.C.J.P. (1983) Peanuts: production, processing, products. *Peanuts: production, processing, products.* (ed. J.G. Woodroofs), AVI Publishing Company, Westport, Connecticut; USA.

Chamberlin, J.R., Todd, J.W., Culbreath, A.K., Johnson, W.C., Iii and Demski, J.W. (1993) Post-harvest management of tobacco thrips (Thysanoptera: Thripidae) overwintering in peanut

fields. *Journal of Entomological Science,* **28**, 433-446.

Charbonnel, N., Pagès, M., Sironen, T., Henttonen, H., Vapalahti, O., Mustonen, J. and Vaheri, A. (2014) Immunogenetic factors affecting susceptibility of humans and rodents to hantaviruses and the clinical course of hantaviral disease in humans. *Viruses,* **6**, 2214-2241.

Chatzivassiliou, E.K., Peters, D. and Katis, N.I. (2002) The efficiency by which Thrips tabaci populations transmit Tomato spotted wilt virus depends on their host preference and reproductive strategy. *Phytopathology,* **92**, 603-609.

Childers, C.C. (1997) Feeding and oviposition injuries to plants. *Thrips as crop pests.* (ed. T. Lewiss), Wallingford, Oxon, UK ; New York, NY, USA : CAB International, c1997.

Costa, A.S. (1941) Uma moléstia de virus de amendoim (Arachis hypogaea L.). *A mancha anular. O Biológico,* **7**, 249-251.

Culbreath, A.K., Baldwin, J., Pappu, H.R., Shokes, F.M., Todd, J.W., Gorbet, D.W. and Brown, S.L. (2000) Reaction of peanut cultivars to spotted wilt. *Peanut science,* **27**, 35-39.

Culbreath, A.K., Csinos, A.S., Brenneman, T.B., Demski, J.W. and Todd, J.W. (1991) Association of tomato spotted wilt virus with foliar chlorosis of peanut in Georgia. *Plant Disease,* **75**, 863-863.

Culbreath, A.K., Holbrook, C.C., Nischwitz, C., Tilman, B.L. and Gorbet, D.W. (2008) Response of New Field-Resistant Peanut Cultivars to Twin-Row Pattern or In-Furrow Applications of Phorate for Management of Spotted Wilt [electronic resource]. *Plant disease: an international journal of applied plant pathology,* **92**, 1307-1312.

Culbreath, A.K. and Srinivasan, R. (2011) Epidemiology of spotted wilt disease of peanut caused by Tomato spotted wilt virus in the southeastern U.S. *Virus Research,* **159**, 101-109.

Culbreath, A.K., Tillman, B.L., Tubbs, R.S., Beasley, J.P., Jr., Kemerait, R.C., Jr. and Brenneman, T.B. (2010) Interactive effects of planting date and cultivar on tomato spotted wilt of peanut. *Plant Disease,* **94**, 898-904.

Culbreath, A.K., Todd, J.W., Branch, W.D., Brown, S.L., Demski, J.W. and Beasley, J.P., Jr. (1994) Effect of new peanut cultivar Georgia Browne on epidemics of spotted wilt. *Plant Disease*, **78**, 1185-1189.

Culbreath, A.K., Todd, J.W. and Brown, S.L. (2003) Epidemiology and management of tomato spotted wilt in peanut. *Annual review of phytopathology,* **41**, 53-75.

Culbreath, A.K., Todd, J.W., Demski, J.W. and Chamberlin, J.R. (1992) Disease progress of spotted wilt in peanut cultivars Florunner and Southern Runner. *Phytopathology,* **82**, 766-771.

Culbreath, A.K., Todd, J.W., Gorbet, D.W., Branch, W.D., Holbrook, C.C., Shokes, F.M. and Demski, J.W. (1996) Variation in susceptibility to tomato spotted wilt virus among advanced breeding lines of peanut (Arachis hypogaea). *Acta Horticulturae*, 402-410.

Diffie, S., Edwards, G. and Mound, L. (2008) Thysanoptera of southeastern U. S. A.: A checklist for Florida and Georgia. *Zootaxa*, 45-62.

Francisco, M.L.D.L. and Resurreccion, A.V.A. (2008) Functional Components in Peanuts. *Critical Reviews in Food Science & Nutrition,* **48**, 715-746.

Funderburk, J., Stavisky, J., Tipping, C., Gorbet, D., Momol, T. and Berger, R. (2002) Infection of Frankliniella fusca (Thysanoptera: Thripidae) in peanut by the parasitic nematode Thripinema fuscum (Tylenchidae: Allantonematidae). *Environmental Entomology,* **31**, 558-563.

Gallo-Megher, M., Chengalrayan, K., Davis, J.M. and Macdonald, G.E. (2001) Phorate-induced

peanut genes that may condition acquired resistance to tomato spotted wilt. *Proc. Am. Peanut Res. Ed. Soc.*, **33**, 29.

German, T.L., Ullman, D.E. and Moyer, J.W. (1992) Tospoviruses: diagnosis, molecular biology, phylogeny, and vector relationships. *Annual Review of Phytopathology,* **30**, 315-348.

Gitaitis, R. (2014) Finding Center Rot and IYSV. *Onion World,* **30**, 20-23.

Gognalons, P., Gebre-Selassie, K. and Marchoux, G. (1998) The range of TSWV hosts continues to increase in size. Further solanaceous plants are now affected. / La gamme d'hôtes du TSWV continue à s'étendre. De nouvelles solanacées sont touchées. *Phytoma*, 47-51.

Groves, R.L., Walgenbach, J.F., Moyer, J.W. and Kennedy, G.G. (2001) Overwintering of Frankliniella fusca (Thysanoptera: Thripidae) on winter annual weeds infected with Tomato spotted wilt virus and patterns of virus movement between susceptible weed hosts. *Phytopathology,* **91**, 891-899.

Groves, R.L., Walgenbach, J.F., Moyer, J.W. and Kennedy, G.G. (2002) The role of weed hosts and tobacco thrips, Frankliniella fusca, in the epidemiology of tomato spotted wilt virus. *Plant Disease,* **86**, 573-582.

Groves, R.L., Walgenbach, J.F., Moyer, J.W. and Kennedy, G.G. (2003) Seasonal dispersal patterns of Frankliniella fusca (Thysanoptera: Thripidae) and tomato spotted wilt virus occurrence in Central and Eastern North Carolina. *Journal of Economic Entomology,* **96**, 1-11.

Haan, P.D., Kormelink, R., Resende, R.D.O., Poelwijk, F.V., Peters, D. and Goldbach, R. (1991) Tomato spotted wilt virus L RNA encodes a putative RNA polymerase. *Journal of General Virology,* **72**, 2207-2216.

Haan, P.D., Wagemakers, L., Goldbach, R. and Peters, D. (1989) Tomato spotted wilt virus, a

new member of the Bunyaviridae ? *Genetics and pathogenicity of negative strand viruses.* eds. D. Kolakosky & B.W.J. Mahys), pp. 287-291. Elsevier Science Publishers, Amsterdam; Netherlands.

Haan, P.D., Wagemakers, L., Peters, D. and Goldbach, R. (1990) The S RNA segment of tomato spotted wilt virus has an ambisense character. *Journal of General Virology,* **71**, 1001-1007.

Hagan, A.K., Weeks, J.R., French, J.C., Gudauskas, R.T., Mullen, J.M., Gazaway, W.S. and Shelby, R. (1990) Tomato spotted wilt virus in peanut in Alabama. *Plant Disease,* **74**, 615-615.

Halliwell, R.S. and Philley, G. (1974) Spotted wilt of peanut in Texas. *Plant Disease Reporter,* **58**, 23-25.

Helms, K., Grylls, N.E. and Purss, G.S. (1961) Peanut plants in Queensland infected with Tomato spotted wilt virus. *Australian Journal of Agricultural Economics,* **12**, 239-246.

Herbert, D.A., Malone, S., Aref, S., Brandenburg, R.L., Jordan, D.L., Royals, B.M. and Johnson, P.D. (2007) Role of insecticides in reducing thrips injury to plants and incidence of tomato spotted wilt virus in Virginia market-type peanut. *Journal of Economic Entomology,* **100**, 1241-1247.

Hunter, W.B., Ullman, D.E. and A., M. (1994) Electronic monitoring: characterizing the feeding behavior of western flower thrips (Thysanoptera: Thripidae). *History, development, and application of AC electronic insect feeding monitors.* eds. M.M. Ellsbury, E.A. Backus & D.L. Ullmans), Lanham, Md. : Entomological Society of America.

Hurt, C.A., Brandenburg, R.L., Jordan, D.L., Kennedy, G.G. and Bailey, J.E. (2005) Management of spotted wilt vectored by Frankliniella fusca (Thysanoptera: Thripidae) in

Virginia market-type peanut. *Journal of Economic Entomology,* **98**, 1435-1440.

Jain, M., Gallo, M., Chengalrayan, K., Shaikh, N.P., Macdonald, G.E. and Davis, J.M. (2015) Phorate-induced host defence responses condition acquired resistance to tomato spotted wilt in cultivated peanut (Arachis hypogaea L.). *Journal of Phytopathology,* **163**, 853-866.

Johnson, C. (2014) Thrips Threat. *Progressive Farmer,* **129**, CL-1.

Johnson, W.C., Iii, Brenneman, T.B., Baker, S.H., Johnson, A.W., Sumner, D.R. and Mullinix, B.G., Jr. (2001) Tillage and pest management considerations in a peanut-cotton rotation in the Southeastern Coastal Plain. *Agronomy Journal*, **93**, 570-576.

Kemerait, R.C. (2013) Georgia crop diseases: What we learned in 2013, what to do next year. *Southeast Farm Press Exclusive Insight*, 4.

Kindt, F., Joosten, N.N., Peters, D. and Tjallingii, W.F. (2003) Characterisation of the feeding behaviour of western flower thrips in terms of electrical penetration graph (EPG) waveforms. *Journal of Insect Physiology,* **49**, 183-191.

King, J.C., Blumberg, J., Ingwersen, L., Jenab, M. and Tucker, K.L. (2008) Tree Nuts and Peanuts as Components of a Healthy Diet. *Journal of Nutrition,* **138**, 1736S-1740S.

Kirk, W.D.J. (1997) Feeding. *Thrips as crop pests.* (ed. T. Lewiss), Wallingford, Oxon, UK ; New York, NY, USA : CAB International, c1997.

Klesser, P.J. (1967) Tomato spotted wilt virus on Arachis hypogaea. *South African Journal of Agricultural Science,* **9**, 731-33.

Kormelink, R., Haan, P.D., Meurs, C., Peters, D. and Goldbach, R. (1992) The nucleotide sequence of the M RNA segment of tomato spotted wilt virus, a bunyavirus with two ambisense RNA segments. *Journal of General Virology,* **73**, 2795-2804.

Kris-Etherton, P.M., Hu, F.B., Ros, E. and Sabaté, J. (2008) The role of tree nuts and peanuts in the prevention of coronary heart disease: multiple potential mechanisms. *Journal of Nutrition,* **138**, 1746S-1751S.

Kritzman, A., Gera, A., Raccah, B., Van Lent, J.W.M. and Peters, D. (2002) The route of tomato spotted wilt virus inside the thrips body in relation to transmission efficiency. *Archives of Virology,* **147**, 2143-2156.

Krstic´, B., Bulajic´, A., Ekic´, I. and Berenji, J. (2008) Tomato spotted wilt virus - one of the most destructive plant viruses. / Virus bronzavosti paradajza - jedan od najdestruktivnijih biljnih virusa. *Pesticidi i Fitomedicina,* **23**, 153-166.

Laviña, A. and Batlle, A. (1994) First report of impatiens necrotic spot virus in Asplenium nidus-avis in Spain. *Plant Disease,* **78**, 316-316.

Lewis, T. (1973) Thrips, their biology, ecology and economic importance. *Thrips, their biology, ecology and economic importance.* pp. xv + 349-xv + 349. London, Academic Press.; UK.

Lewis, T. (1997) Pest thrips in perspective. *Thrips as crop pests.* (ed. T. Lewiss), Wallingford, Oxon, UK ; New York, NY, USA : CAB International, c1997.

Li, Z., Jarret, R.L. and Demski, J.W. (1997) Engineered resistance to tomato spotted wilt virus in transgenic peanut expressing the viral nucleocapsid gene. *Transgenic Research*, **6**, 297-305.

Lowry, V.K., Smith, J.W. and Mitchell, F.L. (1992) Life-Fertility Tables for Frankliniella fusca (Hinds) and F. occidentals (Pergande) (Thysanoptera: Thripidae) on Peanut. *Annals of the Entomological Society of America*, **85**, 744-754.

Lyerly, J.H., Hoffman, K., Moyer, J.W. and Stalker, H.T. (2002) Evaluation of Arachis species

for resistance to tomato spotted wilt virus. *Peanut science,* **29**, 79-84.

Magbanua, Z.V., Wilde, H.D., Roberts, J.K., Chowdhury, K., Abad, J., Moyer, J.W., Wetzstein, H.Y. and Parrott, W.A. (2000) Field resistance to tomato spotted wilt virus in transgenic peanut (Arachis hypogaea L.) expressing an antisense nucleocapsid gene sequence. *Molecular Breeding,* **6**, 227-236.

Mandal, B., Pappu, H.R. and Culbreath, A.K. (2001) Factors affecting mechanical transmission of tomato spotted wilt virus to peanut (Arachis hypogaea). *Plant Disease,* **85**, 1259-1263.

Mandal, B., Pappu, H.R., Culbreath, A.K., Holbrook, C.C., Gorbet, D.W. and Todd, J.W. (2002) Differential response of selected peanut (Arachis hypogaea) genotypes to mechanical inoculation by Tomato spotted wilt virus. *Plant Disease,* **86**, 939-944.

Minton, N.A., Csinos, A.S., Lynch, R.E. and Brenneman, T.B. (1991) Effects of two cropping and two tillage systems and pesticides on peanut pest management. *Peanut Science,* **18**, 41-46.

Monfort, W.S. (2015) 2015 Peanut update. *Peanut Cultivar Options for 2015* Georgia Peanut Commission.

Moritz, G. (1997) Structure, growth and development. *Thrips as crop pests.* (ed. T. Lewiss), Wallingford, Oxon, UK ; New York, NY, USA : CAB International, c1997.

Moritz, G., Kumm, S. and Mound, L. (2004) Tospovirus transmission depends on thrips ontogeny. *Virus Research,* **100**, 143-149.

Mound, L.A. (1997) Biological diversity. *Thrips as crop pests.* (ed. T. Lewiss), Wallingford, Oxon, UK ; New York, NY, USA : CAB International, c1997.

Nagata, T., Inoue-Nagata, A.K., Prins, M., Goldbach, R. and Peters, D. (2000) Impeded thrips transmission of defective tomato spotted wilt virus isolates. *Phytopathology,* **90**, 454-459.

Nagata, T., Inoue-Nagata, A.K., Smid, H.M., Goldbach, R. and Peters, D. (1999) Tissue tropism related to vector competence of Frankliniella occidentalis for tomato spotted wilt tospovirus. *Journal of General Virology,* **80**, 507-515.

Nagata, T. and Peters, D. (2001) Chpater 3: An Anatomical Perspective of Tospovirus Transmission. *Virus-Insect_Plant Interactions.* pp. 51,III-67,III. Elsevier Inc.

Nakao, S., Chikamori, C., Okajima, S., Narai, Y. and Murai, T. (2011) A new record of the tobacco thrips Frankliniella fusca (Hinds) (Thysanoptera: Thripidae) from Japan. *Applied Entomology and Zoology,* **46**, 131-134.

Nuti, R.C., Chen, C.Y., Dang, P.M. and Harvey, J.E. (2014) Peanut cultivar response to tomato spotted wilt over five planting dates. *Peanut Science,* **41**, 32-41.

Odhiambo, C., Venter, M., Chepkorir, E., Mbaika, S., Lutomiah, J., Swanepoel, R. and Sang, R. (2014) Vector competence of selected mosquito species in Kenya for Ngari and Bunyamwera viruses. *Journal of Medical Entomology,* **51**, 1248-1253.

Pappu, H.R. (2008) Tomato Spotted Wilt Virus. *Encyclopedia of Virology (Third Edition).* (ed. B.W.J.M.H.V.V. Regenmortels), pp. 133-138. Academic Press, Oxford.

Pappu, S.S., Todd, J.W., Culbreath, A.K. and Pappu, H.R. (1999) Localization of tomato spotted wilt virus (genus Tospovirus, family Bunyaviridae) in peanut pods. *Peanut science,* **26**, 98-100.

Parrella, G., Gognalons, P., Gebre-Selassiè, K., Vovlas, C. and Marchoux, G. (2003) AN UPDATE OF THE HOST RANGE OF TOMATO SPOTTED WILT VIRUS. p. 227. An International Journal of the Italian Phytopathological Society.

Pereira, M.J., Pfahler, P.L., Knauft, D.A. and Simone, G.W. (1995) Cultivar differences in tomato spotted wilt virus tolerance in peanut. *Proceedings - Soil and Crop Science Society of*

Florida, **54**, 12-16.

Perrings, C., Dehnen-Schmutz, K., Touza, J. and Williamson, M. (2005) How to manage biological invasions under globalization. *Trends in Ecology & Evolution*, **20**, 212-215.

Pittman, H.A. (1927) Spotted wilt of Tomatoes. *J. Council for Sci. and Indus. Res.*, **1**, 74-77.

Prins, M. and Goldbach, R. (1998) The emerging problem of tospovirus infection and nonconventional methods of control. *Trends in Microbiology,* **6**, 31-35.

Reddy, D.V.R., Ratna, A.S., Sudarshana, M.R., Poul, F. and Kumar, I.K. (1992) Serological relationships and purification of bud necrosis virus, a tospovirus occurring in peanut (Arachis hypogaea L.) in India. *Annals of Applied Biology,* **120**, 279-286.

Riley, D.G., Joseph, S.V., Srinivasan, R. and Diffie, S. (2011) Thrips vectors of Tospoviruses. *Journal of Integrated Pest Management,* **2**, I1-I10.

Ruter, J.M. and Gitaitis, R.D. (1993) Impatiens necrotic spot virus in woody landscape plants in Georgia. *Plant Disease*, **77**, 318-318.

Sakimura, K. (1962) The Present Status of Thrips-Borne Viruses11Published with approval of the Director, Pineapple Research Institute of Hawaii, as Technical Paper No. 276. *Biological Transmission of Disease Agents*, 33-40.

Salguero Navas, V.E., Olson, S.M., Mack, T.P., Funderburk, J.E. and Beshear, R.J. (1991) Seasonal patterns of Frankliniella spp. (Thysanoptera: Thripidae) in tomato flowers. *Journal of economic entomology,* **84**, 1818-1822.

Samuel, G., Bald, J.G. and Pittman, H.A. (1930) Investigations on " Spotted Wilt " of Tomatoes. *Bulletin of the Council for Scientific and Industrial Research, Australia.* pp. 64-64. Melbourne.

Scholthof, K.-B.G., Adkins, S., Czosnek, H., Palukaitis, P., Jacquot, E., Hohn, T., Hohn, B.,

Saunders, K., Candresse, T., Ahlquist, P., Hemenway, C. and Foster, G.D. (2011) Top 10 plant viruses in molecular plant pathology. *Molecular Plant Pathology,* **12**, 938-954.

Shrestha, A., Srinivasan, R., Sundaraj, S., Culbreath, A.K. and Riley, D.G. (2013) Second generation peanut genotypes resistant to thrips-transmitted tomato spotted wilt virus exhibit tolerance rather than true resistance and differentially affect thrips fitness. *Journal of Economic Entomology,* **106**, 587-596.

Shrestha, A., Sundaraj, S., Culbreath, A.K., Riley, D.G., Abney, M.R. and Srinivasan, R. (2015) Effects of Thrips Density, Mode of Inoculation, and Plant Age on Tomato Spotted Wilt Virus Transmission in Peanut Plants. *Environmental Entomology,* **44**, 136-143.

Sims, K.R., Funderburk, J.E., Reitz, S.R. and Boucias, D.G. (2009) The impact of a parasitic nematode, Thripinema fuscum, on the feeding behavior and vector competence of Frankliniella fusca. *Entomologia Experimentalis et Applicata,* **132**, 200-208.

Singh, A.P., Surendra, S., Prabhat, B. and Khushbu, Y. (2010) Toxic effect of phorate on the serum biochemical parameters of snake headed fish Channa punctatus (Bloch). *Advances in Bio Research,* **1**, 177-181.

Smith, O.D., Simpson, C.E., Black, M.C. and Besler, B.A. (1998) Registration of 'Tamrun 96' peanut. *Crop Science*, **38**, 1403-1403.

Srinivasan, R., Riley, D., Diffie, S., Shrestha, A. and Culbreath, A. (2014) Winter weeds as inoculum sources of Tomato spotted wilt virus and as reservoirs for its vector, Frankliniella fusca (Thysanoptera: Thripidae) in farmscapes of Georgia. *Environmental Entomology,* **43**, 410-420.

Stalker, H.T. and Pattee, H.E. (1995) Advances in peanut science / edited by Harold E. Pattee and H. Thomas Stalker. Stillwater, OK : American Peanut Research and Education Society,

1995.

Storms, M.M.H., Schoot, C.V.D., Prins, M., Kormelink, R., Lent, J.W.M.V. and Goldbach, R.W. (1998) A comparison of two methods of microinjection for assessing altered plasmodesmal gating in tissues expressing viral movement proteins. *Plant Journal*, **13**, 131-140.

Takeda, A., Sugiyama, K., Nagano, H., Mori, M., Kaido, M., Mise, K., Tsuda, S. and Okuno, T. (2002) Identification of a novel RNA silencing suppressor, NSs protein of Tomato spotted wilt virus. *FEBS Lett*, **532**, 75-9.

Tillman, B.L., Gorbet, D.W. and Andersen, P.C. (2007) Influence of planting date on yield and spotted wilt of runner market type peanut. *Peanut Science.* pp. 79-84. American Peanut Research and Education Society, Stillwater; USA.

Tillman, B.L., Gorbet, D.W., Culbreath, A.K. and Todd, J.W. (2006) Response of peanut cultivars to seeding density and row patterns. *Crop Management*, 1-5.

Todd, J.W., Brown, S.L., Culbreath, A.K., Pappu, H.R. and Gorbet, D.W. (2000) Successes with peanut IPM in the southeastern United States through interdisciplinary approaches. *Int. Congr. Entomol.*, **21**, 648 (Abstr.).

Todd, J.W., Culbreath, A.K. and Brown, S.L. (1996) Dynamics of vector populations and progress of spotted wilt disease relative to insecticide use in peanuts. *Acta Horticulturae*, 483-490.

Todd, J.W., Culbreath, A.K., Chamberlin, J.R., Beshear, R.J. and Mullinix, B.G. (1995) Colonization and population dynamics of thrips in peanuts in the southern United States. *Thrips biology and management: proceedings of the 1993 International Conference on Thysanoptera.* eds. B.L. Parker, M. Skinner & T. Lewiss), pp. 453-460. Plenum

Publishing Co. Ltd, London; USA.

Tsompana, M. and Moyer, J.W. (2008) Tospovirus. *Encyclopedia of Virology (Third Edition).* (ed. B.W.J.M.H.V.V. Regenmortels), pp. 157-163. Academic Press, Oxford.

Tubbs, R.S., Beasley, J.P., Jr., Culbreath, A.K., Kemerait, R.C., Smith, N.B. and Smith, A.R. (2011) Row pattern and seeding rate effects on agronomic, disease, and economic factors in large-seeded runner peanut. *Peanut Science*, **38**, 93-100.

Ullman, D.E., Cho, J.J., Mau, R.F.L., Hunter, W.B., Westcot, D.M. and Custer, D.M. (1992a) Thrips-tomato spotted wilt virus interactions: morphological, behavioral and cellular components influencing thrips transmission. *Advances in Disease Vector Research*, **9**, 195-240.

Ullman, D.E., Cho, J.J., Mau, R.F.L., Westcot, D.M. and Custer, D.M. (1992b) A midgut barrier to tomato spotted wilt virus acquisition by adult western flower thrips. *Phytopathology,* **82**, 1333-1342.

Ullman, D.E., Sherwood, J.L. and German, T.L. (1997) Thrips as vectors of plant pathogens. *Thrips as crop pests.* (ed. T. Lewiss), pp. 539-565. CAB INTERNATIONAL, Wallingford, UK,.

Van De Wetering, F., Goldbach, R. and Peters, D. (1996) Tomato spotted wilt tospovirus ingestion by first instar larvae of Frankliniella occidentalis is a prerequisite for transmission. *Phytopathology,* **86**, 900-905.

Wehtje, G., Weeks, R., West, M., Wells, L., Pace, P., Wehtje, G., Weeks, R., West, M., Wells, L. and Pace, P. (1994) Influence of planter type and seeding rate on yield and disease incidence in peanut. *Peanut Science,* **21**, 16-19.

Wells, M.L., Culbreath, A.K., Todd, J.W., Csinos, A.S., Mandal, B. and Mcpherson, R.M. (2002)

Dynamics of spring tobacco thrips (Thysanoptera: Thripidae) populations: implications for tomato spotted wilt virus management. *Environmental Entomology,* **31**, 1282-1290.

Whitfield, A.E., Ullman, D.E. and German, T.L. (2005) Tospovirus-thrips interactions. *Annu Rev Phytopathol,* **43**, 459-89.

Wijkamp, I. and Peters, D. (1993) Determination of the median latent period of two tospoviruses in Frankliniella occidentalis, using a novel leaf disk assay. *Phytopathology,* **83**, 986-991.

Williams, T. (1997) Silent scourge. *Journal of Pesticide Reform,* **17**, 9-13.

Young, S., Kinzer, R.E., Walton, R.R. and Matlock, R.S. (1972) Field screening for tobacco thrips resistance in peanuts. *Journal of Economic Entomology,* **65**, 828-832.

Yudin, L.S., Cho, J.J. and Mitchell, W.C. (1986) Host range of western flower thrips, Frankliniella occidentalis (Thysanoptera: Thripidae), with special reference to Leucaena glauca. *Environmental Entomology,* **15**, 1292-1295.

CHAPTER 3

EVALUATION OF NEWLY RELEASED PEANUT CULTIVARS WITH ALTERNATIVE INSECTICIDES AND SELECTED CULTURAL PRACTICES IN SPOTTED WILT DISEASE MANAGEMENT IN PEANUT[1]

[1] Lai, P., R. Srinivasan, M. Abney, and A. Culbreath. 2015. To be submitted.

Abstract

Spotted wilt disease caused by *Tomato spotted wilt virus* (TSWV) is a major concern in peanut (*Arachis hypogaea* L.) production in the Southeast. Integrated management options such as TSWV resistant cultivar, cultural practices, and insecticides provide better control of spotted wilt in peanut than any of the tactics alone. Aldicarb and phorate are commonly used insecticides with broad-spectrum toxicity. In this study, field trials were conducted to evaluate various insecticides as alternatives to aldicarb and phorate along with third-generation TSWV-resistant peanut cultivar, Georgia-12Y (GA-12Y). Tillage types and row patterns were also evaluated as components of the integrated management program. Thrips-mediated TSWV transmission experiments in the greenhouse were conducted to investigate the impacts of best field-performing insecticides on TSWV transmission in different cultivars. Results indicated that cyantranilliprole, imidacloprid (in-furrow), and spinetoram provided control of thrips abundance and/or feeding damage as well as aldicarb and phorate in the field, and greenhouse experiments had similar results. Spotted wilt incidence was largely suppressed in GA-12Y, and when phorate was applied. Strip tillage reduced thrips feeding damage and spotted wilt incidence in multiple years but did not affect thrips populations. Row patterns did not affect thrips populations, feeding damage, or spotted wilt incidence. Yields were increased in GA-12Y and when aldicarb and phorate were applied. Results suggest that planting TSWV cultivars with higher field-resistance such as GA-12Y with/without altered modified cultural practices would allow replacing older insecticides that exhibit broad-spectrum toxicity with relatively narrow-spectrum alternatives such as neonicotinoids.

Key words: peanut, spotted wilt disease, insecticides, TSWV resistant cultivars, row patterns, tillage systems.

Introduction

Peanut or groundnut (*Arachis hypogaea* L.) is one of the most important economic crops produced in Georgia. Georgia ranks first in the production of peanut in the United States (Boriss & Kreith, 2013, Flatt, 2004, USDA, 2015). Spotted wilt disease, caused by *Tomato spotted wilt virus* (TSWV) (family *Bunyaviridae*; genus *Tospovirus*), has a worldwide distribution and causes significant losses in yield in many vegetables, ornamental and field crops, including peanut (Culbreath et al., 1992, Pappu et al., 2009). It has been over 40 years since spotted wilt disease of peanut was found in the U.S. (Halliwell & Philley, 1974), and spotted wilt disease remains one of the serious constraints in peanut production in the Southeast (Culbreath & Srinivasan, 2011, Herbert et al., 2007). Losses to spotted wilt disease in peanut increased dramatically from the late 1980s to 1997 in Georgia (Culbreath & Srinivasan, 2011); in Georgia, spotted wilt caused 1.2 million dollars in losses in peanut production in 2010 (Williams-Woodward, 2012). TSWV infection induces symptoms from foliage to roots and pods, including typical concentric ringspots on the leaflets, stunting of all aboveground plant parts, and small/ reduced pods (Culbreath et al., 2003). Consequently, spotted wilt disease leads to severe losses in peanut yields (Culbreath et al., 1992).

Spotted wilt disease is caused by TSWV that is transmitted by thrips. It is one of the most devastating plant diseases, which hinders vegetable and crop production in many parts of the world. Several species of thrips (order Thysanoptera) are exclusive vectors of TSWV (Bragard et al., 2013, German et al., 1992). Nine thrips species are known to be able to transmit TSWV (Riley et al., 2011). Thrips transmit TSWV in a persistent and propagative fashion (Moritz et al., 2004, Sakimura, 1962, Wijkamp et al., 1993). In the southeastern United States, the western flower thrips (*Frankliniella occidentalis* Pergande) and the tobacco thrips (*Frankliniella fusca*

Hinds) are the two major vector species responsible for TSWV epidemics in peanut (Lowry et al., 1992, Todd et al., 1995). The tobacco thrips are confirmed to be the predominant vector species in peanut due to its ability to colonize on peanut seedlings early in the season when peanut plants are vulnerable to virus infection (Lowry et al., 1995, Todd et al., 1995). For spotted wilt disease management in peanut, an integrated management program combining TSWV-resistant cultivars, insecticides, and various cultural tactics is widely adopted. However, none of the options can individually provide sufficient control (Culbreath & Srinivasan, 2011, Culbreath et al., 2003).

Use of resistant peanut cultivars is the most important tactic for spotted wilt management (Branch et al., 2003). Runner-type peanut cultivars are predominantly grown in the southeastern United States. Southern Runner, released in 1984, was the first cultivar that possessed moderate TSWV field resistance (Black, 1991). Georgia Green, released in 1995, had good yield potential and field resistance to TSWV (Branch, 1996, Culbreath et al., 2000); it is one of the first-generation TSWV-resistant cultivars, and was the standard cultivar planted in the southeastern U.S. until 2010 (Cantonwine et al., 2006, Culbreath & Srinivasan, 2011). Georgia-06G, with relatively higher level of field resistance to TSWV (second-generation), is now the major cultivar planted in Georgia, Alabama, and Florida (Monfort, 2015, Branch, 2007). Newer peanut cultivars with higher resistance to TSWV or third-generation resistant cultivars are available as well (Monfort, 2015). Peanut cultivars with field resistance to TSWV not always provide sufficient level of spotted wilt control, as infected plants still show symptoms, and under severe pressure could result in yield losses. Therefore, it is still necessary to combine other management tactics to reduce spotted-wilt induced losses (Culbreath et al., 2013). Most integrated management programs in the Southeast include late planting (mid-May), planting in twin row

patterns, conservation tillage, higher seeding rates, and in furrow application of phorate insecticide at planting (Brown et al., 2005, Culbreath & Srinivasan, 2011).

Cultural practices capable of providing better control of thrips and TSWV were identified to develop integrated management programs, including late planting date, higher plant populations, twin row pattern, and conservational tillage systems. Peanuts are often planted on beds 1.8m wide with two single rows spaced about 91cm apart. Twin rows spaced 18 to 24cm apart on the same bed, instead of two single rows, tend to have lower spotted wilt incidence, even when the total plants per linear unit are similar for both row patterns (Baldwin, 2001, Brown et al., 1996). Thus, the use of twin row pattern is one of the cultural practices that is commonly adopted in the integrated management of spotted wilt disease (Brown et al., 2005). Plant density within the row can have an impact on spotted wilt incidence and final yield in peanuts as well; the higher the plant population, the less the losses due to spotted wilt (Branch et al., 2003, Culbreath et al., 2003). Conventional tillage systems are usually labor-intensive and time-consuming. Conservation tillage is a method of soil cultivation that leaves residues on the soil surface. When conservation tillage is used in peanut fields, suppression of both thrips and feeding injury and reduction in spotted wilt incidence has been documented (Johnson et al., 2001, Minton et al., 1991).

Chemical control of thrips for spotted wilt management has been unsuccessful (Todd et al., 1996). Some insecticides have provided effective control of thrips larvae or adult thrips on peanut, but failed to suppress spotted wilt incidence (Chamberlin et al., 1993, Todd et al., 1996). In-furrow application of the organophosphous insecticide phorate is an exception, as it reduced spotted wilt incidence in peanut (Culbreath et al., 2008, Wiatrak et al., 2000). However, the broad-spectrum toxicity of phorate poses hazards to environment and wildlife that abundance of

non-target effects has occurred (PAN, 2014, Singh et al., 2010). Due to rising environmental awareness, a carbamate insecticide aldicarb, which was the standard insecticide used on peanuts, will be phased out in 2018 due to its toxicity and non-target effects (AgroNews, 2010). Phorate retains many of the properties of aldicarb including broad-spectrum toxicity and its use will potentially be restricted in the near future; several alternatives have been selected and tested for thrips suppression and spotted wilt reduction in peanut (Knight et al., 2015, Marasigan, 2014, Riley, 2007, Wells et al., 2002).

To improve integrated spotted wilt management program of peanut, a newly released TSWV-resistant cultivar Georgia-12Y (GA-12Y) was selected and evaluated with other management options such as insecticides, twin row planting, and strip tillage in comparison with standards such as GA-06G, single row planting, and conventional tillage. Interactions between management tactics, if any, were also investigated.

Materials and Methods

Field trials. Field studies were conducted at the Belflower Farm, Coastal Plain Experimental Station in Tifton, GA in 2013 and 2014 to evaluate the efficacy of various insecticides as alternatives to aldicarb and phorate along with newly released TSWV-resistant peanut cultivars. Tillage types (strip, conventional tillage) with selected TSWV field-resistant peanut cultivars were also evaluated at the Belflower Farm, Coastal Plain Experimental Station in Tifton, GA in 2014 and 2015. Field experiments were conducted in the Attapulgus Research and Education Station in Attapulgus, GA in 2014 and 2015 to evaluate the efficacy of selected insecticides and row patterns on two TSWV-resistant peanut cultivars.

Evaluation of various insecticides as alternatives to aldicarb and phorate along with TSWV-resistant peanut cultivars. A total of 10 insecticides were selected based on documented efficacy against thrips and evaluated in a 2013 trial. Six insecticides, based on their performance in 2013, were selected and evaluated in 2014. Their mode of application and rates are listed in Table 3.1. Two TSWV-resistant peanut cultivars, Georgia-12Y (GA-12Y) (third-generation newly released in 2012) and Georgia- 06G (GA-06G) (second-generation released in 2006) (Branch, 2007), were planted in all trials. For the insecticide trial, peanut plants were planted on April 25th in 2013 and May 8th in 2014 with 6 seeds per foot, single row pattern after conventional tillage for field preparation. A randomized split plot design was adopted. Peanut cultivars served as main plots, while insecticides were considered as subplots. The dimension of plots was 9.14m in length and 5.49m in width with six rows in a plot. Four replications were used to assess main plot effects as well as sub-plot effects.

Thrips samples were first collected about three weeks after planting and were collected for six consecutive weeks in 2013. Quadrifoliate peanut terminals were collected in the first three weeks and peanut blooms were collected in the following three weeks. Ten terminals or blooms were randomly picked from the 2nd and 5th rows in each plot and collected in glass vials containing ~10 ml of 70% ethyl alcohol. Samples were enumerated under a dissecting microscope (40x) (MEIJI TECHNO, Santa Clara, CA) in the vector biology laboratory at UGA Tifton campus. Thrips were identified to species using dichotomous keys (Triplehorn et al., 2005). Thrips feeding damage on peanut plants in the 3rd and 4th rows (harvest rows) was assessed at five weeks after planting. An arbitrary scale from 0 to 10 was adopted, wherein 0 represented no feeding damage and 10 represented a dead plant (Brandenburg et al., 1998, Lynch et al., 1984). Spotted wilt incidence was measured by visual rating based on a standard procedure

created by Culbreath et al. (1997). Plants in the 3rd and 4th rows of each plot were inspected and rated by measuring length range of plant stands showing spotted wilt symptoms in feet with a measuring hit stick bearing 1 foot (30.48cm) long metal at the end (Culbreath et al., 1997); spotted wilt incidence in plots was measured twice (~three months after planting and ~two weeks before harvest) and data were converted to percentages. Only the second spotted wilt incidence rating is presented since the later measurement is usually more representative than the early measurement. At harvest, peanut plants in the 3rd and 4th rows of each plot were dug, inverted, air-dried, picked, and weighted (in kg) according to standard protocols (Baldwin et al., 1998).

Thrips counts and feeding damage rating, spotted wilt incidence, and yields were all subjected to linear mixed models using the GLIMMIX procedure in SAS (SAS Enterprise 4.2, SAS Institute, Cary, NC). Cultivar and insecticide treatment were considered fixed effects, while replication was a random effect. Two-way interactions between cultivar and insecticide, if any, were analyzed. Tukey-Kramer Grouping, as an adjustment for multiple comparisons at $P=0.05$, was used to test the statistical significance of differences among insecticide treatments, and between cultivars.

Evaluation of selected insecticides and tillage with TSWV-resistant peanut cultivars on thrips and spotted wilt incidence. Four insecticides were used in this trial. Their mode of application and application rates are listed in Table 3.2. Strip and conventional tillage were evaluated. Two TSWV-resistant cultivars GA-12Y (newly released) and GA-06G were planted. A randomized split-split plot design was adopted. Tillage was considered as the main plot effect, while cultivars and insecticides were considered as subplot and sub-subplot effects, respectively. The dimension of plots was 9.14m in length and 5.49m in width with six rows in a plot. Peanuts were planted on May 8th in 2014 and April 23th in 2015 with single row pattern at 6 seeds per

foot seeding rate. Conventional tillage plots and strip tillage plots were prepared following standard protocols from Marois and Wright (2003). Strip tillage is one of the conservation tillage methods that tills a narrow band for the seed furrow and leaves the remaining part undisturbed. Four replications were assigned in all trials. Thrips counts and feeding damage, spotted wilt incidence, and yields were obtained as previously described.

All data, including thrips counts, thrips feeding damage rating, spotted wilt incidence, and yield, were subjected to generalized linear mixed models using the GLIMMIX procedure in SAS (SAS Enterprise 4.2, SAS Institute, Cary, NC). Insecticide and tillage type were considered fixed effects, whereas replication was considered a random effect. Tukey-Kramer Grouping, as an adjustment for multiple comparisons at $P=0.05$, was used to examine the statistical significance of differences among insecticide treatments and between tillage types. Interactions between insecticides and tillage, if any, were also analyzed.

Evaluation of row patterns and selected insecticides with TSWV-resistant peanut cultivars on thrips and spotted wilt incidence. Three insecticides (Imidacloprid, Thiamethoxam, phorate) were selected for this trial based on performance in the previous trial. Their mode of application and applied rates are listed in Table 3.2. Single row and twin row patterns were applied on two TSWV-resistant cultivars GA-12Y (newly released) and GA-06G. The single row pattern plot consisted of two single rows spaced 91.44cm apart on a 1.83m wide bed; while the twin row pattern plot was composed of two sets of twin rows on a 7.83m wide bed with 45.72cm between two inner rows and 91.44cm width between two outer rows. A randomized split-split plot design was adopted. Peanut cultivars served as main plots, while row patterns and insecticides served as subplots and sub-subplots, respectively. Peanuts were planted on April 22nd in 2014 and April 28th in 2015 with 6 seeds per foot after conventional tillage for field

preparation. Four replications were assigned in all trials. Thrips counts and feeding damage, spotted wilt incidence, and yields were obtained as previously described.

All data was subjected to linear mixed models using the GLIMMIX procedure in SAS (SAS Enterprise 4.2, SAS Institute, Cary, NC). Peanut cultivars, row patterns, and insecticides were considered fixed effects, while replications were considered random effects. Two-way and three-way interactions between cultivars, row patterns, and insecticides, if any, were analyzed. Tukey-Kramer Grouping, as an adjustment for multiple comparisons at $P=0.05$, was used to test the statistical significance of differences among insecticide treatments, between cultivars, and between row patterns.

Greenhouse experiment

Evaluation of selected insecticides with TSWV-resistant peanut cultivars on TSWV transmission by thrips. The experiment was conducted in a greenhouse at 25- 30°C and 80-90% relative humidity (RH) with a 14:10h (L:D) photoperiod. TSWV-resistant cultivars GA-12Y (newly released) and GA-06G were used. Three selected insecticides (Imidacloprid, Thiamethoxam, phorate) were evaluated; their mode of application and applied rates are listed in Table 3.3. A non-treated control was included as the fourth treatment.

Maintenance of potentially viruliferous *F. fusca*. A colony of potentially viruliferous *F. fusca* was maintained on TSWV-infected Georgia Green leaflets. During the peanut growing season, TSWV-infected Georgia Green foliage was collected from peanut fields in Tifton, GA; while leaflets from mechanically inoculated Georgia Green peanut plants were used in non-growing season. Infected Georgia Green peanut plants were obtained from mechanical inoculation following the standard protocol provided by Mandal et al. (2001). After inoculation, plants were maintained in a greenhouse with the same settings as described above. Thrips were

reared in small petri dishes (60mm x 15mm Polystyrene) (Becton, Dickinson and Company, Falcon™ Labware, Franklin Lakes, NJ), and infected peanut leaflets were placed on a moistened round cotton pad (Swippers Supreme cotton round, Cleveland, Ohio) in each petri dish. Ten female *F. fusca* from a UGA laboratory colony were released in a petri dish and allowed to lay eggs on fresh peanut leaflets, and adult thrips were removed from the dishes after two to three days. Maintenance included adding fresh infected leaflets and watering every two to three days. Thrips were reared in cages from eggs to adults in about two weeks. Old petri dishes were replaced to new ones after one generation was completed. Thrips reared for an entire generation (adult to adult) on TSWV-infected leaflets were considered potentially viruliferous. Adult female thrips (<2 days post eclosion) were used in the experiment. The colony was maintained in a incubator (Percival scientific, Perry, IA) at 29°C with a photoperiod of 14:10 (L:D) h.

Both TSWV infected peanut plants/foliage from field and greenhouse were tested by double antibody sandwich enzyme linked immunosorbent assay (DAS-ELISA) (Clark and Adams 1977) to confirm their infection status.

TSWV detection in plants by double antibody sandwich enzyme linked immunosorbent assay (DAS-ELISA). Fresh leaf tissue (approximately 0.1 g) was obtained from each experimental plant sample and used for DAS-ELISA. The assay was performed in a 96 well microtiter plate (Maxisorp, Nunc, Rochester, NY). Along with samples, two positive controls (TSWV infected peanut leaf tissues) and two negative controls (non-infected peanut leaf tissues) were included in each plate. Primary antibody (anti-TSWV IgG, monoclonal nucleocapsid protein (N)) was used at a dilution ratio of 1:200 and the secondary antibody (anti-TSWV IgG conjugated with alkaline phosphatase) was also used at a 1:200 dilution ratio (Agdia®, Elkhart, IN). Incubation and washing steps were followed as per the manufacturer's instructions. Final

absorbance values were measured at 405 nm in a photometer 1 h after substrate added (Model Elx 800, Bio-Tek®, Kocherwaldstr, Germany). An average absorbance value of negative control samples plus four standard deviations of was considered positive.

Transmission assays. Peanut cultivars GA-12Y and GA-06G were planted in 4-inch diameter plastic pots (Hummert International, St. Louis, MO) with commercial potting mix, Sunshine mix (LT5 Sunshine® mix, Sun Gro® Horticulture Industries, Bellevue, WA). Insecticides were applied while planting. Five plants of both cultivars were used for every insecticide treatment in an experiment, and the experiment was repeated three times (N=15 for each cultivar with each insecticide). About one-week-old peanut plants with first true leaves opened were ready for thrips inoculation. Ten potentially viruliferous adult female *F. fusca* were transferred into a 1.5ml microcentrifuge tube (Fisher Scientific, Pittsburgh, PA) with a paintbrush (fine camel hair #2 with aluminum ferrules, Charles Leonard Inc., Hauppauge, NY). Thrips were subsequently released on peanut plants by placing the microcentrifuges tube at the bottom of the plant (one tube containing ten potentially viruliferous thrips per plant) that had been dusted with approximately 0.05 g of pine (Pinus taeda L.) pollen grains on foliage. Each plant with ten thrips released from the microcentrifuge tube were enclosed in a Mylar® film (Grafix®, Cleveland, PA) cylindrical cage (πr^2h=3.14 x 16 x 39 cm^3) with a copper mesh top (mesh pore size-170 microns) (TWP®, Berkeley, CA). Experimental plants were maintained in thrips-proof cages (47.5 cm^3) (Megaview Science Co., Taichung, Taiwan) in a greenhouse at 25 to 30°C and 80- 90% RH with a 14:10 (L:D) h photoperiod. The evaluation of TSWV infection status was conducted by DAS-ELISA as previously described.

Statistical analyses. A completely randomized design was used for statistical analyses. Peanut cultivars and insecticides were considered fixed effects while experimental replications

were considered random effects. TSWV infection percentage was examined and compared among the insecticide and peanut cultivar. TSWV infection was treated as a binomial response (positive or negative), and data were analyzed using the GENMOD procedure in SAS (SAS Enterprise 4.2, SAS Institute, Cary, NC). Type 3 tests were used to determine the significant differences of fixed effects at P=0.05. Pairwise contrasts at P=0.05 were used to further test the statistical significance between treatment pairs.

Results

Thrips counts

Evaluation of various insecticides as alternatives to aldicarb and phorate along with TSWV-resistant peanut cultivars on thrips and spotted wilt incidence: Number of total thrips, including adult *F. fusca*, adult *F. occidentalis*, other adult thrips, and immatures were enumerated. A significant portion of total thrips collected was thrips larvae. Overall, more thrips were found in the 2013 trial than in the 2014 trial (Fig 3.1). Thrips abundance varied with insecticides across genotypes in 2013 (df=11, 33; F=3.26; P=0.0042), but not in 2014 (df=6, 18; F=0.64; P=0.6954). Cumulative thrips counts were significantly reduced in plots treated with cyantraniliprole than in plots treated with lambda-cyhalothrin and spirotetratmat (Fig.3.1.). Irrespective of insecticides, total thrips numbers varied with genotypes in 2013 trial (df=1, 516; F=4.13; P=0.0425), but not in 2014 trial (df=1, 244; F=0.26; P=0.6121). No interaction between insecticides and genotypes was found in 2013 (df=11, 516; F=0.69; P=0.744) or 2014 (df=6, 244; F=0.14; P=0.9912).

Evaluation of selected insecticides and tillage systems with TSWV-resistant peanut cultivars on thrips and spotted wilt incidence: Number of total thrips, including adult *F. fusca*,

adult *F. occidentalis*, other adult thrips, and immatures were enumerated. A significant portion of total thrips collected was thrips larvae. Overall, fewer thrips were found in 2014 than in 2015 (Fig. 3.5). Irrespective of insecticides and genotypes, thrips abundance did not vary with tillage types in 2014 (df=1, 3; F=3.22; P=0.1705) or 2015 trials (df=1, 3; F=9.83; P=0.0518). Irrespective of insecticides and tillage types, thrips abundance did not vary with genotypes in 2014 (df=1, 373; F=0.72; P=0.3952) or 2015 (df=1, 374; F=1.81; P=0.1798). Thrips abundance did not vary with insecticides in 2014 trial (df=4, 373; F=1.55; P=0.1864), yet it varied with insecticides in 2015 trial (df=4, 374; F=8.99; P<0.0001). In 2015 trials, thrips sampled from plots treated with phorate were significantly fewer than plots treated with thiamethoxam, imidacloprid (at cracking) and non-treated check; thrips sampled from plots treated with imidacloprid (in-furrow application) were significantly fewer than plots treated with thiamethoxam (Fig. 3.5). No significant interaction was found between any of the factors (two-way or three-way) in 2014 and 2015.

Evaluation of row patterns and selected insecticides with TSWV-resistant peanut cultivars on thrips and spotted wilt incidence: Number of total thrips, including adult *F. fusca*, adult *F. occidentalis*, other adult thrips, and immatures were counted. A significant portion of total thrips collected was thrips larvae. Overall, more thrips were found in 2015 than in 2014 (Fig. 3.9). Irrespective of genotypes and insecticides, thrips abundance did not vary with row patterns in either 2014 (df=1, 10; F=0.65; P=0.4396) or 2015 (df=1, 3; F=0.13; P=0.7382) trials. Cumulative thrips number did not vary with genotypes, across row pattern and insecticides, in either 2014 (df=1, 280; F=0.41; P=0.522) or 2015 (df=1, 298; F=0.23; P=0.6296) trials. Irrespective of row patterns and genotypes, thrips abundance did not vary with insecticides in 2014 (df=3, 280; F=2.39; P=0.0689) trial, but in 2015 (df=3, 298; F=2.95; P=0.0332) trial. In

2015 trial, number of thrips found in plots treated with phorate was significantly reduced compared to the non-treated control plots (Fig. 3.9). No two-way or three-way interaction between factors was found in either 2014 or 2015 trials.

Thrips feeding damage

Evaluation of various insecticides as alternatives to aldicarb and phorate along with TSWV-resistant peanut cultivars on thrips and spotted wilt incidence: The severity of thrips feeding damage was affected by insecticide treatments across genotypes in both 2013 (df=11, 33; F=33.94; P<0.0001) and 2014 (df=6, 18; F=70.88; P<0.0001). In 2013, thrips feeding damage was significantly reduced in plots treated with imidacloprid, cyantraniliprole, spinetoram, aldicarb, and phorate than plots treated with remaining insecticides and non-treated check (Fig.3.2). In 2014, thrips feeding damage was significantly reduced in plots treated with phorate than remaining insecticides and non-treated check; excluding phorate treatment, thrips feeding damage was lower in plots treated with cyantraniliprole than treated with other insecticides and non-treated check. Irrespective of insecticides, the degree of thrips feeding damage varied with genotypes in 2013 (df=1, 36; F=17.14; P=0.0002), but not 2014 (df=1, 21; F=0.24; P=0.63). Less thrips feeding damage was observed in genotype GA-06G compared with GA-12Y (Fig. 3.2). Interaction between insecticides and genotypes was found in 2013 trial (df=11, 36; F=4.93; P=0.0001), but not 2014 trial (df=6, 21; F=1.79; P=0.1504).

Evaluation of selected insecticides and tillage systems with TSWV-resistant peanut cultivars on thrips and spotted wilt incidence: Intensity of thrips feeding damage did not vary with tillage systems across genotypes and insecticides in 2014 (df=1, 3; F=0.23; P=0.6629), but in 2015 (df=1, 3; F=74.36; P=0.0033) trials. In 2015 trial, thrips feeding damage was

significantly higher in conventional tillage than strip tillage (Fig. 3.6). Irrespective of tillage systems and insecticides, thrips feeding damage did not vary with genotypes in 2014 (df=1, 54; F=3.86; P=0.0547) or 2015 (df=1, 54; F=0.87; P=0.3539) trials. Intensity of thrips feeding varied with insecticides across tillage systems and genotypes in 2014 (df=4, 54; F=291.26; P<0.0001) and 2015 (df=4, 54; F=177.65; P<0.0001) trials. In 2014 trial, thrips feeding damage was less severe in plots treated phorate than in plots treated with other insecticides and non-treated check; intensity of thrips feeding damage was significantly reduced in plots treated with imidacloprid (in-furrow application) compared with plots treated with imidacloprid (at cracking) and thiamethoxam; intensity of thrips feeding damage was significantly lower in plots treated with imidacloprid (at cracking) than in plots treated with thiamehtoxam (Fig. 3.6). In 2015 trial, intensity of thrips feeding damage was significantly suppressed in plots treated with phorate and imidacloprid (in-furrow) compared with plots treated with other insecticides and non-treated check. A two-way interaction between tillage and insecticides was observed in 2014 trial (df=4, 54; F=7.47; P<0.0001).

Evaluation of row patterns and selected insecticides with TSWV-resistant peanut cultivars on thrips and spotted wilt incidence: Irrespective of genotypes and insecticides, the severity of thrips feeding damage did not vary with row patterns in either 2014 (df=1, 3; F=8.04; P=0.0659) or 2015 (df=1, 3; F=1.26; P=0.3441) trials. Irrespective of row patterns and insecticides, the degree of thrips feeding damage varied with genotypes in 2014 trial (df=1, 42; F=27.02; P<0.0001), but not in 2015 trial (df=1, 42; F=0; P=1). Thrips feeding damage degree was significantly higher in genotype GA-12Y than GA-06G. Irrespective of row patterns and genotypes, the severity of thrips feeding damage varied with insecticides in both 2014 (df=3, 42; F=188.6; P<0.0001) and 2015 (df=3, 42; F=65.68; P<0.0001) trials. In 2014 trial, the intensity

of thrips feeding damage was significantly reduced in plots treated with imidacloprid and phorate when compared with plots treated with thiamethoxam and non-treated check; additionally, thrips feeding damage in plots treated with phorate was significantly lower than plots treated imidacloprid (Fig. 3.10). No specific interactions were found in 2014 and 2015.

Spotted wilt incidence

Evaluation of various insecticides as alternatives to aldicarb and phorate along with TSWV-resistant peanut cultivars on thrips and spotted wilt incidence: Irrespective of genotypes, spotted wilt incidence did not vary with insecticide treatments in either 2013 (df=11, 33; F=1.74; P=0.1081) or 2014 (df=6, 18; F=1.64; P=0.1925) trials. Spotted wilt incidence was affected by genotypes across insecticides in both 2013 (df=1, 36; F=137.8; P<0.0001) and 2014 (df=1, 21; F=27.77; P<0.0001) trials. In both years, spotted wilt incidence was significantly suppressed in genotype GA-12Y compared with GA-06G (Fig. 3.3). Interaction between insecticides and genotypes was observed in both 2013 (df=11, 36; F=2.58; P=0.0158) and 2014 trials (df=6, 21; F=2.78; P=0.0375).

Evaluation of selected insecticides and tillage systems with TSWV-resistant peanut cultivars on thrips and spotted wilt incidence: Irrespective of insecticides and genotypes, spotted wilt incidence varied with tillage system in 2014 (df=1, 3; F=26.11; P=0.0145), but not in 2015 (df=1, 3; F=8.44; P=0.0622) trials. In 2014 trial, spotted wilt incidence was significantly greater in plots with conventional tillage type than with strip tillage type (Fig. 3.7). Spotted wilt incidence varied with genotypes across tillage systems and insecticides in both 2014 (df=1, 54; F=16.43; P=0.0002) and 2015 (df=1, 54; F=41.64; P<0.0001). Spotted wilt incidence was significantly higher in genotype GA-06G than GA-12Y in both of the trials (Fig. 3.7).

Irrespective of tillage systems and genotypes, the degree of spotted wilt incidence varied with insecticides in both 2014 (df=4, 54; *F*=3.32; *P*=0.0168) and 2015 (df=4, 54; *F*=6.07; *P*=0.0004) trials. Spotted wilt incidence was significantly lower in plots treated with phorate than in plots treated with thiamethoxam and non-treated check in 2014 trial (Fig. 3.7). In 2015 trial, spotted wilt incidence of plots treated with phorate was significantly suppressed compared to plots treated with other selected insecticides and the non-treated check. An interaction between tillage type and genotypes was observed in 2014 (df=1, 54; *F*=11.69; *P*=0.0012).

Evaluation of row patterns and selected insecticides with TSWV-resistant peanut cultivars on thrips and spotted wilt incidence: Overall, spotted wilt incidence was higher in 2015 trial than 2014 (Fig.3.11). Irrespective of genotypes and insecticides, spotted wilt incidence did not vary with row patterns in either 2014 (df=1, 3; *F*=0.19; *P*=0.6934) or 2015 (df=1, 3; *F*=4.92; *P*=0.1132) trials. Irrespective of row patterns and insecticides, spotted wilt incidence varied with genotypes in both 2014 (df=1, 42; *F*=16.39; *P*=0.0002) and 2015 (df=1, 42; *F*=13.67; *P*=0.007). In both years, spotted wilt incidence was significantly suppressed in genotype GA-12Y compared with GA-06G (Fig.3.11). Spotted wilt incidence did not vary with insecticides across row patterns and genotypes in either 2014 (df=3, 42; *F*=1.44; *P*=0.2441) or 2015 (df=3, 42; *F*=0.94; *P*=0.4276). No two-way or three-way interactions were found among evaluated factors in both year trials.

Yields

Evaluation of various insecticides as alternatives to aldicarb and phorate along with TSWV-resistant peanut cultivars on thrips and spotted wilt incidence: Irrespective of genotypes, yields varied with insecticide treatments in 2013 trial (df=11, 33; *F*=3.21; *P*=0.0046),

but not in 2014 (df=6, 18; *F*=2.24; *P*=0.865). In 2013 trial, yields from plots treated with aldicarb and phorate were significantly greater than yields from non-treated control plots (Fig. 3.4). Yields varied with genotypes across insecticides in 2013 trial (df=1, 36; *F*=319.07; *P*<0.0001), but not in 2014 trial (df=1, 21; *F*=0.1; *P*=0.7526). Yields from genotype GA-12Y plots were significantly greater when compared with GA-06G plots in 2013 trial (Fig. 3.4). No interaction was found between insecticides and genotypes in 2013 or 2014.

Evaluation of selected insecticides and tillage systems with TSWV-resistant peanut cultivars on thrips and spotted wilt incidence: Irrespective of insecticides and genotypes, yields did not vary with tillage systems in 2014 (df=1, 3; *F*=2.87; *P*=0.1886) or 2015 (df=1, 3; *F*=0; *P*=0.9938). Yields did not vary with genotypes in 2014 (df=1, 54; *F*=1.3; *P*=0.26), but in 2015 (df=1, 54; *F*=30.94; *P*<0.0001) trials, across tillage systems and insecticides. In 2015 trial, yields were significantly greater in genotype GA-12Y than GA-06G (Fig. 3.8). Irrespective of tillage systems and genotypes, yields varied with insecticides in 2014 trial (df=4, 54; *F*=2.71; *P*=0.0393), but not in 2015 trial (df=4, 54; *F*=1.51; *P*=0.2108). In 2014 trial, yields from plots treated with imidacloprid (in-furrow application) were significantly higher than in plots treated with thiamethoxam (Fig. 3.8). No specific interactions between factors were found in both 2014 and 2015 trials.

Evaluation of row patterns and selected insecticides with TSWV-resistant peanut cultivars on thrips and spotted wilt incidence: Overall, yields were greater in 2014 trial than 2015 trial (Fig. 3.12). Irrespective of genotypes and insecticides, yields did not vary with row patterns in either 2014 (df=1, 3; *F*=4.09; *P*=0.1362) or 2015 (df=1, 3; *F*=0.35; *P*=0.652). Yields varied with genotypes across row patterns and insecticides in 2014 (df=1, 42; *F*=26.76; *P*<0.0001) and 2015 (df=1, 42; *F*=98.83; *P*<0.0001) trials. In both years, yields produced in genotype GA-12Y were

greater than in GA-06G (Fig. 3.12). Irrespective of row patterns and genotypes, yields did not vary with insecticides in either 2014 (df=3, 42; F=0.44; P=0.7226) or 2015 (df=3, 42; F=2.21; P=0.101). Two-way interaction between row patterns and genotypes was significant in 2014 trial (df=1, 42; F=4.36; P=0.0429). Two-way interaction between genotype and insecticides was significant (df=3, 42; F=2.96; P=0.0432), and three-way interaction among row pattern, genotypes, and insecticides was significant in 2015 (df=3, 42; F=8; P=0.0002).

Impact of selected insecticides on TSWV transmission in TSWV-resistant peanut cultivars in greenhouse: Overall, TSWV infection percentages of peanut plants irrespective of genotypes after thrips-mediated inoculation were affected by insecticide treatments (df= 3; χ^2=30.59; $Pr<\chi^2$ <0.0001), and TSWV infection percentages did not vary with the experimental repeats (df= 2; χ^2=0.36; $Pr<\chi^2$ =0.8360). Regardless of insecticides, TSWV infection percentage was not affected by cultivars (df= 1; χ^2=3.56; $Pr<\chi^2$=0.0590). In genotype GA-06G, TSWV infection percentages varied with insecticide treatments (df= 3; χ^2=21.47; $Pr>\chi^2$ <0.0001). TSWV infection was significantly suppressed in plants treated with imidacloprid and phorate (Fig. 3.13). In genotype GA-12Y, TSWV infection percentages varied with insecticide treatments as well (df= 3; χ^2=12.37; $Pr>\chi^2$=0.0062). TSWV infection was significantly reduced in plants treated with phorate (Fig. 3.13).

Discussion

In this study, various insecticides were evaluated for their potential to serve as alternatives to aldicarb and phorate along with peanut cultivars that are highly resistant to TSWV in the field. Planting cultivars with field resistance to TSWV is the most important tactic in spotted wilt management in peanut. A newly released peanut cultivar GA-12Y, with higher TSWV field

resistance, was evaluated. Thrips abundance found in peanut terminals or blooms and feeding damage on the foliage were not affected by cultivars. The second-generation peanut cultivar, GA-06G and the third-generation cultivar, GA-12Y, were both infested by thrips. TSWV field resistant cultivars are known to reduce spotted wilt incidence in the field, yet they are not resistant to thrips. Previous studies indicated that attractiveness to thrips, reproduction of thrips, and feeding injury caused by thrips were not affected by cultivars with different levels of field resistance to TSWV (Chamberlin et al., 1992, Culbreath et al., 2000, Culbreath et al., 1999, Culbreath et al., 1994, Culbreath et al., 1996). Besides being the vectors of TSWV in peanut, tobacco thrips can also severely infest peanut fields early in the planting season. Thrips damage on peanut foliage mainly caused by larvae feeding has been a serious seedling problem that would subsequently affect peanut yields and maturity (Lynch et al., 1984, Todd et al., 1993, Young et al., 1972). When insecticides were applied along with TSWV resistant cultivars, alternatives such as imidacloprid (in-furrow), cyantraniliprole, and spinetoram effectively suppressed thrips populations and/or reduced feeding damage similar to aldicarb and phorate. Some of the other alternatives evaluated such as thiamethoxam and azadirachtin were not as effective as phorate in reducing thrips feeding damage. Insecticides, alternative to aldicarb and phorate, were capable of providing sufficient control of thrips populations and reduce feeding damage especially with cultivars possessing higher field resistance to TSWV.

Spotted wilt incidence was suppressed more in cultivar GA-12Y than GA-06G. Branch and Brenneman (2015) also provide evidence of better performance of GA-12Y in reducing TSWV incidence than other cultivars. GA-12Y was released in 2012 with increased field resistance to TSWV than several other second-generation genotypes including GA-06G. Even with higher field resistance, spotted wilt was still observed in GA-12Y in field trials. This reiterates that field

resistant cultivars are not immune to TSWV. Similarly, TSWV infection occurred in both GA-06G and GA-12Y following thrips-mediated transmission in the greenhouse; incidence of spotted wilt was marginally suppressed in GA-12Y when compared with GA-06G. Shrestha et al. (2013) also documented that both TSWV susceptible and resistant genotypes were susceptible to TSWV. The underlying mechanism of field resistance to TSWV in peanut cultivars remains unidentified. In the case of vegetables, where TSWV is also a major pathogen, such as pepper (*Capsicum annuum* L.) and tomato (*Solanum lycopersicon* L.), the mechanism is known. Dominant genes such as Sw5 and Tsw confer resistance in those crops. Resistance dominated by one single gene in pepper and tomato leads to local lesions and avoidance of systemic infection (Hallwass et al., 2014, Moury et al., 1997, Ngoc Huy et al., 2013). It is proposed that field resistance to TSWV is possibly tolerance rather than true resistance (Shrestha et al., 2013).

Thrips are exclusive vectors of TSWV that causes spotted wilt disease in peanut. However, most of the time, reduction of thrips populations and thrips feeding damage does not consequently result in reducing spotted wilt incidence (Todd et al., 1996, Todd et al., 1994). In this study, spotted wilt incidence was only affected by insecticide treatments when there was a high incidence of spotted wilt. Incidence of spotted wilt in peanut plots treated with phorate was significantly reduced compared with plots treated with other insecticides. Imidacloprid (in-furrow) resulted in marginal suppression of spotted wilt incidence in one of the years in both cultivars, while other alternatives did not affect spotted wilt incidence. Overall, the effect of insecticides on spotted wilt incidence was more noticeable in cultivar with less field resistance, which is GA-06G in our case. In GA-12Y, the overall spotted wilt incidence was relatively low that differences between insecticides were limited. Culbreath et al. (2008) also suggested that benefit of spotted wilt suppression by phorate on some second-generation TSWV resistant

cultivars might be limited when compared with cultivars that are more susceptible to TSWV. In greenhouse, spotted wilt incidence was also lower when plants treated with phorate and imidacloprid in both GA-06G and GA-12Y. Imidacloprid could likely serve as an alternative to phorate in combination with a third-generation TSWV-resistant cultivar than previously released TSWV-resistant cultivars.

Phorate application in peanut has been reported to suppress spotted wilt incidence in addition to thrips control (Todd et al., 1996, Wiatrak et al., 2000). Typically, phorate did not provide better thrips control than other insecticides that have little or no effect on the spotted wilt epidemics (Todd et al., 1996). Thus, the mechanism of suppression of spotted wilt incidence by phorate application is not likely related to thrips control. Phorate is phytotoxic to peanut that often causes "peanut burn" referring to marginal chlorosis and necrosis on peanut foliage. Research conducted by Jain et al. (2015) indicated that phorate application triggers defense response in plants, followed by interference of virus-host interactions. Some of the genes encoding for pathogenesis and defense-related proteins as well as membrane-trafficking functions were found regulated in plants treated with phorate; certain gene regulation may subsequently affect virus replication and limit systemic spread of TSWV (Jain et al., 2015).

Overall, results of spotted wilt incidence from field trials and greenhouse experiments showed a consistent trend that lower incidence was observed in GA-12Y as well as plants treated with phorate and imidacloprid irrespective of cultivars. Nevertheless, TSWV infection percentages of both cultivars were greatly higher in greenhouse than in the field. It is likely due to the differences in thrips and TSWV pressures as well as the age of the plants between greenhouse and field conditions. In greenhouse, only one-week-old peanut plants were used when they were at the most vulnerable stage. In addition, a single plant infested by ten thrips could certainly be

different from the field. It is concluded that under high thrips and TSWV pressures peanut cultivars with high resistance to TSWV can still suffer from severe TSWV infection, and the degree of reduction in spotted wilt incidence by insecticides may not be noticeable in cultivar with higher level of field resistance to TSWV.

Insecticide application increased yields in one of the years. Plots treated with aldicarb and phorate had greater yields than plots treated with other insecticides. Alternative insecticides were actuallly possessing similar efficacy as aldicarb and phorate in increasing yields, however, yields were only marginally higher than non-treated check. In-furrow application of aldicarb and phorate has been reported to increase pod yields in peanut by Herbert et al. (2007). Nonetheless, the impact of phorate application in increasing yields was not significant in some cases; especially when cultivars with increased resistance to TSWV (second-generation resistant genotypes) were planted (Culbreath et al., 2008, Marois & Wright, 2003). GA-12 resulted in higher yields than GA-06G. Reduction in spotted wilt incidence as well as the high yielding property in GA-12Y could have contributed to the increase in final yields.

Different tillage systems were evaluated in combination with highly TSWV field resistant cultivar and selected insecticides. Differences in tillage systems did not Whether plant residues left on the soil surface did not affect thrips abundance, but thrips-feeding damage was reduced in plots prepared with strip tillage, which left plant residues between the two rows. Suppression of thrips feeding damage and reduction of immature thrips numbers were documented with reduced tillage in previous studies (Brown et al., 1996, Knight et al., 2015, Minton et al., 1991). Strip-tillage also suppressed spotted wilt incidence in one of the year trial. We found that the impact of strip tillage in reducing spotted wilt incidence was significant in GA-06G, while strip tillage only marginally affected spotted wilt incidence in GA-12Y, the cultivar with increased field resistance

to TSWV. Our findings corroborated with previous studies that conservation tillage system was likely to appear lower TSWV prevalence when compared with conventional tillage, although the difference was not always significant (Hurt et al., 2006, Johnson et al., 2001, Marois & Wright, 2003). Strip tillage is one of the conservation tillage methods that tills a narrow band for the seed furrow and leaving the remaining part undisturbed. Increased ground cover from winter cover crops in conservation tillage systems has resulted in reduction of TSWV incidence in peanut (Hurt et al., 2005, Johnson et al., 2001). The cause of this effect has not been fully understood. It is suggested that ground cover of crop stubbles may interfere with visual locating ability of host plants by migrating thrips (Brown et al., 2005, Culbreath et al., 2003). Tillage system did not affect yields in our study. Increased yields by using conservational tillage system was documented by some previous studies, yet the difference in yields between conventional or conservation tillage systems were not always significant (Drake et al., 2014, Hurt et al., 2006, Johnson et al., 2001, Marois & Wright, 2003). Conservation tillage requires less operation time and labor for planting compared to conventional tillage (Brown et al., 2005). Therefore, it is possible that while using cultivar with increased field resistance to TSWV such as GA-12Y, strip tillage can still be used to improve the management of thrips and TSWV.

Manipulation of row patterns did not affect thrips abundance and thrips feeding damage. In previous studies, less thrips feeding damage was observed in twin row plots (Hurt et al., 2005, Marasigan, 2014). Spotted wilt incidence was not affected by row patterns in this study. In contrast, consistent suppression of spotted wilt incidence when twin row pattern was adopted has been documented in previous studies (Culbreath et al., 2008, Lanier et al., 2004, Tillman et al., 2006, Tubbs et al., 2011). The reason of row patterns affecting spotted wilt incidence was unknown. Peanut plants in twin row pattern cover ground more rapidly than single row, and this

is one of the speculations that ground coverage may affect the abilities of thrips to locate a seedling host (Brown et al., 2005). Research conducted by Lanier et al. (2004) showed that reduction of spotted wilt incidence was observed in TSWV moderately resistant cultivar but not in a more resistant cultivar. Similarly, a tendency of reducing spotted wilt incidence in twin row pattern of GA-06G was observed, while there was no impact of row patterns on TSWV prevalence in GA-12Y. However, Culbreath et al. (2008) suggested that even the more resistant cultivars might benefit from use of twin row pattern for suppressing spotted wilt epidemics when the thrips and/or virus pressure is high. Row patterns did not affect yields in our study. Yield improvement by twin row pattern was observed in some previous studies (Culbreath et al., 2008, Jordan et al., 2010, Tubbs et al., 2011). The impacts of row patterns on yields of GA-06G and GA-12Y were not different in our study. Culbreath et al. (2008) also indicated that there was no interaction between peanut cultivars with varying degrees of field resistance to TSWV and row patterns.

In conclusion, our results corroborated that GA-12Y has higher field resistant to TSWV than GA-06G. Alternative insecticides, possessing less non-target effects, could replace aldicarb and phorate in thrips and spotted wilt management in peanut without compromising yield, especially when cultivars with increased field resistance to TSWV, such as GA-12Y, is used. With relatively narrow-sprectrum toxicity than aldicarb and phorate, insecticides such as imidacloprid and cyantraniliprole were as effective as aldicarb and phorate in thrips management. The different application methods such as in-furrow application and seed treatment of alternative insecticides provide growers with more flexibility. Strip tillage and twin row pattern are suitable cultural tactics that could be readily used along with cultivars that exhibit increased field resistance in order to reinforce integrated management programs.

Table 3.1. List of selected insecticides as alternatives to aldicarb and phorate applied in the field trials.

Active Ingredient	Classification/ Chemical Name	Trade Name	Rate per Acre	Type of Application	Manufacturer	2013	2014
Thiamethoxam	(4A) Neonicotinoids	Actara	2 oz	At cracking	Syngenta	v	
Imidacloprid	(4A) Neonicotinoids	Admire Pro	7.0 fl oz/10 fl oz	In-furrow	Bayer CropScience	v	v
Imidacloprid	(4A) Neonicotinoids	Admire Pro	1.7 fl oz	At cracking	Bayer CropScience	v	v
Thiamethoxam	(4A) Neonicotinoids	Cruiser	4.0 oz	Seed treatment	Syngenta	v	v
Azadirachtin	UN	Azatin XL	1%	At cracking	OHP, Inc.	v	
Cyantraniliprole	Diamides	HGW086 10C	20.4 fl oz	At cracking	Dupont	v	
Cyantraniliprole	Diamides	Exirel	20 fl oz	At cracking	Dupont		v
Lambda-cyhalothrin	(3A) Pyrethroids	Karate	3.5 fl oz	At cracking	Syngenta	v	
Spirotetratmat	Tetronic and Trtramic acid derivatives	Movento 2SC	5 fl oz	At cracking	Bayer CropScience	v	
Spinetoram	Spinosyn	Radiant SC	5 fl oz/ 8 fl oz	At cracking	Dow AgroSciences	v	v
Aldicarb	(1A) Carbamates	Temik 15G	5 lb	In-furrow	Bayer CropScience	v	
Phorate	(1B) Organophosphates	Thimet 10G	5 lb	In-furrow	Amvac	v	v

Table 3.2. List of selected insecticides applied in conjunction with cultural practices in field trials.

Active Ingredient	Classification/ Chemical Name	Trade Name	Rate per Acre	Type of Application	Manufacturer	Field trials
Imidacloprid	(4A) Neonicotinoids	Admire® Pro	1.2 fl oz	At crecking	Bayer CropScience	Tillage system
Imidacloprid	(4A) Neonicotinoids	Admire® Pro	10 fl oz	In-furrow	Bayer CropScience	Tillage system; Row pattern
Thiamethoxam	(4A) Neonicotinoids	Cruiser Maxx™	4.0-5.4 oz[x]	Seed treatment	Syngenta	Tillage system; Row pattern;
Phorate	(1B) Organophosphates	Thimet® 20G	5 lb	In-furrow	Amvac	Tillage system; Row pattern;

[x] Calculation based on the estimation of 648 seeds (GA-06G) in 100lb of seeds.

Table 3.3. List of selected insecticides used for greenhouse experiment.

Treatment No.	Classification/ Chemical Name	Active Ingredient	Trade Name	Rate per Acre	Rate per seed	Type of Application	Manufacturer
1	4A Neonicotinoids	Imidacloprid	Admire® Pro	7.0-10.5 fl oz	3.55µl[x]	In-furrow	Bayer CropScience
2	4A Neonicotinoids	Thiamethoxam	Cruiser Maxx™	4.0-5.4 oz[y]	1.75mg[y]	Seed treatment	Syngenta
3	1B Organophosphates	Phorate	Thimet® 20G	5 lb	0.026g[x]	In-furrow	Amvac

[x] Calculation based on the estimation of 87362 seeds planted per acre (6 seeds per foot).

[y] Calculation based on the estimation of 648 seeds (GA-06G) in 100lb of seeds.

Figures

Fig. 3.1. Mean (±SE) number of cumulative thrips counts in two peanut cultivars with insecticide treatments. Cumulative thrips counts include adult and immature thrips. Thrips samples were collected from quadrifoliate peanut terminals and blooms for five consecutive weeks (six weeks in 2013) from ~three weeks after planting. Treatment means labeled with different letters indicate significant differences at $\alpha = 0.05$.

Fig. 3.2. Mean (±SE) thrips feeding damage rating in two peanut cultivars with insecticide treatments. Feeding damage was evaluated by giving a score using an arbitrary scale from 0 to 10, while 0 represents no feeding and 10 represents a dead plant. Treatment means labeled with different letters indicate significant difference at $\alpha = 0.05$.

Fig. 3.3. Mean (±SE) percentage of spotted wilt incidence in two peanut cultivars with insecticide treatments. Spotted wilt incidence was evaluated by measuring the amount (in feet) of infected plants in two harvested rows. Treatment means labeled with different letters indicate significant difference at $\alpha = 0.05$.

Fig. 3.4. Mean (±SE) of yields (lb/acre) in two peanut cultivars with insecticide treatments. Peanut plants in two harvested rows were weighted. Treatment means labeled with different letters indicate significant difference at $\alpha = 0.05$.

Fig. 3.5. Mean (±SE) number of cumulative thrips counts in two peanut cultivars with four insecticides in two tillage systems. Cumulative thrips counts include adult and immature thrips. Thrips samples were collected from quadrifoliate peanut terminals and blooms for five

consecutive weeks from ~three weeks after planting. Treatment means labeled with different letters indicate significant difference at α= 0.05.

Fig. 3.6. Mean (±SE) of thrips feeding damage rating in two peanut cultivars with four insecticides in two tillage systems. Thrips damage was evaluated by giving a score using an arbitrary scale from 0 to 10, while 0 represents no feeding and 10 represents a dead plant. Treatment means labeled with different letters indicate significant difference at α= 0.05.

Fig. 3.7. Mean (±SE) percentage of spotted wilt incidence in two peanut cultivars with four insecticides in two tillage systems. Spotted wilt incidence was evaluated by measuring the amount (in feet) of infected plants in two harvested rows. Treatment means labeled with different letters indicate significant difference at α= 0.05.

Fig. 3.8. Mean (±SE) of yields (lb/acre) in two peanut cultivars with four insecticides in two tillage systems. Peanut plants in the two harvested rows were weighted. Treatment means labeled with different letters indicate significant difference at α= 0.05.

Fig. 3.9. Mean (±SE) number of cumulative thrips counts in two peanut cultivars with four insecticides in two row patterns. Cumulative thrips counts include adult and immature thrips. Thrips samples were collected from quadrifoliate peanut terminals and blooms for five consecutive weeks from ~three weeks after planting. Treatment means labeled with different letters indicates significant difference at α= 0.05.

Fig. 3.10. Mean (±SE) of thrips feeding damage rating in two peanut cultivars with four insecticides in two row patterns. Thrips damage was evaluated by giving a score using an

arbitrary scale from 0 to 10, while 0 represents no feeding and 10 represents a dead plant. Treatment means labeled with different letters indicate significant difference at α= 0.05.

Fig. 3.11. Mean (±SE) percentage of spotted wilt incidence in two peanut cultivars with four insecticides in two row patterns. Spotted wilt incidence was evaluated by measuring the amount (in feet) of infected plants in two harvested rows. Treatment means labeled with different letters indicate significant difference at α= 0.05.

Fig. 3.12. Mean (±SE) of yields (lb/acre) in two peanut cultivars with four insecticides in two row patterns. Peanut plants in the two harvested rows were weighted. Treatment means labeled with different letters indicate significant difference at α= 0.05.

Fig. 3.13. Mean (±SE) of TSWV infection percentage in two peanut cultivars with four selected insecticides in greenhouse transmission experiment. Five replicates were included in each treatment, and experiment was repeated twice (N=15 for each insecticide in both cultivars). Treatment means labeled with different letters indicate significant difference at α= 0.05.

Fig. 3.1.

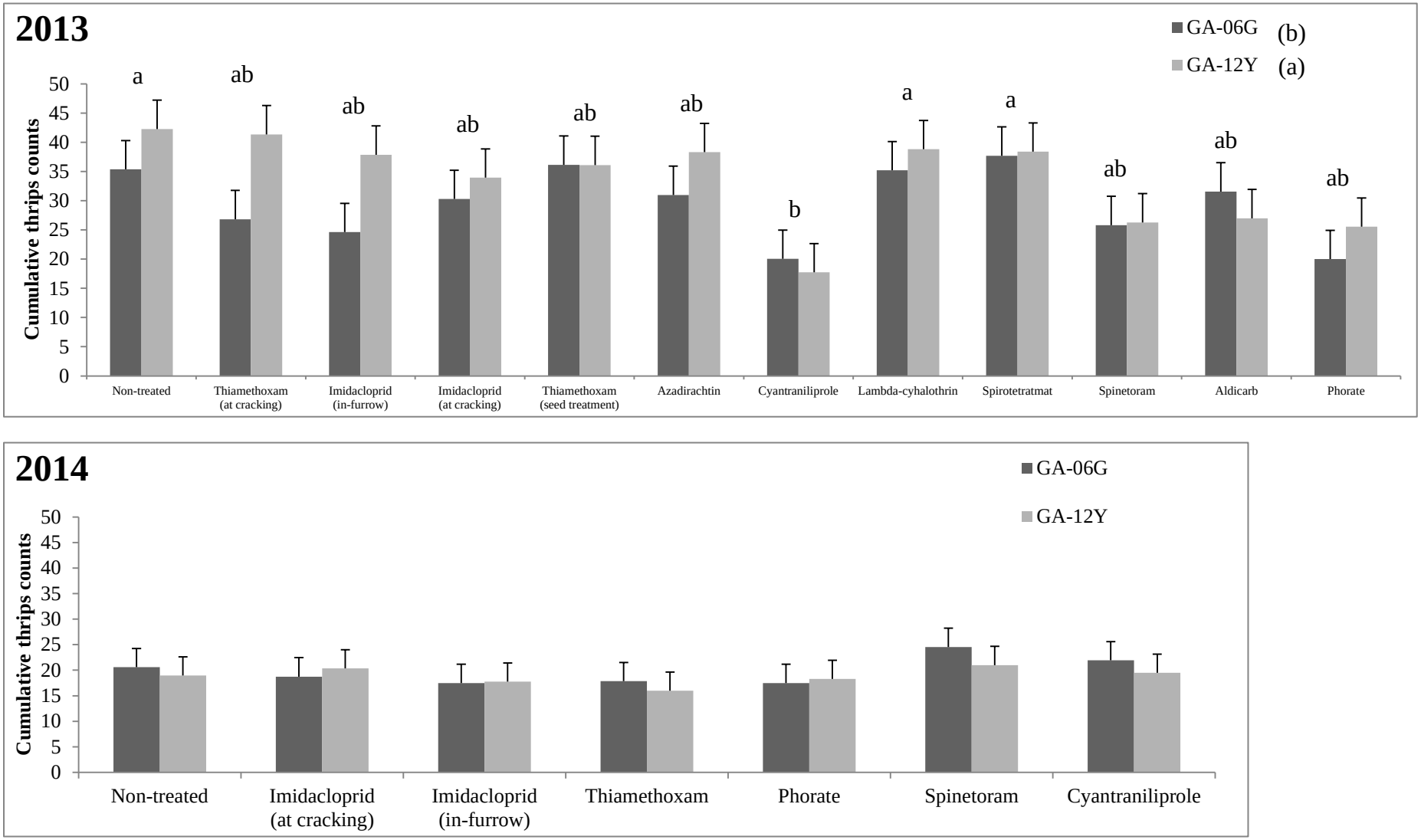
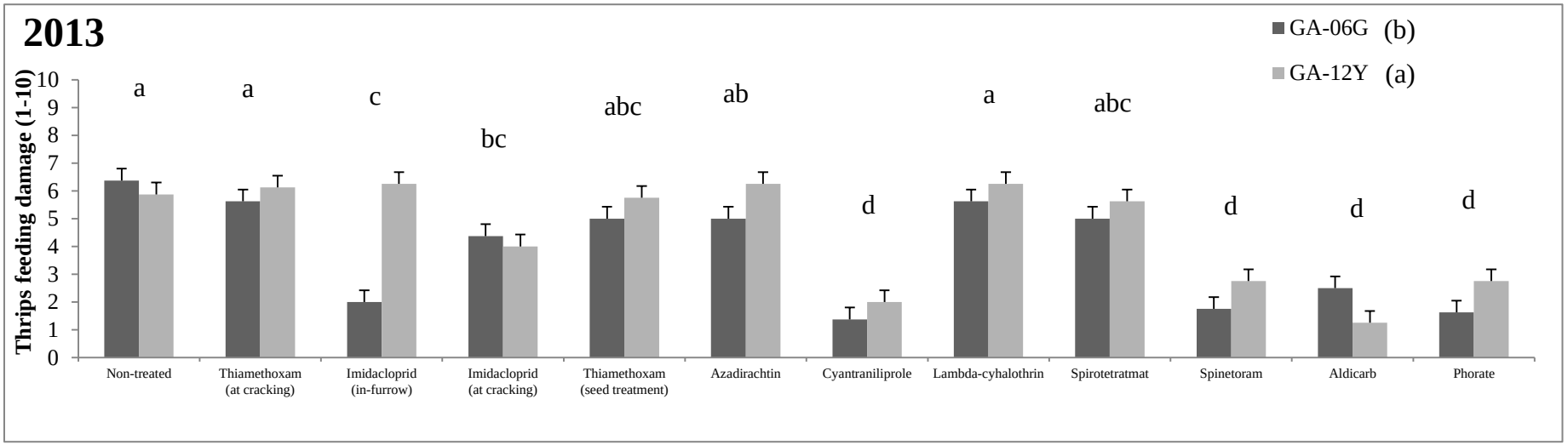

2013
GA-06G (b)
GA-12Y (a)
Cumulative thrips counts
0
5
10
15
20
25
30
35
40
45
50
a
ab
ab
ab
ab
ab
b
a
a
ab
ab
ab
Non-treated
Thiamethoxam (at cracking)
Imidacloprid (in-furrow)
Imidacloprid (at cracking)
Thiamethoxam (seed treatment)
Azadirachtin
Cyantraniliprole
Lambda-cyhalothrin
Spirotetratmat
Spinetoram
Aldicarb
Phorate
2014
GA-06G
GA-12Y
Cumulative thrips counts
0
5
10
15
20
25
30
35
40
45
50
Non-treated
Imidacloprid (at cracking)
Imidacloprid (in-furrow)
Thiamethoxam
Phorate
Spinetoram
Cyantraniliprole

Fig. 3.2.


2013
GA-06G (b)
GA-12Y (a)
Thrips feeding damage (1-10)
0 1 2 3 4 5 6 7 8 9 10
a a c bc abc ab d a abc d d d
Non-treated
Thiamethoxam (at cracking)
Imidacloprid (in-furrow)
Imidacloprid (at cracking)
Thiamethoxam (seed treatment)
Azadirachtin
Cyantraniliprole
Lambda-cyhalothrin
Spirotetratmat
Spinetoram
Aldicarb
Phorate

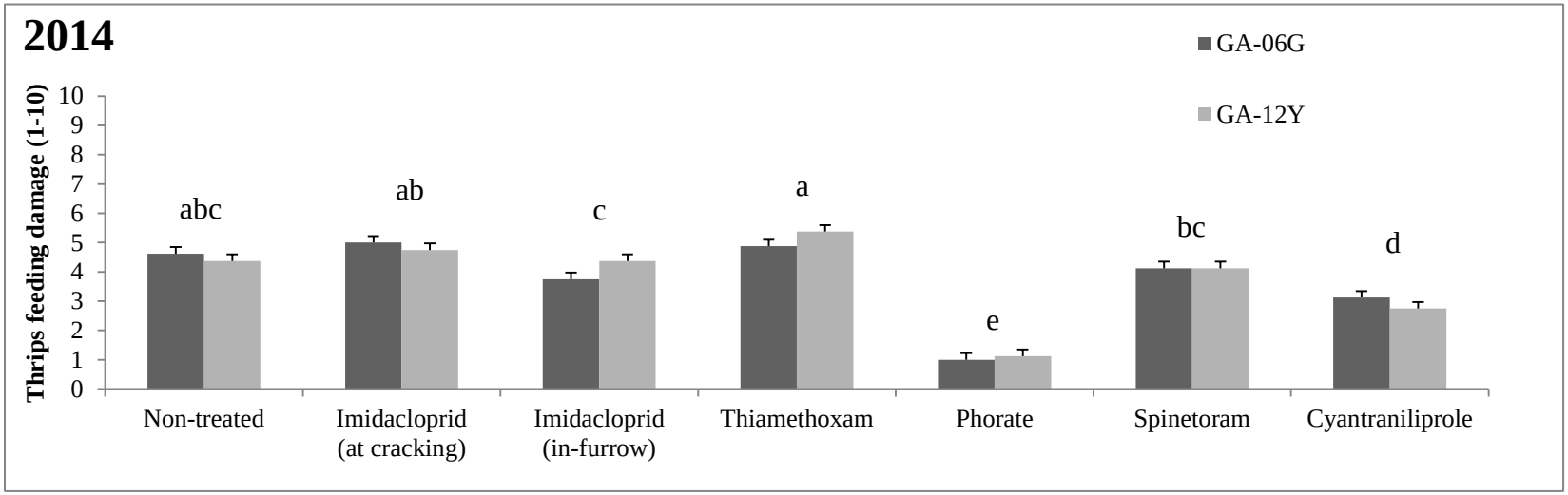
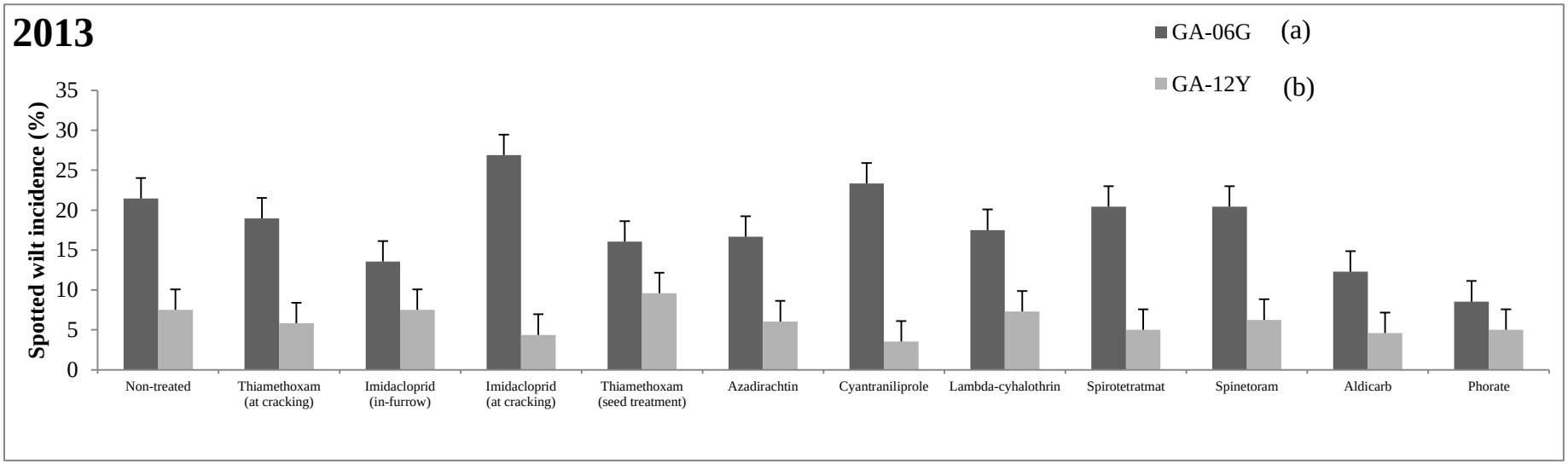

2014
GA-06G
GA-12Y
Thrips feeding damage (1-10)
0 1 2 3 4 5 6 7 8 9 10
abc ab c a e bc d
Non-treated
Imidacloprid (at cracking)
Imidacloprid (in-furrow)
Thiamethoxam
Phorate
Spinetoram
Cyantraniliprole

Fig. 3.3.


2013
GA-06G (a)
GA-12Y (b)
Spotted wilt incidence (%)
35
30
25
20
15
10
5
0
Non-treated
Thiamethoxam (at cracking)
Imidacloprid (in-furrow)
Imidacloprid (at cracking)
Thiamethoxam (seed treatment)
Azadirachtin
Cyantraniliprole
Lambda-cyhalothrin
Spirotetratmat
Spinetoram
Aldicarb
Phorate

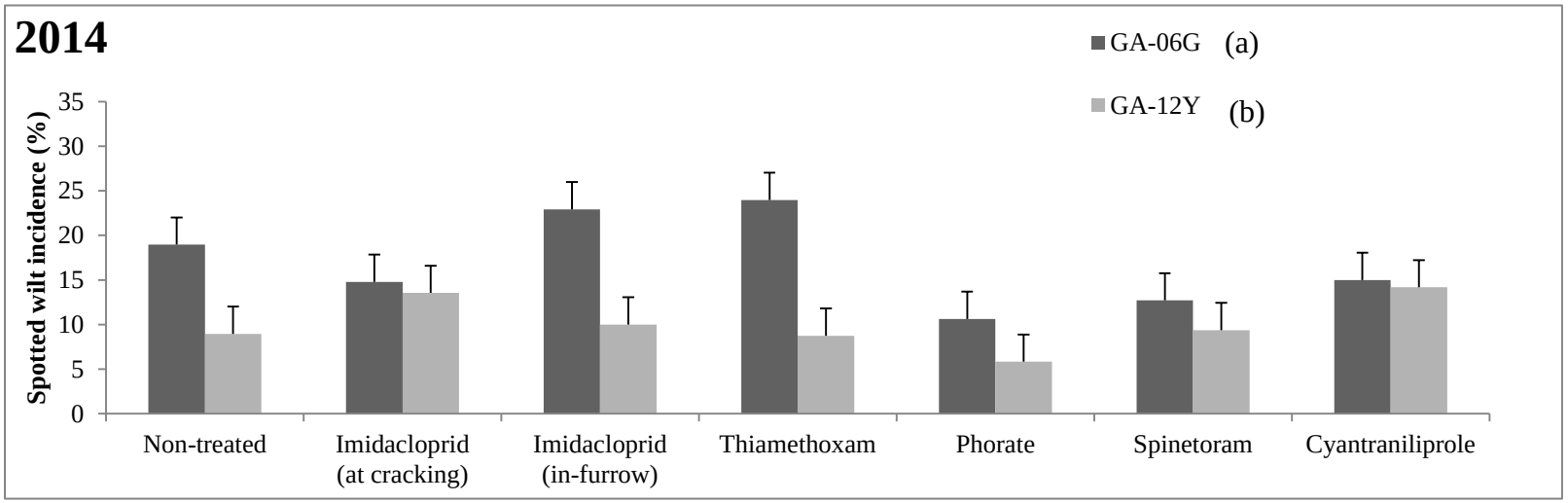

2014
GA-06G (a)
GA-12Y (b)
Spotted wilt incidence (%)
35
30
25
20
15
10
5
0
Non-treated
Imidacloprid (at cracking)
Imidacloprid (in-furrow)
Thiamethoxam
Phorate
Spinetoram
Cyantraniliprole

Fig. 3.4.

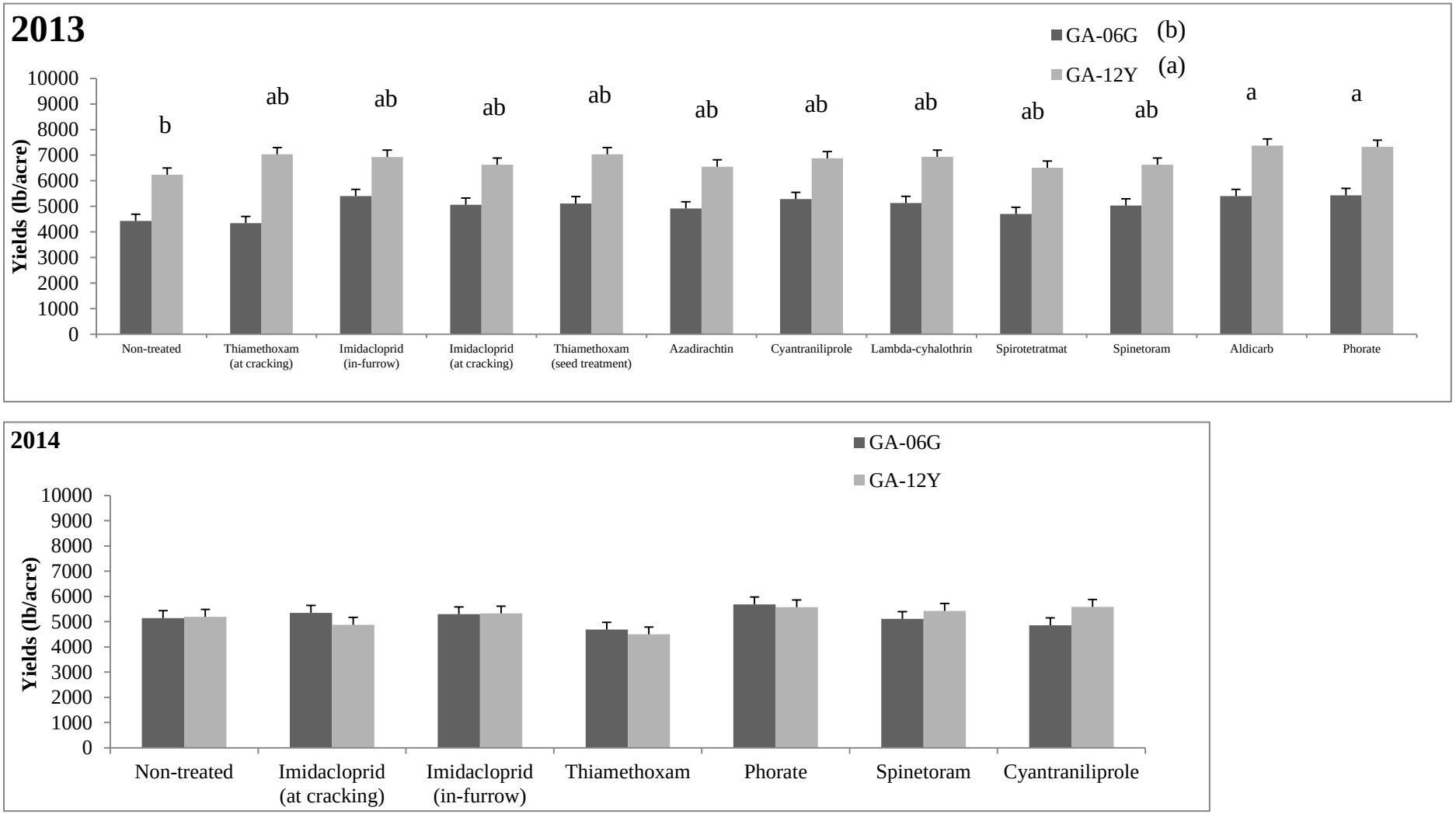
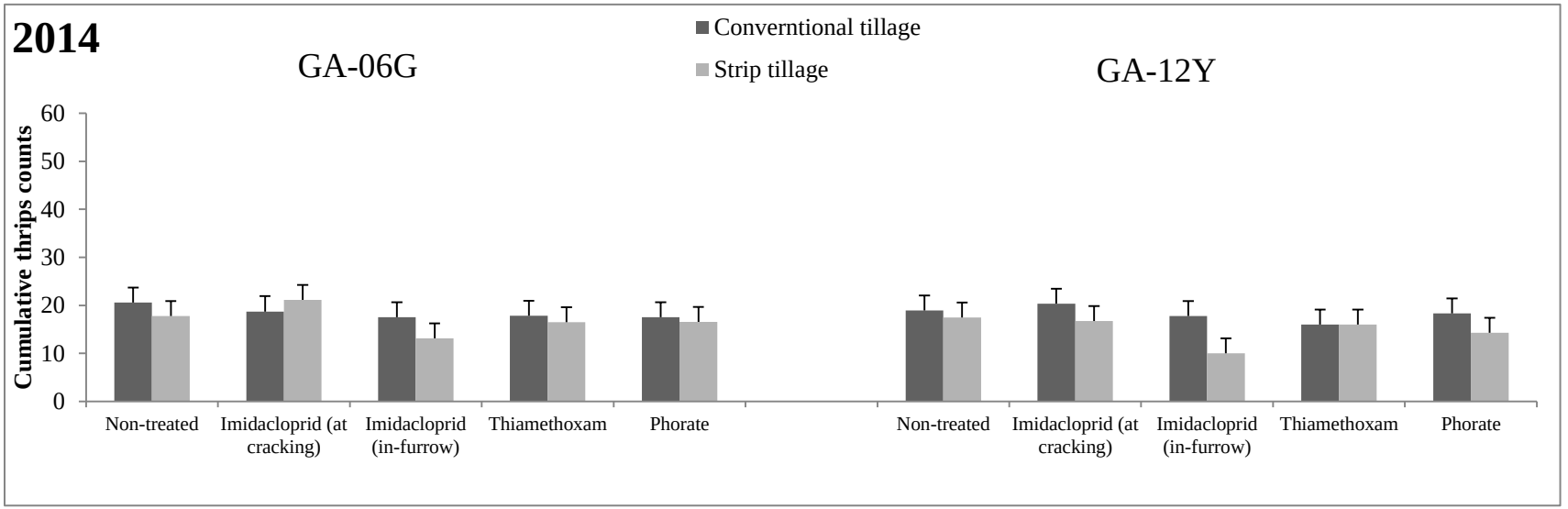

2013
GA-06G (b)
GA-12Y (a)
Yields (lb/acre)
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
b
ab
ab
ab
ab
ab
ab
ab
ab
ab
a
a
Non-treated
Thiamethoxam (at cracking)
Imidacloprid (in-furrow)
Imidacloprid (at cracking)
Thiamethoxam (seed treatment)
Azadirachtin
Cyantraniliprole
Lambda-cyhalothrin
Spirotetratmat
Spinetoram
Aldicarb
Phorate
2014
GA-06G
GA-12Y
Yields (lb/acre)
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
Non-treated
Imidacloprid (at cracking)
Imidacloprid (in-furrow)
Thiamethoxam
Phorate
Spinetoram
Cyantraniliprole

Fig. 3.5.


2014
Converntional tillage
Strip tillage
GA-06G
GA-12Y
Cumulative thrips counts
60
50
40
30
20
10
0
Non-treated
Imidacloprid (at cracking)
Imidacloprid (in-furrow)
Thiamethoxam
Phorate
Non-treated
Imidacloprid (at cracking)
Imidacloprid (in-furrow)
Thiamethoxam
Phorate

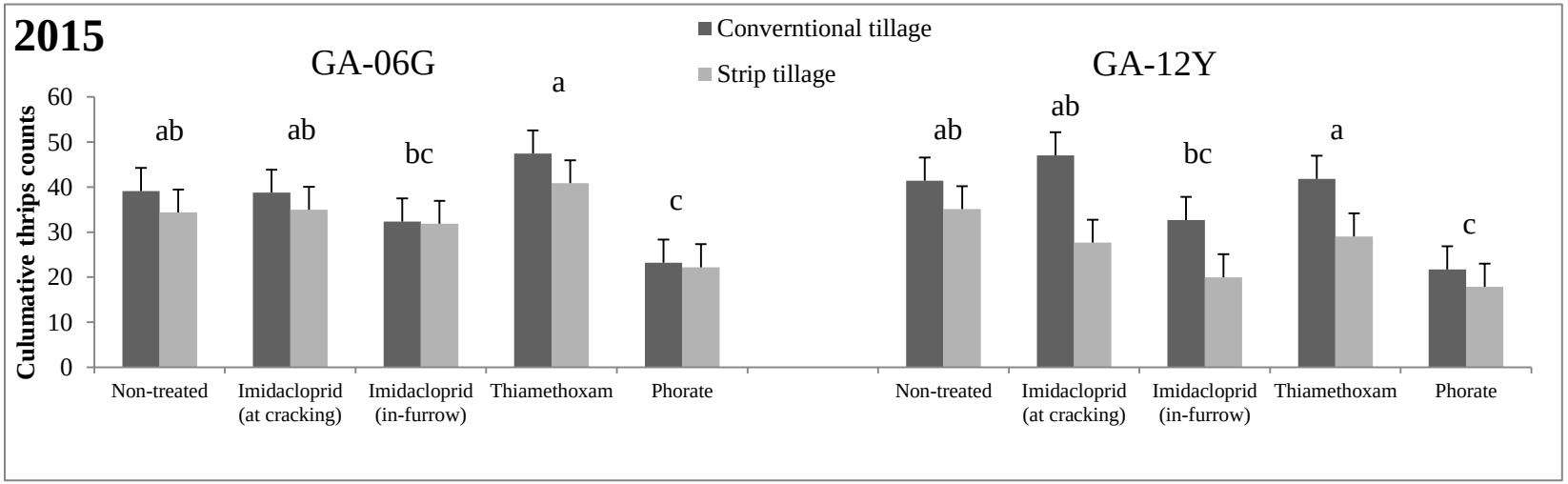

2015
Converntional tillage
Strip tillage
GA-06G
GA-12Y
Culumative thrips counts
60
50
40
30
20
10
0
ab
ab
bc
a
c
ab
ab
bc
a
c
Non-treated
Imidacloprid (at cracking)
Imidacloprid (in-furrow)
Thiamethoxam
Phorate
Non-treated
Imidacloprid (at cracking)
Imidacloprid (in-furrow)
Thiamethoxam
Phorate

Fig. 3.6.

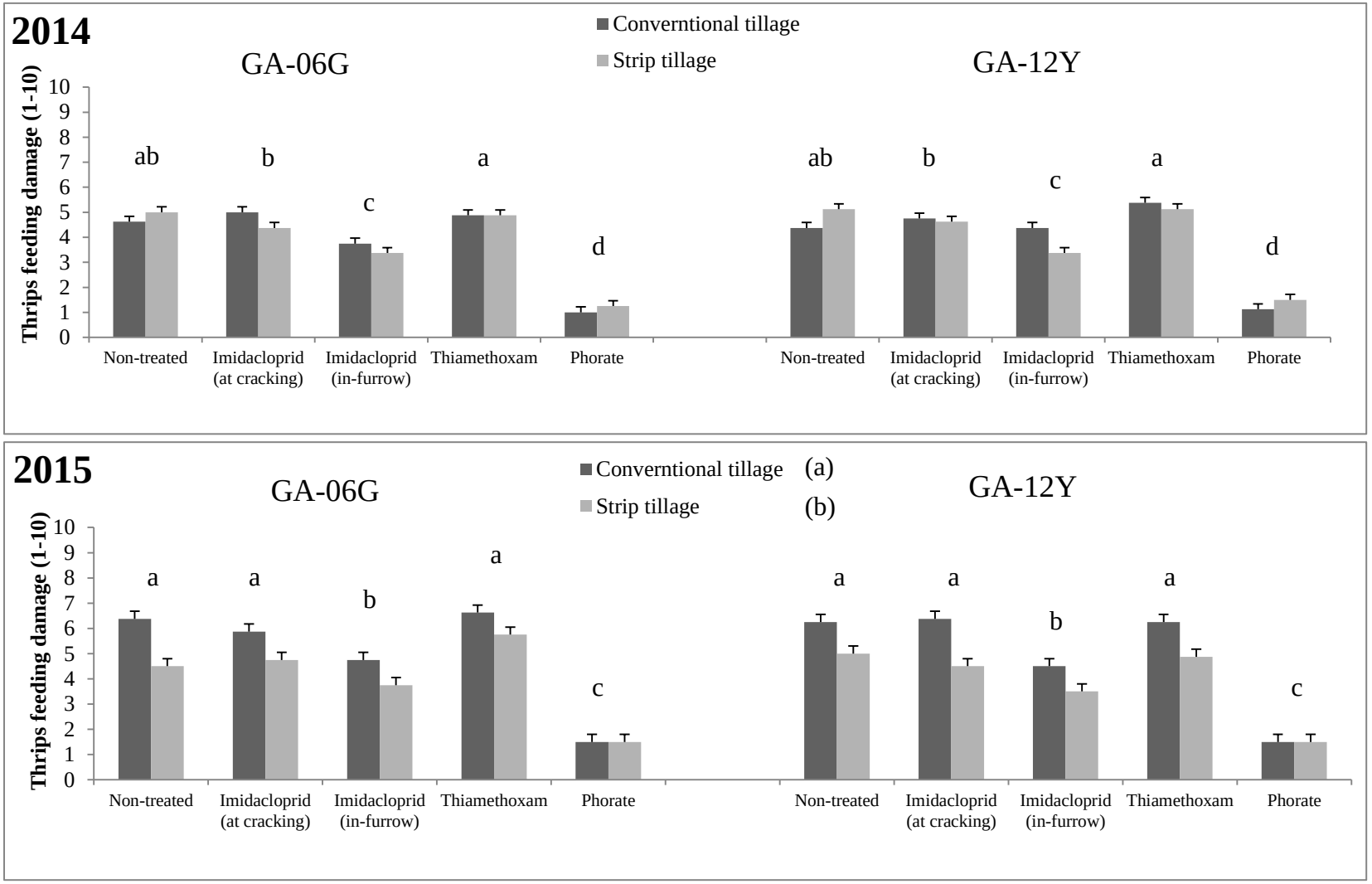

2014
Converntional tillage
Strip tillage
GA-06G
GA-12Y
Thrips feeding damage (1-10)
ab
b
c
a
d
ab
b
c
a
d
Non-treated
Imidacloprid (at cracking)
Imidacloprid (in-furrow)
Thiamethoxam
Phorate
2015
Converntional tillage
Strip tillage
(a)
(b)
GA-06G
GA-12Y
Thrips feeding damage (1-10)
a
a
b
a
c
a
a
b
a
c
Non-treated
Imidacloprid (at cracking)
Imidacloprid (in-furrow)
Thiamethoxam
Phorate

Fig. 3.7.

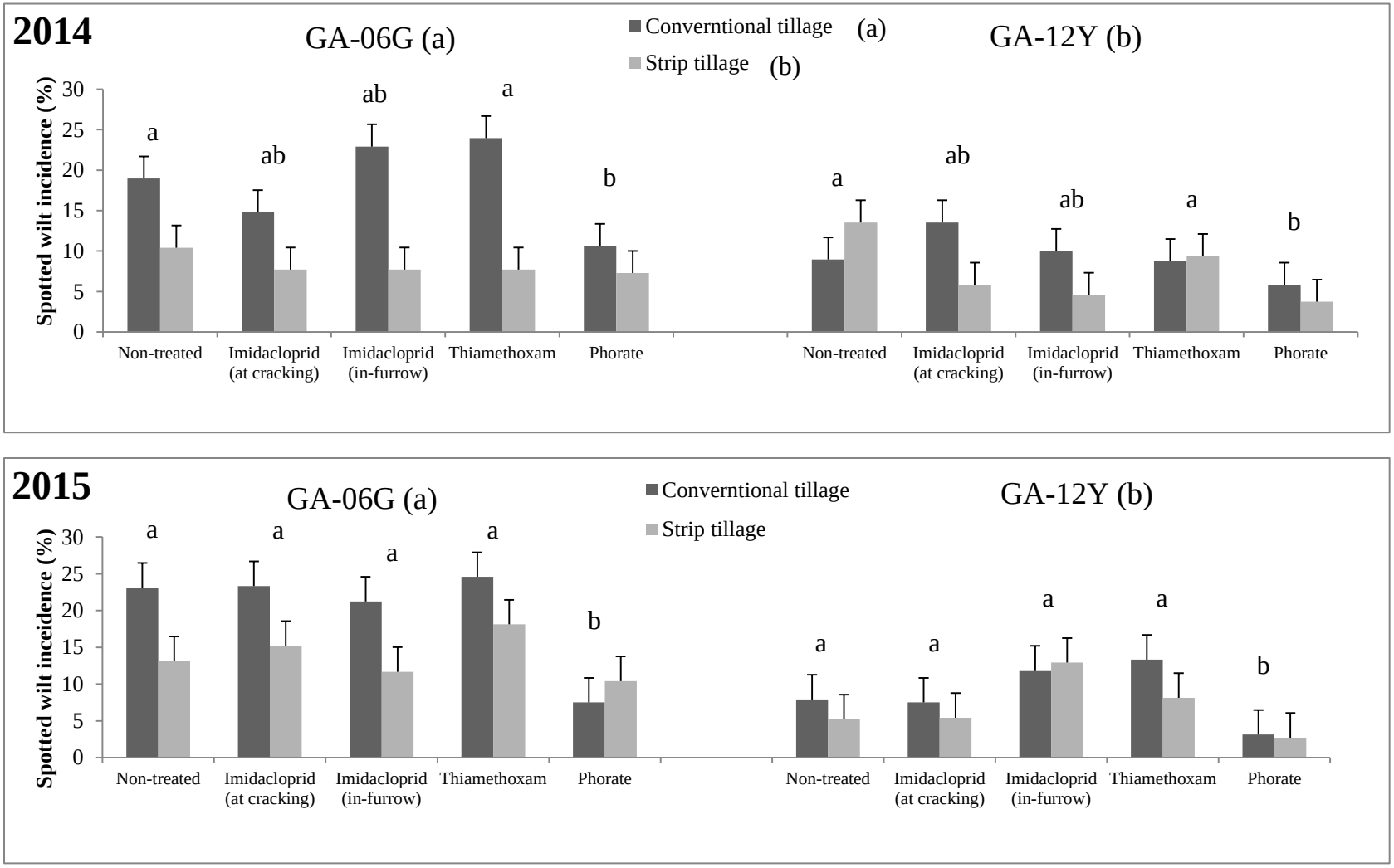

2014
GA-06G (a)
Converntional tillage (a)
Strip tillage (b)
GA-12Y (b)
Spotted wilt incidence (%)
30
25
20
15
10
5
0
a
ab
ab
a
b
a
ab
ab
a
b
Non-treated
Imidacloprid (at cracking)
Imidacloprid (in-furrow)
Thiamethoxam
Phorate
Non-treated
Imidacloprid (at cracking)
Imidacloprid (in-furrow)
Thiamethoxam
Phorate
2015
GA-06G (a)
Converntional tillage
Strip tillage
GA-12Y (b)
Spotted wilt inceidence (%)
30
25
20
15
10
5
0
a
a
a
a
b
a
a
a
a
b
Non-treated
Imidacloprid (at cracking)
Imidacloprid (in-furrow)
Thiamethoxam
Phorate
Non-treated
Imidacloprid (at cracking)
Imidacloprid (in-furrow)
Thiamethoxam
Phorate

Fig. 3.8.

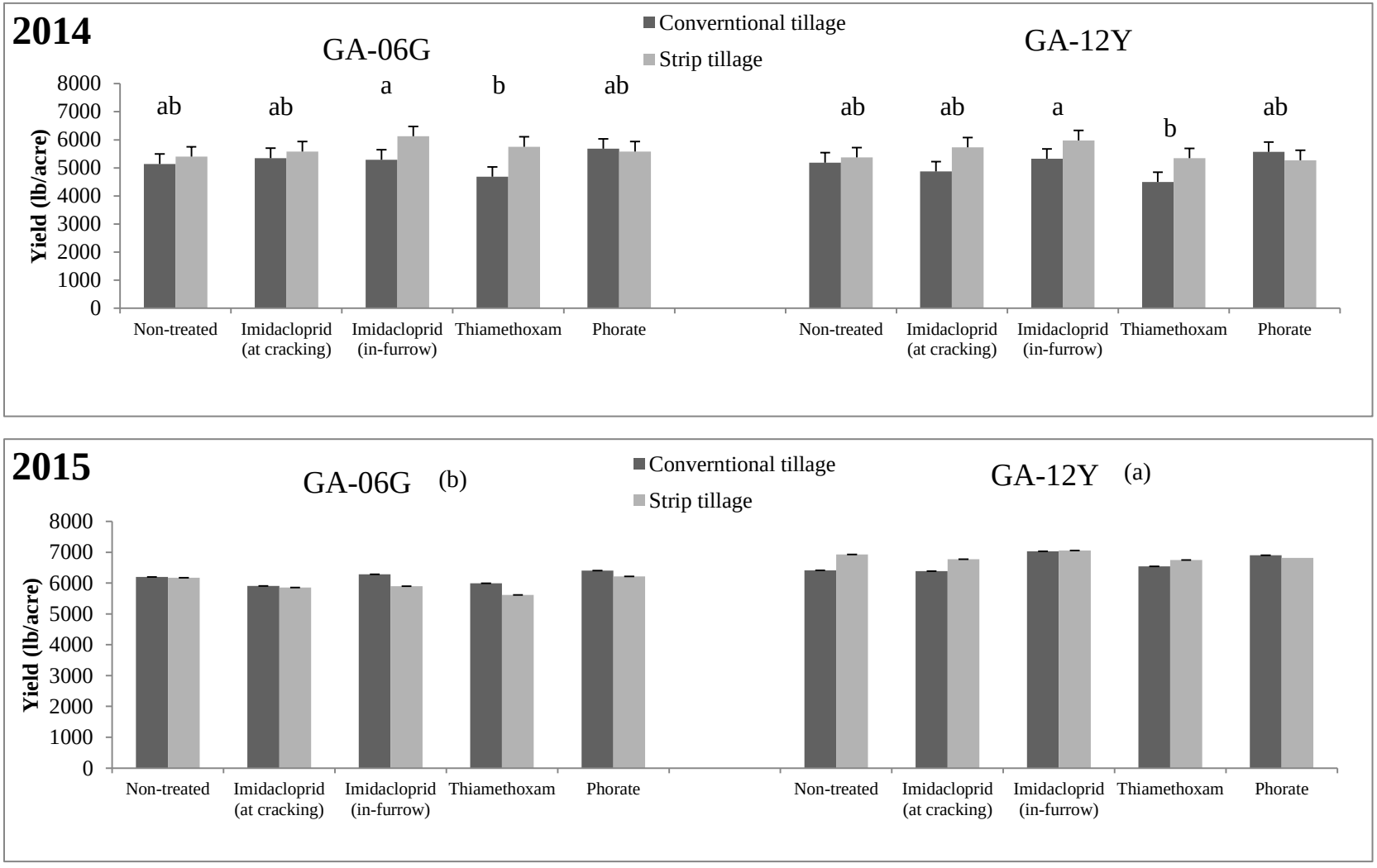

2014
GA-06G
GA-12Y
Converntional tillage
Strip tillage
Yield (lb/acre)
8000
7000
6000
5000
4000
3000
2000
1000
0
ab
ab
a
b
ab
ab
ab
a
b
ab
Non-treated
Imidacloprid (at cracking)
Imidacloprid (in-furrow)
Thiamethoxam
Phorate
2015
GA-06G (b)
GA-12Y (a)
Converntional tillage
Strip tillage
Yield (lb/acre)
Non-treated
Imidacloprid (at cracking)
Imidacloprid (in-furrow)
Thiamethoxam
Phorate

Fig. 3.9.

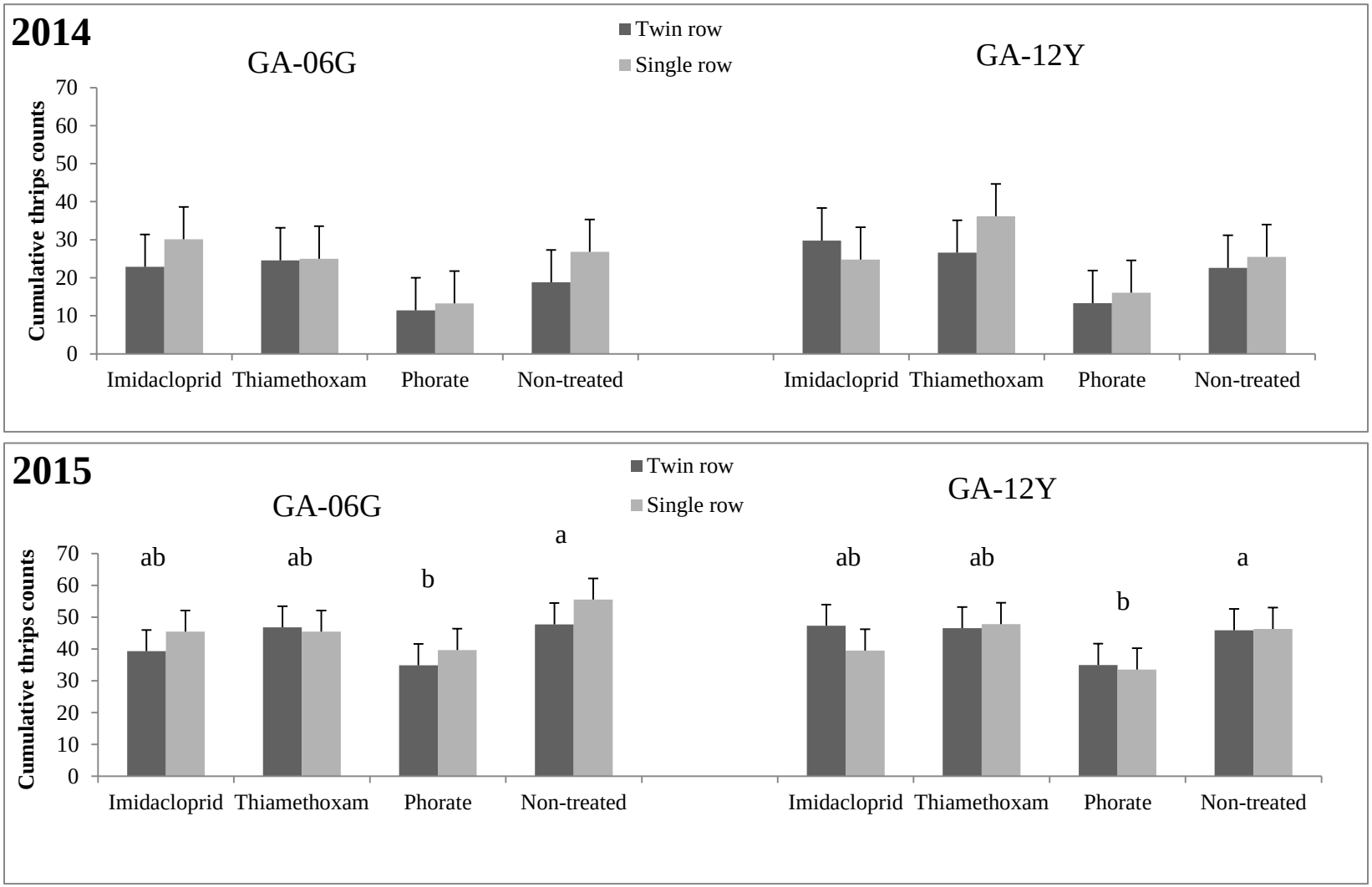

2014
GA-06G
GA-12Y
Twin row
Single row
Cumulative thrips counts
0
10
20
30
40
50
60
70
Imidacloprid
Thiamethoxam
Phorate
Non-treated
2015
GA-06G
GA-12Y
Twin row
Single row
Cumulative thrips counts
ab
ab
b
a
ab
ab
b
a
Imidacloprid
Thiamethoxam
Phorate
Non-treated

Fig. 3.10.

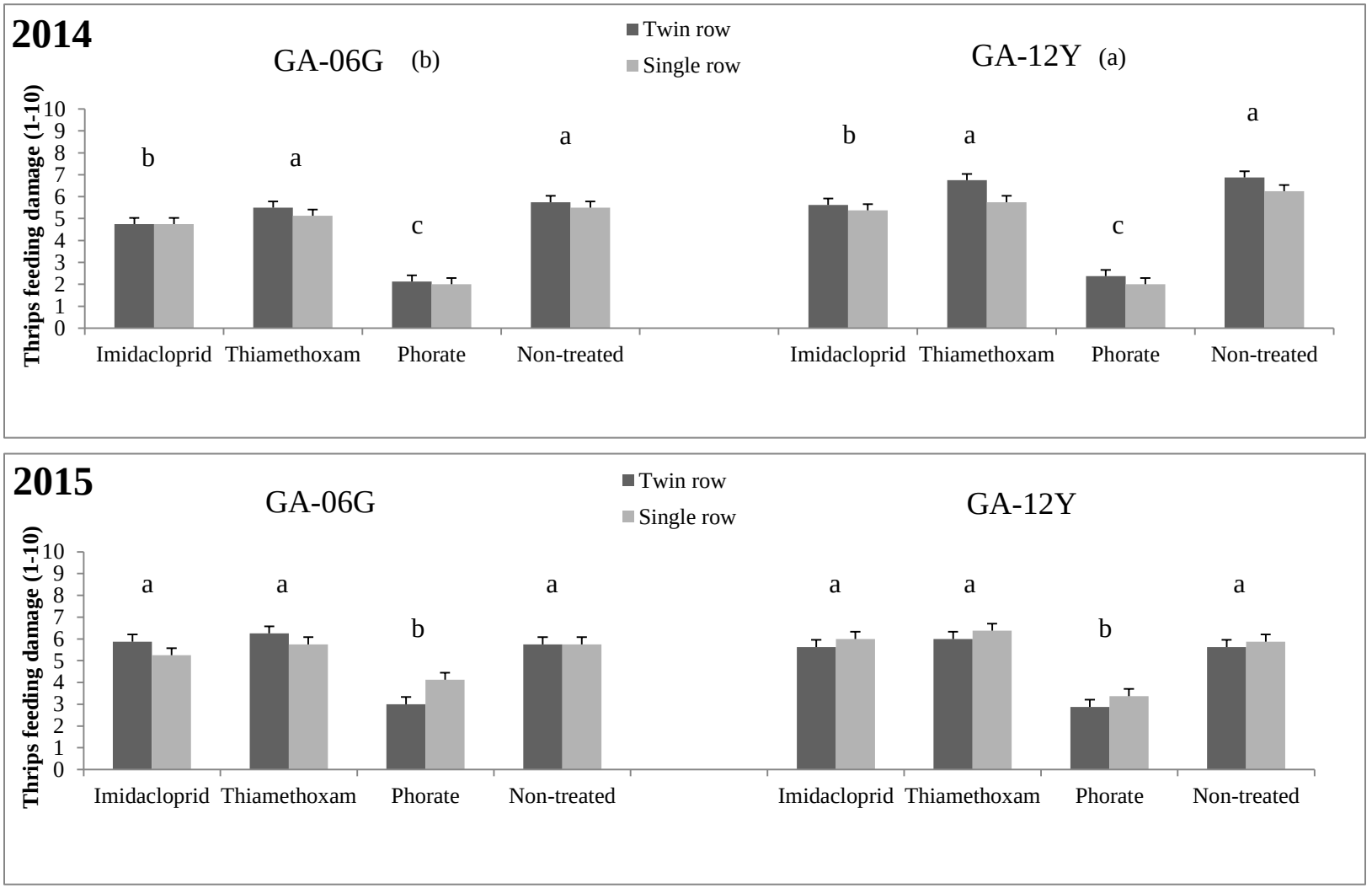

2014
GA-06G (b)
Twin row
Single row
GA-12Y (a)
Thrips feeding damage (1-10)
0 1 2 3 4 5 6 7 8 9 10
b a c a b a c a
Imidacloprid Thiamethoxam Phorate Non-treated Imidacloprid Thiamethoxam Phorate Non-treated
2015
GA-06G
Twin row
Single row
GA-12Y
Thrips feeding damage (1-10)
0 1 2 3 4 5 6 7 8 9 10
a a b a a a b a
Imidacloprid Thiamethoxam Phorate Non-treated Imidacloprid Thiamethoxam Phorate Non-treated

Fig. 3.11.

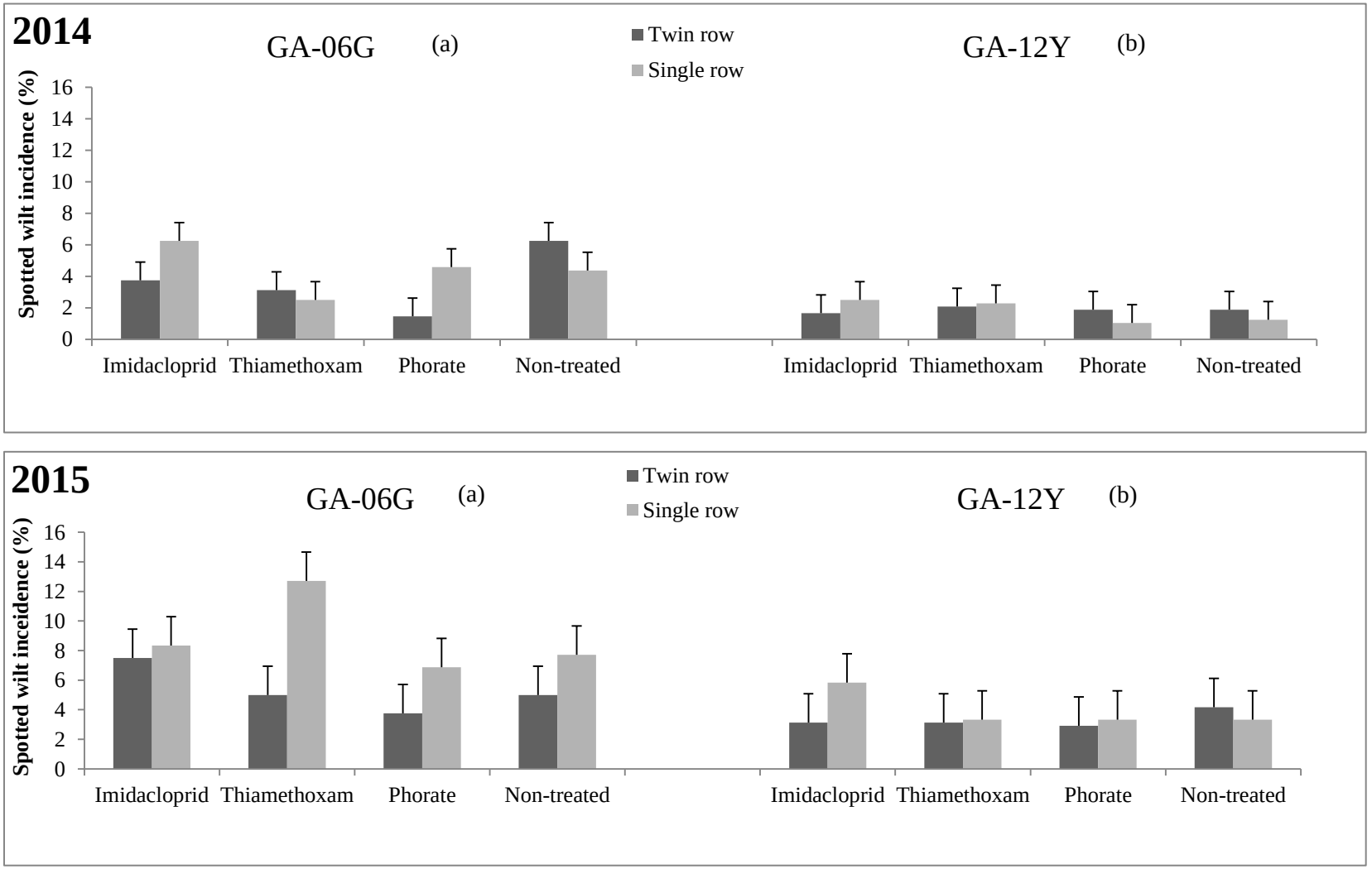

2014
GA-06G
(a)
Twin row
Single row
GA-12Y
(b)
Spotted wilt incidence (%)
16
14
12
10
8
6
4
2
0
Imidacloprid
Thiamethoxam
Phorate
Non-treated
Imidacloprid
Thiamethoxam
Phorate
Non-treated
2015
GA-06G
(a)
Twin row
Single row
GA-12Y
(b)
Spotted wilt inceidence (%)
16
14
12
10
8
6
4
2
0
Imidacloprid
Thiamethoxam
Phorate
Non-treated
Imidacloprid
Thiamethoxam
Phorate
Non-treated

Fig. 3.12.

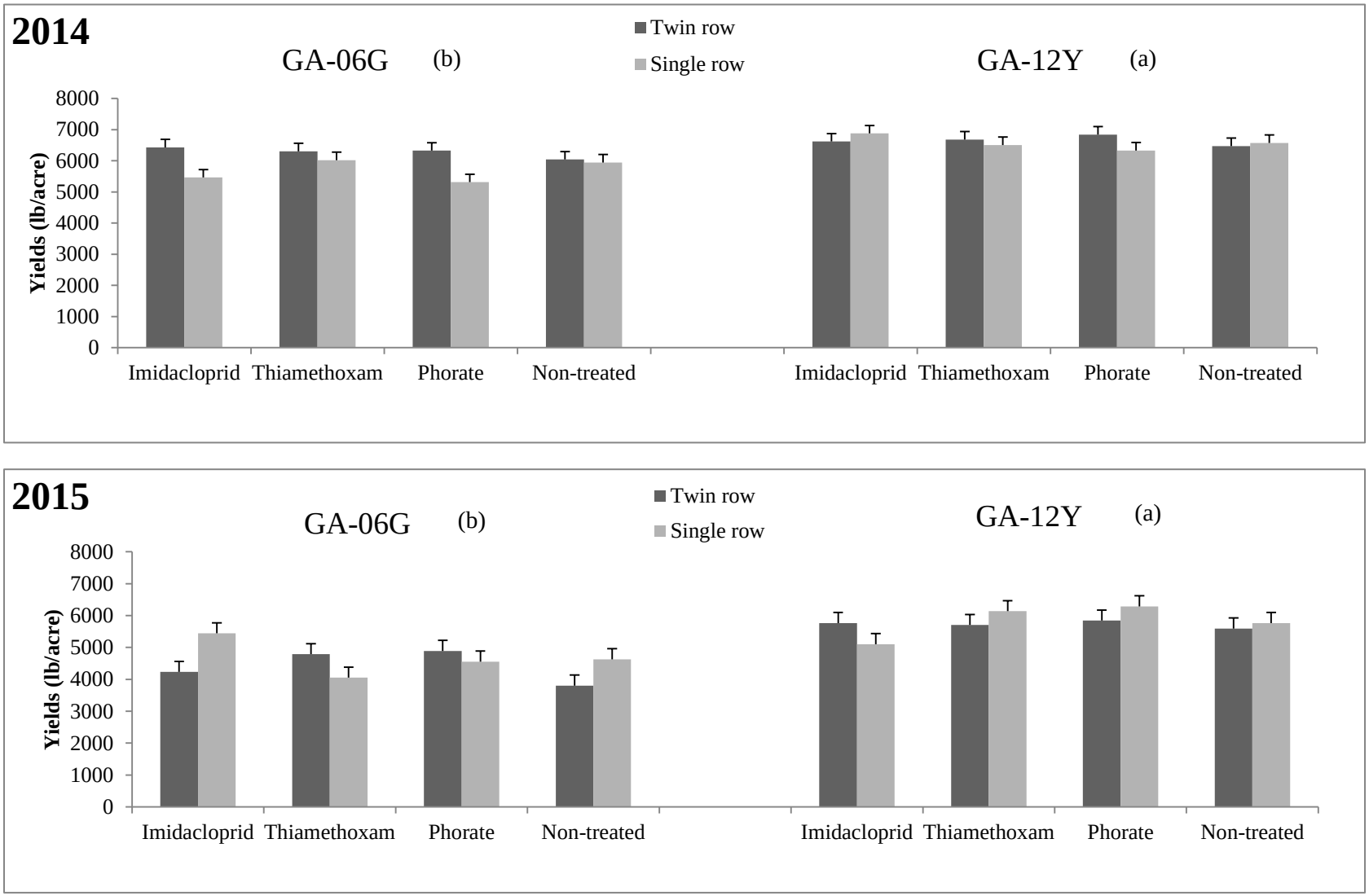
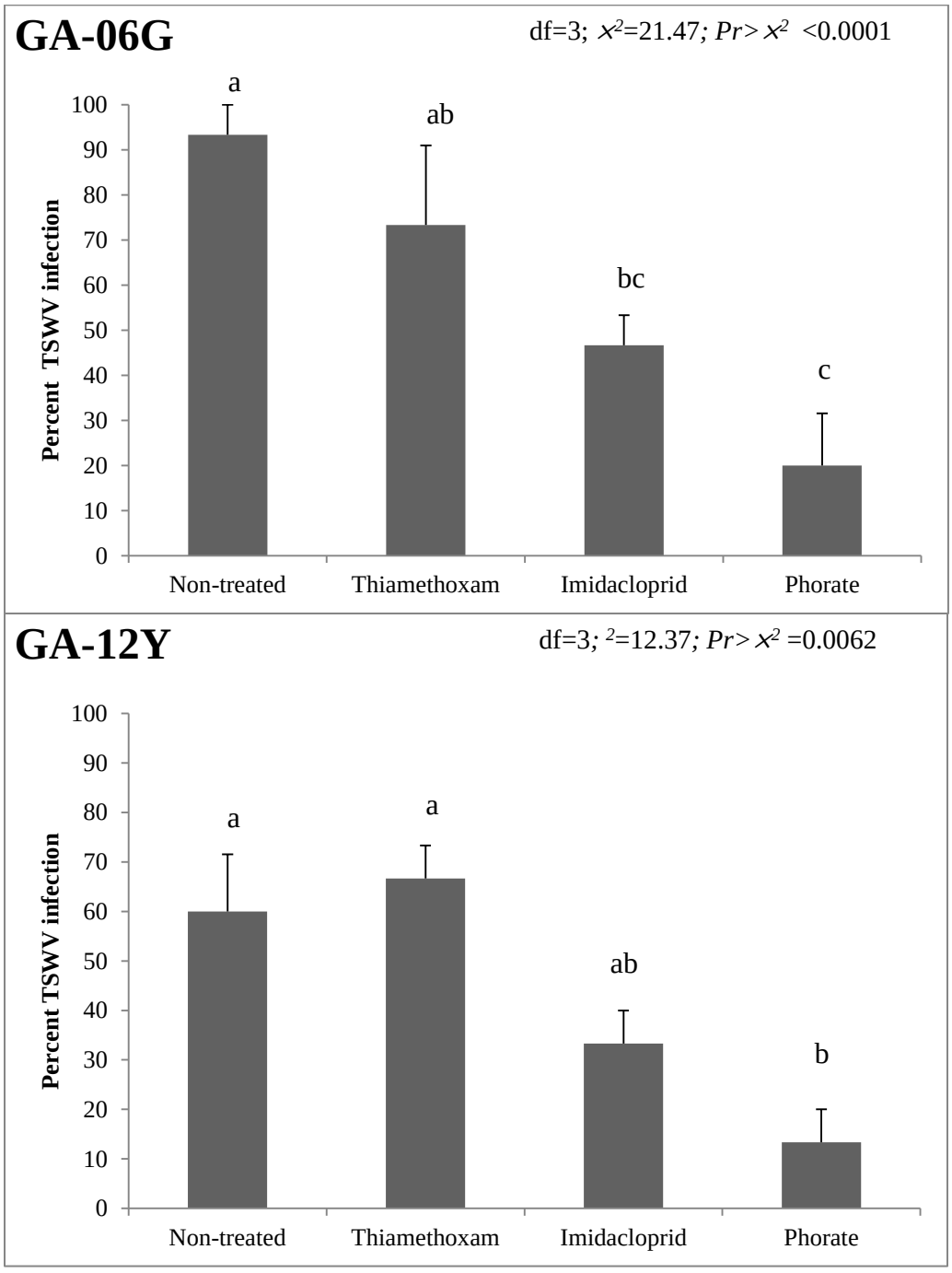

2014
Twin row
Single row
GA-06G (b)
GA-12Y (a)
Yields (lb/acre)
8000
7000
6000
5000
4000
3000
2000
1000
0
Imidacloprid
Thiamethoxam
Phorate
Non-treated
Imidacloprid
Thiamethoxam
Phorate
Non-treated
2015
Twin row
Single row
GA-06G (b)
GA-12Y (a)
Yields (lb/acre)
8000
7000
6000
5000
4000
3000
2000
1000
0
Imidacloprid
Thiamethoxam
Phorate
Non-treated
Imidacloprid
Thiamethoxam
Phorate
Non-treated

Fig. 3.13.


GA-06G
df=3; x^2=21.47; Pr> x^2 <0.0001
Percent TSWV infection
100
90
80
70
60
50
40
30
20
10
0
a
ab
bc
c
Non-treated
Thiamethoxam
Imidacloprid
Phorate
GA-12Y
df=3; ^{2}=12.37; Pr> x^2 =0.0062
Percent TSWV infection
100
90
80
70
60
50
40
30
20
10
0
a
a
ab
b
Non-treated
Thiamethoxam
Imidacloprid
Phorate

Literature cited

Agronews (2010) Bayer CropScience to close a plant as Temik phasing out.

Baldwin, J.A. (2001) A REGIONAL STUDY TO EVALUATE TILLAGE, ROW PATTERNS, IN-FURROW INSECTICIDE, AND PLANTING DATE ON THE YIELD, GRADE, AND TOMATO SPOTTED WILT VIRUS INCIDENCE OF THE GEORGIA GREEN PEANUT CULTIVAR.

Baldwin, J.A., Beasley, J.P., Brown, S.L., Todd, J.W. and Culbreath, A.K. (1998) Yield, grade, and tomato spotted wilt incidence of four peanut cultivars in response to twin vs. single row planting patterns. *Proc. Am. Peanut Res. Ed. Soc.*, **30**, 51 (Abstr.).

Black, M. (1991) Effects of spotted wilt on selected peanut varieties. *Proc. Am. Peanut Res. Ed. Soc. ,* **23**, 52.

Boriss, H. and Kreith, M. (2013) Peanut profile. AgMRC.

Bragard, C., Caciagli, P., Lemaire, O., Lopez-Moya, J.J., Macfarlane, S., Peters, D., Susi, P. and Torrance, L. (2013) Status and prospects of plant virus control through interference with vector transmission. *Annu Rev Phytopathol,* **51**, 177-201.

Branch, W.D. (1996) Registration of 'Georgia Green' peanut. *Crop Science,* **36**, 806-806.

Branch, W.D. (2007) Registration of 'Georgia-06G' peanut. *Journal of Plant Registrations,* **1**, 120-120.

Branch, W.D., Baldwin, J.A. and Culbreath, A.K. (2003) Genotype x Seeding Rate Interaction among TSWV-Resistant, Runner-Type Peanut Cultivars. *PEANUT SCIENCE,* **30**, 108-111.

Branch, W.D. and Brenneman, T.B. (2015) Stem Rot (White Mold) and Tomato Spotted Wilt Resistance among Peanut Genotypes. *Peanut Science,* **42**, 18-22.

Brandenburg, R.L., Naderman, G.C., Wright, S.F., Herbert, D.A., Jr. and Sullivan, G.A. (1998) The impact of tillage practices on thrips injury of peanut in North Carolina and Virginia. *Peanut science,* **25**, 27-31.

Brown, S.L., Culbreath, A.K., Todd, J.W., Gorbet, D.W., Baldwin, J.A. and Beasley, J.P., Jr. (2005) Development of a method of risk assessment to facilitate integrated management of spotted wilt of peanut. *Plant Disease*, **89**, 348-356.

Brown, S.L., Todd, J.W. and Culbreath, A.K. (1996) Effect of selected cultural practices on incidence of tomato spotted wilt virus and populations of thrips vectors in peanuts. *Acta Horticulturae,* 491-498.

Cantonwine, E.G., Culbreath, A.K., Stevenson, K.L., Kemerait, R.C., Jr., Brenneman, T.B., Smith, N.B. and Mullinix, B.G., Jr. (2006) Integrated disease management of leaf spot and spotted wilt of peanut. *Plant Disease,* **90**, 493-500.

Chamberlin, J.R., Todd, J.W., Culbreath, A.K., Johnson, W.C., Iii and Demski, J.W. (1993) Post-harvest management of tobacco thrips (Thysanoptera: Thripidae) overwintering in peanut fields. *Journal of Entomological Science,* **28**, 433-446.

Chamberlin, J.R., Todd, J.W., Farrow, J.M. and Mullinix, B.G., Jr. (1992) Aldicarb residue persistence in leaf terminals of 'Florunner' peanut. *Journal of Economic Entomology,* **85**, 1072-1078.

Culbreath, A.K., Baldwin, J., Pappu, H.R., Shokes, F.M., Todd, J.W., Gorbet, D.W. and Brown, S.L. (2000) Reaction of peanut cultivars to spotted wilt. *Peanut science,* **27**, 35-39.

Culbreath, A.K., Holbrook, C.C., Nischwitz, C., Tilman, B.L. and Gorbet, D.W. (2008) Response of New Field-Resistant Peanut Cultivars to Twin-Row Pattern or In-Furrow Applications of Phorate for Management of Spotted Wilt [electronic resource]. *Plant disease: an*

international journal of applied plant pathology, **92**, 1307-1312.

Culbreath, A.K., Pappu, H.R., Holbrook, C.C., Shokes, F.M., Baldwin, J.A., Todd, J.W., Gorbet, D.W. and Brown, S.L. (1999) Response of early, medium, and late maturing peanut breeding lines to field epidemics of tomato spotted wilt. *Peanut science,* **26**, 100-106.

Culbreath, A.K. and Srinivasan, R. (2011) Epidemiology of spotted wilt disease of peanut caused by Tomato spotted wilt virus in the southeastern U.S. *Virus Research,* **159**, 101-109.

Culbreath, A.K., Todd, J.W., Branch, W.D., Brown, S.L., Demski, J.W. and Beasley, J.P., Jr. (1994) Effect of new peanut cultivar Georgia Browne on epidemics of spotted wilt. *Plant Disease,* **78**, 1185-1189.

Culbreath, A.K., Todd, J.W. and Brown, S.L. (2003) Epidemiology and management of tomato spotted wilt in peanut. *Annual review of phytopathology,* **41**, 53-75.

Culbreath, A.K., Todd, J.W., Demski, J.W. and Chamberlin, J.R. (1992) Disease progress of spotted wilt in peanut cultivars Florunner and Southern Runner. *Phytopathology,* **82**, 766-771.

Culbreath, A.K., Todd, J.W., Gorbet, D.W., Branch, W.D., Holbrook, C.C., Shokes, F.M. and Demski, J.W. (1996) Variation in susceptibility to tomato spotted wilt virus among advanced breeding lines of peanut (Arachis hypogaea). *Acta Horticulturae*, 402-410.

Culbreath, A.K., Todd, J.W., Gorbet, D.W., Shokes, F.M. and Pappu, H.R. (1997) Field Performance of Advanced Runner- and Virginia-Type Peanut Breeding Lines During Epidemics of TSWV. *PEANUT SCIENCE*, **24**, 123-127.

Culbreath, A.K., Tubbs, R.S., Tillman, B.L., Beasley, J.J.P., Branch, W.D., Holbrook, C.C., Smith, A.R. and Smith, N.B. (2013) Effects of seeding rate and cultivar on tomato spotted wilt of peanut. *Crop Protection,* **53**, 118-124.

Drake, W., Jordan, D., Johnson, P., Shew, B., Brandenburg, R. and Corbett, T. (2014) Peanut Response to Planting Date, Tillage, and Cultivar in North Carolin. *AGRONOMY JOURNAL,* **106**, 5.

Flatt, W.P. (2004) Agriculture in Georgia: Overview. University of Georgia, NEW GEORGIA Encyclopedia.

German, T.L., Ullman, D.E. and Moyer, J.W. (1992) Tospoviruses: diagnosis, molecular biology, phylogeny, and vector relationships. *Annual Review of Phytopathology,* **30**, 315-348.

Halliwell, R.S. and Philley, G. (1974) Spotted wilt of peanut in Texas. *Plant Disease Reporter,* **58**, 23-25.

Hallwass, M., Oliveira, A.S., Campos Dianese, E., Lohuis, D., Boiteux, L.S., Inoue-Nagata, A.K., Resende, R.O. and Kormelink, R. (2014) The Tomato spotted wilt virus cell-to-cell movement protein (NSM) triggers a hypersensitive response in Sw-5-containing resistant tomato lines and in Nicotiana benthamiana transformed with the functional Sw-5b resistance gene copy. *Molecular Plant Pathology,* **15**, 871-880.

Herbert, D.A., Malone, S., Aref, S., Brandenburg, R.L., Jordan, D.L., Royals, B.M. and Johnson, P.D. (2007) Role of insecticides in reducing thrips injury to plants and incidence of tomato spotted wilt virus in Virginia market-type peanut. *Journal of Economic Entomology,* **100**, 1241-1247.

Hurt, C.A., Brandenburg, R.L., Jordan, D.L., Kennedy, G.G. and Bailey, J.E. (2005) Management of spotted wilt vectored by Frankliniella fusca (Thysanoptera: Thripidae) in Virginia market-type peanut. *Journal of Economic Entomology,* **98**, 1435-1440.

Hurt, C.A., Brandenburg, R.L., Jordan, D.L., Royals, B.M. and Johnson, P.D. (2006) Interactions of tillage with management practices designed to minimize tomato spotted wilt of peanut

(Arachis hypogaea L.). *Peanut Science.* pp. 83-89. American Peanut Research and Education Society, Stillwater; USA.

Jain, M., Gallo, M., Chengalrayan, K., Shaikh, N.P., Macdonald, G.E. and Davis, J.M. (2015) Phorate-induced host defence responses condition acquired resistance to tomato spotted wilt in cultivated peanut (Arachis hypogaea L.). *Journal of Phytopathology,* **163**, 853-866.

Johnson, W.C., Iii, Brenneman, T.B., Baker, S.H., Johnson, A.W., Sumner, D.R. and Mullinix, B.G., Jr. (2001) Tillage and pest management considerations in a peanut-cotton rotation in the Southeastern Coastal Plain. *Agronomy Journal*, **93**, 570-576.

Jordan, D.L., Place, G., Brandenburg, R.L., Lanier, J.E. and Carley, D.S. (2010) Response of Virginia market type peanut to planting pattern and herbicide program. *Crop Management*, CM-RS.

Knight, I.A., Rains, G.C., Culbreath, A.K. and Toews, M.D. (2015) Conservation tillage and thiamethoxam seed treatments as tools to reduce thrips densities and disease in cotton and peanut. *Crop Protection*, **76**, 92-99.

Lanier, J.E., Jordan, D.L., Spears, J.F., Wells, R., Johnson, P.D., Barnes, J.S., Hurt, C.A., Brandenburg, R.L. and Bailey, J.E. (2004) Peanut response to planting pattern, row spacing, and irrigation. *Agronomy Journal,* **96**, 1066-1072.

Lowry, V.K., Smith, J.W., Jr., Mitchell, F.L. and Crumley, C.R. (1995) Thrips vectors responsible for the secondary spread of tomato spotted wilt virus in south Texas peanut. *Thrips biology and management: proceedings of the 1993 International Conference on Thysanoptera.* eds. B.L. Parker, M. Skinner & T. Lewiss), pp. 167-170. Plenum Publishing Co. Ltd, London; USA.

Lowry, V.K., Smith, J.W. and Mitchell, F.L. (1992) Life-Fertility Tables for Frankliniella fusca (Hinds) and F. occidentals (Pergande) (Thysanoptera: Thripidae) on Peanut. *Annals of the Entomological Society of America*, **85**, 744-754.

Lynch, R.E., Morgan, L.W. and Garner, J.W. (1984) Influence of systemic insecticides on thrips damage and yield of Florunner peanuts in Georgia. *Journal of agricultural entomology,* **1**, 33-42.

Mandal, B., Pappu, H.R. and Culbreath, A.K. (2001) Factors affecting mechanical transmission of tomato spotted wilt virus to peanut (Arachis hypogaea). *Plant Disease,* **85**, 1259-1263.

Marasigan, K.M. (2014) *Alternatives to carbamate and organophosphate insecticides, cultural tactics, and ecological factors that affect Tomato spotted wilt virus epidemics in peanut. [electronic resource]*, 2014.

Marois, J.J. and Wright, D.L. (2003) Effect of tillage system, phorate, and cultivar on tomato spotted wilt of peanut. *Agronomy Journal*, **95**, 386-389.

Minton, N.A., Csinos, A.S., Lynch, R.E. and Brenneman, T.B. (1991) Effects of two cropping and two tillage systems and pesticides on peanut pest management. *Peanut Science,* **18**, 41-46.

Monfort, W.S. (2015) 2015 Peanut update. *Peanut Cultivar Options for 2015* Georgia Peanut Commission.

Moritz, G., Kumm, S. and Mound, L. (2004) Tospovirus transmission depends on thrips ontogeny. *Virus Research*, **100**, 143-149.

Moury, B., Palloix, A., Selassie, K.G. and Marchoux, G. (1997) Hypersensitive resistance to tomato spotted wilt virus in three Capsicum chinense accessions is controlled by a single gene and is overcome by virulent strains. *Euphytica,* **94**, 45-52.

Ngoc Huy, H., Yang, H. and Kang, B. (2013) Identification and inheritance of a new source of resistance against Tomato spotted wilt virus (TSWV) in Capsicum. *Scientia Horticulturae,* **161**, 8-14.

Pan (2014) Phorate - Identification, toxicity, use, water pollution potential, ecological toxicity and regulatory information.

Pappu, H.R., Jones, R.a.C. and Jain, R.K. (2009) Global status of tospovirus epidemics in diverse cropping systems: Successes achieved and challenges ahead. *Virus Research,* **141**, 219-236.

Riley, D.G. (2007) Effect of imidacloprid on settling behavior of Frankliniella occidentalis and Frankliniella fusca (Thysanoptera: Thripidae) on tomato and peanut. *Journal of Entomological Science,* **42**, 74-83.

Riley, D.G., Joseph, S.V., Srinivasan, R. and Diffie, S. (2011) Thrips vectors of Tospoviruses. *Journal of Integrated Pest Management,* **2**, I1-I10.

Sakimura, K. (1962) The Present Status of Thrips-Borne Viruses11Published with approval of the Director, Pineapple Research Institute of Hawaii, as Technical Paper No. 276. *Biological Transmission of Disease Agents*, 33-40.

Shrestha, A., Srinivasan, R., Sundaraj, S., Culbreath, A.K. and Riley, D.G. (2013) Second generation peanut genotypes resistant to thrips-transmitted tomato spotted wilt virus exhibit tolerance rather than true resistance and differentially affect thrips fitness. *Journal of Economic Entomology,* **106**, 587-596.

Singh, A.P., Surendra, S., Prabhat, B. and Khushbu, Y. (2010) Toxic effect of phorate on the serum biochemical parameters of snake headed fish Channa punctatus (Bloch). *Advances in Bio Research,* **1**, 177-181.

Tillman, B.L., Gorbet, D.W., Culbreath, A.K. and Todd, J.W. (2006) Response of peanut cultivars to seeding density and row patterns. *Crop Management*, 1-5.

Todd, J.W., Culbreath, A.K. and Brown, S.L. (1996) Dynamics of vector populations and progress of spotted wilt disease relative to insecticide use in peanuts. *Acta Horticulturae*, 483-490.

Todd, J.W., Culbreath, A.K., Chamberlin, J.R., Beshear, R.J. and Mullinix, B.G. (1995) Colonization and population dynamics of thrips in peanuts in the southern United States. *Thrips biology and management: proceedings of the 1993 International Conference on Thysanoptera.* eds. B.L. Parker, M. Skinner & T. Lewiss), pp. 453-460. Plenum Publishing Co. Ltd, London; USA.

Todd, J.W., Culbreath, A.K., Rogers, D. and Demski, J.W. (1994) Contraindications of insecticide use relative to vector control and spotted wilt disease progress in peanut. *Proc. Am. Peanut Res. Ed. Soc.*, **26**, 42 (Abstr.).

Todd, J.W., Jr, C., Culbreath, A.K. and Demski, J.W. (1993) Timing and duration of vector management in relation to spotted wilt disease incidence in peanut. *Proc. Am. Peanut Res. Ed. Soc*, **25**, 87 (Abstr.).

Triplehorn, C.A., Borror, D.J., Delong, D.M. and Johnson, N.F. (2005) *Borror and DeLong's introduction to the study of insects*, Belmont, CA : Thompson Brooks/Cole, c2005. 7th ed.

Tubbs, R.S., Beasley, J.P., Jr., Culbreath, A.K., Kemerait, R.C., Smith, N.B. and Smith, A.R. (2011) Row pattern and seeding rate effects on agronomic, disease, and economic factors in large-seeded runner peanut. *Peanut Science*, **38**, 93-100.

Usda (2015) Crop Production 2014 Summary. USDA.

Wells, M.L., Culbreath, A.K., Csinos, A.S. and Todd, J.W. (2002) Effects of a plant activator and insecticides on tobacco thrips (Thysanoptera: Thripidae) feeding and survival. *Journal of Agricultural and Urban Entomology,* **19**, 117-120.

Wiatrak, P.J., Wright, D.L., Marois, J.J., Grzes, S., Koziara, W. and Pudelko, J.A. (2000) Conservation tillage and thimet effects on tomato spotted wilt virus in three peanut cultivars. *Proceedings - Soil and Crop Science Society of Florida,* **59**, 109-111.

Wijkamp, I., Lent, J.V., Kormelink, R., Goldbach, R. and Peters, D. (1993) Multiplication of tomato spotted wilt virus in its insect vector, Frankliniella occidentalis. *Journal of General Virology,* **74**, 341-349.

Williams-Woodward, J. (2012) 2010 Georgia Plant Disease Loss Estimates. Univ. Georgia Coop. Ext. , Univ. Georgia Coop. Ext. .

Young, S., Kinzer, R.E., Walton, R.R. and Matlock, R.S. (1972) Field screening for tobacco thrips resistance in peanuts. *Journal of Economic Entomology,* **65**, 828-832.

CHAPTER 4

EVALUATION OF SELECTED INSECTICIDES' RESIDUAL STATUS IN PEANUT AND EFFICACY ON THRIPS CONTROL OVER TIME[1]

[1] Lai, P., R. Srinivasan, M. Abney, and A. Culbreath. 2015. To be submitted.

Abstract

Spotted wilt disease caused by *Tomato spotted wilt virus* (TSWV) is a major concern in peanut production, and tobacco thrips (*Frankliniella fusca*) is the main vector species that transmits TSWV in the southeastern United States. Insecticide application is crucial to manage thrips populations and potentially suppress the incidence of spotted wilt disease in peanut in some cases. Aldicarb, a commonly used insecticide, will be phased out in the near future due to its broad-spectrum toxicity. Phorate, though effective against thrips, has similar toxicity concerns. Alternative insecticides with less non-target effects are available for use in peanut, such as neonicotinoid insecticides. The residual toxicity in plants and insecticide resistance development are some main concerns associated with the alternative insecticides. In the current study, temporal residual toxicity of imidacloprid, thiamethoxam, and phorate in peanut plants and corresponding thrips mortality was assessed. Membrane-based feeding assays were conducted to investigate insecticide resistance development in field populations in comparison with a lab population. Results indicated that insecticide residues detected in leaf tissues declined significantly from 10 days after application at planting. The effectiveness of imidacloprid and phorate to cause thrips mortality largely declined 10 days post treatment. Thiamethoxam seed treatment did not affect thrips mortality or the degree of feeding damage. The median lethal concentration (LC50) values for lab colony thrips were established as a baseline to further assess the resistance status of thrips from field populations. Thrips populations evaluated in this study had similar level of susceptibility to selected insecticides as that of the lab population.

Key words: Tobacco thrips, insecticide effectiveness, residual toxicity, resistance monitoring

Introduction

Thrips (Thysanoptera) are important pests of peanut (Culbreath et al., 2003). Primarily, thrips can injure host plants by direct feeding on plant foliage particularly early in the season. More importantly, thrips can serve as vectors of *tospoviruses* (Pappu et al., 2009, Riley et al., 2011, Whitfield et al., 2005). *Tomato spotted wilt virus* (TSWV) belonging to the genus *Tospovirus* in the family *Bunyaviridae* causes spotted wilt disease in peanut (Culbreath et al., 2003, Pappu et al., 2009) in the southeastern United States. Spotted wilt disease became a severe problem in peanut production during the 1990s (Culbreath & Srinivasan, 2011, Culbreath et al., 2003). Estimated losses to spotted wilt in 1997 were 12% of the entire crop in Georgia alone, representing an approximate value of $40 million (Bertrand, 1998).

In the Southeast, the tobacco thrips, *Frankliniella fusca* Hinds, and the western flower thrips, *Frankliniella occidentalis* Pergande, are two competent vectors of TSWV that commonly occur on peanut. Peanut appears to be a better host of *F. fusca*, as their reproduction and survival rates were higher than *F. occidentalis* (Lowry et al., 1992). *F. fusca* represents more than 80% of the adult thrips population found in peanut fields (Mitchell and Smith 1991, Marasigan 2014). Because of its peanut colonizing abilities early in the season when the plants are very susceptible, *F. fusca* is considered the predominant vector of TSWV in peanut (Culbreath et al., 2003, Lowry et al., 1995). In general, there is no single spotted wilt management tactic. Hence, a number of management options such as TSWV-resistant cultivars, insecticides, and cultural tactics are routinely used (Brown et al., 2005, Culbreath et al., 2003).

Insecticides are commonly used to manage thrips vectors (Todd et al., 1996). Aldicarb, a systemic carbamate insecticide, has been the standard in-furrow insecticide used for thrips control in southeastern United States until the safety concerns have arisen in recent years

(Chamberlin et al., 1992, Culbreath et al., 2003). Studies showed that aldicarb provided excellent control in reducing first generation larvae, but did not reduce TSWV incidence (Chamberlin et al., 1992, Lynch et al., 1984). Phorate, another systemic organophosphate insecticide, is widely used as an in-furrow treatment in peanut. Phorate applications have resulted in suppression of thrips populations as well as in the reduction of spotted wilt incidence (Todd et al., 1996, Wiatrak et al., 2000). However, it is speculated that TSWV reduction may not be directly related to thrips suppression, as phorate applications have been known to trigger defense responses that could potentially interfere with virus-host interactions (Culbreath et al., 2003, Jain et al., 2015). Insecticide applications, despite their effects on thrips, have failed to prevent inoculation of TSWV by viruliferous adult thrips (Chamberlin et al., 1993), as viruliferous thrips can inoculate plants with TSWV in as little as 5 minutes of feeding (Wijkamp, 1995).

Besides being the vectors of TSWV in peanut, thrips can also severely infest peanut fields early in the planting season. Thrips damage on peanut foliage mainly caused by larvae feeding has been a serious seedling problem that would affect yields and maturity (Todd et al., 1993, Lynch et al., 1984, Young et al., 1972). Therefore, despite their inability to reduce spotted wilt incidence in most of the cases, insecticides continue to be used as a part of the integrated management program. The commonly used insecticides aldicarb and phorate are broad-spectrum insecticides and remain an environmental concern (AgroNews, 2010, Singh et al., 2010, Williams, 1997). Aldicarb will be completely phased out in 2018 (AgroNews, 2010). Phorate, on the other hand, is still being extensively used. Several alternative insecticides have been labeled for use in peanut. Neonicotinoid insecticides with relatively less toxicity to non-target organisms than aldicarb and phorate are available for use in peanut production. For example, thiamethoxam seed treatment and in furrow application of imidacloprid are options for peanut growers

(Entomology, 2015). From the practical standpoint, granular products are harder to handle, and this provides another good reason to seek alternative insecticides (Hollis, 2014). A rough estimate indicates that ~40% of peanut acreage planted in North Florida, Georgia, and Alabama was treated with a neonicotinoid insecticide (including thiamethoxam and imidacloprid) in 2015 (M. Abney, personal communication). Preliminary studies indicate that some of the neonicotinoids, particularly thiamethoxam, were not as effective as in suppressing thrips damage as phorate and imidacloprid.

A number of hypotheses have been developedsp to explain the ineffectiveness of certain neonicotinoids against thrips on peanut. One of the concerns is residual toxicity. In the past, field studies indicated that the effectiveness of aldicarb applied while planting to kill adult thrips has lasted for only 1-2 weeks after plants emerged (Lynch et al., 1984). The residual toxicity of neonicotinoids or phorate on peanut plants over time is not known. Another cause for concern, particularly with neonicotinoids, is the development of insecticide resistance. Neonicotinoids are the most sold insecticides of any class in the world (Jeschke et al., 2011). They are also used widely in row crops and in vegetable crops in the Southeastern United States (Groves et al., 2001, Jeschke et al., 2011, Knight et al., 2015). *F. fusca* resistance to thiamethoxam has been reported in cotton in Georgia and elsewhere in the Southeastern United States (Johnson, 2014). Peanut and cotton are typically planted in close proximity in farmscapes in the southeastern United States. Currently, there is no information on the development of insecticide resistance in thrips to neonicotinoids in peanut.

In this manuscript, we evaluated three insecticides that are commonly used in peanut in the Southeast, namely phorate, imidacloprid, and thiamethoxam, for thrips management. Residue levels of each insecticide in peanut foliage were evaluated at different time intervals post

planting. Leaf tissues were subjected to standard pesticide analysis using the Association of Analytical Communities (AOAC) protocol, which includes QuEChERS extraction followed by Gas Chromatography-Mass Spectrometry (GC-MS) and Liquid Chromatography-Mass Spectrometry (LC-MS) for composition analysis. Mortality assays were further conducted to assess the impact of residual toxicity on *F. fusca*. In addition, to evaluate resistance development in thrips against the three insecticides, membrane-based feeding bioassays were conducted to measure the median lethal concentration (LD50) for laboratory and field populations.

Materials and Methods

Peanut Plants. Peanut cultivar Georgia Green (Branch, 1996) was used for thrips colony maintenance. Fungicide (Dynasty PD)-treated seeds were planted in 4-inch diameter plastic pots (Hummert International, St. Louis, MO) with commercial potting mix, Sunshine mix (LT5 Sunshine® mix, Sun Gro® Horticulture Industries, Bellevue, WA). Peanut plants were maintained in thrips-proof cages (47.5 cm^3) (Megaview Science Co., Taichung, Taiwan) in a greenhouse at 25- 30°C and 80- 90% relative humidity (RH) with a 14:10 (L:D) h photoperiod. At least one-week old peanut leaflets were used for thrips rearing. Georgia-06G (Branch, 2007) was used for residual toxicity bioassays. Georgia-06G is a runner-type peanut cultivar with moderate level of field resistance to TSWV, and it is the predominant cultivar planted in the Southeast since 2010.

Insecticides. Three insecticides were selected and evaluated in this study. Phorate, a systemic organophosphate insecticide, is one of the most commonly used insecticides in peanut for thrips and spotted wilt management (Culbreath et al., 2003). Imidacloprid and thiamethoxam belong to systemic neonicotinoid class. All the three insecticides are currently labeled for use in peanut.

The insecticides, classifications, their mode of application and applied rates are listed in Table 4.1.

Maintenance of Non-Viruliferous *F. fusca*. Non-viruliferous thrips from a lab colony of *F. fusca* established in 2009 were used for all experiments in this study. Thrips were originally collected from peanut blooms in Tifton, GA. Leaflets of non-infected Georgia Green peanut plants maintained in greenhouse were used for thrips rearing. Thrips were reared in small petri dishes (60mm x 15mm) (Becton, Dickinson and Company, Falcon™ Labware, Franklin Lakes, NJ) on peanut leaflets that were placed on a moistened round cotton pad (Swippers Supreme cotton round, Cleveland, Ohio). Ten female thrips were released and allowed to lay eggs on fresh peanut leaflets in each cage (a petri dish). Adult thrips were removed from the dishes after two to three days. Afterwards, fresh leaflets and water were added every two to three days. Only adult female thrips up to 2 days old were used for all experiments. The colony was maintained in a growth chamber (Percival Scientific, Inc., Perry, IA) at 29°C with a photoperiod of 14:10 (L:D) h.

Effect of insecticides on adult thrips mortality and feeding damage over time (residual effect bioassays). Phorate, imidacloprid, and thiamethoxam were used in this experiment. Their modes of application and application rates are listed in Table 2.1. Non-treated control check was also included. Peanut cultivar Georgia-06G was planted in the field with respective treatments. Thiamethoxam was directly coated on the seeds; imidacloprid and phorate were applied at planting. Mortality bioassays were conducted using Munger cages (11 x 9 x 2 cm^3) (Munger, 1942) and peanut leaflets from field plots. Ten Muger cages were set up for each treatment, and the experiment was repeated twice (N=20 for each treatment). The first planting was at Aquaculture Research Station in Tifton, GA on June 5th 2015, and peanut leaflets from there

were used for the first experiment. Each insecticide treatment was assigned to one plot with two single rows. The plot was 30 ft (9.14m) long and 6 ft (1.83m) wide. The second planting was at the Belflower Farm, Coastal Plain Experimental Station in Tifton, GA on July 26th 2015, and the peanut leaflets from there were used for the repeated (second) experiment. A total of three plots were assigned to each insecticide in this replicate, and plots of respective treatments were randomly arranged. Each plot was 30 ft (9.14m) long and two rows spaced 6 ft (1.83m) apart. Leaflets were collected from the field plots at 10 days, 17days, 24days, and 31days after planting and treatment application. Younger leaflets on top with no visible thrips feeding injuries were collected for the laboratory experiments. Ten non-viruliferous adult female thrips were released in each Munger cage by a paintbrush (10/0 The fine touch ® Round, Oklahoma City, OK) with two peanut leaflets collected from the field plots treated with each insecticide. Thrips mortality and feeding damage were recorded at 48 and 96 hours post release. The feeding damage was rated by using an arbitrary scale from 0 to 5 with 0 represents no visible feeding scar and 5 represents 100% leaf area coverage by feeding scars.

Statistical analyses were conducted using the GLIMMIX procedure in SAS (SAS Enterprise 4.2, SAS Institute, Cary, NC) to determine the differences between treatments in thrips mortality and feeding damage. Data were pooled from all experiments using experiment as blocking variable, and were subjected to generalized linear mixed models. Treatments were considered as fixed effects, while replications were considered as random effects. Least square means at *P=0.05* were used to compare the statistical significance of differences between treatments. Tukey-Kramer Grouping was used as an adjustment for multiple comparisons at *P=0.05*.

Effect of insecticides on larval thrips mortality and feeding damage over time (residual effect bioassays). Georgia-06G was planted at the Horticultural Hill, Coastal Plain

Experimental Station in Tifton, GA on August 10th 2015. The selected insecticides were applied at planting as previously described, and each insecticide randomly assigned to one plot with two single rows. The plot was 30 ft (9.14m) long and two rows spaced 6 ft (1.83m) apart. The effectiveness of those insecticides on thrips larval mortality and feeding damage was evaluated by the laboratory bioassay as described above. Five Munger cages were set up for each insecticide treatment, and leaflets were collected at 9 days, 16 days, 23 days, and 30 days after planting. Ten non-viruliferous thrips larvae (1st instars to early 2nd instars) were transferred into a Munger cage with leaflets from treated plants in the field by a paintbrush under a stereomicroscope (Leica MZ9.5, Leica Microsystems Inc., Buffalo Grove, IL). Thrips larvae mortality and feeding damage were recorded at 48 and 96 hours post release. Thrips mortality and feeding injury rating were assessed as discussed above.

Data were pooled from all experiments using experiment as blocking variable and subjected to generalized linear mixed models for statistical analyses. Analyses were conducted using the GLIMMIX procedure in SAS (SAS Enterprise 4.2, SAS Institute, Cary, NC) described above

Residue analyses of active ingredients from peanut leaf tissues. Foliage from Georgia-06G treated with selected insecticides at the Belflower farm was used for residue analyses. The average amount of active ingredients for each treatment was estimated from three independent samples. Fresh leaf tissue was collected weekly from plots at dates corresponding to the bioassay described above (10days, 24 days, and 31 days), except for the second week (17 days). Leaf tissue from each plot served as a sample, and a total of three samples were collected from respective plots for each insecticide. Leaf tissue samples were delivered to Pacific Agricultural Laboratory (Portland, OR) for insecticide active ingredient residue analyses using the Association of Analytical Communities (AOAC) official method for comprehensive pesticide

profile (QuEChERS extraction; GC-MS/MS and LC-MS/MS Analysis). QuEChERS was developed using an extraction method for pesticides in fruits and vegetables, coupled with a cleanup method that removes sugars, lipids, organic acids, sterols, proteins, pigments, and excess water (http://www.restek.com/pdfs/805-01-002.pdf). Test samples were then subjected to Gas Chromatography Mass Spectrometry (GC-MS) and Liquid chromatography–mass spectrometry (LC-MS) to identify different substances and the amount. Results were pooled from all samples, and the average amount of active ingredients at each time point was calculated for all insecticide treatments.

Evaluation of susceptibility of *F. fusca* to three insecticides by direct feeding assays. The toxicity of three selected insecticides to thrips were evaluated. Direct feeding assays were conducted using 1.5ml microcentrifuge tubes (Fisher Scientific, Pittsburgh, PA). Stock sucrose solution (3% w/v) with green food dye (McCormick & Company, Inc., Sparks, MD) was used as the control diet. Admire® Pro (liquid form imidacloprid), Cruiser® 5FS (liquid form thiamethoxam), and Thimet® technical insecticide (liquid form; >90% purity phorate) were mixed with stock sucrose solution. Treatment solutions of varying doses were obtained by serially diluting the stock solution with a dilution factor of 10 for each insecticide. Selected insecticides and their dilution rates are listed in Table 4.2. Five (or six) concentrations of each insecticide were evaluated independently. Five tubes were set up for each treatment in an experiment, and experiment was repeated four times (N=20 for each concentration treatment in every insecticide). Ten non-viruliferous thrips were collected in a 1.5ml microcentrifuge tube by a paintbrush (10/0 The fine touch® Round, Oklahoma City, OK). The lid of the microcentrifuge tube was filled up 150µl of treatment solution by a pipette (PIPETMAN P1000, Gilson, Inc., Middleton, WI). After which, the lid was sealed by a stretched paraffin film (1 cm^2) (Parafilm

R℗, Bemis Company, Inc., Oshkosh, WI), and the lid was used to cover the tube with ten thrips inside. Microcentrifuge tubes were maintained in a growth chamber (Percival Scientific, Inc., Perry, IA) at 29°C with a photoperiod of 14:10 (L:D) h for 48 hours, and the mortality in each tube was recorded. Mortality was calculated by the dividing the number of dead thrips divided by the total number of thrips.

Statistical analyses were conducted to determine the median lethal concentration (LC50) value of all the insecticides tested in this study. Data were pooled from all experiments using experiment as the blocking variable. Data were subjected to PROBIT procedure in SAS (SAS Enterprise 4.2, SAS Institute, Cary, NC), which calculated the maximum likelihood estimates of regression parameters and the natural response rate for quantal response data.

Evaluation of insecticide susceptibility in field populations of *F. fusca* by direct feeding assays. The susceptibility of *F.fusca* field populations to three selected insecticides were evaluated by direct feeding assays as described above. Insecticides and their application rates were the same as previously described and are listed in Table 2.2. Adult thrips were collected from peanut blooms in plots without any insecticide treatment in the peanut fields (except for grower's farm), and only *F. fusca* female adults were used in this experiment. Peanut blooms were collected from three experiment station farms and a grower's farm in South Georgia. Peanut blooms were collected from the Attapulgus Research and Education Station in Attapulgus, GA on June 2nd; at the Bowen Farm, Coastal Plain Experimental Station in Tifton, GA on June 8th, at the Belflower Farm, Coastal Plain Experimental Station in Tifton, GA on June 17th; and a grower's farm in Berrien County on June 24th. Ten 1.5ml microcentrifuge tubes were set up for each concentration treatment for every insecticide, except for thrips collected from Berrien County. Only 30 thrips (in 3 microcentrifuge tubes) for each concentration and each

insecticide were used from Berrien County. Microcentrifuge tubes were maintained in a growth chamber (Percival Scientific, Inc., Perry, IA) at 29°C with a photoperiod of 14:10 (L:D) h for 48 hours, and the mortality percentage in each tube was recorded.

Statistical analyses were conducted to determine the median lethal concentration (LC50) value of all the insecticides for thrips from different locations. Data were subjected to PROBIT procedure in SAS as described above. Resistance ratios were used to compare LC50 values between field populations and the lab population. LC50 values obtained from lab-reared insects were used to establish the baseline.

Results

Effect of insecticides on adult thrips mortality and feeding damage over time: At 10 days post-treatment, adult thrips mortality varied with insecticides treatments at 48-hour post release (hpr) ($df=3, 66$; $F=13.65$; $P<0.0001$) and 96 hpr ($df=3, 66$; $F=19.11$; $P<0.0001$). Mortality of adult thrips feeding on leaflets from phorate and imidacloprid treated plots was significantly higher than on leaflets treated with thiamethoxam and non-treated check at 48 hpr and 96 hpr (Fig. 4.1). Adult thrips mortality did not vary with repeats of the experiment at either 48 hpr ($df=1, 66$; $F=0.88$; $P=0.3525$) or 96 hpr ($df=1, 66$; $F=1.04$; $P=0.3120$).

At 17 days post-treatment, adult thrips mortality did not vary with experimental treatments at either 48 hpr ($df=3, 66$; $F=1.75$; $P=0.1661$) or 96 hpr ($df=3, 66$; $F=1.41$; $P=0.2470$). Mortality was not affected by insecticide treatments (Fig. 4.1). Also, mortality percentages did not vary with experimental repeats at 48 hpr ($df=1, 66$; $F=0.29$; $P=0.5950$) or at 96 hpr ($df=1, 66$; $F=0.01$; $P=0.9359$).

At 24 days post-treatment, adult thrips mortality did not vary with insecticide treatments at 48 hpr (df= 3, 66; F=1.35; P=0.2659). However, mortality percentages varied with treatments at 96 hpr (df= 3, 66; F=3.66; P=0.0168). Mortality of adult thrips feeding on leaflets from phorate treated plots was significantly higher than on leaflets treated with thiamethoxam (Fig. 4.1). There was no difference between the results of two experiment repeats at both 48 hpr (df=1, 66; F=1.48; P=0.2282) and 96hpr (df=1, 66; F=1.23; P=0.2712).

At 31 days post-treatment, adult thrips mortality did not vary with experimental treatments at either 48 hpr (df=3, 66; F=1.06; P=0.3731) or 96 hpr (df=3, 66; F=1.42; P=0.2455). There was no impact of phorate, imidacloprid, and thiamethoxam treatments on adult thrips mortality when compared with non-treated check (Fig. 4.1). Results from experiment replicates were consistent at 48 hpr (df=1, 66; F=0.61; P=0.4358); however, there was difference between two repeats at 96 hpr (df=1, 66; F=5.57; P=0.0212).

Overall, adult thrips feeding damage varied with treatments at 48 hpr at 10 days (df=3, 66; F=46.91; P<0.0001), 17 days (df=3, 66; F=15.15; P<0.0001), and 24 days (df=3, 66; F=8.41; P<0.0001) post-treatment, but not 31 days (df=3, 66; F=2.16; P=0.1017). Similarly, adult thrips feeding damage varied with treatments at 96 hpr at 10 days (df=3, 66; F=70.42; P<0.0001), 17 days (df=3, 66; F=14.09; P<0.0001), 24 days (df=3, 66; F=13.01; P<0.0001), and 31 days (df=3, 66; F=4.00; P=0.0112) post-treatment.

At 10 days post-treatment, feeding damage on phorate and imidacloprid treated leaflets was significantly reduced than on leaflets treated with thiamethoxam and non-treated check at both 48 hpr and 96 hpr (Fig. 4.2). The results did not vary with experimental repeats at 48 hpr (df=1, 66; F=2.27; P=0.1366) or 96 hpr (df=1, 66; F=1.78; P=0.1869).

At 17 days post-treatment, similarly, feeding damage on leaflets from phorate and imidacloprid treated plots were significantly reduced compared with feeding on leaflets from thiamethoxam treated and non-treated plots at both 48 hpr and 96 hpr (Fig. 4.2). However, there were significant variations between the results from experimental repeats at 48 hpr ($df=1, 66$; $F=71.96$; $P<0.0001$) and 96 hpr ($df=1, 66$; $F=57.57$; $P<0.0001$).

At 24 days post-treatment, feeding damage on leaflets from imidacloprid treated plots was significantly reduced compared to leaflets from thiamethoxam treated and non-treated plots at 48 hpr. Phorate treatment resulted in reduced feeding damage on leaflets when compared with non-treated check. Feeding damage on thiamethoxam treated leaflets did not differ from that on phorate treated or non-treated leaflets (Fig. 4.2). At 96 hpr, feeding damage on leaflets from imidacloprid treated plots was significantly reduced in comparison with leaflets from thiamethoxam treatment and non-treated check. Leaflets from phorate treated plots resulted in significant reduction of feeding damage compared with leaflets from thiamethoxam treated plots; while feeding damage on phorate treated leaflets did not differ from feeding damage on non-treated leaflets (Fig. 4.2). The results did not vary with experimental replicates at 48-hour reading ($df=1, 66$; $F=3.68$; $P=0.0594$) or 96-hour reading ($df=1, 66$; $F=0.26$; $P=0.6145$).

At 31 days post-treatment, feeding damage on leaflets treated with insecticides did not significantly less than non-treated leaflets at 48 hpr. However, at 96 hpr, leaflets from plots treated with imidacloprid resulted in significant reduction of feeding damage than leaflets from non-treated plots. Feeding damage on leaflets from phorate and thiamethoxam treated plots was not different from feeding damage on leaflets from non-treated plots as well as imidacloprid treated plots (Fig. 4.2). The feeding damage did not vary with the repeats of the experiment at 48

hpr (df=3, 66; F=2.16; P=0.1017); however, it varied with the experimental repeats at 96 hpr (df=3, 66; F=4.00; P=0.0112).

Effect of insecticides on thrips larval mortality and feeding damage over time: At 9 days post-treatment, thrips larvae mortality varied with insecticide treatments at 48 hpr (df=3, 12; F=10.69; P=0.0010) and 96 hpr (df=3, 12; F=40.61; P<0.0001). At both instances, mortality of thrips larvae feeding on leaflets treated with phorate and imidacloprid was significantly higher when compared with leaflets treated with thiamethoxam and non-treated leaflets (Fig. 4.3). At 16, 23, and 30 days post-treatment, thrips larvae mortality did not vary with treatments either at 48 hpr or 96 hpr. Mortality percentages of larval thrips were not affected by insecticide treatment after 9 days post-treatment (Fig. 4.3).

Overall, feeding damage of thrips larvae did not vary with treatments, except for both 48 and 96 hpr at 9 days post-treatment and 96 hpr at 30 days post treatment. At 9 days post-treatment, significant differences in thrips larval feeding damage was observed at 48 hpr (df=3, 12; F=40.13; P<0.0001) and 96 hpr (df=3, 12; F=67.42; P<0.0001). In both readings, feeding damage of leaflets treated with phorate and imidacloprid was significantly reduced than leaflets treated with thiamethoxam and non-treated leaflets (Fig. 4.4). Later at 16 days and 23 days post-treatment, larval thrips feeding damage on non-treated leaflets did not differ from any insecticide treated leaflets. At 30 days post-treatment, feeding damage was not different between insecticide treatments and non-treated check at 48 hpr; however, larval thrips feeding damage varied with insecticide treatments at 96 hpr (df=3, 12; F=4.81; P=0.0201). Among all treatments, only feeding damage on leaflets treated with phorate was significantly reduced than non-treated check; feeding damage on imidacloprid and thiamethoxam treated leaflets was not different from the feeding damage on non-treated leaflets (Fig. 4.4).

Residue analyses of insecticide active ingredients in leaf tissues from treated plants: In imidacloprid-treated plants, the amount of active ingredient ranged from 17.67 to 0.74 mg/kg in leaf tissue from 10 days to 31 days post-treatment (dpt). The amount of imidacloprid in leaf tissue significantly dropped from 17.67 mg/kg to 0.9 mg/kg from 10 to 24 (dpt); in other words, the leaf tissue lost more than 95% of its imidacloprid content in two weeks (Table 4.3). At 31 days post treatment, the residue value of imidacloprid was 0.74 mg/kg (Table 4.3).

For thiamethoxam treatment, the residue values in leaf tissue ranged from 8.73 to 0.007 mg/kg during the experiment. The amount of thiamethoxam in tested leaf tissue was 8.73 mg/kg at 10 dpt, followed by a huge drop to 0.007 mg/kg at 24 days post-treatment; the active ingredient concentration decreased over 99% during the two-week period (Table 4.3). At 31 dpt, the residue value was 0.011 mg/kg, but it was a negligible amount when compared with the value at the beginning of the experiment (Table 4.3).

In phorate-treated plants, the amount of phorate estimated in leaf tissues ranged from 0.09 mg/kg to a non-detectable value. At 10 dpt, the average residue value of phorate detected in leaf tissue was 0.09 mg/kg. At 24 dpt, 0.0057 mg/kg of phorate was detected in the leaf tissue; 94% of the phorate content was lost from 10 to 24 dpt. Finally, there was no detectable amount of phorate left in leaf tissues at 31 dpt (Table 4.3).

Susceptibility of *F. fusca* to three insecticides: In non-treated control check, the mortality of thrips from the lab colony was 3.5-5%, while the mortality of thrips from the field populations was 2-37% when fed with sucrose solution (data not shown). The median lethal concentration (LC50) values of thrips from the lab colony as well as from four field populations were listed in Table 4.4. The confidence intervals, when estimated, were also listed in Table 4.4.

Discussion

Results of the insecticide residue analyses indicated that the amount of active ingredients, including imidacloprid, thiamethoxam, and phorate, detected in leaf tissues declined significantly at 24 days after applications at planting. Investigations on insecticides uptake and persistence after application at planting in peanut were rarely conducted. Chamberlin et al. (1992) estimated the residue levels of aldicarb, one of the standard insecticides, used at that time using gas chromatography. They found that the residue levels of aldicarb in peanut terminals declined rapidly and typically by ≥100-fold every two weeks. Similarly, in our study, peanut leaf tissues lost 94-98% of the amount of active ingredients in all selected insecticides in two weeks (10 days to 24 days post treatment). Under field conditions, several factors can influence the persistence of insecticide residues in plant tissues and subsequently affect the efficacy to control pests. Since all of our insecticides were applied at planting by in-furrow application or seed-treatment, insecticides were probably taken up systemically through roots. At this point, rainfall, water erosion, and soil type might affect the actual amount of insecticide that would be absorbed by plants. Imidacloprid, thiamethoxam, and phorate are systemic insecticides that translocate through the xylem (Lindquist et al., 1961, Sur & Stork, 2003). In addition, the movement of insecticides in plants is likely regulated by transpiration. It is suggested that the insecticide residues dissipated faster in warmer weather due to higher transpiration rates (Chamberlin et al., 1992).

Correspondingly, leaflet residual toxicity bioassays demonstrated that the effectiveness of imidacloprid and phorate on both adults and larvae mortality largely dissipated 10 days post treatment. However, imidacloprid and phorate were more toxic to thrips larvae than adults, the larval mortality was 50% and 72% while adult mortality was 32% and 34% at 9 (or 10) days post

treatment for imidacloprid and phorate, respectively. It is reasonable to assume that thrips in immature stages are usually more vulnerable and susceptible to insecticides due to their limited mobility. Even though the mortality of adult thrips was not affected by insecticides after 10 days, the effectiveness of imidacloprid and phorate to reduce adult thrips feeding damage lasted for at least 24 days in our study. It is likely leaflets from imidacloprid and phorate treated plots still retained some antifeedent properties even at low residue levels, despite the absence of toxicity not sufficient enough to kill adult thrips. Imidacloprid and phorate treatments significantly reduced thrips larvae feeding only up to 9 days after application at planting. The leaf surface exposed to ten 1^{st} to 2^{nd} instars larvae was relatively large that minor differences in feeding damage were difficult to determine. In spotted wilt disease management in peanut, using insecticides to manage thrips is one of the commonly adopted management practices (Culbreath et al., 2003). Phorate has been effective in managing thrips populations and reducing feeding injuries in peanut (Brown et al., 2005, Todd et al., 1996). Our results are consistent with previous studies. In this study, assays also revealed that Imidacloprid was as effective as phorate in causing significant thrips mortality and reducing thrips feeding damage. (Wells et al., 2002) documented that imidacloprid reduced thrips damage and survival was better than phorate. Based on our results, imidacloprid and phorate in-furrow application effectively suppressed thrips in the first two weeks post planting, and both were better than seed-treatment with thiamethoxam.

Phorate is a broad-spectrum insecticide with high toxicity to birds, fish, and mammals including humans (Lakshmaiah & Indi, 2014, Timoroglu et al., 2014, Williams, 1997). Until now, it was also the standard insecticide used in peanut for spotted wilt management for decades. The reason for that is because phorate has provided consistent suppression of spotted wilt disease incidence in peanut (Culbreath et al., 2003, Todd et al., 1996, Wiatrak et al., 2000). The

underlying mechanism by which phorate provides better control of spotted wilt in peanut remains unclear. Thrips control does not appear to be the only factor responsible for the phorate-induced suppression of spotted wilt disease, since other insecticides provided sufficient control of thrips but not spotted wilt disease (Todd et al., 1996, Todd et al., 2005). Phorate causes phytotoxicity in peanut, which typically caused chlorosis and necrosis of young peanut leaves, called 'peanut burn' (Culbreath & Srinivasan, 2011). It is suggested that peanut burn may be related to induction of a host defense response or inhibition of virus replication or movement in the host. Both activation and suppression of certain genes in peanut plants treated with phorate were observed and reported by Jain et al. (2015). Some of the genes encode for pathogenesis and defense-related proteins as well as membrane-trafficking functions that may subsequently affect virus replication and limit systemic spread of TSWV (Jain et al., 2015).

Imidacloprid was the first commercially available neonicotinoid insecticide that was labeled for use in peanut (Bai et al., 1991, Nauen et al., 1998, Sur & Stork, 2003). In general, neonicotinoid insecticides have been widely used due to relatively low toxicity to mammals (Elbert et al., 1998). However, contradictory results were documented with imidacloprid applications in peanut. For instance, seed-treated or in-furrow application of imidacloprid resulted in increased incidence of spotted wilt than other insecticide treatments or no insecticide treatment (Todd et al., 1994).The assumption of reducing thrips feeding could consequently suppress incidence of spotted wilt in peanut became questionable. Imidacloprid-induced changes in feeding behavior on thrips (primarily *F. occidentalis*) were also noticed in tomato (Chaisuekul & Riley, 2001). Imidacloprid has been documented to alter thrips probing and settling behavior depending on thrips species (Joost & Riley, 2005). Tomato plants treated with imidacloprid tended to repel *F. fusca* and reduce the frequency and duration of their probing on leaflets, while

it tended to increase the number of *F. occidentalis* and accelerate their probing (Joost & Riley, 2005). In this study, imidacloprid effectively suppressed thrips survival and feeding damage.

Thiamethoxam is another systemic insecticide in the neonicotinoid class and has broad-spectrum insecticidal activity, especially for sucking insects such as aphid, whiteflies, and thrips. Due to low use rates, flexible application methods, promising efficacy, and a favorable safety profile, thiamethoxam has been largely used in many cropping systems including peanut (Maienfisch et al., 2001). However, in this study thiamethoxam seed-treatment was not better than imidacloprid or phorate treatments. Thrips suppression following thiamethoxam seed treatment was only marginally better than the non-treated check. The reason for poor thrips suppression following thiamethoxam treatment is not clear; one of the speculations has been the development of resistance against the insecticide. This study also evaluated resistance development to all three commonly used insecticides including imidacloprid, phorate and thiamethoxam by conducing membrane-based bioassays using laboratory and field populations.

Imidacloprid, thiamethoxam, and phorate were tested in several dilutions (obtained serially) through membrane-based assays. Thrips mortality was very high when exposed to the insecticides at respective field rates. Every insecticide in corresponding field rate resulted in thrips mortality ranged from 96.5 to 100 % in all different locations or populations (data not shown). Recommended field rates used in the study were all for in-furrow application at planting. Therefore, the actual amount of insecticides that could be absorbed by crops and translocated will largely affect the efficacy of the insecticides against thrips. The uptake rate of crop roots when insecticide applied in soil or seed treatment is possibly very low. When using imidacloprid in soil or as seed, the uptake is only about 5% of the applied dose (Sur & Stork, 2003).

Using the LC50 value of thrips from the lab colony as the baseline, the resistance status of thrips from field populations were monitored by assigning a resistance ratio to every thrips population for each insecticide (Table 4.5). Results of membrane-based bioassays revealed differences in susceptibility to imidacloprid, thiamethoxam, and phorate in *F. fusca* populations from different locations. In general, thrips from the lab colony had higher LC50 values of all the selected insecticides (Table 4.4). Resistance ratios showed that minor variations in insecticide susceptibility among thrips populations tested.

Thrips population collected from Berrien County was the only one that had a slightly higher resistance ratio among all thrips populations; while thrips from Belflower farm was the most susceptible population to imidacloprid in our study. There were much fewer replicates for thrips from Berrien County in the bioassays than thrips from other locations; future investigation will verify the susceptibility to imidacloprid in this thrips population. All the resistance ratios for thiamethoxam were less than 1, which means no resistance observed among thrips populations tested. Some of the thrips populations had about ten times more susceptibility compared with the baseline lab thrips colony. There was no resistance to phorate observed among the field populations either, based on the resistance ratios. Thrips from the Attapulgus research station again were a hundred times more susceptible than thrips from the lab colony. The high survival rate of thrips from lab colony when exposed to insecticides was possibly because their rearing conditions were better than thrips collected from fields. The mortality of thrips feeding on non-treated control solution supported this point. Thrips from the lab colony used in all experiments were not older than two days old; however, ages of thrips collected from fields were not estimable. Generally, older thrips would have higher mortality even under optimal conditions. In addition, due to their mobility

Resistance to insecticides such as emamectin benzoate, imidacloprid, and spinosad were found in China and Australia on different species of thrips (Gao et al., 2014, Herron & James, 2007, Wang et al., 2014). Tobacco thrips, *F. fusca*, is mainly reported in the continental U.S. as an agricultural pest (Riley et al., 2011). There was no documentation of insecticide resistance in *F. fusca* until 2014. Awareness of insecticide resistance in tobacco thrips populations has increased because widespread use of neonicotinoid insecticides (Funderburk, 2015, Herbert & Kennedy, 2015). A neonicotinoid resistance-monitoring program for *F. fusca* has been established in the U.S., led by Dr. George Kennedy at North Carolina State University. Thrips samples collected by researchers across the Southeast were subjected to initial bioassay. Using the upper 95% confidence limit of susceptible NCSU population as the baseline, potentially resistant populations were determined and further tested to establish a dose-response relationship. Thiamethoxam resistance has been identified in thrips populations from Arkansas, Georgia, Louisiana, Mississippi, North Carolina, and Tennessee; imidacloprid resistance has also been identified in Arkansas, Georgia, Mississippi, North Carolina, Tennessee, and Florida (Funderburk, 2015, Herbert & Kennedy, 2015). It is suggested that resistance is established in thrips populations at the local landscape level, since resistance levels were similar across populations collected from cotton, wheat, and weeds (Herbert & Kennedy, 2015). In contrast to the region-wide study from the neonicotinoid resistance-monitoring program, our results indicated that there was no insecticide resistance found in our study.

In conclusion, the residue levels of imidacloprid, thiamethoxam, and phorate applied at planting in the field dramatically declined after 24 days post treatments. Relatively, the insecticide effectiveness to cause thrips mortality lasted no longer than 10 days after treatment at planting. Among the selected insecticides, imidacloprid was as effective as phorate in controlling

thrips, and both were better than thiamethoxam in reducing thrips damage and population. Feeding bioassays in the laboratory indicated that all the insecticides with rates corresponding to field rates provided sufficient control of thrips; there was no insecticide resistance observed in this study. Imidacloprid and thiamethoxam are both in the neonicotinoid class of insecticides; however, the effectiveness of imidacloprid was significantly higher than thiamethoxam in thrips control when applied in the field. Thiamethoxam was coated on the seeds, while imidacloprid was applied directly at planting. The application method could have caused the difference as well. Overall, the current study suggests that thrips control could be affected by timing of thrips occurrence in the field and the corresponding insecticide residues available within the plant. Though, resistance could influence thrips susceptibility, preliminary data does not seem to indicate so. Future studies will help address the resistance phenomenon in thrips in peanut.

Tables

Table 4.1. List of insecticides used in the residue bioassay

Treatment No.	Classification/ Chemical Name	Active Ingredient	Trade Name	Rate per Acre[x]	Type of Application	Manufacturer
1	4A Neonicotinoids	Imidacloprid	Admire® Pro	7.0-10.5 fl oz	In-furrow	Bayer CropScience
2	4A Neonicotinoids	Thiamethoxam	Cruiser Maxx™	4.0- 5.4 oz[y]	Seed treatment	Syngenta
3	1B Organophosphates	Phorate	Thimet® 20G	5 lb	In-furrow	Amvac

[x] Based on the manufacturer recommended field rates.

[y] Recommended rate of 3.0-4.0oz per 100 lb of seeds. Conversion was based on the estimation of 648 seeds (GA-06G) for one pound; 6 seeds planted per foot; 87362 seeds planted in one acre.

Table 4.2. List of insecticides used in feeding assays

Treatment No.	Active Ingredient	Trade Name	Rate per Acre[x]	Highest rate in the series (ppm) (µl/L)	Manufacturer
1	Imidacloprid	Admire® Pro	10.5 fl oz	164100[y]	Bayer CropScience
2	Thiamethoxam	Cruiser 5FS	0.48µl per seed	3600[z]	Syngenta
3	Phorate	Thimet® (Technical form)	1 lb (a.i.)	205000[y]	Amvac

[x] Based on the manufacturer recommended field rates.

[y] 10 times field rate calculated based on recommended rate and 5 gallon water usage per acre.

[z] Based on a preliminary study; calculated field rate was 2216 ppm with the assumption of 87362 seeds planted per acre and 5 gallon water used per acre.

Table 4.3. Residue analyses of insecticide active ingredients in peanut plant leaf tissue

Residue value (AI/plant tissues) (mg/kg)					
Treatment No.	Trade Name[x]	Active Ingredient[y]	10 days[z]	24 days[z]	31days[z]
1	Admire® Pro	Imidacloprid	17.66667	0.903333	0.743333
2	Cruiser Maxx™	Thiamethoxam	8.733333	0.007	0.011
3	Thimet®	Phorate	0.089667	0.005667	NA

[x] Selected insecticides used for residual toxicity bioassays. The application rates used are listed in Table 2.1.

[y] Plant tissue samples collected were subjected to active ingredient residue analyses, including QuEChERS extraction, GC-MS/MS and LC-MS/MS analysis by Pacific agricultural laboratory (Portland, OR).

[z] Plant tissue samples were collected at 10 days, 24 days, and 31 days after planting and treatment application.

Table 4.4. *Franklineilla fusca* susceptibility to selected insecticides

Treatments	Lab colony	
	LC50[x] (ppm)	95% CI[y]
Admire® Pro (Imidacloprid)	7.08	1.16-20.72
Cruiser® 5FS (Thiamethoxam)	15.15	10.96-20.97
Thimet® (Phorate)	15.1	12.66-18.05
Treatments	Attapulgus research station	
	LC50[x] (ppm)	95% CI[y]
Admire® Pro (Imidacloprid)	6.38	0.84-16.77
Cruiser® 5FS (Thiamethoxam)	13.57	10.06-18.39
Thimet® (Phorate)	11.28	
Treatments	Belflower farm	
	LC50[x] (ppm)	95% CI[y]
Admire® Pro (Imidacloprid)	3.22	0.28-7.02
Cruiser® 5FS (Thiamethoxam)	3.93	2.73-5.58
Thimet® (Phorate)	8.33	

[x] Mortality data were subjected to the PROBIT procedure in SAS (SAS Enterprise 4.2, SAS Institute, Cary, NC) to evaluate the median lethal concentration (LC50) for each insecticide.

[y] 95% confidence interval.

Treatments	Bowen farm	
	LC50[x] (ppm)	95% CI[y]
Admire® Pro (Imidacloprid)	7.14	
Cruiser® 5FS (Thiamethoxam)	6.21	3.53-10.48
Thimet® (Phorate)	0.02	
Treatments	Berrien County	
	LC50[x] (ppm)	95% CI[y]
Admire® Pro (Imidacloprid)	13.74	
Cruiser® 5FS (Thiamethoxam)	1.68	0.35-4.91
Thimet® (Phorate)		

[x] Mortality data were subjected to the PROBIT procedure in SAS (SAS Enterprise 4.2, SAS Institute, Cary, NC) to evaluate the median lethal concentration (LC50) for each insecticide.

[y] 95% confidence interval.

Table 4.5. Resistance ratios of thrips populations in different locations

	Resistance ratio[x]				
Treatment	Lab colony	Attapulgus research station	Belflower farm	Bowen farm	Berrien County
Admire® Pro (Imidacloprid)	1	0.90	0.45	1.01	1.94
Cruiser® 5FS (Thiamethoxam)	1	0.90	0.26	0.41	0.11
Thimet® (Phorate)	1	0.75	0.55	0.01	NA

[x] Resistance ratios were calculated by comparing LC50 values of field-collected thrips with LC50 values of laboratory thrips.

Figures

Fig. 4.1. Mean (±SE) adult thrips mortality (%) in residual toxicity bioassay. Peanut leaflets treated with selected insecticides at 10 days, 17 days, 24 days, and 31 days post treatment. Ten female adult *F. fusca* were released in a Munger cage with two respective treatment leaflets. Thrips mortality was evaluated at 48 hours and 96 hours post thrips release. Ten cages were set up for an experiment and the experiment was repeated once (N=20). Treatment means labeled with different letters indicates significant differences, while the same letter indicates no difference.

Fig. 4.2. Mean (±SE) adult feeding damage rating in residual toxicity bioassay. Peanut leaflets treated with selected insecticides were collected at 10 days, 17 days, 24 days, and 31 days post treatment. Ten female adult *F. fusca* were released in a Munger cage with two respective treatment leaflets. Thrips feeding damage was evaluated at 48 hours and 96 hours post thrips released using an arbitrary scale from 0 to 5 (0 represents no feeding while 5 represents 100% coverage of feeding injuries on the leaf surface). Ten cages were set up for an experiment and the experiment was repeated once (N=20). Treatment means labeled with different letters indicates that there was significant difference, while the same letter indicates no difference.

Fig. 4.3. Mean (±SE) thrips larval mortality (%) in residual toxicity bioassay. Peanut leaflets treated with selected insecticides were collected at 10 days, 17 days, 24 days, and 31 days post treatment. Ten thrips larvae were released in a Munger cage with two respective treatment leaflets. Thrips larvae mortality was evaluated at 48 hours and 96 hours post thrips release. Ten cages were set up for each experiment (N=10). Treatment means labeled with different letters indicates that there was significant difference, while the same letter indicates no difference.

Fig. 4.4. Mean (±SE) thrips larvae feeding damage rating in residual toxicity bioassay. Peanut leaflets treated with selected insecticides were collected at 10 days, 17 days, 24 days, and 31 days post treatment. Ten thrips larvae were released in a Munger cage with two respective treatment leaflets. Thrips larval feeding damage was evaluated at 48 hours and 96 hours post thrips release using an arbitrary rating scale based on the percentage of feeding injury coverage of the leaf surface. Ten cages were set up for the experiment (N=10). Treatment means labeled with different letters indicates that there was significant difference, while the same letter indicates no difference.

Fig. 4.1.

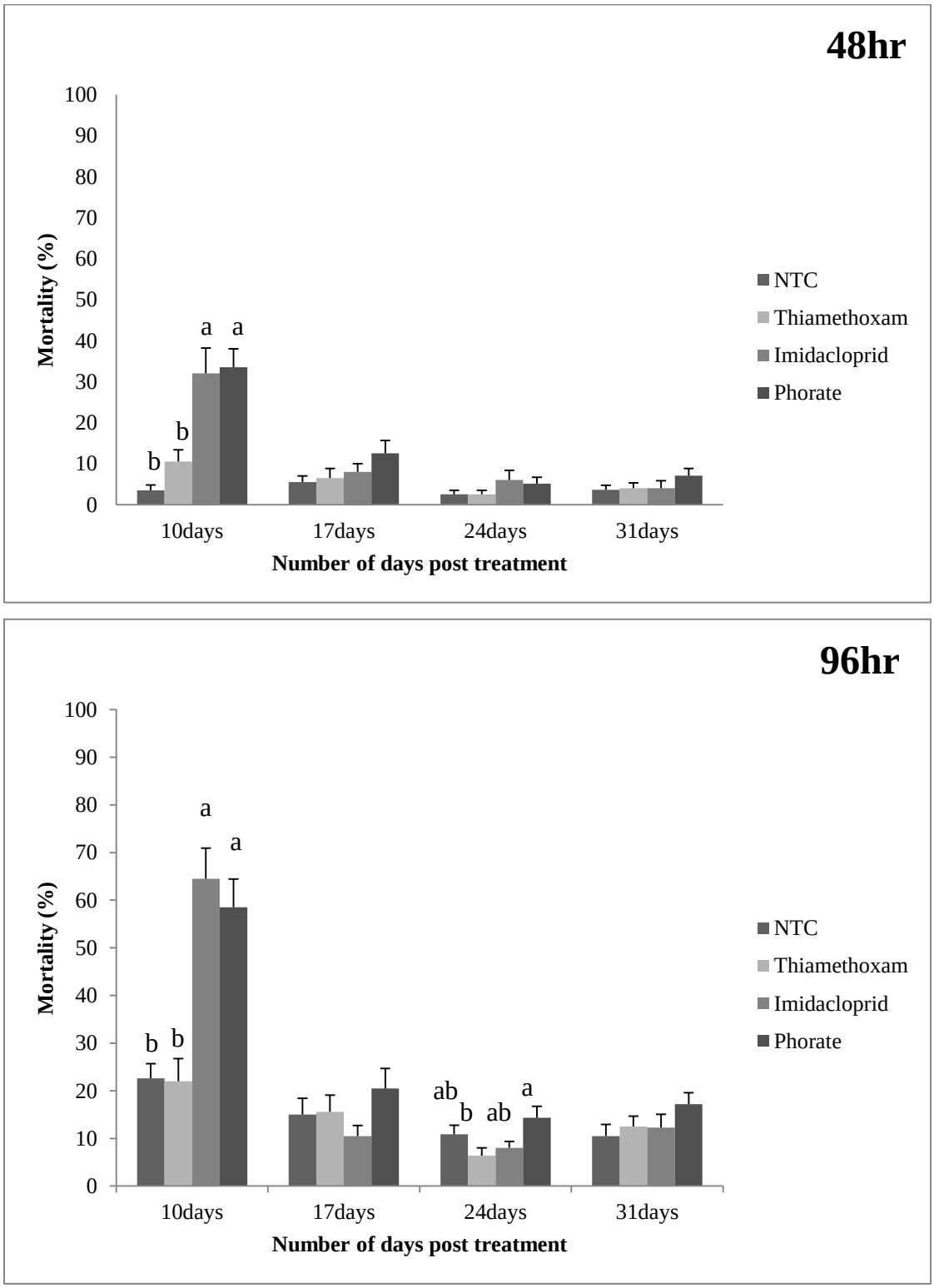
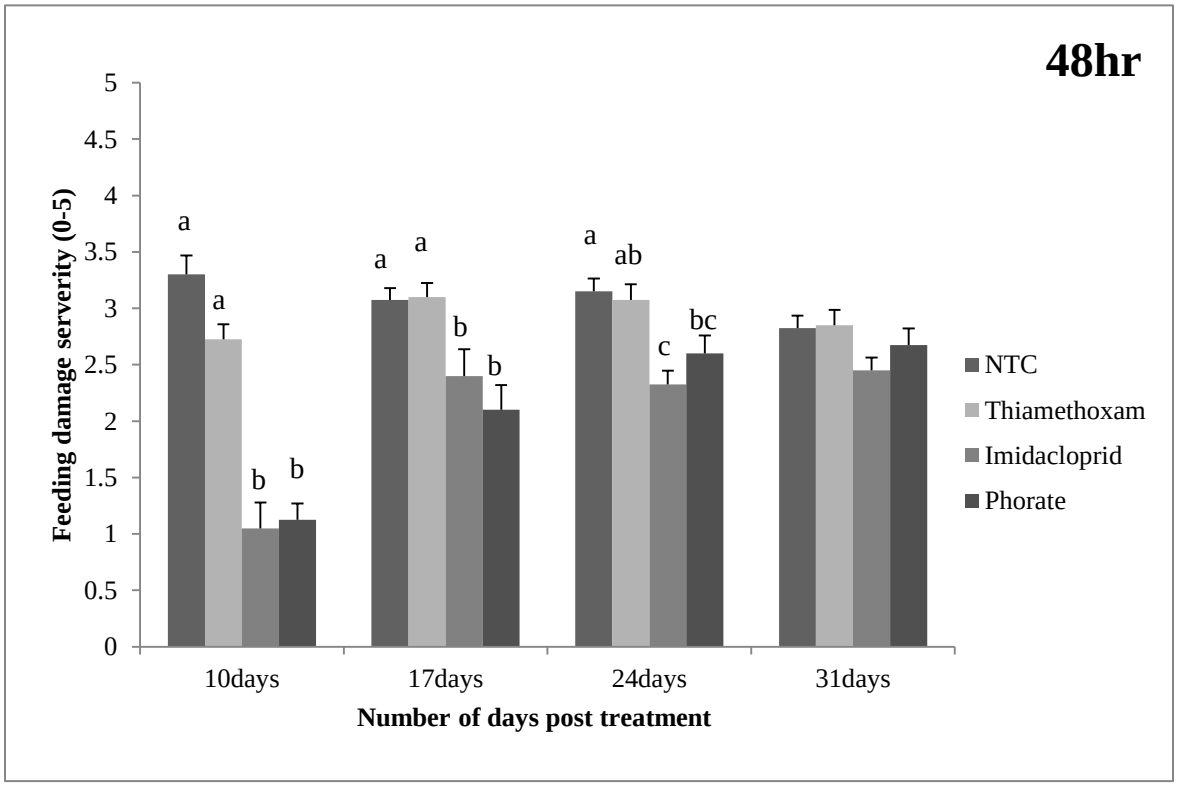

48hr
Mortality (%)
100
90
80
70
60
50
40
30
20
10
0
b
b
a
a
10days
17days
24days
31days
Number of days post treatment
NTC
Thiamethoxam
Imidacloprid
Phorate
96hr
Mortality (%)
b
b
a
a
ab
b
ab
a
10days
17days
24days
31days
Number of days post treatment
NTC
Thiamethoxam
Imidacloprid
Phorate

Fig. 4.2.


48hr
Feeding damage serverity (0-5)
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
a a b b
a a b b
a ab c bc
10days
17days
24days
31days
Number of days post treatment
NTC
Thiamethoxam
Imidacloprid
Phorate

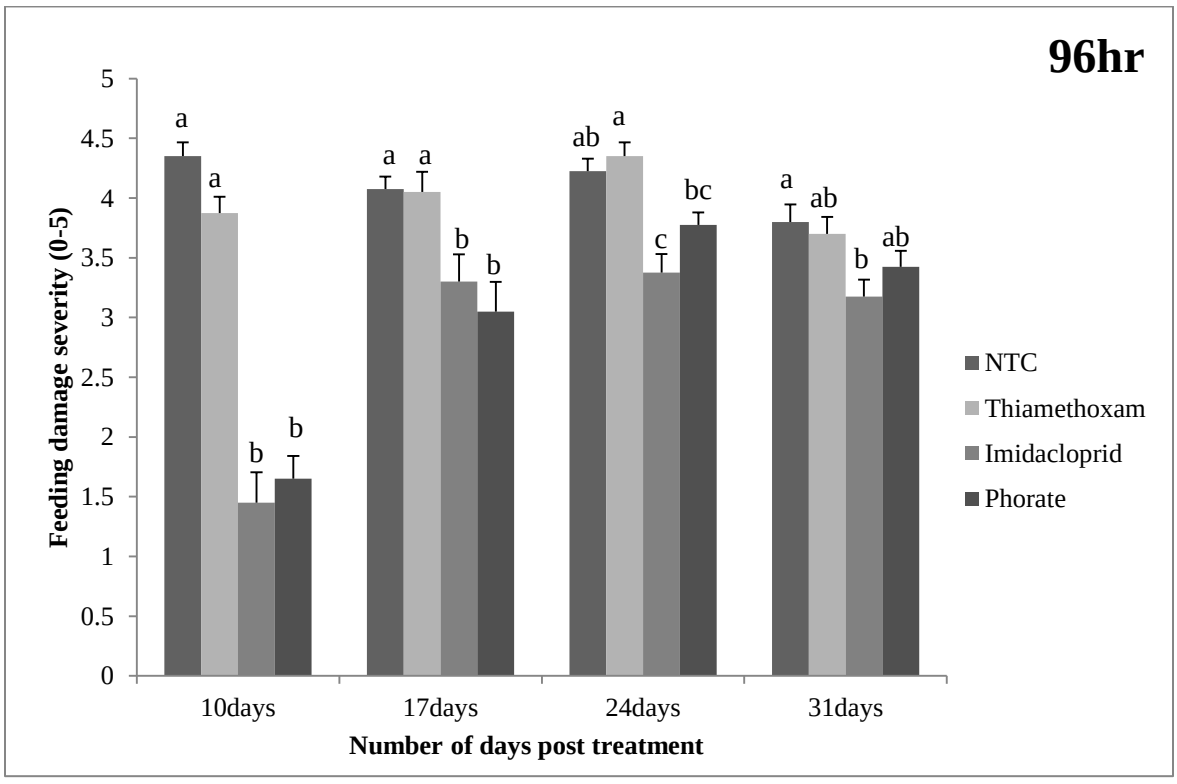

96hr
Feeding damage severity (0-5)
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
a a b b
a a b b
ab a c bc
a ab b ab
10days
17days
24days
31days
Number of days post treatment
NTC
Thiamethoxam
Imidacloprid
Phorate

Fig. 4.3.

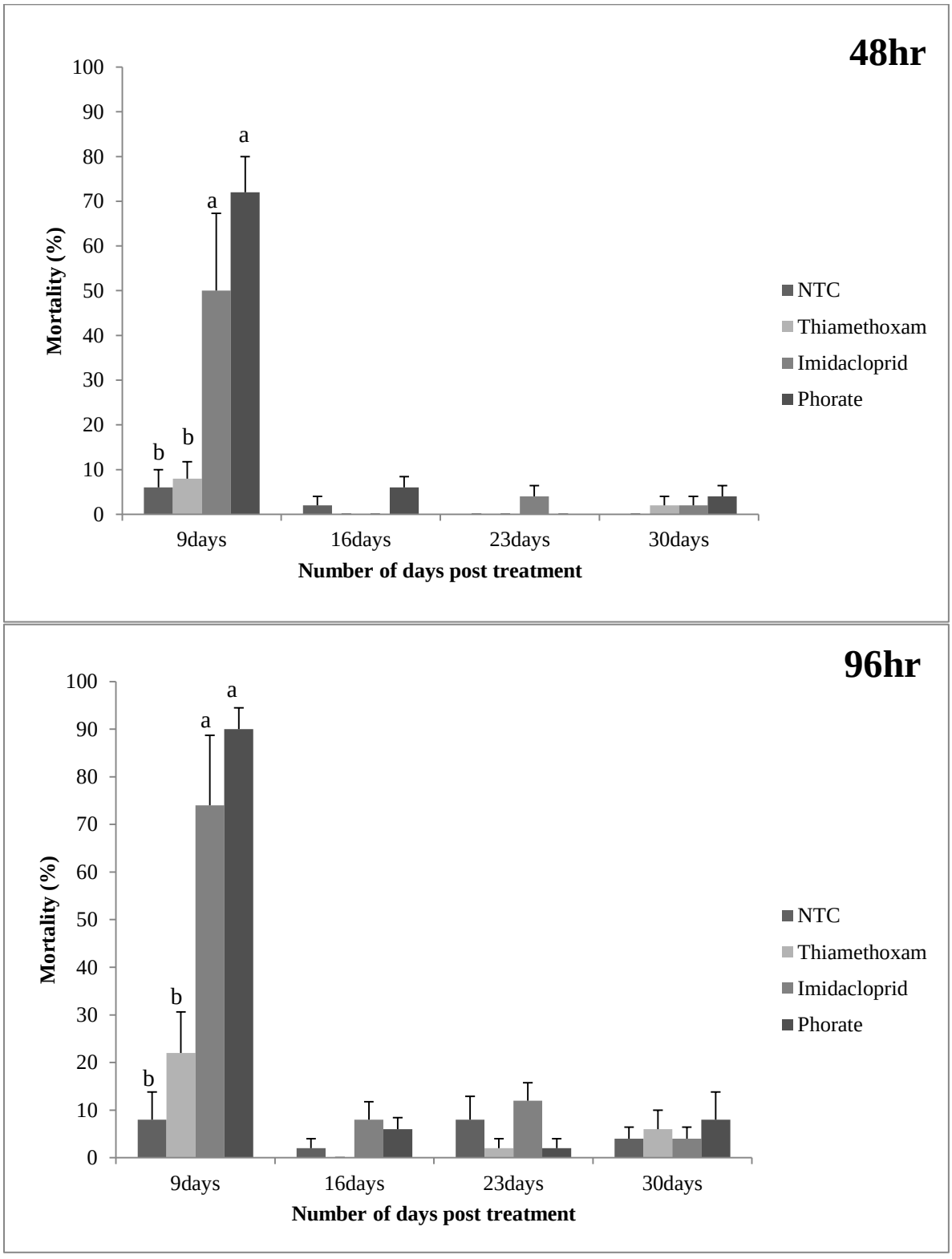
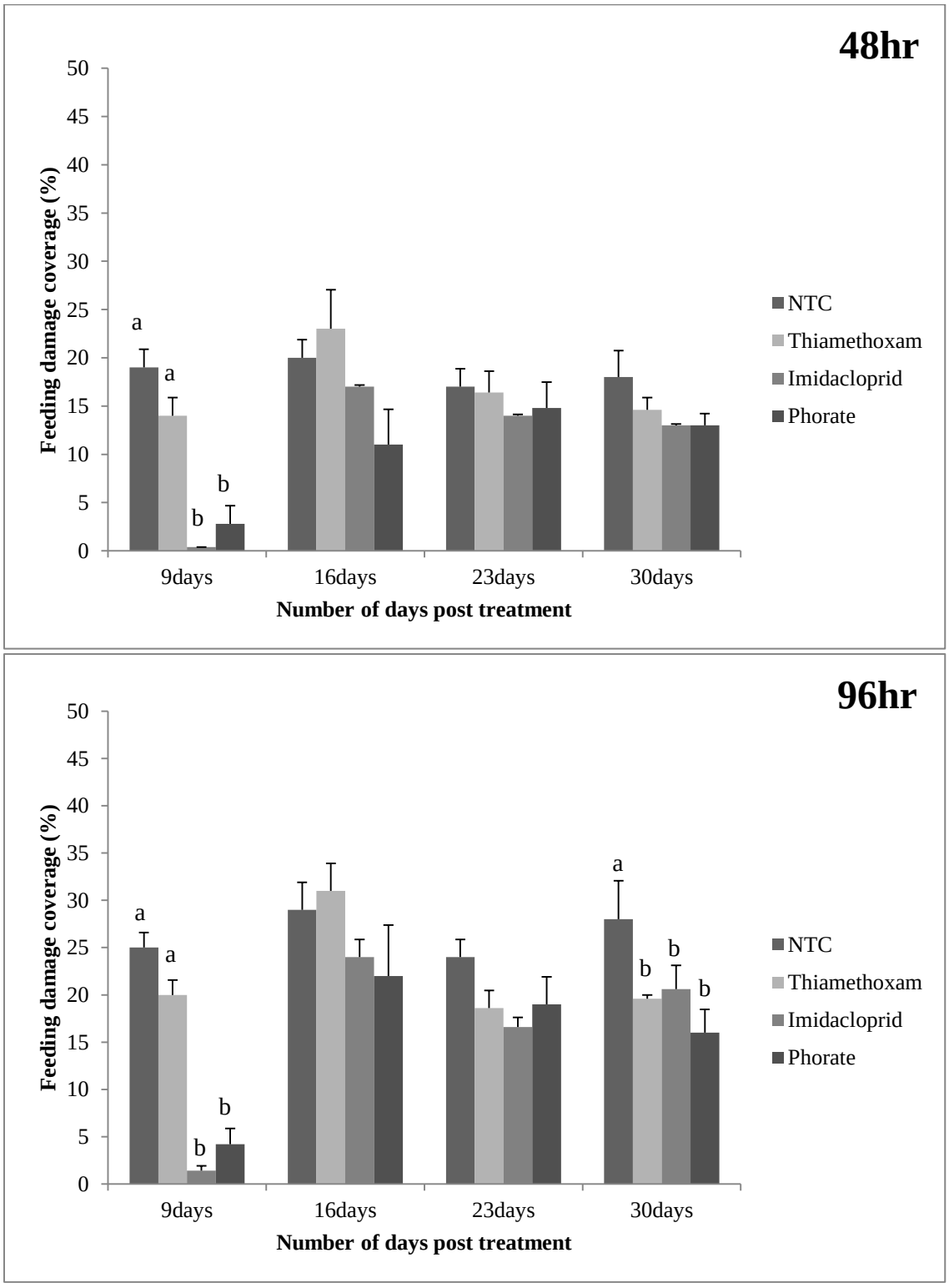

48hr
Mortality (%)
100
90
80
70
60
50
40
30
20
10
0
a
a
b
b
9days
16days
23days
30days
Number of days post treatment
NTC
Thiamethoxam
Imidacloprid
Phorate
96hr
Mortality (%)
100
90
80
70
60
50
40
30
20
10
0
a
a
b
b
9days
16days
23days
30days
Number of days post treatment
NTC
Thiamethoxam
Imidacloprid
Phorate

Fig. 4.4.


48hr
Feeding damage coverage (%)
50
45
40
35
30
25
20
15
10
5
0
a
a
b
b
9days
16days
23days
30days
Number of days post treatment
NTC
Thiamethoxam
Imidacloprid
Phorate
96hr
Feeding damage coverage (%)
a
a
b
b
a
b
b
9days
16days
23days
30days
Number of days post treatment
NTC
Thiamethoxam
Imidacloprid
Phorate

Literature cited

Agronews (2010) Bayer CropScience to close a plant as Temik phasing out.

Bai, D., Lummis, S.C.R., Leicht, W., Breer, H. and Sattelle, D.B. (1991) Actions of imidacloprid and a related nitromethylene on cholinergic receptors of an identified insect motor neurone. *Pesticide Science*, **33**, 197-204.

Bertrand, P.F. (1998) 1997 Georgia Plant Disease Loss Estimates. Univ. Georgia Coop. Ext. , Pub. Path. 98-007.

Branch, W.D. (1996) Registration of 'Georgia Green' peanut. *Crop Science*, **36**, 806-806.

Branch, W.D. (2007) Registration of 'Georgia-06G' peanut. *Journal of Plant Registrations*, **1**, 120-120.

Brown, S.L., Culbreath, A.K., Todd, J.W., Gorbet, D.W., Baldwin, J.A. and Beasley, J.P., Jr. (2005) Development of a method of risk assessment to facilitate integrated management of spotted wilt of peanut. *Plant Disease*, **89**, 348-356.

Chaisuekul, C. and Riley, D.G. (2001) Thrips (Thysanoptera: Thripidae) feeding response to concentration of imidacloprid in tomato leaf tissue. *Journal of Entomological Science*, **36**, 315-315.

Chamberlin, J.R., Todd, J.W., Culbreath, A.K., Johnson, W.C., Iii and Demski, J.W. (1993) Post-harvest management of tobacco thrips (Thysanoptera: Thripidae) overwintering in peanut fields. *Journal of Entomological Science*, **28**, 433-446.

Chamberlin, J.R., Todd, J.W., Farrow, J.M. and Mullinix, B.G., Jr. (1992) Aldicarb residue persistence in leaf terminals of 'Florunner' peanut. *Journal of Economic Entomology*, **85**, 1072-1078.

Culbreath, A.K. and Srinivasan, R. (2011) Epidemiology of spotted wilt disease of peanut caused

by Tomato spotted wilt virus in the southeastern U.S. *Virus Research,* **159**, 101-109.

Culbreath, A.K., Todd, J.W. and Brown, S.L. (2003) Epidemiology and management of tomato spotted wilt in peanut. *Annual review of phytopathology,* **41**, 53-75.

Elbert, A., Nauen, R. and Leicht, W. (1998) Imidacloprid, a Novel Chloronicotinyl Insecticide: Biological Activity and Agricultural Importance. *Insecticides with Novel Modes of Action.* eds. I. Ishaaya & D. Degheeles), pp. 50-73. Springer Berlin Heidelberg.

Entomology, U.P. (2015) Thrips Management for 2015. UGA Extension.

Funderburk, J.E. (2015) Evaluation of Thrip Resistance to Neonicotinoid Insecticides. UF IFAS Extension, Panhandle Ag e-NEWS.

Gao, C.-F., Ma, S.-Z., Shan, C.-H. and Wu, S.-F. (2014) Thiamethoxam resistance selected in the western flower thrips Frankliniella occidentalis (Thysanoptera: Thripidae): Cross-resistance patterns, possible biochemical mechanisms and fitness costs analysis. *Pesticide Biochemistry & Physiology,* **114**, 90-96.

Groves, R.L., Sorenson, C.E., Walgenbach, J.F. and Kennedy, G.G. (2001) Effects of imidacloprid on transmission of tomato spotted wilt tospovirus to pepper, tomato and tobacco by Frankliniella fusca Hinds (Thysanoptera: Thripidae). *Crop Protection,* **20**, 439-445.

Herbert, A. and Kennedy, G.G. (2015) New Survey Shows High Level and Widespread Resistance of Thrips to Neonicotinoid Insecticides

Herron, G.A. and James, T.M. (2007) Insecticide resistance in Australian populations of Western Flower Thrips, Frankliniella occidentalis (Pergande) (Thysanoptera: Thripidae). *General and Applied Entomology.* pp. 1-5. Entomological Society of New South Wales, Sydney; Australia.

Hollis, P.L. (2014) Peanut growers expand battle against thrips. (cover story). *Southeast Farm Press,* **41**, 1.

Jain, M., Gallo, M., Chengalrayan, K., Shaikh, N.P., Macdonald, G.E. and Davis, J.M. (2015) Phorate-induced host defence responses condition acquired resistance to tomato spotted wilt in cultivated peanut (Arachis hypogaea L.). *Journal of Phytopathology,* **163**, 853-866.

Jeschke, P., Nauen, R., Schindler, M. and Elbert, A. (2011) Overview of the Status and Global Strategy for Neonicotinoids. *JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY,* **59**, 2897-2908.

Johnson, C. (2014) Thrips Threat. *Progressive Farmer,* **129**, CL-1.

Joost, P.H. and Riley, D.G. (2005) Imidacloprid effects on probing and settling behavior of Frankliniella fusca and Frankliniella occidentalis (Thysanoptera: Thripidae) in tomato. *Journal of Economic Entomology,* **98**, 1622-1629.

Knight, I.A., Rains, G.C., Culbreath, A.K. and Toews, M.D. (2015) Conservation tillage and thiamethoxam seed treatments as tools to reduce thrips densities and disease in cotton and peanut. *Crop Protection,* **76**, 92-99.

Lakshmaiah, G. and Indi, P. (2014) Investigation of acute toxicity of phorate (an organophosphate) on fresh water fish Cyprinus carpio. *International Journal of Pharma and Bio Sciences,* **5**, B-110.

Lindquist, D.A., Hacskaylo, J. and Davich, T.B. (1961) Absorption and Translocation of Phorate and Phosphorus by Cotton Seedlings. p. 137. The University of Chicago Press.

Lowry, V.K., Smith, J.W., Jr., Mitchell, F.L. and Crumley, C.R. (1995) Thrips vectors responsible for the secondary spread of tomato spotted wilt virus in south Texas peanut. *Thrips*

biology and management: proceedings of the 1993 International Conference on Thysanoptera. eds. B.L. Parker, M. Skinner & T. Lewiss), pp. 167-170. Plenum Publishing Co. Ltd, London; USA.

Lowry, V.K., Smith, J.W. and Mitchell, F.L. (1992) Life-Fertility Tables for Frankliniella fusca (Hinds) and F. occidentals (Pergande) (Thysanoptera: Thripidae) on Peanut. *Annals of the Entomological Society of America*, **85**, 744-754.

Lynch, R.E., Morgan, L.W. and Garner, J.W. (1984) Influence of systemic insecticides on thrips damage and yield of Florunner peanuts in Georgia. *Journal of agricultural entomology,* **1**, 33-42.

Maienfisch, P., Angst, M., Brandl, F., Fischer, W., Hofer, D., Kayser, H., Kobel, W., Rindlisbacher, A., Senn, R., Steinemann, A. and Widmer, H. (2001) Chemistry and biology of thiamethoxam: a second generation neonicotinoid. *Pest Manag Sci,* **57**, 906-13.

Munger, F. (1942) A Method for rearing Citrus Thrips in the Laboratory. *Journal of Economic Entomology,* **35**, 373-375.

Nauen, R., Hungenberg, H., Tollo, B., Tietjen, K. and Elbert, A. (1998) Antifeedant effect, biological efficacy and high affinity binding of imidacloprid to acetylcholine receptors in Myzus persicae and Myzus nicotianae. *Pesticide Science,* **53**, 133-140.

Pappu, H.R., Jones, R.a.C. and Jain, R.K. (2009) Global status of tospovirus epidemics in diverse cropping systems: Successes achieved and challenges ahead. *Virus Research,* **141**, 219-236.

Riley, D.G., Joseph, S.V., Srinivasan, R. and Diffie, S. (2011) Thrips vectors of Tospoviruses. *Journal of Integrated Pest Management,* **2**, I1-I10.

Singh, A.P., Surendra, S., Prabhat, B. and Khushbu, Y. (2010) Toxic effect of phorate on the serum biochemical parameters of snake headed fish Channa punctatus (Bloch). *Advances in Bio Research,* **1**, 177-181.

Sur, R. and Stork, A. (2003) Uptake, translocation and metabolism of imidacloprid in plants. *Bulletin of Insectology,* **56**, 35-40.

Timoroglu, I., Yüzbasoglu, D., Ünal, F., Ylmaz, S., Aksoy, H. and Celik, M. (2014) Assessment of the genotoxic effects of organophosphorus insecticides phorate and trichlorfon in human lymphocytes. *Environmental Toxicology,* **29**, 577-587.

Todd, J.W., Culbreath, A.K. and Brown, S.L. (1996) Dynamics of vector populations and progress of spotted wilt disease relative to insecticide use in peanuts. *Acta Horticulturae*, 483-490.

Todd, J.W., Culbreath, A.K., Rogers, D. and Demski, J.W. (1994) Contraindications of insecticide use relative to vector control and spotted wilt disease progress in peanut. *Proc. Am. Peanut Res. Ed. Soc.*, **26**, 42 (Abstr.).

Todd, J.W., Gorbet, D.W., Culbreath, A.K., Brown, S.L. and Weeks, J.R. (2005) Comparisonof final TSWV severity and yield in peanuts treated with acephate, aldicarb, or phorate insecticide at planting. *Proc. Am. Peanut Res. Educ. Soc.*, **37**, 79-80 (Abstr.).

Todd, J.W., Jr, C., Culbreath, A.K. and Demski, J.W. (1993) Timing and duration of vector management in relation to spotted wilt disease incidence in peanut. *Proc. Am. Peanut Res. Ed. Soc*, **25**, 87 (Abstr.).

Wang, S., Zhang, A., Li, L., Men, X., Zhou, X., Zhai, Y., Liu, Y., Wei, S. and Yu, Y. (2014) Insecticide resistance status of field populations of Frankliniella occidentalis (Thysanoptera: Thripidae) in China and its control strategies. *Acta Entomologica Sinica,*

57, 621-630.

Wells, M.L., Culbreath, A.K., Csinos, A.S. and Todd, J.W. (2002) Effects of a plant activator and insecticides on tobacco thrips (Thysanoptera: Thripidae) feeding and survival. *Journal of Agricultural and Urban Entomology,* **19**, 117-120.

Whitfield, A.E., Ullman, D.E. and German, T.L. (2005) Tospovirus-thrips interactions. *Annu Rev Phytopathol,* **43**, 459-89.

Wiatrak, P.J., Wright, D.L., Marois, J.J., Grzes, S., Koziara, W. and Pudelko, J.A. (2000) Conservation tillage and thimet effects on tomato spotted wilt virus in three peanut cultivars. *Proceedings - Soil and Crop Science Society of Florida,* **59**, 109-111.

Wijkamp, I. (1995) Virus-vector relationships in the transmission of tospoviruses. *Agric.* p. 143. Univ. Wageningen, The Netherlands.

Williams, T. (1997) Silent scourge. *Journal of Pesticide Reform,* **17**, 9-13.

Young, S., Kinzer, R.E., Walton, R.R. and Matlock, R.S. (1972) Field screening for tobacco thrips resistance in peanuts. *Journal of Economic Entomology,* **65**, 828-832.

CHAPTER 5

EFFECTS OF *TOMATO SPOTTED WILT VIRUS* (TSWV)-RESISTANT TETRAPLOID AND DIPLOID PEANUT GENOTYPES ON TSWV TRANSMISSION BY *FRANKLINIELLA FUSCA* AND ITS BIOLOGICAL FITNESS[1]

[1] Lai, P., R. Srinivasan, M. Abney, and A. Culbreath. 2015. To be submitted.

Abstract

Thrips-transmitted *Tomato spotted wilt virus* (TSWV) is a devastating pathogen that causes spotted wilt disease epidemics in peanut in southeastern United States. Peanut genotypes resistant to TSWV are vital for spotted wilt disease management. Breeding programs have developed a suite of peanut genotypes with appreciable levels of field resistance to TSWV. Cultivated peanut, *Arachis hypogaea* (L.), is a tetraploid species and its wild species relatives are diploids. In this study, several newly released runner genotypes (with field resistance to TSWV), a Virginia genotype, its diploid (*Arachis diogoi*) hybrid, and *A. diogoi* were evaluated through thrips-mediated TSWV transmission assays. *A. diogoi* possesses high level of resistance to TSWV, and is used as a resistance source in breeding programs. TSWV infection percentages in all selected genotypes were estimated three weeks post-inoculation using DAS-ELISA. Viral loads in infected plants were assessed using quantitative PCR. Acquisition of TSWV by thrips from infected genotypes was evaluated to investigate the genotypes as TSWV inoculum sources. Furthermore, effects of tetraploid and diploid genotypes on thrips fitness were assessed using microcosms. Results indicated that TSWV infection percentages did not differ between resistant genotypes and a susceptible check, except for *A. diogoi*. TSWV loads varied with genotypes. Particularly, genotypes with *A. diogoi* in their pedigree exhibited reduced virus accumulation. Acquisition assays revealed that most evaluated genotypes were viable as TSWV inoculum sources. Net reproduction rate and median developmental time of thrips varied with peanut host genotypes. Biological fitness of thrips was considerably poor on the diploid genotype *A. diogoi*.

Key words: TSWV resistant peanut genotype, *Arachis diogoi*, TSWV transmission.

Introduction

Thrips are important pests of peanut in the southeastern United States. Besides causing foliar feeding injuries on peanut seedlings, they also transmit *Tomato spotted wilt virus* (TSWV) (family *Bunyaviridae*; genus *Tospovirus*). TSWV infection in peanut leads to spotted wilt disease, which is one of the most important diseases of peanut (*Arachis hypogaea* L.) (Cantonwine et al., 2006, Culbreath & Srinivasan, 2011). Economic losses in peanut due to spotted wilt disease in Georgia were first noted in 1990 (Hadden, 1991). Since then, spotted wilt disease has been a major constraint in peanut production, and is responsible for millions of dollars in losses every year (Culbreath & Srinivasan, 2011). Several species of thrips (Thysanoptera, Thripidae) transmit TSWV in a persistent and propagative manner (Pappu et al., 2009, Ullman et al., 1997, Whitfield et al., 2005). Among all thrips species that transmit TSWV, the western flower thrips, *Frankliniella occidentalis* (Pergande), and the tobacco thrips, *Frankliniella fusca* (Hinds), are the two major vectors of TSWV in peanut in the Southeast (Todd et al., 1995). Of the two, *F. fusca* is considered the predominant vector due to its abilities to colonize peanut plants at the early seedling stage when the plants are most vulnerable to TSWV infection (Culbreath et al., 2003, Lowry et al., 1992).

To manage tomato spotted wilt disease in peanut, multidisciplinary investigations have developed an integrated management package. Use of TSWV field-resistant cultivars along with insecticide applications and altered cultural practices has provided better control than any single tactic alone (Brown et al., 2005, Culbreath et al., 2003). Among all the factors affecting spotted wilt disease incidence in peanut, cultivar selection is considered to be the most important factor (Culbreath et al., 2008, Culbreath et al., 2003).

When spotted wilt disease first emerged in peanut in 1980s in the Southeast, the predominant cultivars planted such as Florunner, SunOleic 97R, and GK-7, were very susceptible to TSWV (Culbreath et al., 2000). The peanut cultivar "Southern Runner", released in 1984, was the first cultivar that possessed field resistance to TSWV (Black, 1991, Culbreath et al., 1992). Since then, great efforts went into screening of cultivars and breeding lines followed by the development of a series of cultivars with appreciable levels of field resistance to TSWV (Culbreath et al., 2000, Culbreath et al., 1999, Culbreath et al., 1996a). Several resistant genotypes such as Georgia Browne, and Georgia Green were released in the following two decades (Culbreath et al., 1994, Culbreath et al., 1996a, Gorbet et al., 1987). Using TSWV-resistant cultivars has consistently suppressed spotted wilt incidence and reduced yield losses. Georgia Green became the standard Runner type cultivar grown in Georgia, Alabama, and Florida from 1998 until 2010 (Culbreath et al., 2000). Subsequently, second generation TSWV-resistant cultivars with greater resistant levels were developed and released such as Georgia-06G (Branch, 2007), Tifguard (Holbrook et al., 2008), Georganic (Holbrook & Culbreath, 2008). Georgia-06G, a second-generation peanut cultivar with high yielding and a high level of resistance to TSWV, has been the predominant cultivar grown in the Southeast since 2010 (Beasley J. P., 2011, Monfort, 2015). Recently, third-generation TSWV-resistant cultivars, which are believed to be more resistant to TSWV than second-generation cultivars, have become available such as Georgia-10T (Branch & Culbreath, 2011), Georgia-12Y (Branch, 2013). All the peanut cultivars that have been developed via breeding so far possess varying levels of resistance to TSWV, but none of them has completely resistance to TSWV (Culbreath et al., 1997). Runner type peanut accounts for 80 percent of the peanuts grown in the United States., whereas, Virginia type peanut accounts for 15 percent of total U.S. production (NPB, 2014).

Gregory is a Virginia type peanuts grown in significant percentage is categorized as moderately resistant to TSWV, as it exhibited lower incidence of spotted wilt than other susceptible Virginia type peanuts (Isleib et al., 1999).

Cultivated peanut, *Arachis hypogaea* L., is a tetraploid (2n=4x=40) species. Low genetic diversity makes this tetraploid peanut very vulnerable to plant pathogens (Ratnaparkhe et al., 2011). Breeding attempts have been made to introgress TSWV resistance from wild species into cultivated peanut. One such example is *Arachis diogoi* that possesses substantial resistance to TSWV (Lyerly et al., 2002). Despite identifying resistance, the underlying mechanism of resistance to TSWV in either tertraploid or diploid peanut still remain unidentified (Culbreath et al., 2003). In the case of other crops such as pepper (*Capsicum annuum* L.) and tomato (*Solanum lycopersicon* L.), the mechanisms of TSWV resistance have been identified. Single dominant genes such as *Sw-5* in tomato and *Tsw* in pepper have been demonstrated to trigger hypersensitive reaction (HR) after TSWV infection leading to local lesions and avoidance of systemic infection (Hallwass et al., 2014, Moury et al., 1997, Ngoc Huy et al., 2013). In peanut, no hypersensitive response was documented, and no resistance conferring genes have been identified either. Typically cultivars that possess field-resistance to TSWV get symptomatically infected (Shrestha et al., 2013). Based on the results from current studies, it is suggested that field resistance to TSWV in peanut is mainly induced against TSWV infection, and the resistance trait is possibly tolerance rather than true resistance (Shrestha et al., 2013). Not much is known about the mechanism of resistance in wild species as well. There are noticeable differences in cultivar susceptibility, however, that is not believed to be due to differential preference by thrips vectors; thrips populations on resistant genotypes do not appear to be significantly lower than those on susceptible ones (Culbreath et al., 2000, Culbreath et al., 1992,

Culbreath et al., 1996b). Currently, no significant resistance to thrips in available genotypes has been documented. It is not clear if there is any resistance to thrips in wild diploid species as well.

In this study, we attempted to examine the impact of TSWV resistant genotypes on TSWV transmission by thrips, so the efficiency of TSWV transmission in a number of resistant and susceptible tetraploid and diplpoid, and runner and Virginia, genotypes was assessed. We further examined TSWV acquisition ability of the vector *F. fusca* from all selected genotypes to determine the ability of genotypes to serve as inoculum sources. To act as inoculum sources, host plants must be able to support reproduction of the vector species (Culbreath et al., 2003). Therefore, the effect of TSWV resistant genotypes on reproduction and development of TSWV's predominant vector *F. fusca* was also evaluated.

Materials and Methods

Peanut Plants. Peanut genotype Georgia Green was used for laboratory thrips colony maintenance. Four runner-type peanut genotypes Georgia-12Y, Georgia-10T, FloRun '107', and SunOleic 97R and two Virginia-type peanut genotypes Gregory, Gregory x *Arachis diogoi* (GKP 10602) were selected and used for all experiments. GKP10602 accession *Arachis diogoi* is a diploid wild type species, a close relative of the cultivated peanut that possesses TSWV resistance, was also included in some experiments (Table 3.1). Two sets of experiments were conducted i.e., one with Runner-type peanuts and the other one with Virginia-type peanuts. In Runner group, SunOleic 97R –a susceptible cultivar released in 1997 (Gorbet & Knauft, 2000) and FloRun '107' –a TSWV-resistant cultivar released in 2010 (Tillman & Gorbet, 2015) by University of Florida Research Station, FL, and two other resistant cultivars Georgia-10T and Georgia-12Y released in 2010 (Branch & Culbreath, 2011) and in 2012 (Branch, 2013),

respectively by University of Georgia's Coastal Plain Experimental Station in Tifton, GA were selected. In Virginia-type peanuts, Gregory –a moderately TSWV-resistant released in 1997 (Isleib et al., 1999); and the interspecific hybrid derived from Gregory x *Arachis diogoi* (GKP 10602) were selected. A diogoi was also included along with the Virginia group. Seeds were pre-germinated in moistened paper towels and incubated in a growth chamber (Thermo scientific, Dubuque, IA) at 25-30°C for two days. Budded peanut seeds were transplanted into 4-inch diameter plastic pots (Hummert International, St. Louis, MO) with commercial potting mix, Sunshine mix (LT5 Sunshine® mix, Sun Gro® Horticulture Industries, Bellevue, WA). Peanut plants were maintained in thrips-proof cages (47.5 cm^3) (Megaview Science Co., Taichung, Taiwan) in a greenhouse keeping the temperature at 25-30°C and 80-90% relative humidity (RH) with a 14:10 (L:D) h photoperiod. One to two weeks old peanut plants were used for all experiments.

Maintenance of non-viruliferous *F. fusca*. Non-viruliferous thrips from a laboratory colony of *Frankliniella fusca* established in 2009 were used for all experiments. Thrips were originally collected from peanut blooms in Tifton, GA. Leaflets of non-infected Georgia Green peanut planted in greenhouse were used for thrips rearing. Thrips were reared in small petri dishes (60mm x 15mm Polystyrene) (Becton, Dickinson and Company, Falcon™ Labware, Franklin Lakes, NJ), and peanut leaflets were placed on a moistened round cotton pad (Swippers Supreme cotton round, Cleveland, Ohio) in each petri dish. Ten female thrips were released in a petri dish and allowed to lay eggs on fresh peanut leaflets for two to three days, followed by removal of adult thrips from the dishes. Fresh leaflets and water were added into the cage every two to three days. Thrips were reared from eggs to adults in about two weeks. The colony was maintained in

a growth chamber (Percival Scientific, Inc., Perry, IA) at 29°C with a photoperiod of 14:10 (L:D) h. Only adult female thrips up to 2 days old were used for all experiments.

Maintenance of potentially viruliferous *F. fusca*. Similar to non-viruliferus thrips colony, a colony of potentially viruliferous thrips was maintained on TSWV-infected Georgia Green leaflets. During peanut growing season, TSWV-infected Georgia Green foliage were collected from peanut fields in Tifton, GA; while leaflets from mechanically inoculated Georgia Green peanut plants were used in the non-growing season. Infected Georgia Green peanut plants were obtained from mechanical inoculation followed the standard protocol provided by Mandal et al. (2001). After inoculation, plants were maintained in a greenhouse as described previously. Thrips were reared in small petri dishes in the same way as described above, but maintained in a separate growth chamber (Percival Scientific, Inc., Perry, IA) with the same settings. Thrips reared for an entire generation on TSWV-infected leaflets were considered potentially viruliferous. For all experiments only up to 2 days old adult female thrips were used.

Both TSWV infected peanut plants from field or greenhouse were tested by double antibody sandwich enzyme linked immunosorbent assay (DAS-ELISA) (Clark & Adams, 1977) to confirm their infection status.

TSWV detection in plants by double antibody sandwich enzyme linked immunosorbent assay (DAS-ELISA). Fresh leaf tissue (approximately 0.1 g) was obtained from each experimental plant and used for DAS-ELISA. The assay was performed in a 96 well microtiter plate (Maxisorp, Nunc, Rochester, NY). Along with samples, two positive controls (TSWV infected peanut leaf tissues) and two negative controls (non-infected peanut leaf tissues) were included in each plate. Primary antibody (anti-TSWV IgG, monoclonal nucleocapsid protein (N)) was used at a dilution ratio of 1:200 and the secondary antibody (anti-TSWV IgG

conjugated with alkaline phosphatase) was also used at a 1:200 dilution ratio (Agdia®, Elkhart, IN). Incubation and washing steps were followed as per the manufacturer's instructions. Final absorbance values were measured at 405 nm in a photometer 1 h after substrate added (Model Elx 800, Bio-Tek®, Kocherwaldstr, Germany).

Thrips mediated inoculation and TSWV-infected peanut plants. Selected genotypes of peanut plants were all inoculated by potentially viruliferous thrips from the laboratory colony. One-week old peanut plants were maintained in thrips-proof cages in the greenhouse as described previously. Potentially viruliferous thrips were released into those cages for inoculation. Three weeks after thrips inoculation,,TSWV infection status in thrips-inoculated plants was assessed by DAS-ELISA as previously described. TSWV infected plants were subsequently generated by thrips-mediated inoculation, and leaflets with obvious TSWV symptoms were used for experiments.

TSWV transmission to resistant and susceptible genotypes. A total of seven peanut genotypes, including six TSWV-resistant genotypes (Georgia-12Y, Georgia-10T, FloRun '107', Gregory, Gregory x A. diogoi, A. diogoi) and one susceptible genotype SunOleic 97R, were evaluated for TSWV susceptibility. All genotypes were divided into two groups, which were the Runner-type and the Virginia-type; experiments and analyses were done for both groups independently. Ten plants (up to one-week old) of each genotype were used in the experiment, and the experiment was repeated once (N = 20 plants for each genotype). Ten potentially viruliferous *F. fusca* female adults reared on TSWV-infected leaflets were colleted into a 1.5ml microcentrifuge tube (Fisher Scientific, Pittsburgh, PA) by a paintbrush (10/0 The fine touch ® Round, Oklahoma City, OK). Thrips were subsequently released on peanut plants by placing the microcentrifuge tubes at the bottom of the plants (one tube containing ten potentially viruliferous

thrips per plant) that have been dusted with approximately 0.05 g of pine (*Pinus taeda* L.) pollen grains on foliage. Each plant with ten thrips released from the microcentrifuge tube were enclosed in a cylindrical Mylar® film (Grafix®, Cleveland, PA) cage (πr^2h=3.14 x 16 x 39 cm^3) with a copper mesh top (mesh pore size-170 microns) (TWP®, Berkeley, CA). Plants were maintained in thrips-proof cages in the greenhouse for two to three weeks, followed by assessing TSWV infection status with DAS-ELISA procedure as described previously.

Statistical analyses were performed to identify the differences of TSWV infection percentages among selected genotypes. Data were pooled from all repeats of the experiment and using experiment as the blocking variable. TSWV infection was regarded as a binomial response (positive or negative), and differences among treatments were estimated by logistic regression analyses using the GENMOD procedure with logit link function in SAS (SAS Enterprise 4.2, SAS Institute, Cary, NC). Treatments (genotypes) were considered as fixed effects and replications were considered as random effects. The statistical significance of differences between treatment pairs was estimated using pairwise contrasts at α= 0.05.

TSWV loads in TSWV-resistant and susceptible peanut genotypes. Virus copy numbers loaded in peanut plants were estimated using real time quantitative reverse transcriptase polymerase chain reaction (qRT-PCR). Leaf samples from plants that tested TSWV positive by DAS-ELISA. TSWV N-gene was partially amplified by qRT-PCR. Symptomatic leaflet tissues (approximately 0.1g) were collected from top one-third above ground section of each infected plant for RNA extraction. Total RNA was extracted by using RNeasy plant mini kit (Qiagen®, Valencia, CA) as per manufacturer's instructions. The extracted RNA products were subsequently used for complementary DNA (cDNA) synthesis. Complementary DNA was synthesized using the Go-Script™ reverse transcription system (Promega corporation, Madison,

WI) following manufacturer's instructions. Oligo (dT) (5 µM) was used as primers for cDNA synthesis. Three µl of RNA, 1 µl of Oligo (dT), and 1 µl of nuclease-free water were mixed and preheated to 70°C for 5 min in a thermocycler (Bio-Rad, Hercules, CA). Next, 4 µl of Go-Script reaction buffer, 2 µl of $Mgcl^2$, 1 µl of deoxynucleotide triphosphates (dNTP), 0.5 µl of RNase inhibitor, 1 µl of reverse transcriptase, and 6.5 µl of nuclease-free water, were added to the reaction mix to a total volume of 20 µl. The reaction mix was placed in the same thermocycler (Bio-Rad, Hercules, CA) at 25°C for 5 min, 42°C for 1 h, and at 70°C for 15 min. Obtained cDNA was used as templates for qRT-PCR.

Quantitative RT-PCR was conducted using TSWV N-gene specific primers. The forward and reverse primers, 5′GCTTCCCACCCTTTGATTC3′ and 5′ATAGCCAAGACAACACTGATC3', respectively, were used (Rotenberg et al., 2009). The reaction mix for qRT-PCR comprised 1 µl of synthesized cDNA, 12.5 µl of GoTaq qPCR MasterMix (Promega Corporation, Madison, WI), 0.5 µl of each of the forward and reverse primers, and the final volume of reaction mix was brought to 25 µl by adding nuclease-free water. The reaction was run at 95° C for 2 min, followed by 40 cycles of a three-step program including 95°C for 15 s, 55°C for 1 min, and 72°C for 20 min in a Realplex Mastercycler (Eppendorf, Hamburg, Germany). Melting curve analysis was applied to the reaction mix right after the final PCR cycle by incubating the reaction at 95°C for 15 s, 60°C for 15 s and then increasing the temperature by 0.5°C per min for 20 min. Each sample was duplicated per PCR run. Also, negative (non-infected peanut leaf tissue) and positive (TSWV infected peanut leaf tissue) controls were included in each PCR run as well as blank controls containing master mix without cDNA and water alone.

Linearized plasmids with N-gene inserted were used as external standards. ~800 bp amplicons produced by RT-PCR with TSWV N-gene specific primers (Jain et al., 1998) were used for

cloning. Plasmids with N-gene inserts were obtained by TOPA cloning following manufacturer's instructions (Invitrogen, Carlsbad, CA). Plasmids with cloned N-gene inserts were purified using GeneJet, Plasmid Miniprep Kit (Fermentas Inc. Glen Burnie, MD), and followed by restriction enzyme (HindIII) digestion for linearization. Linearized plasmids were purified using Qiaquick PCR purification kit (Qiagen®, Valencia, CA); restriction digestion and the presence of the insert were confirmed by gel electrophoresis analysis (0.1% gel). Linearized plasmids were quantified using Nanodrop (Thermo Scientific, Wilmington, DE) and virus copy numbers were estimated using a formula based on the assumption that the average weight of a base pair (bp) is 650 Daltons (URI Genomics & Sequencing Center ' http://www.uri.edu/research/gsc/resources/cndna.html '). Subsequently, nine steps of serial dilution procedure with dilution factor of 10 were applied to plasmids with TSWV N-gene inserts, which were used as external standards. A standard curve was generated based on the threshold cycle (Ct) of each standard. TSWV copy numbers in leaf tissue samples were estimated by using the standard curve (Rotenberg et al., 2009, Shrestha et al., 2013, Sundaraj et al., 2014).

Copy numbers from samples were subjected to Generalized linear mixed models using PROC GLIMMIX in SAS (SAS Enterprise 4.2, SAS Institute, Cary, NC) for evaluation of statistical differences in TSWV N-gene copy numbers among genotypes. Treatments (genotypes) were considered as fixed effects and replications were considered as random effects. Least squares means were used to identify statistically significant differences in N-gene copy numbers among genotypes at α=0.05. Tukey-Kramer Grouping was used as an adjustment for multiple comparisons at α=0.05.

TSWV acquisition by *F. fusca* from TSWV-infected resistant and susceptible genotypes. Experiments were conducted using Munger cages (11.43 x 8.89 x 1.77 cm^3) (Munger 1942). TSWV susceptible genotype (SunOleic 97R) and resistant genotypes (Georgia-10T, Georgia-12Y, Gregory, and Gregory x *A.diogoi*) were used for the acquisition experiment. Non-viruliferous thrips from the lab colony were released in each Munger cage with respective TSWV-infected peanut leaflets from thrips-mediated transmission. Thrips were allowed to lay eggs for 48h and were removed from the Munger cages. Thrips were maintained on TSWV-infected leaflets of each genotype independently in separate cages for an entire generation (adult to adult). The next generation potentially viruliferous adult female thrips reared on each genotype (up to 2d old) were subjected to qRT-PCR to examine TSWV infection status and subsequently determine their ability to acquire TSWV from different peanut genotypes. Five individual potentially viruliferous female thrips (the next generation) were pooled to serve as one sample. Ten samples were collected from cages for each genotype and subjected to qRT-PCR (N=50 thrips for each genotype). Total RNA extraction, cDNA synthesis, and qRT-PCR were all conducted as described previously.

Data were pooled from all repeats of the experiment and analyzed by generalized mixed linear models using the GLIMMIX procedure in SAS (SAS Enterprise 4.2, SAS Institute, Cary, NC). Least squares means were used to determine statistically significant differences of TSWV N-gene copy numbers in adult thrips reared on selected peanut genotypes at α=0.05. Treatments (genotypes) were considered as fixed effects and replications were considered as random effects. Tukey-Kramer Grouping was used as an adjustment for multiple comparisons at α=0.05.

Impact of TSWV-resistant genotypes on thrips reproduction and development. Leaflets from all selected peanut genotypes were used for thrips biology fitness assays. Ten Munger

cages were set up for each genotype with two non-infected leaflets of respective genotypes (two weeks old). The experiment was repeated once (N=20 cages for each genotype). Cages were maintained in a growth chamber (Thermo scientific, Dubuque, IA) at 25- 30°C with a photoperiod of 14:10 (L:D) h. Ten non-viruliferous female adult thrips were released in each Munger cage and allowed to lay eggs for three successive days. After thrips were removed from the Munger cages, cages were monitored daily under a compound microscope (MEIJI TECHNO, Santa Clara, CA) and the date the first newly hatched larvae appeared in each cage was recorded. The number of adults emerging from every cage was recorded at 24 h interval and removed. The cages were monitored until there were no more adults emerging from the same generation. The median developmental time required for thrips to grow from larvae to adult was estimated on each genotype.

Data were pooled from all repeats of the experiment and using experiment as blocking variable. All data were subjected to generalized mixed linear models using the GLIMMIX procedure in SAS to analyze differences in the number of adults produced by single thrips reared on various peanut genotypes. Treatments (genotypes) were assigned as fixed effects while replications were considered as random effects. Tukey-Kramer Grouping was used as an adjustment for multiple comparisons at $\alpha=0.05$. The results of the median developmental time were subjected to median one-way analysis ($\alpha=0.05$) using NPAR1WAY procedure to determine significant differences between treatments and repeats.

Results

TSWV transmission to resistant and susceptible genotypes. Overall, thrips-mediated TSWV transmission resulted in incidence of spotted wilt in both susceptible and resistant

genotypes. Typical symptoms were observed in all tested Runner-type peanut genotypes, except for FloRun '107' (Fig. 5.1); symptoms were also observed in Virginia-type peanuts Gregory and Gregory x *A.diogoi*, but not in *A. diogoi* (Fig. 5.2). Symptoms appeared in about 14-20 days post inoculation. Initial symptoms such as chlorotic spots were usually first observed in newly grown terminals. As the disease progressed, severe chlorotic and necrotic spots were found on mottled leaflets, followed by droopy leaves quickly dropping off. Typical concentric ring spots were also observed (Fig. 5.2).

In the Runner-type peanut group, TSWV infection percentage did not vary with genotypes (df= 3, 76; χ^2=5.91; *P*=0.1161). The incidence of TSWV infection in resistant genotypes Georgia-10T (40±14.14%), Georgia-12Y (50±7.07%), and Florun '107' (35±10.61%) were not significantly different from susceptible genotype SunOleic 97R (70±7.07%). In the Virginia-type peanut group, incidence of TSWV infection varied with genotypes (df= 2, 57; χ^2=11.71; P>χ^2=0.0029). The incidence of TSWV infection in Gregory (75±3.54%) and Gregory x *A. diogoi* (65±3.54%) was significantly greater than the incidence in *A. diogoi* (25±10.6%)(Table 5.2). The incidence of TSWV infection in the runner group (df= 1, 78; χ^2=0.49; *P*=0.4858) and in the Virginia group (df=1, 58; χ^2=0.08; *P*=0.7732) did not vary with repeats of the experiment.

TSWV copies in TSWV-resistant and susceptible peanut genotypes. In the Runner-type peanut group, TSWV copy numbers did not vary with genotypes (df= 3, 35; F=2.82; P=0.0530). The amount of TSWV copy numbers accumulated in plants was not affected by genotypes (Fig. 5.3). In the Virginia-type peanut group, TSWV copies in the plants varied with genotypes (df= 2, 29; F=4.19; P=0.0253). TSWV N-gene copy numbers were significantly greater in Gregory than in Gregory x *A. diogoi* and *A. diogoi* (Fig. 5.4).

TSWV acquisition by *F. fusca* from TSWV-infected resistant and susceptible genotypes and TSWV loads in potentially viruliferous *F. fusca*.

TSWV copies in viruliferous *F. fusca* emerging from infected foliage did not vary with genotypes in the Runner-type peanut group (df=2, 18; F=2.63; P=0.0998). TSWV N-gene copy numbers accumulated in viruliferous thrips was not affected by the genotypes with different TSWV susceptibility (Fig. 5.5). In the Virginia-type peanut group, TSWV N-gene copy numbers in viruliferous thrips varied with genotypes (df=1, 9; F=5.43; P=0.0447). TSWV load in viruliferous thrips reared on Gregory x *A. diogoi* was significantly greater than thrips reared on Gregory (Fig. 5.6).

Impacts of TSWV-resistant genotypes on thrips reproduction and development. In the Runner-type peanut group, the number of adults produced by one female thrips varied with genotypes (df=3, 75; F=10.31; P<0.0001). The number of adult thrips emerged from Florun'107' (8.52±0.90) was significantly greater than Georgia-10T (5.48±0.55), SunOleic 97R (4.29±0.55), and Georgia-12Y (3.8±0.58) (Fig.5.7). There was statistical difference between the repeats of the experiment in the number of offspring produced per thrips (df=1, 66; F=14; P=0.0004). In the Virginia-type peanut group, the number of adults emerged also varied with peanut genotypes (df=2, 56; F=66.10; P<0.0001). The numbers of emerging adults on Gregory (11.55±0.94) and Gregory x *A. diogoi* (9.65±0.73) were significantly greater than thrips reared on *A. diogoi* (0.75±0.14) (Fig. 5.8). The number of adults emerged varied with the repeats of the experiment in this group as well (df=1, 47; F=9.26; P=0.0038).

The median developmental time required for thrips to grow from larvae to adults did not vary with genotypes (df= 3; χ^2=1.8405; P>χ^2=0.6062) in the Runner-type peanut group. Thrips reared on SunOleic 97R required 10 days to develop from larvae to adult, while it only required

9 days for thrips reared on Georgia-10T, Georgia-12Y, and Florun'107' in average (Table 5.3). The median developmental time in the repeats of the experiment varied (df=1, 78; F=34.8586; P<0.0001). In the Virginia-type peanut group, the median developmental time varied with genotypes (df= 2; χ^2=29.4655; P>χ^2 <0.0001). *F. fusca* reared on *A. diogoi* required longer time (10 days) to grow from larvae to adults when compared with Gregory (8 days) and Gregory x *A. diogoi* (8 days) (Table 5.4). The results of the median developmental time were consistent with repeats of the experiment (df=1, 55; F=0.0625; P=0.8035).

Discussion

Both susceptible and resistant peanut genotypes in our study were susceptible to *Tomato spotted wilt virus* (TSWV). All the selected genotypes showed typical spotted wilt symptoms after thrips inoculation, except for FloRun '107' and the wild diploid species *Arachis diogoi*. Symptoms observed on the leaflets of Georgoa-10T, one of the resistant genotypes, was milder than the other susceptible and resistant genotypes in this study. Comparing the symptoms observed in the Runner-type peanut and the Virginia-type peanut, there was not any noticeable difference. Genotypes in both groups showing typical concentric ring spots and mottled pattern on leaf surface.

TSWV infection percentages were not suppressed in newer Runner-type resistant genotypes, which were inconsistent to previous study. Shrestha et al. (2013) documented that some second-generation Runner-type peanut genotypes resistant to TSWV such as Georgia-06G and Tifguard had lower infection rates when compared with susceptible genotype in thrips-mediated transmission experiments. In Virginia-type peanut, a wild species *Arachis diogoi* is used in breeding programs for introgressing TSWV resistance. *A. diogoi* is a diploid relative to the

cultivated peanut *A. hypogaea*. It is documented to possess high levels of resistance to multiple diseases caused by fungi and viruses that commonly occur in cultivated peanut as well as insect pests such as thrips and aphids (Rao et al., 2003). Lyerly et al. (2002) documented that *A. diogoi* (accession GKP10602) is highly resistant to TSWV based on TSWV symptoms evaluation after mechanical inoculation. Our study corroborated the previous findings. TSWV incidence was significantly reduced in *A. diogoi* when compared with Gregory. Overall across the two peanut groups, *A. diogoi* exhibited the lowest TSWV infection percentage while Gregory had the highest incidence of infection. Being the only susceptible genotype in the runner group,

Under field condition, TSWV resistant peanut cultivars usually had less severity of spotted wilt disease and higher yields than susceptible cultivars when TSWV epidemic occurred (Culbreath et al., 2012, Culbreath et al., 2008, Culbreath et al., 2013, Tillman et al., 2006). The underlying mechanism of field resistance to TSWV in peanut cultivars remains unknown. In thrips-mediated transmission experiment, TSWV resistant genotypes were under high thrips pressure as opposed to plants in the field. It is suggested that under high pressure of thrips and TSWV, resistant peanut genotypes can still suffer from severe TSWV infection just like susceptible genotypes. In other words, TSWV field resistant genotypes may be able to tolerate the amount of TSWV inoculated by ten potentially viruliferous thrips simultaneously. It is suggested that TSWV resistance in peanut cultivars is tolerance rather than true resistance (Shrestha et al., 2013), and our results concur with this point of view. In contrast to peanut, the resistance to TSWV in tomato and pepper has been found to be manipulated by single dominant genes, namely *Sw-1* and *Tsw* in tomato and pepper, respectively (Hallwass et al., 2014, Moury et al., 1997). Resistance genes in tomato and pepper both induce hypersensitive reaction (HR) at the inoculation site leading to necrotic local lesions and the abscission of inoculation part, and

subsequently prevent the movement of TSWV (Hallwass et al., 2014, Moury et al., 1997, Ngoc Huy et al., 2013). Evaluations of TSWV resistance levels in peanut genotypes were monitored by mechanical inoculation; local lesion symptoms were used to classify the degree of resistance as opposed to systemic infection (Lyerly et al., 2002, Mandal et al., 2006, Mandal et al., 2002). Genotypes showing localized symptoms without systemic infection appeared after mechanical inoculation of field resistant genotypes; it is suggested that the localized infection of resistant genotype was due to restriction of long distance movement of TSWV at the inoculation site (Lyerly et al., 2002, Mandal et al., 2002). Similar assumption was also documented in genotypes resistant to *Peanut bud necrosis virus*, another Tospovirus (Reddy et al., 2000). Nonetheless, local lesion is not a typical symptom of TSWV infection in peanut under natural conditions (Culbreath et al., 2003).

A. diogoi was highly resistant to TSWV(accession GKP10602) when examined by mechanical inoculation; by conducting thrips-mediated transmission experiment, we further confirmed the high resistance level of *A. diogoi*. Efforts have been put upon isolation of expressed resistance gene analogs (RGAs) from peanut based on identified resistance genes (R gene) conferring resistance to various diseases in different plant species (Bertioli et al., 2003, Ratnaparkhe et al., 2011, Yuksel et al., 2005). Numbers of RGAs from peanut expressed sequence tags (ESTs) have also been identified (Liu et al., 2013). Intensive genome mapping research could soon help identify peanut resistance genes and decipher the role in conferring resistance.

Quantification of TSWV loads in resistant and susceptible genotypes showed that TSWV N-gene copy numbers in the infected peanut plants were affected by genotypes; however, there was no certain correlation found between TSWV loads and susceptibility of the genotypes to TSWV.

Surprisingly, TSWV copies load in the susceptible genotype SunOleic 97R did not significantly differ from any of the resistant genotypes. In another study, TSWV loads in several second generation resistant cultivars were significantly greater than a susceptible genotype, Georgia Green (Shrestha et al. 2013). In the Virginia-type genotypes we found that the accumulation of TSWV loaded in plants was significantly reduced in Gregory x *A. diogoi* and *A. diogoi* when compared with Gregory. It is confirmed that *A. diogoi* is highly resistant to TSWV; however, it is not immune to TSWV. Although the incidence of TSWV was not significantly suppressed, TSWV copies were greatly reduced in Gregory x *A. diogoi*. Suppression TSWV replication and accumulation in the host might account for the resistance. Peanut plants with less accumulation of TSWV will potentially be a bad inoculum and further affect TSWV epidemics. Therefore, it is assumed that incorporation of resistance genes from wild species *A. diogoi* with existing peanut cultivar enhanced the resistant level to TSWV; however, the degree of enhancement is somehow limited in our study. Further transmission studies in the field will be helpful for determining the resistance level of Gregory x *A. diogoi*. Studies have attempted to incorporate TSWV resistant genes from wild species into the pedigree of cultivated peanut breading lines, but failed to prevent systemic infection. It is assumed that the genes conferring TSWV resistance might be lost due to gene segregation occurring during self-pollination without selection (Lyerly et al., 2002) Comparing TSWV copies between Runner-type and Virginia-type peanuts, the accumulation of TSWV copies was in general higher in Runner-type peanut genotypes. It is suggested that TSWV incidence and TSWV copies in the host plant were two independent parameters that might not be correlated.

F. fusca acquired TSWV from infected peanut plants in both susceptible and resistant genotypes. Shrestha et al. (2013) also documented that TSWV acquisition and replication in *F.*

fusca in various peanut genotypes despite their susceptibility status. In this study we not only determined the ability of thrips to acquire TSWV from different genotype but also quantified TSWV copies within potentially viruliferous thrips. Viruliferous thrips reared on Georgia-10T tended to harbor more TSWV copies than other genotypes in the group, however, the variation of TSWV loads in thrips samples emerged from Georgia-10T was in a large range. TSWV successfully acquired by *F. fusca* reared on infected Gregory and Gregory x *A. diogoi*, which indicated that both of them are competent TSWV inoculum. Unexpectedly, TSWV N-gene copies acquired by thrips from infected Gregory x *A. diogoi* were greater than acquired from infected Gregory. The difference of TSWV copies observed in viruliferous thrips after acquired TSWV from infected plants of various genotypes can be related to two factors; one is the amount of TSWV harbored in the plant inoculum, and the other one is the efficiency of acquisition and TSWV accumulation in thrips. Based on our results from thrips-mediated transmission experiments, TSWV accumulation varied with peanut genotypes. It is speculated whether the amount of TSWV in the peanut genotype affected the TSWV copies acquired by thrips, and subsequently the amount of TSWV copies in viruliferous thrips. In either Runner-type peanut or Virginia-type peanut group, the amount of TSWV copies in plants were not correlated with the amount of TSWV loads in thrips reared on the respective plant. Nevertheless, when comparing TSWV copies harbored in thrips reared on peanut genotypes in either Runner-type or Virginia-type, similar results were observed as the TSWV accumulation in infected peanut genotypes that thrips reared on Runner-type peanuts harbored more TSWV copies than thrips reared on Virginia-type peanuts. It is indicated that the amount of TSWV copies in the infected inoculum affects the subsequent TSWV amount accumulated in thrips.

Evaluation of impacts of peanut genotypes on thrips vectors can provide crucial information to determine if a certain genotype is a viable host plant for the vectors. The host plant will be a dead-end host if the plant cannot support reproduction of the vector species. Several field trials attempted to investigate the impact of peanut genotypes on *F. fusca* and *F. occidentalis* (Culbreath et al., 2000, Culbreath et al., 1992, Culbreath et al., 1996b). In those studies, the numbers of thrips were not affected by the peanut genotypes; in other words, there was no correlation between thrips population and spotted wilt incidence among genotypes (Brown et al., 1996). Evaluation of the impact of selected Runner-type as well as Virginia-type peanut genotypes on reproductive efficacy of *F. fusca* was conducted in this study. In Runner-type peanuts, similar numbers of offspring emerged from resistant and susceptible genotypes per each female *F. fusca* released have been observed, except for FloRun '107'. The higher net reproduction rate of *F. fusca* reared on FloRun '107', a TSWV resistant genotype, is an unexpected result. In the previous study, *F. fusca* reared on the TSWV susceptible genotype had higher net reproduction rate than resistant genotypes (Shrestha et al., 2013) In this study, the development of *F. fusca* did not vary with the selected Runner-type peanut genotypes. The cause of higher reproduction rate of *F. fusca* on FloRun '107' is unknown; it is possibly related to the nutritional composition in this high-oleic peanut genotype. Studies have shown that the nutrition composition of the host plant species had largely affected the population of *F. occidentalis* (Brown et al., 2002). Other than genotype differences, the physiological traits of peanut foliage such as the thickness and wax content could affect fitness affecting traits such as oviposition preference (Bergh & Le Blanc, 1997). In Virginia-type peanuts, *A. diogoi was* not a suitable host of *F. fusca* when compared with cultivated peanut genotypes. The number of offspring produced per female *F. fusca* released was significantly lower and the median developmental time was

longer when reared on the leaflets of *A. diogoi* compared with reared on Gregory and Greogry x *A. diogoi*. Thrips resistance in *A. diogoi* was previously speculated (Rao et al., 2003). *F. fusca* also produced slightly fewer offspring when reared on Gregory x *A. diogoi* than on Gregory, but the median developmental time was the same. In our case, the net reproductive rate and median developmental time of *F. fusca* indirectly support the hypothesis that the field resistance of peanut genotypes is not related to thrips populations or the preference of hosts in thrips.

In conclusion, thrips-mediated TSWV transmission resulted in mostly no difference in the incidence of spotted between susceptible and resistant peanut genotypes; only the peanut relative wild species *A. diogoi* was highly resistant to TSWV. TSWV can infect all the genotypes tested in this study. The amount of virus loads slightly varied with peanut genotypes, especially in genotypes having A. diogoi in their pedigree. *A. diogoi* again was the most resistant genotype with significantly lower TSWV copy numbers in our study. *F. fusca* acquired TSWV from most of the genotypes in this study, including both susceptible and resistance genotypes. Thrips biological traits evaluation did not show any specific trend in line with TSWV resistance, except for *A. diogoi*. Development of new cultivars with greater field resistance to TSWV is one of the major research emphases that have the most potential to improve spotted wilt disease management in peanut (Culbreath et al., 2003). In cultivated peanut, high level of resistance to TSWV has not been found; and the sources of resistance reported so far are limited (Lyerly et al., 2002, Rao et al., 2003). Wild species relatives provide important source of disease resistance genes, especially when the genetic variations in the cultivated peanut (*Arachis hypogaea* L.) are limited (Rao et al., 2003, Ratnaparkhe et al., 2011).

Tables

Table 5.1. Selected peanut genotypes evaluated in this study.

Peanut genotypes			
Runner-type	**Registration (released)**	**Development unit**	**Characteristics**
SunOleic 97R	1997 (Reg. no. CV-65, PI 596800) (Gorbet & Knauft, 2000)	University of Florida, Agricultural Experiment Station	Favorable oil chemistry (high-oleic) Longer shelf-life Susceptible to *Tomato spotted wilt virus* (TSWV)
Georgia-10T	2010 (Reg. No. CV-113, PI 660315) (Branch & Culbreath, 2011)	University of Georgia, Coastal Plain Experiment Station	High-yielding and large-seeded Resistant to TSWV
Georgia-12Y	2012 (Reg. no. CV-119, PI 667552) (Branch, 2013)	University of Georgia, Coastal Plain Experiment Station	High-yielding and medium-seeded Resistant to TSWV and white mold or stem rot
FloRun '107'	2010 (Reg. No. CV-127, PI 663993) (Tillman & Gorbet, 2015)	University of Florida, Agricultural Experiment Station	Improved oil chemistry (high-oleic) Longer shelf-life Resistant to late leaf spot, white mold, and TSWV
Virginia-type	**Registration (released)**	**Development unit**	**Characteristics**
Gregory	1997 (Reg. no. CV-62, PI 608666) (Isleib et al., 1999)	North Carolina Agricultural Research Service (NCARS)	Large-seeded Resistant to several diseases common to the Virginia-Carolina region, including TSWV
Gregory x *A. diogoi*			
Arachis diogoi	Accession GKP 10602 (Wild species)		Resistant to peanut diseases caused by various fungi, viruses, and nematodes (Rao et al., 2003)

Table 5.2. Pair-wise comparison of TSWV infection rate in Virginia-type genotypes.

Pair-wise comparison			
	df	$\times^2$	P>$\times^2$
Gregory vs. Gregory x A. diogoi	1, 18	0.48	0.4891
Gregory vs. A.diogoi	1, 18	10.48	0.0012*
Gregory x A. diogoi vs. A.diogoi	1, 18	6.67	0.0098*

*Results indicated that there was significant difference between genotypes in logistic regression analysis at α=0.05.

Table 5.3. Median developmental time (the value range in parenthesis) in days required for *Frankliniella fusca* to development from larvae to adult on the leaflets of Tomato spotted wilt virus-resistant and susceptible peanut genotypes.

Peanut genotypes	N	Median developmental time (range) (d)	Sum of scores[z]	Expected under Ho[z]	Std Dev under Ho[z]	Mean score[z]
SunOleic 97R[x]	20	10 (8-11)	12.5	10	1.732	0.625
Georgia-10T[y]	20	9 (8-11)	8.9	10	1.732	0.445
Georgia-12Y[y]	20	9 (7-12)	8.7	10	1.732	0.435
FloRun '107'[y]	20	9 (7-14)	9.9	10	1.732	0.495
Median One-Way Analysis						
χ^2	2.29					
Df	3					
P	0.5144					

[x] TSWV-susceptible peanut genotype.

[y] TSWV-resistant peanut genotypes.

[z] Sum of scores for median one-way analysis, sum of scores expected under null hypothesis that developmental time in all genotype is not different, standard deviation from null hypothesis and mean scores.

Table 5.4. Median developmental time (the value range in parenthesis) in days required for *Frankliniella fusca* to development from larvae to adult on the leaflets of Tomato spotted wilt virus-resistant genotypes incorporating resistance source from wild species (*A. diogoi*).

Peanut genotypes	N	Median developmental time (range) (d)	Sum of scores[x]	Expected under Ho[x]	Std Dev under Ho[x]	Mean score[x]
Gregory	20	8 (8-9)	2	9.825	1.817	0.1
Gregory x A.diogoi	20	8 (8-10)	9	9.825	1.817	0.45
A. diogoi	17	10 (9-12)	17	8.351	1.742	1
Median One-Way Analysis						
χ^2	29.4655					
Df	2					
P	<0.0001*					

[x] Sum of scores for median one-way analysis, sum of scores expected under null hypothesis that developmental time in all genotype is not different, standard deviation from null hypothesis and mean scores.

*Results indicated that there was significant difference between genotypes in median developmental time of *F. fusca* at α=0.05.

Figures

Fig. 5.1. Symptoms of *Tomato spotted wilt virus* (TSWV) infection in susceptible (SunOleic 97R) and resistant (Georgia-10T and Georgia-12Y) Runner-type genotypes after thrips-mediated transmission. Experiments were conducted by thrips-mediated TSWV transmission. Viruliferous *Frankliniella fusca* were released (10 thrips per plant) on peanut genotypes for TSWV inoculation. Ten plants of each genotype were tested and the experiment was repeated once (N=20).

Fig. 5.2. Symptoms of *Tomato spotted wilt virus* (TSWV) infection in resistant Virginia-type genotypes (Gregory, Gregory x *A. diogoi*, *A. diogoi*) after thrips-mediated transmission. Experiments were conducted by thrips-mediated TSWV transmission. Viruliferous *Frankliniella fusca* were released (10 thrips per plant) on peanut genotypes for TSWV inoculation. Ten plants of each genotype were tested and the experiment was repeated once (N=20).

Fig. 5.3. Mean (±SE) TSWV N-gene loads in leaflet samples of TSWV-infected peanut genotypes, including susceptible (SunOleic 97R) and resistant (Georgia-10T, Georgia-12Y, and FloRun '107') Runner type genotypes. Peanut plants in each genotype tested positive with DAS-ELISA were subjected to real time quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) using primer set specific to TSWV N-gene. Threshold cycle (Ct) for each sample was estimated and a standard curve was generated using linearized plasmids with N-gene inserts. TSWV N-gene copy numbers in leaflet samples were estimated from the standard curve. Bars labeled with different letters indicate statistically significant differences between genotypes.

Fig. 5.4. Mean (±SE) TSWV N-gene loads in leaflet samples of TSWV-infected Virginia type peanut genotypes, including Gregory, Gregory x *A. diogoi*, and *A. diogoi*. Peanut plants in each genotype tested positive with DAS-ELISA were subjected to real time quantitative reverse

transcriptase polymerase chain reaction (qRT-PCR) using primer set specific to TSWV N-gene. Threshold cycle (Ct) for each sample was estimated and a standard curve was generated using linearized plasmids with N-gene inserts. TSWV N-gene copy numbers in leaflet samples were estimated from the standard curve. Bars labeled with different letters indicate statistically significant differences between genotypes.

Fig. 5.5. Mean (±SE) TSWV N-gene loads in viruliferous thrips (*F. fusca*) reared on leaflets of TSWV-infected peanut genotypes, including susceptible (SunOleic 97R) and resistant (Georgia-10T and Georgia-12Y) genotypes. Peanut plants were inoculated by viruliferous thrips and the infection status was examined by DAS-ELISA. Leaflets from infected plants were used for thrips oviposition and used to rear thrips. Adult female *F. fusca* emerged from infected foliage of different genotypes were collected and subjected to qRT-PCR using primer set specific to TSWV N-gene. TSWV N-gene copy numbers in leaflet samples were estimated from the standard curve. Bars labeled with different letters indicate statistically significant differences between genotypes.

Fig. 5.6. Mean (±SE) TSWV N-gene loads in viruliferous thrips (*F. fusca*) reared on foliage of TSWV-infected peanut genotypes, including susceptible (Gregory) and hybrid (Gregory x *A. diogoi*) genotypes. Peanut plants were inoculated by viruliferous thrips and the infection status was examined by DAS-ELISA. Leaflets from infected plants were used for thrips oviposition and used to rear thrips. Adult female *F. fusca* emerged from infected foliage of different genotypes were collected and subjected to qRT-PCR using primer set specific to TSWV N-gene. TSWV N-gene copy numbers in leaflet samples were estimated from the standard curve. Bars labeled with different letters indicate statistically significant differences between genotypes.

Fig. 5.7. Mean (±SE) number of adults produced per female released on leaflets of TSWV-resistant (Georgia-10T, Georgia-12Y, and FloRun ‘107’) and susceptible (SunOleic 97R)

genotypes. Ten nonviruliferous thrips were released in a Munger cage with leaflets of respective genotype, and ten cages were set up for each genotype. The experiment was repeated once (N=200 thrips released for each genotype). The number of adults emerging from each cage was observed and recorded at 24-hour intervals. Bars labeled with different letters indicate statistically significant differences between genotypes.

Fig. 5.8. Mean (±SE) number of adult produced per female released on leaflets of TSWV-resistant genotypes, including Gregory, Gregory x *A. diogoi*, and *A. diogoi*. Ten nonviruliferous thrips were released in a Munger cage with leaflets of respective genotype, and ten cages were set up for each genotype. The experiment was repeated once (N=200 thrips released for each genotype). The number of adults emerging from each cage was observed and recorded at 24-hour intervals. Bars labeled with different letters indicate statistically significant differences between genotypes.

Fig. 5.1.

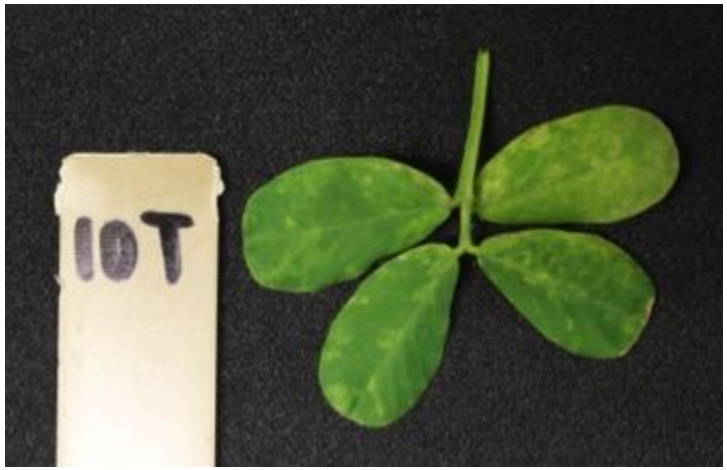


Fig. 5.2.

Fig. 5.3.

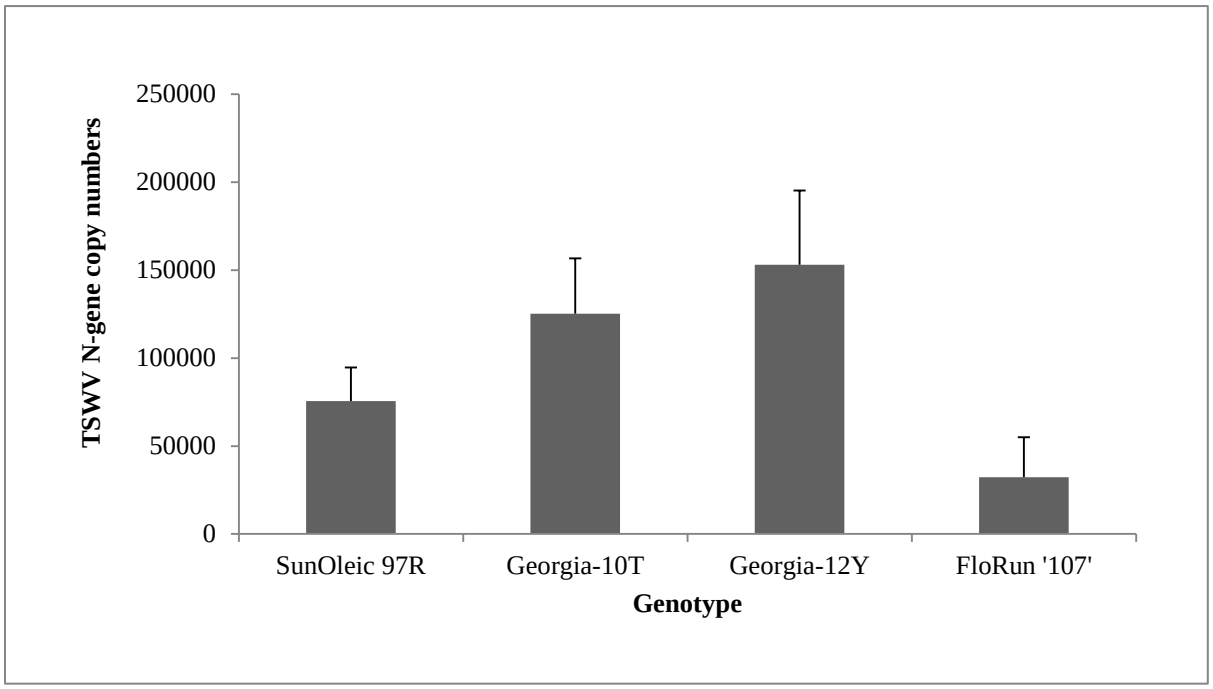

250000
200000
150000
100000
50000
0
TSWV N-gene copy numbers
SunOleic 97R
Georgia-10T
Georgia-12Y
FloRun '107'
Genotype

Fig. 5.4.

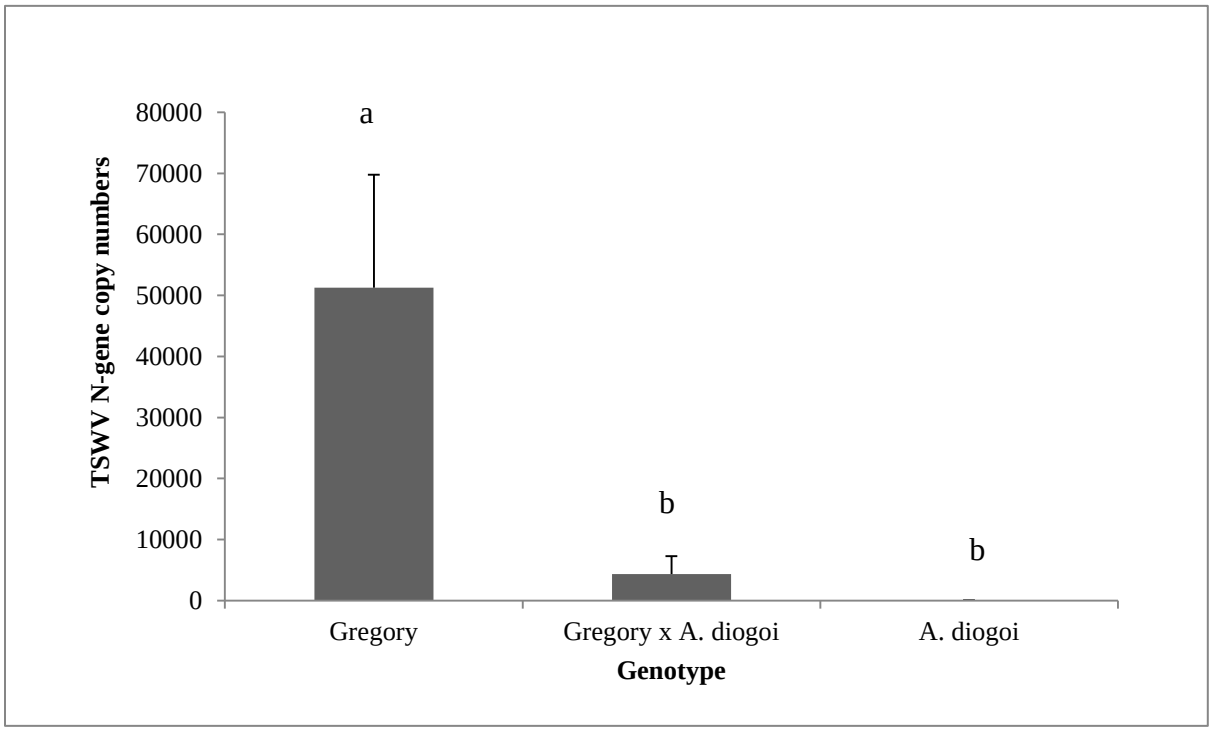

80000
70000
60000
50000
40000
30000
20000
10000
0
TSWV N-gene copy numbers
a
b
b
Gregory
Gregory x A. diogoi
A. diogoi
Genotype

Fig. 5.5.

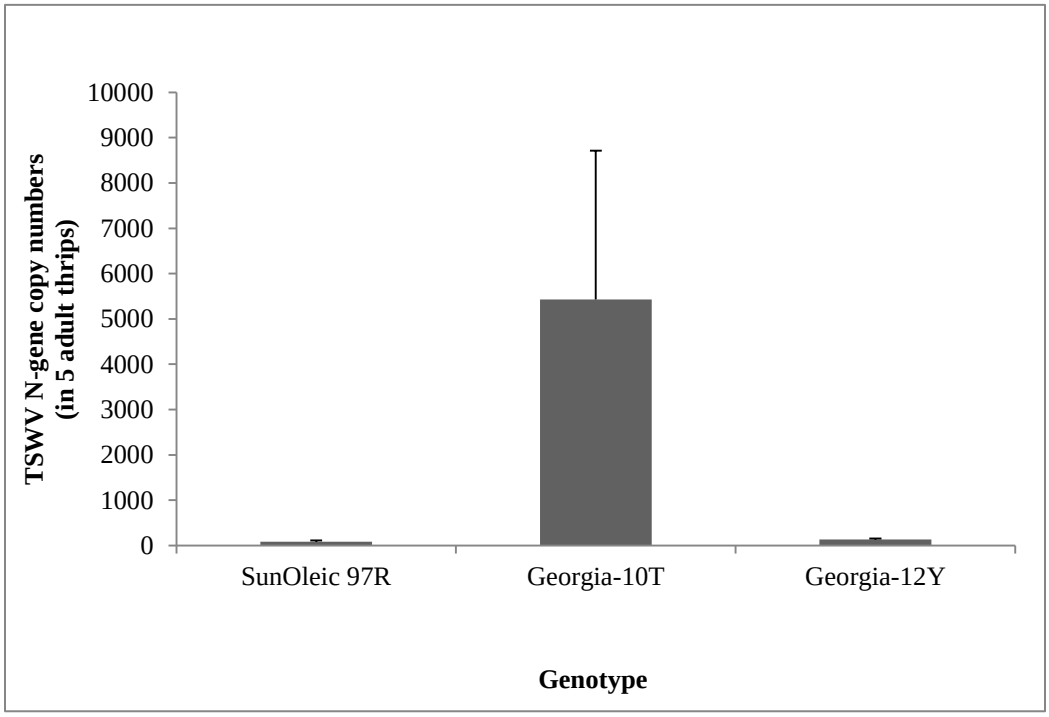

TSWV N-gene copy numbers (in 5 adult thrips)
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
SunOleic 97R
Georgia-10T
Georgia-12Y
Genotype

Fig. 5.6.

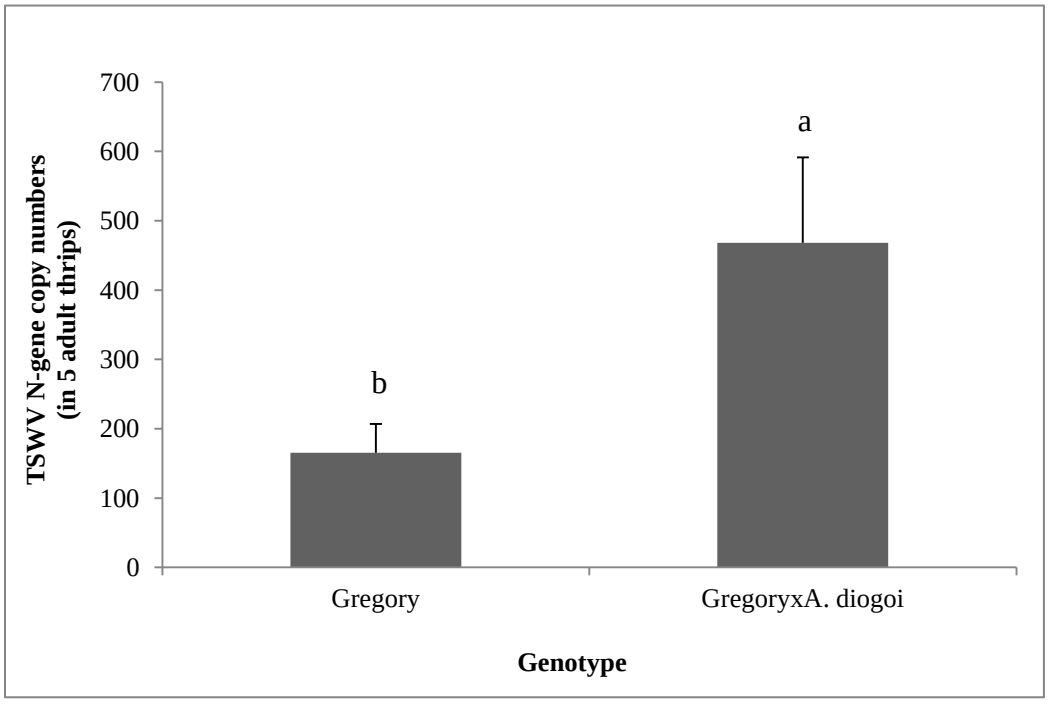

TSWV N-gene copy numbers (in 5 adult thrips)
700
600
500
400
300
200
100
0
b
a
Gregory
GregoryxA. diogoi
Genotype

Fig. 5.7.

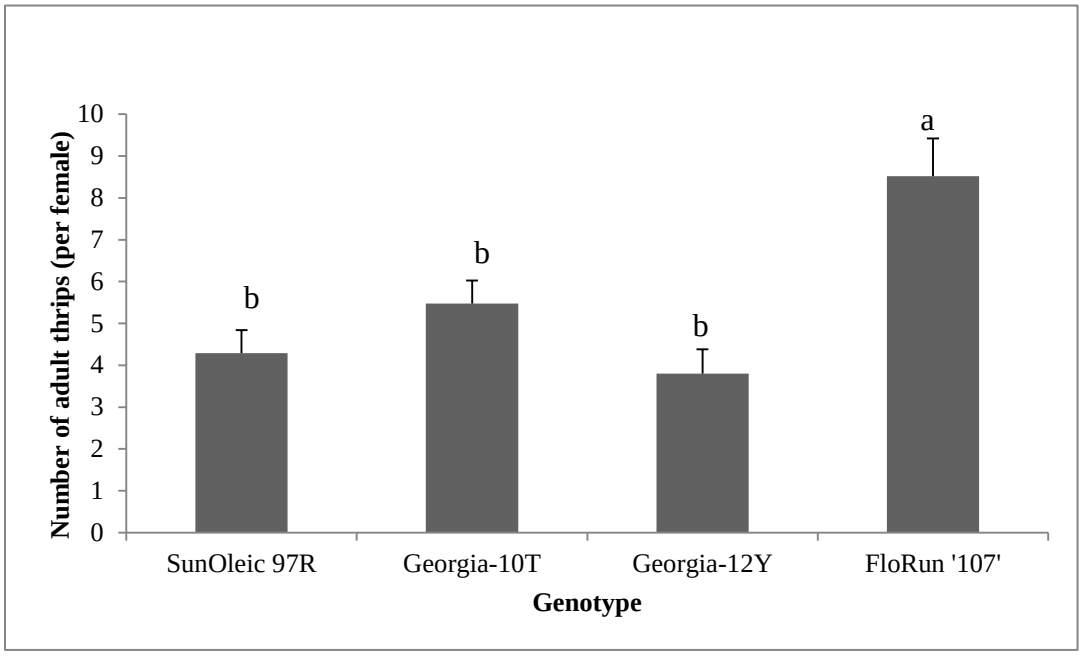
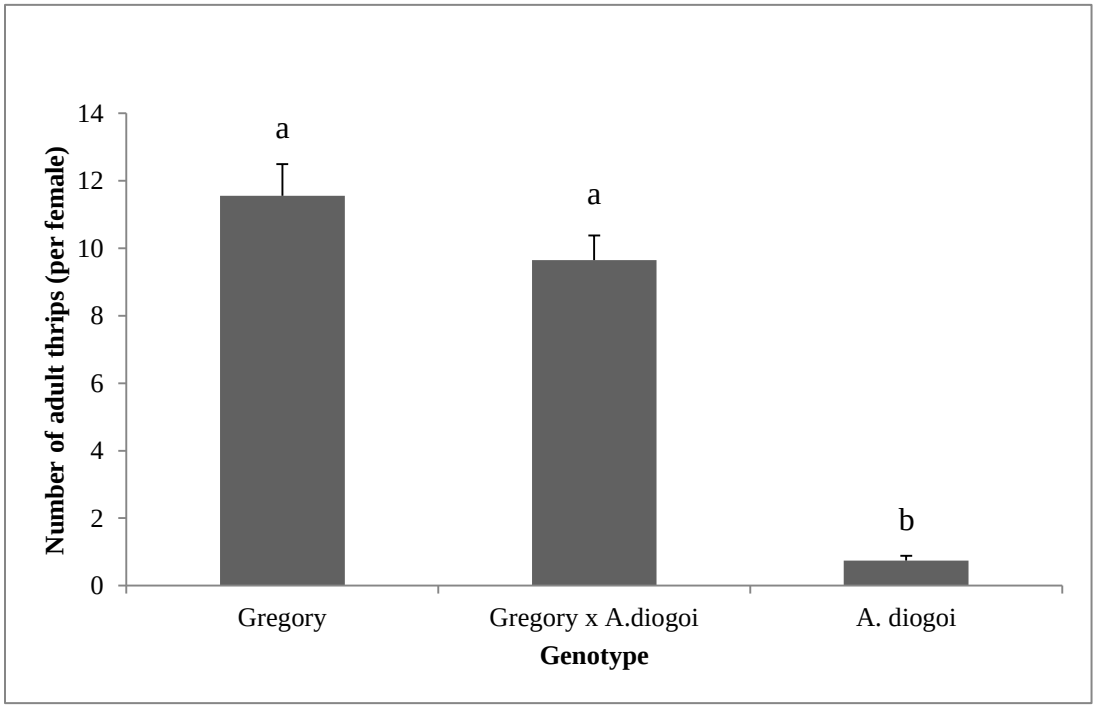

Number of adult thrips (per female)
10
9
8
7
6
5
4
3
2
1
0
b
b
b
a
SunOleic 97R
Georgia-10T
Georgia-12Y
FloRun '107'
Genotype

Fig. 5.8.


Number of adult thrips (per female)
14
12
10
8
6
4
2
0
a
a
b
Gregory
Gregory x A.diogoi
A. diogoi
Genotype

Literature cited

Beasley J. P., J. (2011) 2011 Peanut update. *Cultivar Options for 2011* Univ. Georgia Coop. Ext. .

Bergh, J.C. and Le Blanc, J.P.R. (1997) Performance of western flower thrips (Thysanoptera: Thripidae) on cultivars of miniature rose. *Journal of economic entomology,* **90**, 679-688.

Bertioli, D.J., Leal-Bertioli, S.C.M., Lion, M.B., Santos, V.L., Pappas, G., Jr., Cannon, S.B. and Guimarães, P.M. (2003) A large scale analysis of resistance gene homologues in Arachis. *Molecular Genetics and Genomics,* **270**, 34-45.

Black, M. (1991) Effects of spotted wilt on selected peanut varieties. *Proc. Am. Peanut Res. Ed. Soc.* , **23**, 52.

Branch, W.D. (2007) Registration of 'Georgia-06G' peanut. *Journal of Plant Registrations,* **1**, 120-120.

Branch, W.D. (2013) Registration of 'Georgia-12Y' peanut. *Journal of Plant Registrations,* **7**, 151-153.

Branch, W.D. and Culbreath, A.K. (2011) Registration of 'Georgia-10T' peanut. *Journal of Plant Registrations,* **5**, 279-281.

Brown, A.S.S., Simmonds, M.S.J. and Blaney, W.M. (2002) Relationship between nutritional composition of plant species and infestation levels of thrips. *Journal of Chemical Ecology,* **28**, 2399-2409.

Brown, S.L., Culbreath, A.K., Todd, J.W., Gorbet, D.W., Baldwin, J.A. and Beasley, J.P., Jr. (2005) Development of a method of risk assessment to facilitate integrated management of spotted wilt of peanut. *Plant Disease*, **89**, 348-356.

Brown, S.L., Todd, J.W. and Culbreath, A.K. (1996) Effect of selected cultural practices on

incidence of tomato spotted wilt virus and populations of thrips vectors in peanuts. *Acta Horticulturae*, 491-498.

Cantonwine, E.G., Culbreath, A.K., Stevenson, K.L., Kemerait, R.C., Jr., Brenneman, T.B., Smith, N.B. and Mullinix, B.G., Jr. (2006) Integrated disease management of leaf spot and spotted wilt of peanut. *Plant Disease,* **90**, 493-500.

Clark, M.F. and Adams, A.N. (1977) Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. *The Journal Of General Virology,* **34**, 475-483.

Culbreath, A.K., Baldwin, J., Pappu, H.R., Shokes, F.M., Todd, J.W., Gorbet, D.W. and Brown, S.L. (2000) Reaction of peanut cultivars to spotted wilt. *Peanut science*, **27**, 35-39.

Culbreath, A.K., Branch, W.D., Beasley, J.P., Jr., Tubbs, R.S. and Holbrook, C.C. (2012) Peanut genotype and seeding rate effects on spotted wilt. *Plant Health Progress*, PHP-RS.

Culbreath, A.K., Holbrook, C.C., Nischwitz, C., Tilman, B.L. and Gorbet, D.W. (2008) Response of New Field-Resistant Peanut Cultivars to Twin-Row Pattern or In-Furrow Applications of Phorate for Management of Spotted Wilt [electronic resource]. *Plant disease: an international journal of applied plant pathology,* **92**, 1307-1312.

Culbreath, A.K., Pappu, H.R., Holbrook, C.C., Shokes, F.M., Baldwin, J.A., Todd, J.W., Gorbet, D.W. and Brown, S.L. (1999) Response of early, medium, and late maturing peanut breeding lines to field epidemics of tomato spotted wilt. *Peanut science,* **26**, 100-106.

Culbreath, A.K. and Srinivasan, R. (2011) Epidemiology of spotted wilt disease of peanut caused by Tomato spotted wilt virus in the southeastern U.S. *Virus Research,* **159**, 101-109.

Culbreath, A.K., Todd, J.W., Branch, W.D., Brown, S.L., Demski, J.W. and Beasley, J.P., Jr. (1994) Effect of new peanut cultivar Georgia Browne on epidemics of spotted wilt. *Plant*

Disease, **78**, 1185-1189.

Culbreath, A.K., Todd, J.W. and Brown, S.L. (2003) Epidemiology and management of tomato spotted wilt in peanut. *Annual review of phytopathology*, **41**, 53-75.

Culbreath, A.K., Todd, J.W., Demski, J.W. and Chamberlin, J.R. (1992) Disease progress of spotted wilt in peanut cultivars Florunner and Southern Runner. *Phytopathology*, **82**, 766-771.

Culbreath, A.K., Todd, J.W., Gorbet, D.W., Branch, W.D., Holbrook, C.C., Shokes, F.M. and Demski, J.W. (1996a) Variation in susceptibility to tomato spotted wilt virus among advanced breeding lines of peanut (Arachis hypogaea). *Acta Horticulturae*, 402-410.

Culbreath, A.K., Todd, J.W., Gorbet, D.W., Branch, W.D., Sprenkel, K., Shokes, F.M. and Demski, J.W. (1996b) Disease progress of tomato spotted wilt virus in selected peanut cultivars and advanced breeding lines. *Plant Disease*, **80**, 70-73.

Culbreath, A.K., Todd, J.W., Gorbet, D.W., Shokes, F.M. and Pappu, H.R. (1997) Field Performance of Advanced Runner- and Virginia-Type Peanut Breeding Lines During Epidemics of TSWV. *PEANUT SCIENCE*, **24**, 123-127.

Culbreath, A.K., Tubbs, R.S., Tillman, B.L., Beasley, J.J.P., Branch, W.D., Holbrook, C.C., Smith, A.R. and Smith, N.B. (2013) Effects of seeding rate and cultivar on tomato spotted wilt of peanut. *Crop Protection*, **53**, 118-124.

Gorbet, D.W. and Knauft, D.A. (2000) Registration of 'SunOleic 97R' Peanut. *Crop Science*, **40**, 1190-1191.

Gorbet, D.W., Norden, A.J., Shokes, F.M. and Knauft, D.A. (1987) Registration of 'Southern Runner' peanut. *Crop Science*, **27**, 817-817.

Hadden, J.F. (1991) 1990 Georgia Plant Disease Loss Estimates. Univ. Georgia Coop. Ext. Serv.,

Pub. Path. 91-005.

Hallwass, M., Oliveira, A.S., Campos Dianese, E., Lohuis, D., Boiteux, L.S., Inoue-Nagata, A.K., Resende, R.O. and Kormelink, R. (2014) The Tomato spotted wilt virus cell-to-cell movement protein (NSM) triggers a hypersensitive response in Sw-5-containing resistant tomato lines and in Nicotiana benthamiana transformed with the functional Sw-5b resistance gene copy. *Molecular Plant Pathology,* **15**, 871-880.

Holbrook, C.C. and Culbreath, A.K. (2008) Registration of 'Georganic' peanut. *Journal of Plant Registrations*, **2**, 17-17.

Holbrook, C.C., Timper, P., Culbreath, A.K. and Kvien, C.K. (2008) Registration of 'Tifguard' peanut. *Journal of Plant Registrations,* **2**, 92-94.

Isleib, T.G., Rice, P.W., Mozingo, R.W., Mozingo, R.W., Ii and Pattee, H.E. (1999) Registration of 'Gregory' peanut. *Crop Science,* **39**, 1526-1526.

Jain, R.K., Pappu, S.S., Pappu, H.R., Culbreath, A.K. and Todd, J.W. (1998) Molecular Diagnosis of Tomato Spotted Wilt Tospovirus Infection of Peanut and Other Field and Greenhouse Crops. *Plant Disease,* **82**, 900-904.

Liu, Z., Culbreath, A.K., Varshney, R.K., Guo, B., Feng, S., Pandey, M.K. and Chen, X. (2013) Identification of Expressed Resistance Gene Analogs from Peanut (Arachis hypogaea L.) Expressed Sequence Tags [electronic resource]. *Journal of integrative plant biology,* **55**, 453-461.

Lowry, V.K., Smith, J.W. and Mitchell, F.L. (1992) Life-Fertility Tables for Frankliniella fusca (Hinds) and F. occidentals (Pergande) (Thysanoptera: Thripidae) on Peanut. *Annals of the Entomological Society of America*, **85**, 744-754.

Lyerly, J.H., Hoffman, K., Moyer, J.W. and Stalker, H.T. (2002) Evaluation of Arachis species

for resistance to tomato spotted wilt virus. *Peanut science,* **29**, 79-84.

Mandal, B., Pappu, H.R., Csinos, A.S. and Culbreath, A.K. (2006) Response of Peanut, Pepper, Tobacco, and Tomato Cultivars to Two Biologically Distinct Isolates of Tomato spotted wilt virus. *Plant Disease,* **90**, 1150-1155.

Mandal, B., Pappu, H.R. and Culbreath, A.K. (2001) Factors affecting mechanical transmission of tomato spotted wilt virus to peanut (Arachis hypogaea). *Plant Disease,* **85**, 1259-1263.

Mandal, B., Pappu, H.R., Culbreath, A.K., Holbrook, C.C., Gorbet, D.W. and Todd, J.W. (2002) Differential response of selected peanut (Arachis hypogaea) genotypes to mechanical inoculation by Tomato spotted wilt virus. *Plant Disease,* **86**, 939-944.

Monfort, W.S. (2015) 2015 Peanut update. *Peanut Cultivar Options for 2015* Georgia Peanut Commission.

Moury, B., Palloix, A., Selassie, K.G. and Marchoux, G. (1997) Hypersensitive resistance to tomato spotted wilt virus in three Capsicum chinense accessions is controlled by a single gene and is overcome by virulent strains. *Euphytica*, **94**, 45-52.

Ngoc Huy, H., Yang, H. and Kang, B. (2013) Identification and inheritance of a new source of resistance against Tomato spotted wilt virus (TSWV) in Capsicum. *Scientia Horticulturae,* **161**, 8-14.

Npb (2014) Peanut types.

Pappu, H.R., Jones, R.a.C. and Jain, R.K. (2009) Global status of tospovirus epidemics in diverse cropping systems: Successes achieved and challenges ahead. *Virus Research,* **141**, 219-236.

Rao, N.K., Reddy, L.J. and Bramel, P.J. (2003) Potential of wild species for genetic enhancement of some semi-arid food crops. *GENETIC RESOURCES AND CROP EVOLUTION,* **50**,

707-721.

Ratnaparkhe, M.B., Wang, X., Li, J., Compton, R.O., Rainville, L.K., Lemke, C., Kim, C., Tang, H. and Paterson, A.H. (2011) Comparative analysis of peanut NBS-LRR gene clusters suggests evolutionary innovation among duplicated domains and erosion of gene microsynteny. p. 164. John Wiley & Sons.

Reddy, A.S., Reddy, L.J., Nalini, M., Abdurahman, M.D., Reddy, Y.V., Bramel, P.J. and Reddy, D.V.R. (2000) Identification of resistance to Peanut bud necrosis virus (PBNV) in wild Arachis germplasm. *Annals of Applied Biology,* **137**, 135-139.

Rotenberg, D., Krishna Kumar, N.K., Ullman, D.E., Montero-Astua, M., Willis, D.K., German, T.L. and Whitfield, A.E. (2009) Variation in Tomato spotted wilt virus titer in Frankliniella occidentalis and its association with frequency of transmission. *Phytopathology,* **99**, 404-10.

Shrestha, A., Srinivasan, R., Sundaraj, S., Culbreath, A.K. and Riley, D.G. (2013) Second generation peanut genotypes resistant to thrips-transmitted tomato spotted wilt virus exhibit tolerance rather than true resistance and differentially affect thrips fitness. *Journal of Economic Entomology,* **106**, 587-596.

Sundaraj, S., Srinivasan, R., Culbreath, A.K., Riley, D.G. and Pappu, H.R. (2014) Host plant resistance against Tomato spotted wilt virus in peanut (Arachis hypogaea) and its impact on susceptibility to the virus, virus population genetics, and vector feeding behavior and survival. *Phytopathology,* **104**, 202-210.

Tillman, B.L. and Gorbet, D.W. (2015) Registration of 'FloRun '107'' peanut. *Journal of Plant Registrations*, **9**, 162-167.

Tillman, B.L., Gorbet, D.W., Culbreath, A.K. and Todd, J.W. (2006) Response of peanut cultivars

to seeding density and row patterns. *Crop Management*, 1-5.

Todd, J.W., Culbreath, A.K., Chamberlin, J.R., Beshear, R.J. and Mullinix, B.G. (1995) Colonization and population dynamics of thrips in peanuts in the southern United States. *Thrips biology and management: proceedings of the 1993 International Conference on Thysanoptera.* eds. B.L. Parker, M. Skinner & T. Lewiss), pp. 453-460. Plenum Publishing Co. Ltd, London; USA.

Ullman, D.E., Sherwood, J.L. and German, T.L. (1997) Thrips as vectors of plant pathogens. *Thrips as crop pests.* (ed. T. Lewiss), pp. 539-565. CAB INTERNATIONAL, Wallingford, UK,.

Whitfield, A.E., Ullman, D.E. and German, T.L. (2005) Tospovirus-thrips interactions. *Annu Rev Phytopathol*, **43**, 459-89.

Yuksel, B., Estill, J.C., Schulze, S.R. and Paterson, A.H. (2005) Organization and evolution of resistance gene analogs in peanut. *Molecular Genetics And Genomics: MGG*, **274**, 248-263.

CHAPTER 6

SUMMARY

The goal of this research was to evaluate numbers of management tactics against tobacco thrips (*Frankliniella fusca* Hinds) and *Tomato spotted wilt virus* (TSWV) in spotted wilt disease of peanut in Georgia. TSWV field resistant cultivars, insecticides, and cultural practices are important components in integrated management programs of spotted wilt disease in peanut. Three objectives were set for the research. The first objective focused on evaluation of various insecticides alternatives to aldicarb and phorate, which are two commonly used conventional insecticides with broad-spectrum toxicity, along with newly released third-generation TSWV field resistant cultivar Georgia-12Y (GA-12Y) in management of spotted wilt in peanut. In addition, cultural practices, namely row patterns and tillage systems, were also assessed in the integrated management programs cooperating with TSWV field resistant peanut cultivars and selected alternative insecticides for thrips control and spotted wilt management. Numbers of insecticides with less non-target effects were identified as alternatives to aldicarb and phorate. When higher TSWV field resistant cultivar GA-12Y was planted, imidacloprid (in-furrow), cyantraniliprole, and spinetoram were as effective as aldicarb and phorate in reducing thrips populations, thrips feeding damage, and suppressing spotted wilt incidence without compromising yields. Thrips-mediated experiments in greenhouse supported the findings in the field trials. Twin row pattern and strip tillage had potential to aid with the control of thrips and TSWV when used in combination with higher TSWV field resistant cultivar GA-12Y and selected alternative insecticides such as imidcaloprid (in-furrow) in management of spotted wilt

in peanut. These results together indicated that when peanut cultivar with higher TSWV field resistance was planted, insecticides such as imidacloprid and cyantraniliprole were capable to replace older highly toxic insecticides such as aldicarb and phorate for controlling thrips and TSWV in management programs of spotted wilt in peanut with or without cultural practices; incorporating twin row pattern and strip tillage with high TSWV field resistant cultivar and effective insecticide alternatives would potentially enhance the efficacy of integrated management programs for spotted wilt disease of peanut.

The second objective was to evaluate the effectiveness of selected insecticides, including phorate and alternatives, against thrips. Assessments of residual toxicity in plants as well as insecticide resistance development in thrips were conducted. Results indicated that residue levels of selected insecticides, namely imidacloprid, thiamethoxam, and phorate, detected in plants greatly declined in 24 days after application at planting. The effectiveness of imidacloprid and phorate to cause thrips mortality largely declined 10 days post treatment, while it lasted from 9 to 24 days to reduce thrips feeding. Thiamethoxam seed treatment did not affect thrips mortality or the degree of feeding damage. The median lethal concentration (LC50) representing the susceptibility to the selected insecticides of thrips populations has been evaluated. Results indicated that thrips populations evaluated in this study had similar susceptibility to the insecticides. It is concluded that imidacloprid and phorate are most effective to control thrips in 24 days after application at planting; thrips populations evaluated were all susceptible to thiamethoxam, imidacloprid, and phorate by direct feeding.

The third objective focused on investigation of the impact of TSWV resistant tetraploid and diploid peanut genotypes on TSWV transmission by tobacco thrips (*Frankliniella fusca* Hind) and its biological fitness. A set of Runner-type peanuts including susceptible (SunOleic 97R) and

resistant (Georgia-10T, Georgia-12Y, FloRun ‘107’) genotypes was evaluated through thrips-mediated TSWV transmission assays. On the other hand, a Virginia-type peanut (Gregory), its diploid (*Arachis diogoi*) hybrid, and *A. diogoi* were also evaluated. Thrips-mediated TSWV inoculation resulted in spotted wilt incidence in all the genotypes. All the genotypes exhibited TSWV symptoms, except for FloRun ‘107’ and A. diogoi. TSWV infection percentages and TSWV copies did not vary with genotypes in Runner-type peanuts. When comparing Gregory, *A. diogoi*, and the hybrid genotype, incidence of TSWV infection was reduced in A. diogoi. TSWV copies accumulated in *A. diogoi* and the hybrid were lower than in Gregory. Thrips biological fitness including reproduction and developmental time when reared on selected genotypes was investigated. Number of thrips emerged from FloRun ‘107’ leaflets was higher than other genotypes in Runner-type peanuts; while the developmental time required for thrips to grow from larvae to adults did not vary with genotypes in this group. Number of adult thrips emerged from *A. diogoi* was lower than from Gregory and the hybrid genotype; and thrips required longer developmental time when reared on *A. diogoi*. All the results indicated that TSWV field resistant genotypes were not immune to either TSWV or thrips. *A. diogoi* is potentially a good source of resistance to TSWV as well as thrips.