ALTERNATIVES TO CARBAMATE AND ORGANOPHOSPHATE INSECTICIDES, CULTURAL TACTICS, AND ECOLOGICAL FACTORS THAT AFFECT *TOMATO SPOTTTED WILT VIRUS* EPIDEMICS IN PEANUT

by

KATHLEEN MONFERO MARASIGAN

(Under the Direction of Rajagopalbabu Srinivasan)

ABSTRACT

Selected management tactics, including insecticides and cultural practices, against *Frankliniella fusca* (Hinds) and *Tomato spotted wilt virus* (TSWV) in peanut were evaluated. Effects of pine pollen on thrips fitness and TSWV transmission and the role of cotton as alternative host of thrips and TSWV were also assessed. The first objective focused on evaluating alternative insecticides to carbamate and organophosphate usage in peanut production. Efficacy of alternative insecticides was assessed based on four parameters: thrips density and feeding injury, spotted wilt incidence, and yield. The second objective investigated the integration of selected alternative insecticides with different cultural practices: tillage systems, row patterns, and seeding rates. The third objective focused on the effects of pine pollen, as supplemental source of amino acids, on thrips populations and TSWV transmission. The fourth objective assessed the role of cotton as a thrips reservoir and as a TSWV inoculum source.

INDEX WORDS: Tobacco thrips, spotted wilt, management, pine pollen, cotton

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by

KATHLEEN MONFERO MARASIGAN

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by

KATHLEEN MONFERO MARASIGAN

Major Professor:	Rajagopalbabu Srinivasan
Committee:	Michael Toews
	Robert Kemerait, Jr.

Electronic Version Approved:

Maureen Grasso
Dean of the Graduate School
The University of Georgia
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To my parents, thank you for your endless love and support.

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CHAPTER 1

INTRODUCTION

Peanut, *Arachis hypogaea* L., is one of the most economical row crops in the United States. It ranked 3rd in production volume and 4th in production area of approximately 1,893,376 metric tons and 1,067,000 acres, respectively, in 2013 (NASS 2014). Next to China and India, being the world's 1st and 2nd largest peanut producers, respectively, the United States is the third largest in peanut production (Pattee and Stalker 1995, FAS 2013). Georgia is the number one peanut producing state in the United States. In 2013, approximately 40% of overall planted area in the United States, equivalent to 430,000 acres, was in Georgia. Georgia, Alabama (140,000 acres), and Florida (140,000 acres) together have 67% of the total production acreage in the United States (NASS 2014). Georgia recorded an average yield record of 4,430 pounds per acre (lbs/acre) followed by Florida (3,950 lbs/acre) and Alabama (3,550 lbs/acre). Georgia has approximately 3,500 peanut farmers on 14,000 individual farms. In 2012, peanut production contributed over 50,000 jobs to the state (GPC 2012).

Peanut is known mainly for its high protein content and unsaturated fatty acids (Venkatachalam and Sathe 2006, USDA 2013a). It provides vitamins (e.g., folic acid, niacin, vitamin B6, and vitamin E), minerals (e.g., calcium, magnesium, and potassium), and antioxidant polyphenolics such as *p*-coumaric acid (Talcott et al. 2005, Ros 2010). It is grown for several food products and confections like peanut butter, peanut flour, peanut oil, boiled peanuts, and roasted peanuts (APC 2013, Soyatech 2013).

Peanut production in the United States is greatly affected by spotted wilt, a viral disease caused by *Tomato spotted wilt virus* (TSWV) in the family *Bunyaviridae*, genus *Tospovirus* (Culbreath et al. 2003, Whitfield et al. 2005, Culbreath and Srinivasan, 2011). The disease was first reported in Texas during 1971, where nearly 100% of the crop was lost (Halliwell and Philley 1974, Black et al. 1986). A few years later, spotted wilt also occurred in peanut fields in nearby southeastern states including Georgia. The disease incidence became widespread in the late 1980s through 1997, which led to extensive yield losses valued at approximately $40 million (Bertrand 1998). Under natural conditions, TSWV is transmitted by thrips (Thysanoptera: Thripidae) in a persistent-propagative manner (German et al. 1992, Jones 2005, Whitfield et al. 2005, Pappu et al. 2009). There are 1,710 known species of thrips in the family Thripidae. Of these, only 9 and 14 species of thrips have been confirmed as vectors of TSWV and *Tospoviruses*, respectively (Riley et al. 2011b). In Georgia, tobacco thrips, *Frankliniella fusca* (Hinds), and western flower thrips, *Frankliniella occidentalis* (Pergande), are the most important vectors of TSWV (Todd et al. 1995, 1996, 1997). TSWV-infected plants show prominent symptoms on the foliage such as concentric ringspots, chlorotic patterns, and deformation. In addition, noticeable overall stunting of the plant can be observed as well as abnormalities in pegs, roots, pods and kernels (Culbreath et al. 2003, Culbreath and Srinivasan 2011).

Many runner type cultivars (e.g., Florunner, Georgia Runner, SunOleic 97R, and GK-7) were extremely susceptible to TSWV during the late 1980s and early 1990s. Intensive screening of breeding lines has led to development of moderate sources of field resistance to TSWV. This subsequently led to the release of several cultivars such as Georgia Browne, Southern Runner, Georgia Green, Florida MDR 98 (formerly known as UF 91108), and C-99R (Culbreath et al. 1992, 1994, 1996, 1997, 2000, 2003; Wells et al. 2002). The performance of the said moderately

resistant cultivars has been consistent when compared with the susceptible cultivars such as Georgia Runner and SunOleic 97R. In 2002, Georgia Green was planted on >90% of the peanut acreage in Georgia (Culbreath et al. 2003). More recently, other runner type peanut cultivars with higher levels of field resistance as compared to Georgia Green have been released. These include Georgia-06G, Georgia Greener, Georgia-07W, Georgia-09B, Georgia-10T, and Georgia-12Y (Branch 2007a, 2007b, 2010; Branch and Brenneman 2008, Holbrook and Culbreath 2008, Holbrook et al. 2008, Branch and Culbreath 2011, Beasley 2013, Branch 2013). At present, these next generation cultivars are being compared to Georgia Green, which was formerly one of the moderately resistant cultivars. In 2013, Georgia-06G was planted on 77% of the acreage in southeastern states (Beasley 2013).

Even with the identification of the field resistance from the newly released cultivars, the mechanism underlying the resistance to TSWV is still unclear. *Sw5* and *Tsw* are the two major genes that confer resistance to TSWV in other crops such as tomato and pepper, respectively, but these have not been identified in peanut (Adkins 2000, Pappu et al. 2009, Riley and Joseph 2011). However, inoculation of newer peanut cultivars with TSWV under greenhouse conditions produced TSWV symptoms (Shrestha et al. 2013). These results indicate that the high levels of resistance observed under field situations may not be attributed to major gene resistance but rather due to a phenomenon similar to tolerance (Culbreath and Srinivasan 2011, Shrestha et al. 2013).

Generally, viruses such as TSWV are difficult to manage. The planting of virus-resistant varieties would be the most effective tactic to manage the virus spread. However, planting of TSWV-resistant peanut alone is not enough to manage the virus spread. Another approach is chemical management (Todd et al. 1994a, 1994b, 1995, 1996; Todd and Culbreath 1995). During

early season of peanut production, thrips feeding can severely affect seedlings performance in establishing a stable plant. It may lead to delayed maturity which affects the final yield performance of the peanut (Todd et al. 1996, 1997; Culbreath et al. 2003, Tubbs et al. 2013). Insecticides such as aldicarb and phorate are effective in reducing the virus spread (Todd et al. 1996, Culbreath et al. 2003, 2008; Brown et al. 2005, Ames Herbert et al. 2007, Culbreath and Srinivasan 2011). Due to the high mammalian toxicity and other non-target effects of older insecticides and due to phasing out of aldicarb by 2018, alternatives are sought (AgroNews 2010, Digiuseppe 2010, GFB News 2010). Insecticides with low non-target effects and lower toxicity levels when compared to the abovementioned chemicals include imidacloprid and cyantraniliprole (Todd et al. 1994a, 1994b; Culbreath et al. 2003, Jacobson and Kennedy 2011). The effects of these insecticides, together with other newer insecticides, were examined to assess their suitability as alternatives to phorate and aldicarb. Normally, they are applied during planting (in-furrow) or at the time of emergence of young plants, "at cracking" (Culbreath et al. 2003, 2008).

Besides planting moderately resistant cultivars and application of insecticides, cultural practices such as planting date, plant density, row patterns, seeding rates, and tillage systems are effective in reducing thrips populations and spotted wilt incidence in peanut (Culbreath et al., 2003, 2008, 2010, 2013; Marois and Wright 2003, Tillman et al. 2006). The use of reduced or strip tillage also suppressed thrips populations and feeding injuries when compared with the conventional tillage (Minton et al. 1991, Brandenburg et al. 1998, Baldwin et al. 1998, 2001a). Similar benefits to the previously mentioned parameters were also observed with the use of greater seeding rates and with the use of twin/double rows when compared with single rows

(Baldwin et al. 2001a, 2001b; Tillman et al. 2006, Culbreath et al. 2008, 2013; Tubbs and Beasley 2009, Tubbs et al. 2011).

Though a substantial number of studies have focused on management of TSWV in peanut, other ecological factors in the farmscape that might contribute to its spread have largely been ignored. In addition to peanut, thrips species feed on several host plants such as tomato, onion, and cotton (Culbreath et al. 2003, Angelella and Riley 2010, Toews et al. 2010). Typically, thrips feed on many parts of the plant including the foliage and pollen from the blooms. Previous experiments showed that supplemental food source from *Pinus elliottii* Engelm., *P. sylvestris* L., *Acer saccharum* Marsh., and *Brassica napus* L. increased the daily and lifetime fecundity and oviposition in many thrips species such as *F. fusca*, *F. occidentalis*, *Taeniothrips inconsequens* (Uzel), and *Thrips fuscipennis* (Haliday), respectively (Kirk 1985, Leskey et al. 1997, Hulshof et al. 2003, Riley et al. 2007, Wäckers et al. 2007, Angelella and Riley 2010).

In Georgia, pollen release from different crops and trees can be heavy especially during the spring season (Riley et al. 2011a). Pollen dehiscence from pine (*Pinus* spp.) trees is known to influence thrips populations in the spring season preceding planting of peanuts. Pine species in the southeastern states include slash pine (*P. elliottii* Engelm), longleaf pine (*P. palustris* Mill.), loblolly pine (*P. taeda* L.), pond pine (*P. serotina* Michx.), and shortleaf pine (*P. echinata* Mill.) (Dorman and Barber 1956, Riley and Pappu 2004, USDA 2013b). Studies on *Frankliniella* species, such as *F. occidentalis* and *F. fusca*, indicated effects of pine pollen on their fitness and settling behavior (Trichilo and Leigh 1988, Hulshof and Vänninen et al. 2001, Chitturi et al. 2006, Riley et al. 2007, Angelella and Riley 2010). Increase in oviposition rate and reproduction were also documented (Kirk 1984, 1985; Murai and Loomans 2001, Chitturi et al. 2006, Riley et

al. 2007, Angelella and Riley 2010). The increased fitness effects might be influenced by the enhanced nutritional content in pollen grains such as free amino acids, and soluble sugars (Stanley and Linskins 1974, Kirk 1997, Lundgren 2009).

Cotton (*Gossypium hirsutum* L.) is often grown in rotation with or in proximity to peanut (Johnson et al. 2001). It is also considered an excellent reservoir of thrips (*F. fusca*) and host for TSWV (Schuster and Haliwell 1994, Groves et al. 1998, Toews et al. 2010). However, its role in TSWV epidemics in peanut is not yet clearly understood. Identifying the possible role of cotton in thrips population and TSWV epidemics could help to understand TSWV epidemics better. Only a single study indicated that thrips could transmit TSWV to cotton (Groves et al. 1998). Groves et al. (1998) demonstrated that cotton plants could be infected following thrips (*F. fusca*) inoculation under greenhouse conditions (Groves et al. 1998). However, no in depth studies were done to evaluate cotton as source of TSWV inoculum and its effect on TSWV transmission.

Therefore, this current study has four objectives. In the first objective, we evaluated alternative insecticides to aldicarb (Temik®) and phorate (Thimet®) under field and greenhouse conditions. This is to identify the mechanisms associated with the suppression of thrips populations and feeding and TSWV incidence in TSWV-resistant (Georgia-06G) and susceptible (Georgia Green) peanut cultivars. In the second objective, we evaluated cultural practices such as row patterns, seeding rates, and tillage systems with selected insecticides to assess their effects based on suppression of thrips populations and thrips feeding, and reduction of spotted wilt incidence in Georgia-06G and/or in Georgia Green. In the third objective, we examined the effects of pine pollen grains on thrips (*F. fusca*) fitness, feeding behavior, settling preference, and TSWV transmission. In the fourth objective, we evaluated the role of cotton as a thrips reservoir and as a TSWV inoculum source.

Literature Cited

Adkins, S. 2000. *Tomato spotted wilt virus* – positive steps towards negative success. Mol. Plant Pathol. 1: 151-15.

AgroNews. 2010. Bayer CropScience to close a plant as Temik phasing out. http://news.agro pages.com/News/NewsDetail---2766.htm.

Ames Herbert, D., S. Malone, S. Aref, R. L. Brandenburg, D. L. Jordan, B. M. Royals, and P. D. Johnson. 2007. Role of insecticides in reducing thrips injury to plants and incidence of *Tomato spotted wilt virus* in Virginia market-type peanut. J. Econ. Entomol. 100: 1241-1247.

Angelella, G. M., and D. G. Riley. 2010. Effects of pine pollen supplementation in an onion diet on *Frankliniella fusca* reproduction. Environ. Entomol. 39: 505-512.

APC. 2013. American Peanut Council. http://www.peanutusa.com/about-peanuts/the-peanut-industry3/15-value-added-products.html.

Baldwin, J. A., and J. Hook. 1998. Reduced tillage systems for peanut production in Georgia. Proc. Amer. Peanut Res. Ed. Soc. 30:48.

Baldwin, J. A., J. W. Todd, J. R. Weeks, D. W. Gorbet, A. K. Culbreath, A. S. Luke-Morgan, S. M. Fletcher, and S. L. Brown. 2001a. 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. Proc. Ann. Southern Cons. Till. Conf. Sust. Agric. 24: 26-34.

Baldwin, J. A., R. McDaniel, D. E. McGriff, and B. Tankersley. 2001b. Yield, grade and tomato spotted wilt incidence of Georgia Green and AT201 peanut when planted in twin vs. single row pattern. Abstr. Proc. Am. Peanut Res. Ed. Soc. 33: 31.

Beasley, J. P. 2013. Cultivar options in 2013. *In* J.P. Beasley (ed.), 2013 Peanut Production Update. The University of Georgia Cooperative Extension Service, Athens, GA.

Bertrand, P. 1998. 1997 Georgia plant disease loss estimates. Univ. GA. Coop. Ext. Pub. Path.: 98-1007.

Black, M. C., P. F. Lummus, D. H. Smith, and J. W. Demski. 1986. An epidemic of spotted wilt disease in south Texas peanuts in 1985. Proc. Am. Peanut Res. Educ. Soc. 18: 66.

Branch, W. D. 2007a. Registration of 'Georgia-06G' peanut. J. Plant Reg. 1: 120.

Branch, W. D. 2007b. Registration of 'Georgia Greener' peanut. J. Plant Reg. 1: 121.

Branch, W. D. 2010. Registration of 'Georgia-09B' peanut. J. Plant Reg. 4: 175-178.

Branch, W. D. 2013. Registration of 'Georgia-12Y' peanut. J. Plant Reg. 7: 151-153.

Branch, W. D., and A. K. Culbreath. 2011. Registration of 'Georgia-10T' peanut. J. Plant Reg. 5: 279-281.

Branch, W. D., and T. B. Brenneman. 2008. Registration of 'Georgia-07W' peanut. J. Plant Reg. 2: 88-91.

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

Brown, S. L., A. K. Culbreath, J. W. Todd, D. W. Gorbert, J. A. Baldwin, and J. P. Beasley. 2005. Development of a method of risk assessment to facilitate integrated management of spotted wilt of peanut. Plant Dis. 89: 348-356.

Chitturi, A. D. G. Riley, and P. Houston Joost. 2006. Effect of pine pollen on settling behavior of *Frankliniella occidentalis* and *Frankliniella fusca* (Thysanoptera: Thripidae) on tomato and peanut. Environ. Entomol. 35: 1396-1403.

Cho, J. J., R. F. L. Mau, T. L. German, R. W. Hartmann, L. S. Yudin, D. Gonsalves, and R. Provvidenti. 1989. A multidisciplinary approach to management of *Tomato spotted wilt virus* in Hawaii. Plant Dis. 73: 375-383.

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

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

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

Culbreath, A. K., J. W. Todd, D. W. Gorbet, F. M. Shokes, and H. R. Pappu. 1997. Field response of new peanut cultivar UF 91108 to *Tomato spotted wilt virus*. Plant Dis. 81: 1410-1415.

Culbreath, A. K., J. W. Todd, D. W. Gorbet, and S. L. Brown. 2000. Reaction of peanut cultivars to spotted wilt. Peanut Sci. 27: 35-39.

Culbreath, A. K., J. W. Todd, and S. L. Brown 2003. Epidemiology and management of tomato spotted wilt in peanut. Annu. Rev. Phytopathol. 41: 53-75.

Culbreath, A. K., B. L. Tillman, D. W. Gorbet, C. C. Holbrook, and C. Nischwitz. 2008. Response of new field-resistant peanut cultivars to twin-row pattern or in-furrow applications of phorate for management of spotted wilt. Plant Dis. 92: 1307-1312.

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

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

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

Digiuseppe, G. 2010. Bayer to phase out use of Temik. *In* Crop Production. http://magissues.farmprogress.com/MDS/MS11Nove10/mds024.pdf.

Dorman, K. W., and J. C. Barber. 1956. Time of flowering and seed ripening in southern pines. South Eastern Forest Expt. Sta. Paper No. 72.

FAS. 2013. Foreign Agricultural Service. World agricultural production: World crop production summary April 2013. United States Department of Agriculture.

German, T. L., D. E. Ullman, and J. W. Moyer. 1992. *Tospoviruses*: diagnosis, molecular biology, phylogeny, and vector relationships. Annu. Rev. Phytopathol. 30: 315-348.

GFB News. 2010. Georgia Farm Bureau: the voice of Georgia farmers. Bayer CropScience to phase out pesticide aldicarb, close Georgia plant. http://www.gfb.org./gfbnews/gfbnews moreinfo.asp?recordid=284.

Groves, R. L., G. G. Kennedy, and J. F. Walgenbach. 1998. Inoculation of *Tomato spotted wilt virus* into cotton. Disease notes in Plant Dis. 82: 959.

GPC. 2012. Georgia Peanut Commission. 2012 Georgia the state, peanut facts sheet. http://www.americanpeanuts.com/gapeanuts/about/2012crop_gapnutsfactsheet.pdf.

Halliwell, R. S., and G. Philley. 1974. Spotted wilt of peanut in Texas. Plant Dis. Rep. 58: 23-25.

Holbrook, C. C., and A. K. Culbreath. 2008. Registration of 'Georganic' peanut. J. Plant Reg. 2: 17.

Holbrook, C. C.,, P. Timper, A. K. Culbreath, and C. K. Kvien. 2008. Registration of 'Tifguard' peanut. J. Plant Reg. 2: 92-94.

Hulshof, J., and I. Vänninen. 2001. Western flower thrips feeding on pollen, and its implications for control, pp. 173-179. *In* R. Marullo and L. Mound (eds.), Thrips and Tospoviruses: Proceedings of the 7th International Symposium on Thysanoptera.

Hulshof, J., E. Ketoja, and I. Vänninen. 2003. Life history characteristics of *Frankliniella occidentalis* on cucumber leaves with and without supplemental food. Entomol. Exp. Appl. 108:19-32.

Jacobson, A. L., and G. G. Kennedy. 2011. The effect of three rates of cyantraniliprole on the transmission of *Tomato spotted wilt virus* by *Frankliniella occidentalis* and *Frankliniella fusca* (Thysanoptera: Thripidae) to *Capsicum annuum*. Crop Protection 30: 512-515.

Jones, D. R. 2005. Plant viruses transmitted by thrips. Eur. J. Plant Pathol. 113: 119-157.

Johnson, W. C., T. B. Brenneman, S. H. Baker. A. W. Johnson, D. R. Summer, and B. G. Mullinix. 2001. Tillage and pest management consideration in a peanut-cotton rotation in the southeastern coastal plain. Agron. J. 93: 570-576.

Kirk, W. D. J. 1984. Pollen feeding in thrips (Insecta: Thysanoptera). J. Zoo. 204: 107-117.

Kirk, W. D. J. 1985. Pollen-feeding and the host specificity and fecundity of flower thrips (Thysanoptera). Ecol. Entomol. 10: 281-289.

Kirk, W. D. J. 1997. Feeding. thrips as crop pests. T. L. Lewis (ed.). CAB International, London, UK.

Leskey, T. C., D. A. J. Teulon, and E. A. 1997. Effects of temperature and sugar maple pollen on oviposition and longevity of pear thrips (Thysanoptera: Thripidae). Environ. Entomol. 26: 566-571.

Lundgren, J. G. 2009. Relationships of natural enemies and non-prey foods. Springer, USA.

Marois, J. J., and D. L. Wright. 2003. Effect of tillage system, phorate, and cultivar on tomato spotted wilt of peanut. Agron. J. 95: 386-389.

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

Murai, T., and A. J. M. Loomans. 2001. Evaluation of an improved method for mass-rearing of thrips and a thrips parasitoid. Entomologia Experimentalis et Applicata 101: 281-289.

Murai, T., and T. Ishii. 1982. Simple rearing method for flower thrips (Thysanoptera: Thripidae) on pollens. Jap. J. Appl. Ent. Zool. 26: 149-154.

NASS. 2014. National Agricultural Statistics Service. Crop production 2013 summary: updated January 2014. United States Department of Agriculture.

Pappu, H. R., R. A. C. Jones, and R. K. Jain. 2009. Global status of *Tospovirus* epidemics in diverse cropping systems: successes achieved and challenges ahead. Vir. Res. 141: 219-236.

Pattee, H. E., and H. T. Stalker. 1995. Advances in peanut science. American Peanut Research and Education Society, Inc., Stillwater, OK, USA.

Riley, D. G., and H. R. Pappu. 2004. Tactics for management of thrips (Thysanoptera, Thripidae) and *Tomato spotted wilt tospovirus* in tomato. J. Econ. Entomol. 97: 1648-1658.

Riley, D. G., A. Chitturi, and A. N. Sparks. 2007. Does natural deposition of pine pollen affect the ovipositional behavior of *Frankliniella occidentalis* and *Frankliniella fusca*? Entomologia Experimentalis et Applicata 124: 133-141.

Riley, D. G., and S. V. Joseph. 2011. Host plant resistance to *Tomato spotted wilt virus* (*Bunyaviridae*:*Tospovirus*) in tomato. Hort. Sci. 46: 1626-1633.

Riley, D. G., G. M. Angelella, and R. M. McPherson. 2011a. Pine pollen dehiscence relative to thrips population dynamics. Entomologia Experimentalis et Applicata 138: 223-233.

Riley, D. G., S. V. Joseph, R. Srinivasan, and S. Diffie. 2011b. Thrips vectors of tospoviruses. J. Integ. Pest Mngmt. 1: 1-10.

Ros, E. 2010. Health benefits of nut consumption. Nutrients 2: 652-682.

Schuster, G. L., and R. S. Haliwell. 1994. Six new hosts of *Tomato spotted wilt virus* in Texas. Disease Notes in Plant Dis. 78:100.

Shrestha, A., R. Srinivasan, S. Sundaraj, A. K. Culbreath, and D. G. Riley. 2013. Second generation peanut genotypes resistant to thrips-transmitted *Tomato spotted wilt virus* exhibit tolerance rather than true resistance and differentially affect thrips fitness. J. Eco. Ent. 106: 587-596.

Smith, N., and A. Smith. 2013. Peanut outlook and cost analysis for 2013. *In* J.P. Beasley (ed.), 2013 Peanut Production Update. The University of Georgia Cooperative Extension Service, Athens, GA.

Soyatech. 2013. Peanut facts. http://www.soyatech.com/ peanut_facts.htm.

Stanley, R. G. and H. F. Linskins. 1974. Pollen: biology, biochemistry, management. Springer, New York.

Talcott, S. T., S. Passeretti, C. E. Duncan, and D. W. Gorbet. 2005. Polyphenolic content and sensory properties of normal and high oleic acid peanuts. Food Chem. 90: 379-388.

Tillman, B. L., D. W. Gorbet, A. K. Culbreath, and J. W. Todd. 2006. Response of peanut cultivars to seeding density and row patterns. Crop Management, doi: 10.1094/CM-2006-0711-01-RS.

Todd, J.W., J. R. Chamberlin, A. K. Culbreath, and J. W. Demski. 1994a. Timing and duration of vector management in relation to spotted wilt disease incidence in peanut. Proc. Am. Peanut Res. Ed. Soc. 25: 86.

Todd, J.W., J. R. Chamberlin, A. K. Culbreath, D. Rogers, and J. W. Demski. 1994b. Contradictions of insecticide use relative to vector control and spotted wilt disease progress in peanut. Proc. Am. Peanut Res. Ed. Soc. 26: 42.

Todd, J.W., and A. K. Culbreath. 1995. Thrips populations and spotted wilt disease progress on resistance/susceptible cultivars treated with various insecticides. Proc. Am. Peanut Res. Ed. Soc. 27: 35.

Todd, J. W., A. K. Culbreath, J. R. Chamberlin, R. J. Beshear, and B. G. Mullinix. 1995. Colonization and population dynamics of thrips in peanuts in the southern United States, pp. 453-460. *In* B. Parker, M. Skinner and T. Lewis (eds.), Thrips Biol. Mgt. Plenum Press, New York.

Todd, J. W., A. K. Culbreath, and S. L. Brown. 1996. Dynamics of vector populations and progress of spotted wilt disease relative to insecticides use in peanuts. Acta Hortic. 431: 483-490.

Todd, J. W., A. K. Culbreath, H. R. Pappu, and S. L. Brown. 1997. Thrips as *Tospovirus* vectors in peanut. Proc. Am. Peanut Res. Ed. Soc. 29: 17.

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

Trichilo, P. J., and T. F. Leigh. 1988. Influence of resource quality on the reproductive fitness of flower thrips (Thysanoptera, Thripidae). Annals Entomol. Soc. America 81: 64-70.

Tubbs, R. S., and J. P. Beasley. 2009. Tillage and row pattern update *In* 2009 Peanut Update. The University of Georgia, College of Agriculture and Environmental Science.

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

Tubbs, R., K. Balkcom, M. Toews, and R. Srinivasan. 2013. Effects of fertilization, tillage, and phorate on thrips and TSWV incidence in early planted peanuts (abstr.). Southern Cons. Agric. Sys. Conf.. http//:www.ars.usda.gov/research/publications/publications.htm.

USDA. 2013a. United States Department of Agriculture. National nutrient database for standard reference, release 26, nutrient data laboratory.

USDA. 2013b. United States Department of Agriculture. Natural Resources Conservation Service. Plants database updated by national plant data team.

Venkatachalam, M., and S. K. Sathe. 2006. Chemical composition of selected edible nut seeds. J. Agri. Food Chem. 54: 4705-4714.

Wäckers, F. L., J. Romeis, and P. van Rijn. 2007. Nectar and pollen feeding by insect herbivores and implications for multitrophic interactions. Annu. Rev. Entomol. 52: 301-323.

Wells, M. L., A. K. Culbreath, and J. W. Todd. 2002. The effect of in-furrow applications of acibenzolar-S-methyl on *Tomato spotted wilt virus* and thrips in peanut. Peanut Sci. 29: 136-141.

Whitfield, A. E., D. E. Ullman, and T. L. German. 2005. Tospovirus-thrips interactions. Annu. Rev. Phytopathol. 43: 459-489.

CHAPTER 2

LITERATURE REVIEW

Peanut: Its Benefits, Origin, and Current Production in the United States

Peanut (*Arachis hypogaea* L.), commonly known as groundnut, is considered one of the most economically important members of the family *Fabaceae* (Pattee and Young 1982, Pattee and Stalker 1995). It is also known for its high protein (≥25%) and oil (≥50%) contents (Phillips 1997, USDA 2013a). Peanut seeds contain vitamins such as folic acid, riboflavin, niacin, vitamin B6, and vitamin E. Additionally, minerals such as calcium, iron, magnesium, potassium, and zinc are also found in peanut (Venkatachalam and Sathe 2006, USDA 2013a).

Peanut is native to South American countries such as Peru, Brazil and Argentina (Pattee and Young 1982, NPB 2013). Spaniards discovered peanuts as they explored the New World, and peanut traveled with them to Spain. From Spain, traders and explorers for spread the crop to other continents such as Asia and Africa. However, it is believed that peanut reached North America through the African people during slave trading periods in the 1700s (APC 2013, NPB 2013).

World statistics in April 2013 showed that the United States produced 3.06 million metric tons (mmt) of peanut as compared to China (16.50 mmt) and India (5.00 mmt) which ranked first and second, respectively (FAS 2013, NASS 2013). Georgia is the number one peanut producing state in the United States. In 2013, approximately 41% of the acres were peanut productions in Georgia alone. Alabama (8.8 hundred million), Florida (7.8 hundred million), and Texas (5.1

hundred million) ranked second, third and fourth, respectively, in terms of production (lbs), when compared with Georgia (3.3 billion) (NASS 2013).

However, the occurrence of pests and diseases limit the full potential of peanut production in the United States, especially during the 1980s. Among the existing diseases, spotted wilt of peanut, caused by *Tomato spotted wilt virus* (TSWV), is one of the most important diseases which severely limits peanut production. The use of a single management strategy is not enough to solve the problem which makes the pest and the disease difficult to deal with (Culbreath et al. 2003). Thrips transmit TSWV in a persistent and propagative manner (German et al. 1992, Culbreath et al. 2003, Whitfield et al. 2005). TSWV transmission is influenced by unique interactions between the host, the vector, and the virus.

History, Geographical Distribution, and Economic Importance of *Tomato spotted wilt virus* (TSWV)

Spotted wilt disease was first reported in tomato (*Lycopersicon esculentum* Miller) in Australia in 1915 (Brittlebank 1919). Then the causal agent as a plant virus was discovered in 1930 (Samuel et al. 1930). Thrips species, such as the onion thrips (*Thrips tabaci* Lindeman), common blossom thrips (*Frankliniella schultzei* Trybom), and western flower thrips (*Frankliniella occidentalis* Pergande), were identified as vectors of TSWV (Pittman 1927, Samuel et al. 1930, Gardner et al. 1935, Cho et al. 1987). Spotted wilt disease became widespread worldwide among several agricultural and ornamental crops, to even include weeds species. This resulted in the recognition of more species of thrips as vectors of TSWV. At least 1,090 host-plants in 15 monocotyledonous and 69 dicotyledonous were confirmed as hosts of tospoviruses transmitted by thrips (Riley et al. 2011b). TSWV caused significant losses in

production of some economically important crops such as peanut (Cho et al. 1987, Schuster and Haliwell 1994, Groves et al. 2001, Culbreath et al. 2003, Whitfield et al. 2005).

Tomato spotted wilt caused by TSWV was first confirmed in peanut by Costa as early as 1941 in Brazil (Costa 1941). After three decades (1971), it was first reported in the United States in Texas (Halliwell and Philley 1974). This was the beginning of one of the most serious threats to peanut production in North America. In the 1980s, yield losses up to 100% were attributed to TSWV in Southern Texas (Black et al. 1986, Culbreath et al. 1992). Spotted wilt also caused severe yield losses in nearby peanut producing states such as Georgia, Florida, and Alabama. In Georgia alone, yield losses due to TSWV from 1990 to 1997 were estimated to be at 12%, which was equivalent to approximately $40 million (Bertrand 1998, Culbreath et al. 2003). Remarkably, great reduction on percent yield, equivalent to more or less $20 million or 5%, started after 1997 until 2004 (Culbreath and Srinivasan 2011). Yield losses of around 7%, which is about $30 million, occurred in 2005. Fortunately, after 2005, losses (less than 3% or $10 million) due to TSWV continue to decrease (Culbreath and Srinivasan, 2011).

At present, there is no single management tactic to completely eliminate spotted wilt in peanut, as well as its causal virus, and insect vector. However, development of peanut cultivars with higher magnitude of field resistance to TSWV and integration of various management practices such as cultural and chemical tactics, reduced risks induced by TSWV (Culbreath et al. 2003, 2008, 2010).

Host Range of TSWV and Symptoms of Spotted Wilt

More than 1000 plant species in 84 combined families of monocotyledons and dicotyledons can be infected by TSWV (Pappu et al. 2009, Riley et al. 2011b). Symptoms among different agricultural crops can be variable (Culbreath et al. 2003). In general, viral diseases such as spotted wilt may induce stunting especially in younger plants. Chlorosis,

necrotic rings or concentric ringspot patterns are very common in both foliage and fruits of infected hosts. To further describe the disease, there are two general symptoms from which the name was derived. The first one is the bronzing of young leaves that eventually leads to small, dark spots. The second one is the sagging of the leaves that shows a wilt-like appearance similar to TSWV-infected tomato plants (Goldberg 2000). Other abnormalities include die-back of the growing tips, black streak patterns on petioles or terminal stems, deformation of leaves and fruits, and great reduction in fruit quality and yield (Goldberg 2000, Culbreath et al. 1997, 2003; Whitfield et al. 2005) which are often seen in infected tomatoes, peppers, tobacco (Chatzivassiliou 2008), chrysanthemum (Matsuura et al. 2004), and peanuts (German et al. 1992, Riley and Pappu 2004, Culbreath and Srinivasan 2011, Funderburk et al. 2011).

TSWV-infected peanut plants express diverse symptoms on leaflets, pegs, pods, seed coats, and roots (Culbreath et al. 2003). Concentric ringspots, malformation, chlorotic and oakleaf patterns on leaflets, and overall stunting of above-ground plant parts are very common (Culbreath et al. 1997, Culbreath and Srinivasan 2011). In some cases, infections are asymptomatic (Culbreath and Srinivasan 2011). The geocarpophores, or commonly known as "pegs", may be distorted while pods and kernels may be reduced in size. Also, reddish discoloration of seeds and cracking of seed coats may occur. Noticeable stunting of the whole plant can affect the seed quality and yield depending on the time of the appearance of symptoms. Usually those peanut plants that show early symptoms will generate fewer and poorer quality pods and kernels as compared to plants showing symptoms at later stage. The root systems may have different levels of necrosis that can lead to death of the plant. TSWV infections could also increase the risk of infection by other pathogens (Culbreath et al. 1992, 2003).

TSWV: Taxonomy and Genome Organization

TSWV is a type member in the plant-infecting genus *Tospovirus* in the family *Bunyaviridae* (German et al. 1992, Adkins 2000, Hull 2002). The virions are ~80-120 nm in diameter. Other members of the family, namely *Orthobunyavirus, Hantavirus*, *Nairovirus* and *Phlebovirus*, mainly infect vertebrates and arthropods (German et al. 1992, Adkins 2000, Whitfield et al. 2005). The genome of TSWV consists of three single-stranded RNAs known as L (Large), M (Medium), and S (Small). The L RNA has negative sense orientation while M and S RNAs are both ambisense in nature. Individually, they are encapsidated and bounded by a host origin membrane envelope, which makes them unique from most other plant viruses (German et al. 1992, Sherwood et al. 2003, Whitfield et al. 2005, Pappu et al. 2009, Kulshrestha et al. 2013). First, the L RNA (8.9 kb) encodes the protein RNA polymerase or RNA-dependent RNA polymerase (RdRp) (330-kDa), which catalyzes the replication of RNA from an RNA template during the initial stage of the replication process of the RNA genome (de Haan et al. 1991, Pappu et al. 2009, Kulshrestha et al. 2013). Second, the M RNA (4.8 kb) has a viral (v) sense that encodes a 34-kDA non-structural protein known as NSm, which is believed to involve in cell-to-cell movement of the virus (Storms et al. 2001, Pappu et al. 2009, Kulshrestha et al. 2013). NSm also acts as an avirulence determinant between TSWV and resistant pepper carrying *Tsw* (Margaria et al. 2007, Kulshrestha et al. 2013). The M RNA also has a viral complementary (vc) sense that encodes approximately 127-kDa-protein that is processed into 2 polypeptides each of which is glycosylated: G_N (75-kDa) and G_C (46-kDa) (de Haan et al. 1989, Whitfield et al. 2005, Kulshrestha et al. 2013). G_N is believed to be involved in virus binding and/or entry in thrips' midgut cells, while G_C acts as possible fusion protein, which is significant in pH dependent virus entry. To add, the pH dependent entry begins with the attachment of virus followed by a direct

fusion of viral membrane with the host's plasma membrane (Whitfield et al. 2005). Finally, the S RNA (2.9 kb) encodes for NSs (52-kDa) in the v sense that is believed to act as a silencing suppressor against the host defense. In the vc sense, it has open reading frame (ORF) that encodes for the nucleocapsid or N protein (29-kDa) of the virus as protective envelope (de Haan et al. 1990, Bandla et al. 1994, Bucher et al. 2003).

The Vector: Taxonomy and Biology of Thrips

Thrips are small, cylindrical, slender-bodied hemimetabolous insects. Their size ranges from 0.5 to 5.0 mm long (Mound 1997, Triplehorn and Johnson 2005, Gullan and Cranston 2010). The body color is diverse. It ranges from yellow to orange to brown and to black (Morse and Hoddle 2005, Triplehorn and Johnson 2005, Riley et al. 2011b). Thrips are in the order Thysanoptera, which has two suborders namely Tubulifera and Terebrantia (Ananthakrishnan 1993, Mound 2005). There are 10 families in the said order in which only one, the family Phlaeothripidae, is within the suborder Tubulifera. Hence, majority of the economically important pests belong to the suborder Terebrantia. The family Thripidae in Terebrantia is considered very important due to its association with economically important agricultural crops (Jones 2005, Mound 2005).

Thrips species have unique mouthparts for feeding which are often described as "punch and suck" (Triplehorn and Johnson 2005). The feeding apparatus have four stylets, with a vestigial right mandible (Triplehorn and Johnson 2005, Gullan and Cranston 2010). The remaining three functional stylets include the left mandible and two maxillary stylets (laciniae). The left mandible is commonly used in piercing the plant epidermis followed by the breakage of individual plant cells (Mound 2005, Gullan and Cranston 2010, Riley et al. 2011b). Paired maxillary laciniae are located immediately posterior to the mandibles and are used for

penetration and ingestion of plant juices or other food sources such as pollen, blooms, and fungal spores (Kirk 1984, 1997; Triplehorn and Johnson 2005, Whitfield et al. 2005, Riley et al. 2011b).

Mechanical damage brought about by thrips feeding can give several varieties of injury in different parts of the host plant. In peanuts, early infestation may lead to severe injuries more specifically on tender parts of the plant such as the terminal foliage (Riley et al. 2005, Niyomsil et al. 2007). Later it may show "burning of tips" appearance at early stage of the seedlings. Silvery patches, leaf deformation, and browning of the edges are very common signs of thrips feeding activity in vegetative parts (Culbreath et al. 2003, Riley et al. 2005). In general, the discoloration and abnormalities can reduce the overall aesthetic value which leads to economic losses of the crops (Funderburk et al. 1998, Culbreath et al. 2003, Harper and Horne 2012). Thrips as vectors can also have secondary or indirect damage via the mechanical injury in the plants. Those entry points serve as an opportunity for plant pathogen such as viruses (Ananthakrishnan 1993).

The life cycle of thrips comprises six major stages, which include an egg stage, two wingless larval stages, two non-feeding pupal stages, and an adult stage (Whitfield et al. 2005, Riley et al. 2011b). These stages are also important to the fate of TSWV transmission, as it is dependent on whether the adult, male or female, acquires the virus or not during its life stage (Ullman et al. 1992, Whitfield et al. 2005). The life cycle of thrips begins when the adults oviposit eggs into the plant tissue. Normally, eggs hatch after 2-3 days, which depends on the environmental condition such as the current temperature and the availability of host species (McDonald et al. 1998, Hansen et al. 2003, Whitfield et al. 2005). After hatching, there will be two feeding larval stages wherein the 1st instar larval stage can successfully acquire the virus depending on the availability of TSWV-infected foliage. Virus passage from larval stages to

pupal stages is transstadial, i.e. pathogen remains with the vector from one life stage to the next stage particularly from the 2nd instar larval to pre-pupal stage (Whitfield et al. 2005). Usually, the larval stage lasts for 5-7 days after which there are two non-feeding and immobile pupal stages. In some cases, the thrips drop off from the host to pupate on a certain medium such as soil as in *F. occidentalis*, and foliage, as in *T. tabaci* (Broadbent et al. 2003, Whitfield et al. 2005). Approximately 2-4 days after, pupae emerge as adults. The life cycle takes about 20-30 days from egg to adult depending on the environmental conditions (Ananthakrishnan 1993, Whitfield et al. 2005). TSWV can only be transmitted if the 1st instar or early 2nd instar larval stage acquired the virus and successfully molt into the final stage. Thus, as thrips mature during its life cycle, it decreases its potential to acquire and transmit the plant virus. Environmental factors such as wind and weather conditions may contribute to thrips dispersal in the field (German et al. 1992, Ullman et al. 1992, Jones 2005, Whitfield et al. 2005, Pappu et al. 2009).

In total, there are about 5,500 to 6,000 thrips species worldwide including those unidentified (Mound 2005, Pappu et al. 2009). Of those, 1,710 species were identified in the family Thripidae. As of 2011, 14 and 9 species in this family exclusively transmit tospoviruses and TSWV, respectively (Riley et al. 2011b). Most of the thrips species that transmit TSWV are in the genus *Frankliniella* such as *F. fusca* (Hinds), *F. occidentalis*, *F. schultzei*, *F. intosa*, *F. bispinosa* (Morgan), *F. gemina* (Bagnall), and *F. cephalica* (Crawford) (Bautista et al. 1995, Riley et al. 2011b). Other species from different genus include *T. tabaci* and *T. setosus* (Moulton) (Chatzivassiliou et al. 2002, Riley et al. 2011b).

Among the abovementioned species, tobacco thrips (*F. fusca*) and western flower thrips (*F. occidentalis*) are considered the most significant for peanut production in Georgia (Todd et al. 1995, 1996, 1997; Culbreath et al. 2003, Culbreath and Srinivasan 2011). Between the two

species, *F. fusca* is a more efficient vector of TSWV than *F. occidentalis*. The former colonizes and reproduces efficiently on peanut foliage than the latter (Weeks and Hagan 1991, Todd et al. 1995, 1996, 1997; Culbreath et al. 2003, Culbreath and Srinivasan 2011).

Management of Thrips, TSWV, and Spotted Wilt in Peanut

Currently, there is no single management tactic to completely eliminate spotted wilt infection in the field. However, availability of several disease management options can potentially reduce vector populations and disease incidence (Culbreath et al. 1999, 2003; Baldwin et al. 2001, Culbreath and Srinivasan 2011). The use of peanut cultivars with higher field tolerance or resistance to TSWV is considered one of the best approaches. The integration of chemical use with cultural practices is also widely practiced. Individual sections for each tactic are presented in the next few pages.

Development of TSWV-resistant Peanut Cultivars

Several previously grown peanut cultivars such as Florunner, Georgia Runner, SunOleic 97R, and GK-7 were extremely susceptible to TSWV (Culbreath et al. 2003). Due to losses brought about by the disease, peanut researchers and breeders from southeastern states did intensive screening of breeding lines which led to the release of first generation TSWV-resistant peanut cultivars. Some of these were released in the mid 80s to late 90s including Southern Runner, Georgia Browne, Georgia Green, Tamrun 96, UF MDR 98 (formerly known as UF 91108), and C-99R (Gorbet et al. 1987, Culbreath et al. 1992, 1994, 1996, 1997, 2000, 2003; Smith et al. 1998; Gorbet and Shokes 2002). These genotypes had moderate levels of resistance to TSWV and showed better performance in the suppression of spotted wilt when compared with the previously used peanut cultivars such as Florunner and SunOleic 97R (Culbreath et al. 2003). In 2002, 90% of the peanut acreage in Georgia was planted to Georgia Green alone. In addition,

Georgia Green was the leading cultivar grown in other southeastern states (Culbreath et al. 2003).

Thereafter, the second generation of TSWV-resistant cultivars was released with higher field resistance to TSWV than Georgia Green. These included Georgia-06G, Georganic, Georgia Greener, Georgia-07W, Tifguard, and Georgia-09B (Branch 2007a, 2007b, 2010; Branch and Brenneman 2008, Holbrook and Culbreath 2008, Holbrook et al. 2008). These genotypes produced better yields than first generation TSWV-resistant cultivars (Culbreath and Srinivasan 2011). In 2013, about 77% of the acreage in southeastern states was planted to Georgia-06G (Beasley 2013).

More recently, third generation TSWV-resistant peanut cultivars were released such as Georgia-10T and Georgia-12Y (Branch and Culbreath 2011, Beasley 2013, Branch 2013). Based on the three-year field studies, the former was noted as an excellent cultivar for early planting date option in the southeast peanut production area (Branch and Culbreath 2011). Overall, the third generation cultivars had significantly lower TSWV incidence, higher yield, and better grade as compared with some second generation TSWV-resistant peanut cultivars (Branch and Culbreath 2011, Branch 2013).

Integration of Cultural Practices

The incorporation of several cultural practices to peanut cultivars with moderate to high resistance to TSWV decreased thrips populations and spotted wilt incidence and increased pod yield (Culbreath et al. 2003, Culbreath and Srinivasan 2011). They include reduced tillage instead of conventional tillage, twin rows instead of single rows, and increased seeding rate (Gorbet and Shokes 1994, Baldwin et al. 1998, 2001a, 2001b;, Branch et al. 2003, Marois and Wright 2003, Cantonwine et al. 2006, Tillman et al. 2006, Culbreath et al. 2008, 2013).

Based on the definition from USDA (2012), conventional tillage is defined as the system, which involves the utilization of moldboard or heavy disks as primary tillage followed by the secondary tillage involving planting and row cultivation practices that tend to bury nearly all previous crop residues (USDA 2012). On the other hand, conservation tillage, such as no tillage, strip tillage, minimum tillage, use of cover crops, and even light disking, can be referred to as tillage practices that leave 30% of the soil while cover crop residues remain on the surface (NCCE 2010, Mathew et al. 2012). In terms of soil disturbance, conventional tillage normally adopts full soil disturbance while conservation tillage such as strip tillage tends to leave the soil and crop residue undisturbed (NCCE 2010, USDA 2012). Both tillage methods have pros and cons. Benefits from conventional tillage include physical weed control and warming of seedbed for faster crop germination. In contrast, some disadvantages include soil erosion, germination of new weed seeds, and faster soil dryness (Tubbs and Beasley 2009). As for conservation tillage, some advantages include conservation of soil moisture, higher soil organic matter, less cultivation cost, and reduced incidence of pests and diseases (Tubbs and Beasley 2009, Mathew et al. 2012).

In the field study conducted by Baldwin et al. (2001a), the use of strip tillage led to lower incidence of TSWV and higher pod yield when compared with conventional tillage (Baldwin et al. 2001a). Other studies showed that minimum or reduced tillage led to lower insect pests, thrips feeding injury, and TSWV incidence than conventional tillage (Minton et al. 1991, Brown et al. 1996, Baldwin and Hook 1998, Brandenburg et al. 1998, Hurt et al. 2006). Similarly, Tubbs et al. (2013) observed an approximate two-fold reduction of adult tobacco thrips on plots under strip tillage than on plots under conventional tillage. Also, TSWV incidence was more than twice as much in plots under conventional tillage than in plots under strip tillage (Tubbs et al. 2013). The

abovementioned effects might be due to plant debris present in conservation or strip tillage which serves as hindrance for thrips' vision in locating the suitable host plant (Culbreath et al. 2003, Culbreath and Srinivasan 2011).

Another cultural practice that has become more popular in peanut production is using twin or double rows, with a measurement of 18-24 cm x 91 cm of inner and outer row, respectively (Culbreath et al. 2003, Culbreath and Srinivasan 2011). Several studies indicated that twin rows provide more advantages than single rows, they include increased pod yields, reduction of TSWV incidence, and suppression of thrips populations (Baldwin et al. 1998, 2001a, 2001b; Tillman et al. 2006, Culbreath et al. 2008, Tubbs and Beasley 2009, Tubbs et al. 2011). Tubbs et al. (2011) observed a 10% increase, 50% decrease, and 25% improvement in pod yield, TSWV incidence, and plant stand, respectively (Tubbs et al. 2011). Other studies also indicated that planting in twin rows resulted in increased yield and grade and reduced TSWV incidence than planting in single rows (Baldwin et al. 2001, Tillman et al. 2006, Culbreath et al. 2008). Lanier et al. (2004) in North Carolina also observed similar results. These outcomes may be due to the proximity of the seeds in the single row pattern which often results in higher competition for space, light, water, and nutrients (Tubbs et al. 2011). On the other hand, seeds in twin rows have better spacing and even distribution of nutrients (Tubbs et al. 2011). Planting in twin rows could lead to earlier seed germination, better plant coverage, less competition with emerging weeds than planting in single rows. All of these circumstances are commonly associated towards better yield (Tubbs et al. 2011). Another possible explanation for the lower disease incidence is the visual interference of the potentially viruliferous thrips in recognizing suitable host plants for acquiring and transmitting plant virus (Culbreath et al. 2003, Culbreath and Srinivasan 2011).

Use of Insecticides as Chemical Management

Often times, planting of peanut cultivars with high levels of resistance to TSWV is considered the first line of defense against spotted wilt. However, these currently available peanut cultivars are not immune to the virus and are still susceptible to virus infection (Culbreath and Srinivasan 2011). Even with the integration of cultural practices, it is not sufficient to manage TSWV and thrips. Insecticides against insect pests are still widely used in peanut production. In-furrow application of carbamate (aldicarb) and organophosphate (phorate) are commonly used in peanut. A number of studies have evaluated the effects of these insecticides on thrips populations, thrips feeding, and TSWV transmission (Todd et al. 1994a, 1994b; Todd and Culbreath 1995, Baldwin et al. 2001a, Ames Herbert et al. 2007, Culbreath et al. 2008, Tubbs et al. 2013). Although, these insecticides are known to suppress thrips populations, as well as their feeding, their role in reducing TSWV transmission seems to be minimal.

Aldicarb and phorate are commonly used due to their low costs and ease of application when compared with other insecticides (Digiuseppe 2010). However, aldicarb and phorate possess broad-spectrum toxicity on mammals and can potentially cause undesirable non-target effects (AgroNews 2010, Digiuseppe 2010). Recently, United States Environmental Protection Agenccy (US-EPA) and Bayer CropScience agreed to stop the production of aldicarb and phase out its usage by 2018 (AgroNews 2010, Digiuseppe 2010, GFB News 2010). Since phorate has similarities to aldicarb in terms of toxicity and non-target effects, it is possible for it to be phased out as well. Hence, it is important to identify alternatives to carbamate and organophosphate insecticides for spotted wilt management in peanut. Also, these insecticides should be less toxic to the environment and induce less non-target effects than organophosphates and carbamates.

Pollen Dehiscence in the Southeastern United States: Effects on Thrips Population and TSWV Epidemics

In Georgia, it is not unusual to observe heavy pollen deposition from various crops and trees during spring season (Riley et al. 2011a). Pollen dehiscence from different pine (*Pinus* spp.) trees is known to be one of the major contributors in this scenario. Common pine species in the southeastern states include slash pine (*P. elliottii* Engelm), longleaf pine (*P. palustris* Mill.), loblolly pine (*P. taeda* L.), pond pine (*P. serotina* Michx.), and shortleaf pine (*P. echinata* Mill.) (Dorman and Barber 1956, Riley and Pappu 2004, USDA 2013b). Pine pollen dehiscence in the southeast is believed to influence thrips populations in the spring season prior to the growing season of most crops including peanut (Riley et al. 2011a). Peaks of pollen dehiscence from various pine trees occur before the occurrence of thrips peaks (Riley et al. 2011a). For example, loblolly pine sheds pollen around 2nd to 3rd week of April which precedes the seasonal population peak of tobacco thrips by two to five weeks (Dorman and Barber 1956, Groves et al. 2003, Riley et al. 2011a). Therefore, this could benefit thrips, in general, given that a nutritious food source is available in the field. It is more likely that thrips feeding might contribute to increased TSWV transmission if pollen dehiscence enhances thrips feeding particulary for viruliferous thrips.

Importance of Pollen Nutrition Being a Part of the Diet

Pollen grains, in general, are known to have high levels of protein and other nutrients (Andrewartha 1935, Erhardt and Baker 1990, Lundgren 2009). Based on the quantity of pollen dry weight, the nutritional value of protein, lipids, and carbohydrates measures approximately 12-61%, 1.5-18.9%, and 15%, respectively (Lundgren 2009). Pollen grains typically have substantial amounts of amino acids including proline (3% of pollen dry weight) (Erhardt and Baker 1990, Lundgren 2009). Other nutrients including simple sugars, fatty acids, vitamins, and

minerals are also present in pollens (Stanley and Linskins 1974, Kirk 1997, Lundgren 2009). With the nutrient content of pollen, it is possible that pollen feeding by insects such as thrips will lead to fitness benefits. Besides potential direct effects on thrips fitness, pollen supplementation is known to improve utilization of otherwise unsuitable hosts. For example, *F. occidentalis* was able to reproduce on poinsettia (*Euphorbia pulcherrima* Wild ex. Klotzsch) only in the presence of pine pollen (Hulshof and Vänninen 2001).

Role of Pine Pollen on Thrips Oviposition

Several studies documented that the addition of pine pollen to thrips diet enhanced thrips oviposition (Andrewartha 1935, Tsai et al. 1996, Morita et al. 2008). In the study conducted by Morita et al. (2008), *Haplothrips brevitubus* (Karny) produced eggs in the presence of pollen from strawberry, eggplant (*Solanum melongena* L.), pine, and pepper (*Capsicum annum* L.). Another study conducted by Riley et al. (2007) showed that the addition of slash pine pollen led to 1.6- and 2.9-fold increase in egg production by *F. fusca* and *F. occidentalis*, respectively. Addition of tea (*Camellia sinensis* (L.) O. Kuntze) pollen revealed an increased in egg production by *F. intosa* (Murai and Loomans 2001). Increased in egg production with the supplementation of pollen might be due to availability of protein and other nutrients such as amino acids (Andrewartha 1935, Erhardt and Baker 1990, Lundgren 2009). Amino acids such as proline are also associated with vitellogenesis, oogenesis, and egg maturation (Wheeler 1996, Rojas et al. 1998, Carter et al. 2006, Lundgren 2009).

Role of Pollen on Thrips Fitness and Settling Preference

Besides the role of pollen in oviposition of thrips, it is also known to enhance other fitness parameters (Murai and Ishii 1982, Tsai et al. 1996, Hulshof et al. 2003, Angelella and Riley 2010). The addition of scots pine (*P. sylvestris* L.) pollen to cucumber (*Cucumis sativus* L.) and downy birch (*Betula pubescens* Ehrh.) led to more than 60% increase *F. occidentalis*

larvae (Hulshof et al. 2003). Another study conducted by Angelella and Riley (2010), showed that adult emergence rates of *F. fusca* had a 3.4- to 8.0- fold increase with the addition of slash pine pollen to onion foliage. Developmental time of *F. occidentalis* was also shortened with the addition of pollen to cucumber and cotton foliage (Trichilo and Leigh 1988, Hulshof and Vänninen et al. 2001, Hulshof et al. 2003). Addition of pine pollen also influenced the settling behavior of thrips. Chitturi et al. (2006) observed an increase in *F. fusca* and *F.occidentalis* settling with the addition of slash pine pollen (*P. elliottii*) on tomato and peanut foliage.

Overview of Cotton Production in the United States

Cotton (*Gossypium hirsutum* L.) is one of the most important crops in the United States. In 2013, around 10 M acres of upland cotton were planted in the United States (NASS 2013). Georgia is the number one southeastern producing state of cotton in the United States. Georgia harvested of 1.34 M acres of upland cotton in 2013 (NASS 2013). Given that a huge scale of cotton is being produced in Georgia, this crop is often grown in proximity or in crop rotation to peanut (Johnson et al. 2001). Unlike cotton, peanut is highly susceptible to TSWV. If indeed cotton is a host of TSWV, then thrips dispersal from cotton to peanut could increase TSWV spread in peanut.

Role of Cotton as a Thrips Reservoir and as a TSWV Host

Besides peanut, cotton is documented as host of some thrips species including *F. fusca*, *F. occidentalis*, and *F. tritici* (Fitch) (Watts 1936, Toews et al. 2010). Among the three, *F. fusca* is considered the most predominant species in cotton (Toews et al. 2010). Thrips infestation normally occurs at seedling stage up to five true leaf stage of cotton (Toews et al. 2010). In general, early stages of plant are more susceptible to pests and diseases. Hence, presence of viruliferous thrips in cotton fields might be a potential risk to TSWV infection. Only two studies

have demonstrated that cotton could be a host of TSWV. These studies confirmed TSWV infection serological detection technique using double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) and by mechanical inoulation on an indicator host (Schuster and Haliwell 1994, Groves et al. 1998). The incidences of TSWV infection, following thrips-mediated inoculations, on four cotton varieties (DP 20, DP 52, DP 5409, and HS 46) were 10, 63, and 5%, at the cotyledon, first true leaf satge, and four true leaf stage, respectively. This clearly showed that seedling or early stages of cotton were more susceptible to TSWV than at later stages. However, due to limited studies related to this issue, the role of cotton in TSWV epidemics is not yet clearly understood. It would be interesting to further examine the potential role of cotton as inoculum source of TSWV as well as its effect on TSWV transmission.

Scope of Investigation

There were four main objectives in this study. The first objective involved evaluating alternatives to aldicarb and phorate in relation to thrips and spotted wilt management. Insecticides that were effective in the field trials were subjected to additional greenhouse tests. The goal was to screen and select the insecticides that were comparable to the currently used insecticides in managing thrips and TSWV without compromising the yield in peanut production. The second objective involved the evaluation of selected insecticides with the integration of different cultural practices on TSWV-susceptible and/or –resistant peanut cultivars. The third objective was to identify the effects of pine pollen grains, as a source of protein, on thrips biology and behavior, as well as on the transmission of TSWV. The fourth objective was to assess the role of cotton as a thrips and TSWV reservoir and also evaluate if cotton could serve as an inoculum source for TSWV (Cho et al. 1989, Schuster and Haliwell 1994, Groves et al. 1998).

Literature Cited

Adkins, S. 2000. *Tomato spotted wilt virus* – positive steps towards negative success. Mol. Plant Pathol. 1: 151-157.

AgroNews. 2010. Bayer CropScience to close a plant as Temik phasing out. http://news.agropages.com/News/NewsDetail---2766.htm.

Ames Herbert, D., S. Malone, S. Aref, R. L. Brandenburc, D. L. Jordan, B. M. Royals, and P. D. Johnson. 2007. Role of insecticides in reducing thrips injury to plants and incidence of *Tomato spotted wilt virus* in Virginia market-type peanut. J. Econ. Entomol. 100: 1241-1247.

Andrewartha, H. G. 1935. Thrips investigation 7. On the effect of temperature and food upon egg production and the length of adult life of *Thrips imaginis* Bagnall. J. Coun. Scient. Ind. Res. Aust. 8: 281-288.

Ananthakrishnan, T. N. 1993. Binomics of Thrips. Annu. Rev. Entomol. 38**:** 71- 92.

Angelella, G. M. and D. G. Riley. 2010. Effects of pine pollen supplementation in an onion diet on *Frankliniella fusca* reproduction. Environ. Entomol. 39: 505-512.

APC. 2013. American Peanut Council. About peanut: the peanut industry. http://www.peanutsusa.com/about-peanuts/the-peanut-industry3.html.

Baldwin, J. A., and J. Hook. 1998. Reduced tillage systems for peanut production in Georgia. Proc. Amer. Peanut Res. Ed. Soc. 30:48.

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

Baldwin, J. A., J. W. Todd, J. R. Weeks, D. W. Gorbet, A. K. Culbreath, A. S. Luke-Morgan, S. M. Fletcher, and S. L. Brown. 2001a. 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. Proc. Ann. Southern Cons. Till. Conf. Sust. Agric. 24: 26-34.

Baldwin, J. A., R. McDaniel, D. E. McGriff, and B. Tankersley. 2001b. Yield, grade and tomato spotted wilt incidence of Georgia Green and AT201 peanut when planted in twin vs. single row pattern. Abstr. Proc. Am. Peanut Res. Ed. Soc. 33: 31.

Bandla, M. D., D. M. Westcot, K. D. Chenault, D. E. Ullman, T. L. German, and J. L. Sherwood. 1994. Use of monoclonal antibody to the nonstructural protein encoded by the small RNA of *Tomato spotted wilt tospovirus* to identify viruliferous thrips. Phytopathol. 84: 1427-1431.

Beasley, J. P. 2013. Cultivar options in 2013. *In* J.P. Beasley (ed.), 2013 Peanut Production Update. The University of Georgia Cooperative Extension Service, Athens, GA.

Bertrand, P. F. 1998. 1997 Georgia plant disease loss estimates. Univ. Georgia Coop. Ext. Pub. Path: 98-107..

Black, M. C., P. F. Lummus, D. H. Smith, and J. W. Demski. 1986. An epidemic of spotted wilt disease in south Texas peanuts in 1985. Proc. Am. Peanut Res. Educ. Soc. 18: 66.

Branch, W. D. 1996. Registration of ‘Georgia Green’ peanut. Crop Sci. 36: 803-806.

Branch, W. D. 2007a. Registration of ‘Georgia-06G’ peanut. J. Plant Reg. 1: 120.

Branch, W. D. 2007b. Registration of ‘Georgia Greener’ peanut. J. Plant Reg. 1: 121.

Branch, W. D. 2010. Registration of ‘Georgia-09B’ peanut. J. Plant Reg. 4: 175-178.

Branch, W. D. 2013. Registration of ‘Georgia-12Y’ peanut. J. Plant Reg. 7: 151-153.

Branch, W. D., and A. K. Culbreath. 2011. Registration of 'Georgia-10T' peanut. J. Plant Reg. 5: 279-281.

Branch, W. D., and T. B. Brenneman. 2008. Registration of 'Georgia-07W' peanut. J. Plant Reg. 2: 88-91.

Branch, W. D., J. A. Baldwin, and A. K. Culbreath. 2003. Genotype x seeding rate interaction among TSWV-resistant, runner-type peanut cultivars. Peanut Sci. 30: 108-111.

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

Brittlebank, C. C. 1919. Tomato diseases. J. Dept. Agric.Victoria 17: 231-235.

Broadbent, A. B., M. Rhainds, L. Shipp, G. Murphy, and L. Wainman. 2003. Pupation behavior of western flower thrips (Thysanoptera: Thripidae) on potted chrysanthemum. Can. Entomol. 135: 741–744.

Brown, S. L., J. W. Todd, and A. K. Culbreath. 1996. Effect of selected cultural practices on incidence of *Tomato spotted wilt virus* and populations of thrips vectors in peanuts. Acta Hortic. 431: 491-498.

Bucher, E., T. Sijen, P. de Haan, R. Goldbach, and M. Prins. 2003. Negative-strand tospoviruses and tenuiviruses carry a gene for a suppressor of gene silencing at analogous genomic positions. J. Virol. 77: 1329-1336.

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

Carter, C., S. Shafir, L. Yehonatan, R. G. Palmer, and R. Thornburg. 2006. A novel role for proline in plant floral nectars. Naturwissenschafter 93: 72-79.

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

Chatzivassiliou, E. K. 2008. Management of the spread of *Tomato spotted wilt virus* in tobacco crops with insecticides based on estimate of thrips infestation and virus incidence. Plant Dis. 92: 1012-1020.

Cho, J. J., R. F. L. Mau, W. C. Mitchell, D. Gonsalves, and L. S. Yudin. 1987. Host list of plants susceptible to *Tomato spotted wilt virus* (TSWV). Research Extension Series 078, Hawaii Institute of Tropical Agriculture and Human Resources, Honolulu, Hawaii. 1-10.

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

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

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

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

Culbreath, A. K., J. W. Todd, D. W. Gorbet, F. M. Shokes, and H. R. Pappu. 1997. Field response of new peanut cultivar UF 91108 to *Tomato spotted wilt virus*. Plant Dis. 81: 1410-1415.

Culbreath, A. K., J. W. Todd, D. W. Gorbet, and S. L. Brown. 2000. Reaction of peanut cultivars to spotted wilt. Peanut Sci. 27: 35-39.

Culbreath, A. K., J. W. Todd, and S. L. Brown. 2003. Epidemiology and management of tomato spotted wilt in peanut. Annu. Rev. Phytopathol. 41: 53-75.

Culbreath, A. K., B. L. Tillman, D. W. Gorbet, C. C. Holbrook, and C. Nischwitz. 2008. Response of new field-resistant peanut cultivars to twin-row pattern or in-furrow applications of phorate for management of spotted wilt. Plant Dis. 92: 1307-1312.

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

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

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

de Haan, P., L. Wagemakers, D. Peters, and R. Goldbach. 1989. Molecular cloning and terminal sequence determination of the S and M RNAs of *Tomato spotted wilt virus.* J. Gen. Virol. 70: 3469-3473.

de Haan, P., L. Wagemakers, D. Peters, and R. Goldbach. 1990. Th S RNA segment of *Tomato spotted wilt virus* has an ambisense character. J. Gen. Virol. 71: 1001-1007.

de Haan, P., R. Kormelink, R. O. de Resende, F. van Poelwijk, D. Peters, and R. Goldbach. 1991. *Tomato spotted wilt virus* L RNA encodes a putative RNA polymerase. J. Gen. Virol. 72: 2207-2216.

Digiuseppe, G. 2010. Bayer to phase out use of Temik. *In* Crop Production. http://magissues.farmprogress.com/MDS/MS11Nove10/mds024.pdf.

Dorman, K. W., and J. C. Barber. 1956. Time of flowering and seed ripening in southern pines. South Eastern Forest Expt. Sta. Paper No.72.

Erhardt, A. and I. Baker. 1990. Pollen amino acids – an additional diet for a nectar feeding butterfly? Plant Syst. Evol. 169: 111-121.

FAS. 2013. Foreign Agricultural Service. World agricultural production: World crop production summary April 2013. United States Department of Agriculture.

Funderburk, J. E., D. W. Gorbet, I. D. Teare, and J. Stavisky. 1998. Thrips injury can reduce peanut yield and quality under conditions of multiple stress. Agron. J. 90: 563-565.

Funderburk, J., S. Reitz, S. Olson, P. Stansly, H. Smith, G. McAvoy, O. Demirozer, C. Snodgrass, M. Paret, and N. Leppla. 2011. Managing thrips and tospoviruses in tomato. Entomology and Nematology Department, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida.

Gardner, M. W., C. M. Tompkins, and O. C. Whipple. 1935. Spotted wilt of truck crops and ornamental plants. Phytopathol. 25: 17.

German, T. L., D. E. Ullman, and J. W. Moyer. 1992. *Tospoviruses*: diagnosis, molecular biology, phylogeny, and vector relationships. Annu.Rev. Phytopathol. 30: 315-348.

GFB News. 2010. Georgia Farm Bureau: the voice of Georgia farmers. Bayer CropScience to phase out pesticide aldicarb, close Georgia plant. http://www.gfb.org./gfbnews/gfbnews moreinfo.asp?recordid=284.

Goldberg, N. P. 2000. *Tomato spotted wilt virus*: guide H-242. Cooperative Extension Service, College of Agriculture and Home Economics, New Mexico State University.

Gorbet, D. W., A. J. Norden, F. M. Shokes, and D. A Knauft. 1987. Registration of 'Southern Runner' peanut. Crop Sci. 27:817.

Gorbet, D. W., and F. M. Shokes. 1994. Plant spacing and *Tomato spotted wilt virus*. Abstr. Proc. Am. Peanut Res. Ed. Soc. 26: 50.

Gorbet, D. W., and F. M. Shokes. 2002. Registration of 'Florida MDR 98' peanut. Crop Sci. 42: 2207-2208.

Groves, R. L., G. G. Kennedy, and J. F. Walgenbach. 1998. Inoculation of *Tomato spotted wilt virus* into cotton. Disease notes in Plant Dis. 82: 959.

Groves, R. L., J. F. Walgenbach, J. W. Moyer, and G. G. Kennedy. 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. Phytopathol. 91:891-899.

Groves, R.L., J. F. Walgenbach, J. W. Moyer, and G. G. Kennedy. 2003. Seasonal dispersal patterns of Frankliniella fusca (Thysanoptera: Thripidae) and tomato spotted wilt virus occurrence in central and eastern North Carolina. J.Econ. Entomol. 96: 1-11.

Gullan, P. J., and P. S. Cranston. 2010. The insects: an outline of entomology 4th ed. Wiley-Blackwell, A John Wiley and Sons, Ltd., U.K.

Halliwell, R. S., and G. Philley. 1974. Spotted wilt of peanut in Texas. Plant Dis. Rep. 58: 23-25.

Hansen, E. A., J. E. Funderburk, S. R. Reitz, S. Ramachandran, J. E. Eger, and H. McAuslane. 2003. Within-plant distribution of *Frankliniella* species (Thysanoptera: Thripidae) and *Orius insidiosus* (Heteroptera: Anthocoridae) in field pepper. Environ. Entomol. 32: 1035–1044.

Harper, S., and P. A. Horne. 2012. The feeding effects of western flower thrips (*Frankliniella occidentalis* (Pergande)) and wind damage on French beans (*Phaseolus vulgaris* L.). Aust. J. Entomol. 51: 205-208.

Holbrook, C. C., and A. K. Culbreath. 2008. Registration of 'Georganic' peanut. J. Plant Reg. 2: 17.

Holbrook, C. C.,, P. Timper, A. K. Culbreath, and C. K. Kvien. 2008. Registration of 'Tifguard' peanut. J. Plant Reg. 2: 92-94.

Hull, R. 2002. Matthews' plant virology, 4th ed. Elsevier Academic Press, San Diego, CA.

Hulshof, J., and I. Vänninen. 2001. Western flower thrips feeding on pollen, and its implications for control, pp. 173-179. *In* R. Marullo and L. Mound (eds.), Thrips and Tospoviruses: Proceedings of the 7th International Symposium on Thysanoptera.

Hulshof, J., E. Ketoja, and I. Vaenninen. 2003. Life history characteristics of *Frankliniella occidentalis* on cucumber leaves with and without supplemental food. Entomologia Experimentalis et Applicata 108: 19-32.

Hurt, C. A., R. L. Brandenburg, D. L. Jordan, B. M. Royals, and P. D. Johnson. 2006. Interactions of tillage with management practices designed to minimize tomato spotted wilt of peanut (*Arachis hypogaea* L.). Peanut Sci. 33: 83-89.

Johnson, W. C., T. B. Brenneman, S. H. Baker. A. W. Johnson, D. R. Summer, and B. G. Mullinix. 2001. Tillage and pest management consideration in a peanut-cotton rotation in the southeastern coastal plain. Agron. J. 93: 570-576.

Jones, D. R. 2005. Major review: plant viruses transmitted by thrips. Eur. J. Plant Pathol. 113: 119-157.

Kirk, W. D. J. 1984. Pollen feeding in thrips (Insecta: Thysanoptera). J. Zoo. 204: 107-117.

Kirk, W. D. J. 1997. Feeding. *In* T. L. Lewis (ed.). Thrips as crop pests. CAB International, London, UK.

Kulshrestha, S., A. Sharma, and C. A. Seth. 2013. Molecular biology of *Tomato spotted wilt virus*: an update. J. Appl. Hort. 15: 71-80.

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

Lundgren, J. G. 2009. Relationships of natural enemies and non-prey foods. Springer, USA.

Margaria, P., M. Ciuffo, D. Pacífico, and M. Turina. 2007. Evidence that the nonstructural protein of *Tomato spotted wilt virus* is the avirulence determinant in the interaction with resistant pepper carrying the *Tsw* gene. Mol. Plant Microbe Interact. 20: 547-558.

Marois, J. J., and D. L. Wright. 2003. Effect of tillage system, phorate, and cultivar on tomato spotted wilt of peanut. Agron. J. 95: 386-389.

Mathew, R. P., Y. Feng, L. Githinji, R. Ankumah, and K. S. Balkcom. 2012. Impact of no-tillage and conventional tillage systems on soil microbial communities. App. Environ. Soil Sci. DOI:10.1155/2012/548620.

Matsuura, S., S. Ishikura, N. Shigemoto, S. Kajihara, and K. Hagiwara. 2004. Localization of *Tomato spotted wilt virus* in chrysanthemum stock plants and efficiency of viral transmission from infected stock plants to cuttings. J. Phytopathol. 152: 219-223.

McDonald, J. R., J. S. Bale, and K. F. A. Walters. 1998. Effect of temperature on development of the western flower thrips, *Frankliniella occidentalis* (Thysanoptera: Thripidae). Eur. J. Entomol. 95: 301-306.

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

Morita, S., T. Kashio, H. Inoue, and K. Kakimoto. 2008. Development and fecundity of *Haplothrips brevitubus* (Karny) (Thysanoptera: Phlaeothripinae) on the pollen of some fruit vegetables. Kyushu Pl. Prot. Res. 54: 78-84.

Mound, L. 1997. Biological diversity. *In* T. Lewis (ed.), Thrips as crop pests. CAB International, New York.

Mound, L. A. 2005. Thysanoptera: diversity and interactions. Annu. Rev. Entomol. 50: 247-269.

Morse, J. G., and M. S. Hoddle. 2005. Invasion biology of thrips. Annu. Rev. Entomol 51: 67-89.

Murai, T., and T. Ishii. 1982. Simple rearing method for flower thrips (Thysanoptera, Thripidae) on pollens (in Japanese with English abstract). Jpn. J. Appl. Entomol. Zool. 26: 149-154.

Murai, T., and A. J. M. Loomans. 2001. Evaluation of an improved method for mass-rearing of thrips and a thrips parasitoid. Entomologia Experimentalis et Applicata 101: 281-289.

NASS. 2013. National Agricultural Statistics Service. Crop production 2012 summary: September 2013. United States Department of Agriculture.

Niyomsil, K., S. Jogloy, M. Keerati-kasikorn, C. Akkasaeng, T. Kesmala, and A. Patanothai. 2007. Generation means analysis for thrips (Thysanoptera: Thripidae) number and leaf damage by thrips feeding in peanut. Asian J. Plant Sci. 6: 269-275.

NCCE. 2010. North Carolina Cooperative Extension.Soil facts: conservation tillage use in peanut production. NC State University and A&T State University Cooperative Extension.

NPB. 2013. National Peanut Board. History of peanuts and peanut butter. http://national peanutboard.org/the-facts/history-of-peanuts-peanut-butter/

Pappu, H. R., R. A. C. Jones, and R. K. Jain. 2009. Global status of tospovirus epidemics in diverse cropping systems: successes achieved and challenges ahead. Virus Res. 141: 219-236.

Pattee, H. E., and C. T. Young. 1982. Peanut science and technology. American Peanut Research and Education Society, Inc., TX, USA.

Pattee, H. E., and H. T. Stalker. 1995. Advances in peanut sciemce. American Peanut Research and Education Society, Inc., Stillwater, OK, USA.

Phillips, S. L. 1997. The incredible peanut. Ethnobotanical Leaflets, Southern Illinois University Carbondale, USA.

Pittman, H. A. 1927. Spotted wilt of tomatoes: preliminary note concerning the transmission of spotted wilt of tomatoes by an insect vector (*Thrips tabaci* Lindeman). Aust. Coun. Sci. Ind. Res. 1: 74-77.

Riley, D. G., and H. R. Pappu. 2004. Tactics for management of thrips (Thysanoptera: Thripidae) and *Tomato spotted wilt virus* in tomato. J. Econ. Entomol. 97: 1648-1658.

Riley, D., R. McPherson, and L. Wells. 2005. Thrips vectors of TSWV. *In* Tospoviruses: In Solanaceae and Other Crops in The Coastal Plain of Georgia, The University of Georgia, College of Agricultural and Environmental Sciences, Research Report Number 704.

Riley, D. G., A. Chitturi, and A. N. Sparks. 2007. Does natural deposition of pine pollen affect the ovipositional behavior of *Frankliniella occidentalis* and *Frankliniella fusca*? Entomologia Experimentalis et Applicata 124: 133-141.

Riley, D. G., G. M. Angelella, and R. M. McPherson. 2011a. Pine pollen dehiscence relative to thrips population dynamics. Entomologia Experimentalis et Applicata 138: 223-233.

Riley, D. G., S. V. Joseph, R. Srinivasan, and S. Diffie. 2011b. Thrips vectors of tospoviruses. J. Integ. Pest Mngmt. 1: 1-10.

Rojas, M.G., J. A. Morales-Ramos, E. G. King, G. Saldana, and S. M. Greenberg. 1998. Use of a factitious host and supplemented adult diet to rear and induce oogenesis in *Catolaccus grandis* (Hymenoptera:Pteromalida). Environ. Entomol. 27: 499–507.

Sakimura, K. 1962. *Frankliniella occidentalis* (Thysanoptera: Thripidae), a vector of the *Tomato spotted wilt virus*, with special references to the color forms. Annu. Entomol. Soc. Am. 55: 387-389.

Sakimura, K. 1963. *Frankliniella fusca*, an additional vector for the *Tomato spotted wilt virus*, with notes on *Thrips tabaci*, another vector. Phytopathol. 53.

Samuel, G., J. G. Bald, and H. A. Pittman. 1930. Investigations on “spotted wilt” of tomatoes. Aust. Commonw. Counc. Sci. Ind. Res. Bull. No. 44.

Schuster, G. L., and R. S. Haliwell. 1994. Six new hosts of tomato spotted wilt virus in Texas. Disease notes in Plant Dis. 78: 100.

Sherwood, J. L., T. L. German, J. W. Moyer, and D. E. Ullman. 2003. Tomato spotted wilt. The Plant Health Instructor. DOI:10.1094/PHI-I-2003-0613-02.

Shrestha, A., R. Srinivasan, S. Sundaraj, A. K. Culbreath, and D. G. Riley. 2013. Second generation peanut genotypes resistant to thrips-transmitted *Tomato spotted wilt virus* exhibit tolerance rather than true resistance and differentially affect thrips fitness. J. Eco. Ent. 106: 587-596.

Smith, O. D., C. E. Simpson, M. C. Black, and B. A. Besler. 1998. Registration of Tamrun 98 peanut. Crop Sci. 38: 1403.

Stanley, R. G. and H. F. Linskins. 1974. Pollen: biology, biochemistry, management. Springer, New York.

Storms, M. M. H., T. Nagata, R. Kormelink, R. W. Goldbach, and J. W. M. van Lent. 2001. Expression of the movement protein of *Tomato spotted wilt virus* in its insect vector *Frankliniella occidentalis*. Arch. Virol. 147: 825-831.

Tillman, B. L., D. W. Gorbet, A. K. Culbreath, and J. W. Todd. 2006. Response of peanut cultivars to seeding density and row patterns. Crop Management. DOI:10.1094/CM-2006-0711-01-RS.

Todd, J.W., J. R. Chamberlin, A. K. Culbreath, and J. W. Demski. 1994a. Timing and duration of vector management in relation to spotted wilt disease incidence in peanut. Proc. Am. Peanut Res. Ed. Soc. 25: 86.

Todd, J.W., J. R. Chamberlin, A. K. Culbreath, D. Rogers, and J. W. Demski. 1994b. Contradictions of insecticide use relative to vector control and spotted wilt disease progress in peanut. Proc. Am. Peanut Res. Ed. Soc. 26: 42.

Todd, J.W., and A. K. Culbreath. 1995. Thrips populations and spotted wilt disease progress on resistance/susceptible cultivars treated with various insecticides. Proc. Am. Peanut Res. Ed. Soc. 27: 35.

Todd, J. W., A. K. Culbreath, J. R. Chamberlin, R. J. Beshear, and B. G. Mullinix. 1995. Colonization and population dynamics of thrips in peanuts in the southern United States. *In* B. Parker, M. Skinner and T. Lewis (eds.), Thrips Biol. Mgt. Plenum Press, New York.

Todd, J. W., A. K. Culbreath, and S. L. Brown. 1996. Dynamics of vector populations and progress of spotted wilt disease relative to insecticides use in peanuts. Acta Hortic. 431: 483-490.

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

Trichilo, P. J., and T. F. Leigh. 1988. Influence of resource quality on the reproductive fitness of flower thrips (Thysanoptera, Thripidae). Annals Entomol. Soc. America 81: 64-70.

Triplehorn, C. A., and N. F. Johnson. 2005. Borror and Delong's introduction to the study of insects, 7th ed. Brooks/Cole, Cengage Learning, U.S.A.

Tsai, H. J., B. S. Yue, J. E. Funderburk, and S. E. Webb. 1996. Effect of plant pollen on growth and reproduction of *Frankliniella bispinosa*. Acta Horticulturae 431: 535-541.

Tubbs, R. S., and J. P. Beasley. 2009. Tillage and row pattern update *In* 2009 Peanut Update. The University of Georgia, College of Agriculture and Environmental Science.

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

Tubbs, R. S., K. Balkcom, M. Toews, R. Srinivasan. 2013. Effects of fertilization, tillage, and phorate on thrips and TSWV incidence in early planted peanuts. Abstract, Southern Conservation Agricultural Systems Conference. http://www.ars.usda.gov/research/ publication /publication.htm?seq_no_115=296575.

Ullman, D. E., J. J. Cho, R. F. L. Mau, W. B. Hunter, D. M. Westcot, D. M. Custer. 1992. Thrips-*Tomato spotted wilt virus* interactions: morphological, behavioral and cellular components influencing thrips transmission. Adv. Dis. Vect. Res. 9: 195-240.

Ullman, D. E., R. Meideros, L. R. Campbell, A. E. Whitfield, J. L. Sherwood, and T. L. German. 2002. Thrips as vectors of tospoviruses. Adv. Bot. Res., Vol. 36. Academic Press.

USDA. 2012. United States Department of Agriculture. Natural Resources Conservation Service. http://www.ecat.sc.egov.usda.gie/Help.aspx.

USDA. 2013a. United States Department of Agriculture. National nutrient database for standard reference, release 26, nutrient data laboratory.

USDA. 2013b. United States Department of Agriculture. Natural Resources Conservation Service. Plants database updated by national plant data team.

Venkatachalam, M., and S. K. Sathe. 2006. Chemical composition of selected edible nut seeds. J. Agri. Food Chem. 54: 4705-4714.

Watts, J. G. 1936. A study of the biology of the flower thrips *Frankliniella tritici* (Fitch) with special reference to cotton. Bull. S. C Agric. Exp. Stn. 306: 1-46.

Wheeler, D. 1996. The role of nourishment in oogenesis. Annual Review of Entomology 41: 407-431.

Weeks, J. R., and A. K. Hagan. 1991. The effects of planting date and insecticide treatments on thrips populations, *Tomato spotted wilt virus* and yield of peanut in Alabama. . Proc. Am. Peanut Res. Ed. Soc. 23: 41.

Whitfield, A. E., D. E. Ullman, and T. L. German. 2005. Tospovirus-thrips interactions. Annu. Rev. Phytopathol. 43: 459-C-5.

CHAPTER 3

EVALUATION OF ALTERNATIVES TO CARBAMATE AND ORGANOPHOSPHATE INSECTICIDES AGAINST THRIPS AND *TOMATO SPOTTED WILT VIRUS* IN PEANUT PRODUCTION[1]

[1]Marasigan, K., M. Toews, R. Kemerait, Jr., and R. Srinivasan. 2014.

Abstract

Planting peanut cultivars that display field resistance to *Tomato spotted wilt virus* (TSWV) in the family *Bunyaviridae* and genus *Tospovirus* is vital for TSWV management. However, these cultivars are not immune to the virus. TSWV is exclusively transmitted by thrips (Thysanoptera: Thripidae) in a persistent and propagative manner. These cultivars also do not possess any resistance against thrips. Therefore, planting of resistant cultivars alone is not sufficient for suppressing virus incidence. Consequently, peanut growers use insecticides such as aldicarb (Temik®) and phorate (Thimet®) for managing thrips and reducing TSWV transmission by thrips. Both aldicarb and phorate are carbamate and organophosphate insecticides, respectively, and possess broad-spectrum insecticidal effects. Due to a recent agreement, aldicarb usage in peanut will be phased out in 2018. Hence, alternatives are critical to thrips and TSWV management in peanut. In this study, eight alternative insecticides were evaluated in replicated field trials from 2011 through 2013. Of which, imidacloprid (Admire® Pro), thiamethoxam (Actara®), spinetoram (Radiant®), and cyantraniliprole (HGW086) were as effective as aldicarb and phorate in suppressing thrips and reducing spotted wilt incidence. Spotted wilt incidences did not differ between TSWV-resistant cultivar, 'Georgia-06G', and TSWV-susceptible cultivar, 'Georgia Green', from 2011 to 2013. Further examination in the greenhouse substantiated that effects of selected alternative insecticides such as imidacloprid, thiamethoxam, spinetoram, and cyantraniliprole were more than or equal to aldicarb and phorate in suppressing thrips feeding and reducing TSWV transmission. Together, these results suggest that alternatives to aldicarb and phorate could be used in peanut without significantly compromising yields.

Additional Key Words: Tobacco thrips, spotted wilt, management

Introduction

Tomato spotted wilt virus (TSWV) in the family *Bunyaviridae* and genus *Tospovirus* causes spotted wilt disease in peanut (*Arachis hypogaea* L.) (Adkins 2000, Culbreath et al. 2003, Whitfield et al. 2005). The disease is one of the most destructive diseases affecting peanut production in the southeastern United States (Culbreath et al. 1992, Bertrand 1998, Culbreath et al. 2003, Culbreath and Srinivasan 2011). Spotted wilt from the late '80s to late '90s severely reduced average annual yields. In 1997 alone, approximately 12% of the peanut crop (>$40 million) was lost due to the disease in Georgia (Bertrand 1998, Culbreath and Srinivasan 2011). TSWV is only transmitted by thrips in the family Thripidae (Order: Thysanoptera) in a persistent and propagative manner (German et al. 1992, Ullman et al. 1992, Jones 2005, Whitfield et al. 2005, Pappu et al. 2009). Out of 1,710 known species of thrips in Thripidae, only ten species are known to transmit TSWV (Pappu et al. 2009, Riley et al. 2011). Only two species of thrips are primarily responsible for spotted wilt epidemics in the southeastern United States. They include the tobacco thrips, *Frankliniella fusca* (Hinds), and the western flower thrips, *Frankliniella occidentalis* (Pergande) (Todd et al. 1995). Between the two species, the former is known to colonize and reproduce efficiently on peanut foliage especially in the early season when compared with the latter (Todd et al. 1995, 1996, 1997). Peanut plants infected at early stage are more susceptible to TSWV when compared with plants infected at a later stage (Todd et al. 1997, Culbreath et al. 2003).

Peanut cultivars with high levels of resistance to TSWV are often planted as a first line of defense to manage spotted wilt. Even though the currently available cultivars possess substantial amounts of resistance against the virus, they are still not immune to the virus (Culbreath et al. 2003, Culbreath and Srinivasan 2011). The resistant cultivars are still susceptible to the virus and

display typical TSVW symptoms upon infection (Culbreath et al. 2003, Culbreath and Srinivasan 2011). None of the currently available cultivars possess any resistance to thrips infestations (Culbreath et al. 2003). Under substantial thrips and TSWV pressure, these cultivars might also succumb to TSWV. Thus, planting of TSWV-resistant cultivars alone is not sufficient to manage spotted wilt in peanut. Growers utilize TSWV-resistant cultivars in conjunction with insecticides and cultural tactics (Culbreath et al. 2003, Culbreath and Srinivasan 2011).

Insecticides such as aldicarb (Temik®) and phorate (Thimet®) are commonly applied in-furrows at planting (Culbreath et al. 2003, Culbreath et al. 2008). Numerous studies have evaluated the impact of these two insecticides on thrips population, thrips feeding, and transmission of TSWV (Baldwin et al. 2001, Ames Herbert et al. 2007, Culbreath et al. 2008, Tubbs et al. 2013). Even though these insecticides are known to suppress thrips feeding and reduce thrips populations, their ability to reduce virus transmission is minimal (Culbreath et al. 2003). This might be due to the fact that viruliferous thrips landing on a plant could transmit the virus with a brief inoculation access period, lasting only a few minutes. Hence, insecticides that cause rapid cessation of feeding with a strong repellent effect would be beneficial. Besides virus transmission, thrips feeding can severely affect seedlings performance in peanut. Peanut seedlings with severe thrips injuries might take longer to develop than seedlings with little or no feeding injuries. In general, peanut plants recover significantly following thrips peak incidence, but it is not clear if the recovery at the early stage would lead to delayed maturity and yield losses (Todd et al. 1997, Culbreath et al. 2003, Culbreath and Srinivasan 2011, Tubbs et al. 2013). Application of insecticides such as phorate is known to boost yields, but such increases do not appear to be directly related to thrips feeding suppression and/or virus transmission (Todd et

al. 1994a, 1994b, 1997; Todd and Culbreath 1995, Marois and Wright 2003, Culbreath et al. 2008).

Aldicarb and phorate are preferred due to their low costs and ease of application (Digiuseppe 2010). Nevertheless, aldicarb and phorate posses high mammalian toxicity and cause other undesirable non-target effects (Digiuseppe 2010). Recently, United States Environmental Protection Agency (US-EPA) and Bayer CropScience have agreed to halt the production of aldicarb and phase out its usage by 2018 (AgroNews 2010, Digiuseppe 2010, GFB News 2010). Phorate also possesses broad-spectrum toxicity and causes undesirable non-target effects. Thus, it is critical to identity alternatives to carbamate and organophosphate insecticides in peanut production. The currently available peanut cultivars exhibit higher levels of TSWV resistance than previous cultivars. Therefore, it is possible to identify and use alternatives to aldicarb and phorate without significantly compromising yields.

The authors evaluated eight alternative insecticides to aldicarb and phorate under field conditions from 2011 through 2013. Their effects on thrips populations, thrips feeding damage, and spotted wilt incidence were documented on TSWV-susceptible and TSWV-resistant peanut cultivars. Alternatives were also evaluated as seed treatment, in-furrow, and at-crack applications. To further examine the effects of alternative insecticides on thrips feeding as well as TSWV transmission, few insecticides that performed effectively under field conditions were examined in depth under laboratory or greenhouse conditions.

Materials and Methods

Evaluation of various insecticides as alternatives to aldicarb and phorate. Trials were conducted at the Belflower Farm, Coastal Plain Experimental Station, Tifton, GA to evaluate alternative insecticides on two peanut cultivars, Georgia Green (TSWV-susceptible) and

Georgia-06G (TSWV-resistant) from 2011 to 2013. Eight alternative insecticides were evaluated. The insecticides, their mode of application, as well as their dosage/acre are included in Table 3.1. A split plot design was adopted. Peanut cultivars and insecticides (treatments) were assigned as main plot effects and subplot effects, respectively. Each plot was 9.14 m long and 5.49 m wide. There were six rows in each plot. In 2011, peanuts were planted in June 20. In 2012 and 2013, peanuts were planted in April 25 and April 27, respectively. Thrips samples were collected ~three weeks after planting for six consecutive weeks except for 2011. In 2011, they were sampled only for five consecutive weeks due to delayed planting. In the first three weeks, quadrifoliate peanut terminals were collected while in the next three weeks, peanut blooms were collected. Ten terminals or blooms were randomly collected for each plot from the 2nd and 5th rows. The samples were placed in glass vials containing ~10 ml of 70% ethyl alcohol. The samples were brought to the vector biology laboratory in the University of Georgia at the Tifton Campus. Thrips were enumerated under a dissecting microscope (40x) (MEIJI TECHNO, Santa Clara, CA) and were identified to species using dichotomous keys (Triplehorn and Johnson 2005). In 2011, negligible thrips damage was observed and the plots were not rated for thrips damage. Thrips feeding injuries were assessed only for 2012 and 2013 using an arbitrary scale that measured from 0 to 10 (wherein 0 represented no feeding injuries and 10 represented a dead plant) (Lynch et al. 1984, Brandenburg et al. 1998). Feeding injuries were assessed on peanut plants from the 2nd and 5th rows of each plot at five weeks after planting.

Spotted wilt incidence was rated visually using a standard procedure (Culbreath et al. 1997). Plants exhibiting spotted wilt symptoms on 3rd and 4th rows of each plot were identified and rated (Culbreath et al. 1997). In every plot, TSWV-infected plants in row cm were obtained using a 30.48-cm hit stick and converted to percentages. Plots were rated for spotted wilt ~two

weeks prior to harvest. At harvest, peanut plants in the 3rd and 4th rows of each plot were dug, inverted, air-dried, picked, and weighed (in kg) following standard protocols (Baldwin et al. 1998).

Thrips injury rating and counts, spotted wilt incidence, and yield data were subjected to linear mixed models analyses using PROC GLIMMIX in SAS (SAS Enterprise 4.2, SAS Institute, Cary, NC). Peanut cultivars and insecticides were considered fixed effects while replications were considered random effects. Interactions between insecticides and peanut cultivars, if any, were also analyzed. Tukey-Kramer Grouping, as an adjustment for multiple comparisons at P=0.05, was used to test the statistical significance of differences among treatments and between cultivars.

Greenhouse and laboratory experiments. Greenhouse and laboratory experiments were conducted at the Department of Entomology, UGA Tifton Campus, Tifton, GA.

Non-infected peanut plants. Two peanut cultivars, Georgia Green and Georgia-06G, were used for all experiments. Seeds were pre-germinated on moistened paper towels and incubated in a growth chamber with a temperature of 25 to 27^{o}C for one week. Germinated peanut seeds were transplanted into 10.16-cm diameter plastic pots (Hummert International, St. Louis, MO) containing commercial potting mix (LT5 Sunshine mix, Sun Gro Horticulture Industries, Bellevue, WA). Peanut plants were placed in 47.5-cm^3 insect proof cages (Megaview Science Co., Taichung, Taiwan) and maintained in a greenhouse at 25 to 30^{o}C with 80 to 90% relative humidity (RH) and 14:10 (L:D) h photoperiod.

Collection of pine pollen grains. Pine (*Pinus taeda* L.) needles with pollen grains were placed inside a brown paper bag collected in February 2012 at Tifton, GA. The brown paper bag was shook vigorously followed by the removal of pine needles. Pine pollen grains were collected in a

glass vial using a paintbrush (2 Silver 5300 S Round, India) after which, pine pollen grains were stored at 4^{o}C.

Maintenance of non-viruliferous *F. fusca*. In 2012, a colony of *F. fusca* was established by collecting *F. fusca* from peanut blooms from Belflower Farm, Coastal Plain Experimental Station, Tifton, GA. Thrips were transferred and maintained in Munger cage (11.43 x 8.89 x 1.77 cm^{3}) (Munger 1942) containing healthy non-infected peanut leaflets dusted with pine (*Pinus taeda* L.) pollen grains. The Munger cages were placed in a growth chamber (Thermo scientific, Dubuque, IA) at 25 to 27^{o}C with 14:10 (L:D) h photoperiod. New foliage was added to the Munger cages every two to three days.

Maintenance of potentially viruliferous *F. fusca.* A colony of potentially viruliferous thrips was initiated and maintained on TSWV-infected peanut foliage in Munger cages as described for non-viruliferous thrips. TSWV-infected peanut foliage was initially obtained from the Belflower Farm, Coastal Plain Experimental Station, Tifton, GA. The cages were routinely replaced with infected foliage obtained either from the Belflower farm or from the greenhouse. Infection status of peanut foliage was confirmed by double antibody sandwich enzyme linked immunosorbent assay (DAS-ELISA). Thrips reared for an entire generation (adult to adult) on TSWV-infected leaflets were alone considered potentially viruliferous.

Evaluation of alternative insecticides on thrips feeding. Two peanut cultivars and four alternative insecticides were evaluated for thrips feeding. Insecticides were applied as foliar sprays. Details of insecticide applications are included in Table 3.2. Five one-week old peanut plants for each cultivar were placed separately in a 47.5-cm^{3} insect proof cage (Megaview Science Co., Taichung, Taiwan). Approximately 0.05g of pine pollen grains were dusted on each plant. Ten non-viruliferous female adult *F. fusca* (up to two days old) were collected in 0.6 ml

microcentrifuge tubes using a paintbrush (2 Silver 5300 S Round, India) and then released at the base of each plant. Thrips feeding damage was assessed on each plant every two days for a month after initial thrips release. The feeding damage index (FDI) was calculated based on the formula deduced by Maris et al. (2003) with minor adjustments.

$$\text{FDI} = \frac{\text{No.of leaflets with feeding damage}}{\text{Total no. of leaflets in a plant}} \text{ x Intensity of feeding scar}$$

The intensity of feeding scars was based on arbitrary scale (1 = 0-20%, 2 = 20-40%, 3 = 40-60%, 4 = 60-80% and 5 = 80-100%). The experiment was repeated twice (N=15 plants for each treatment and for each cultivar).

A completely randomized design (CRD) was used as a treatment structure. Peanut cultivars and insecticides were considered fixed effects while replications were considered random effects. Statistical differences in feeding damage at three time intervals, 10, 20, and 30 days after thrips release were assessed. PROC GLIMMIX in SAS was used for the analysis. The same plant population was observed for the entire sampling period and the observations were considered as repeated measures. Least square means at P=0.05 was used to compare the statistical significance of differences between treatments.

Evaluation of alternative insecticides on TSWV transmission. Two peanut cultivars were evaluated for TSWV susceptibility. The same four insecticides identified in the feeding assay were used for transmission assay. Insecticides were applied as foliar sprays. Details of insecticide application are included in Table 3.2. Five one-week old peanut plants for each treatment were used in the experiment, and the whole experiment was repeated twice (N=15 plants for each treatment and for each cultivar). Each plant was individually 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). Each plant was dusted with

approximately 0.05 g. of pine pollen grains. Ten potentially viruliferous thrips placed in a 0.6 ml microcentrifuge tube (Fisher Scientific, Pittsburgh, PA) using a paintbrush (2 Silver 5300 S Round India) and then released at the base of each peanut plant. TSWV infection status of plants was confirmed by DAS-ELISA using antibodies specific to the nucleocapsid protein and following manufacturer's recommendations (Clark and Adams 1977, Shrestha et al. 2012).

TSWV detection using DAS-ELISA. Leaf tissue (approximately 0.1 g) was used for DAS-ELISA. The assay was performed in a 96 well microtiter plate (Maxisorp, Nunc, Rochester, NY) with suitable positive and negative controls. 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) also was 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 microplate reader 1 h after substrate addition (Model Elx 800, Bio-Tek®, Kocherwaldstr, Germany). An average absorbance value of negative control samples plus four standard deviations was considered positive.

A CRD was used as a treatment structure. Peanut cultivars and insecticides were considered fixed effects while replications were considered random effects. Incidence of TSWV infection was compared among the treatments and peanut cultivars. TSWV incidence was treated as a binomial response (positive or negative) and data were analyzed using PROC GENMOD in SAS. Pairwise contrasts at P=0.05 were used to test the statistical significance of differences among treatments.

Results

Evaluation of various insecticides as alternatives to aldicarb and phorate: Thrips on peanut terminal foliage and blooms were counted and identified. In 2011, only *F. fusca* adults and immatures were counted. Whereas in 2012 and 2013, thrips were further classified as immatures, adult non-vector, or adult vector species. The percentages of immatures ranged from 31.70 ± 0.76 to 72.25 ± 0.31. The percentages of vector adults ranged from 25.89 ± 0.49 to 68.30 ± 0.76. The non-vector adult percentages ranged from 1.49 ± 0.18 to 4.68 ± 0.20 (Fig. 3.1). Among vectors, *F. fusca* was the most predominant species. The percentage of *F. fusca* sampled ranged from 95.37 ± 0.74 to 98.57 ± 0.08 (Fig. 3.2). In general, irrespective of thrips species, fewer thrips were found in 2012 than in 2011 and in 2013 (Fig. 3.3). The treatment effects on thrips populations, thrips feeding injuries, spotted wilt incidence, and yields are described below.

Thrips counts: Thrips counts on Georgia Green plots were not different from thrips counts on Georgia-06G plots in 2011 (df=1,6; F=0.00; P=0.9662), in 2012 (df=1,6; F=3.78; P=0.0997), and in 2013 (df=1,6; F=5.78; P=0.0531). In 2011, thrips populations were affected by insecticide treatments (df=11,354; F=5.36; P<0.0001). Thiamethoxam, imidacloprid (in-furrow), and cyantraniliprole applications were as effective as aldicarb and phorate in suppressing thrips (Fig. 3.3). In 2012, fewer thrips were collected from aldicarb treated plots and cyantraniliprole treated plots compared with untreated plots (df=11,426; F=5.16; P<0.0001) (Fig. 3.3). In 2013, fewer thrips were observed on cyantraniliprole treated compared to the remaining treatments (df=11,426; F=11.83; P<0.0001). However, thrips counts on cyantraniliprole treated plots were not different from thrips counts on spinetoram and phorate treated plots (Fig. 3.3). No interactions were observed between treatments and cultivars in 2011 (df=11,354; F=0.82;

P=0.6231) and in 2012 (df=11,426; *F*=0.73; *P*=0.7146) but not in 2013 (df=11,426; *F*=1.86; *P*<0.0426).

Thrips feeding damage: Thrips feeding damage varied with insecticide treatments in 2012 (df=11,66; *F*=16.80; *P*<0.0001) as well as in 2013 (df=11,66; *F*=50.48; *P*<0.0001) (Fig. 3.4). In 2013 (df=1,6; *F*=18.40; *P*=0.0052), significantly more feeding damage was observed on Georgia Green plots than on Georgia-06G plots but not in 2012 (df= 1,6; *F*=1.53; *P*=0.2626). Irrespective of cultivars, imidacloprid (in-furrow), spinetoram, and cyantraniliprole were as effective as aldicarb and phorate in reducing thrips feeding damage in 2012 (Fig. 3.4). However, feeding damage on spinetoram treated plots were not different from the feeding damage recorded on azadirachtin, acetamiprid, and imidacloprid (at-cracking) treated plots (Fig. 3.4). Similarly, in 2013, feeding damage in spinetoram and cyantraniliprole treated plots was less than feeding damage in plots treated with other insecticides, except for aldicarb and phorate treated plots. Interactions were observed between treatments and cultivars in 2012 (df=11,66; *F*=2.56; *P*=0.0093) and in 2013 (df=11,66; *F*=5.52; *P*<0.0001).

Spotted wilt incidence: Irrespective of insecticides and cultivars evaluated, spotted wilt incidence in 2013 was higher in 2011 and in 2012. Spotted wilt incidence was not affected by insecticide treatments in 2011 (df=11,66; *F*=1.17; *P*=0.3244) or 2012 (df=11,66; *F*=1.84; *P*=0.0652). Spotted wilt incidence did not vary between Georgia Green plots and Georgia-06G plots in 2011 (df=1,6; *F*=5.54; *P*=0.0568), 2012 (df=1,6; *F*=0.45; *P*=0.5294), and in 2013 (df=1,6; *F*=0.26; *P*=0.6270). In 2013, spotted wilt incidences in imidacloprid (at-cracking) and cyantraniliprole treated plots were significantly higher when compared with phorate treated plots (df=11,66; *F*=2.22; *P*=0.0235) (Fig. 3.5). Spotted wilt incidences in plots treated with other insecticides were not different from spotted wilt incidence in untreated plots. No interactions

were observed between insecticides and cultivars in 2011 (df=11,66; *F*=1.29; *P*=0.2519), 2012 (df=11,66; *F*=1.15; *P*=0.3389) and in 2013 (df=11,66; *F*=1.20; *P*=0.3021).

Yields: Irrespective of insecticide treatments, yields in 2012 were greater on Georgia-06G plots than on Georgia Green plots (df=1,6; *F*=10.53; *P*=0.0176) but not in 2011 (df=1,6; *F*=1.10; *P*=0.3340) and in 2013(df=1,6; *F*=1.85; *P*=0.2226). Irrespective of the cultivars, yields were not influenced by insecticide treatments in 2011 (df=11,66; *F*=1.57; *P*=0.1286) and in 2012 (df=11,66; *F*=1.25; *P*=0.2751). However, in 2013, yields varied with insecticide treatments (df=11,66; *F*=4.90; *P*<0.0001). Lowest yield was recorded from untreated plots. In contrast, the highest yield was recorded from phorate treated plots (Fig. 3.6). Yields from lambda-cyhalothrin and thiamethoxam (at cracking) treated plots were less than yields obtained from phorate treated plots. Yields obtained from plots treated with various insecticides, such as aldicarb, imidacloprid, spinetoram, cyantraniliprole, spirotetramat, and thiamethoxam (seed treatment) were not different from yields obtained from phorate treated plots (Fig. 3.6). No interactions were observed between treatments and cultivars in 2011 (df=11,66; *F*=0.85; *P*=0.5909), 2012 (df=11,66; *F*=0.42; *P*=0.9420) and in 2013 (df=11,66; *F*=1.29; *P*=0.2518).

Greenhouse evaluations

Evaluation of alternative insecticides on thrips feeding: Feeding damage indices were observed for each cultivar separately**.** Significant differences in thrips feeding damage indices at 10, 20, and 30 days were observed on both cultivars (Fig. 3.7 and Table 3.3). Feeding damage indices observed on Georgia Green peanut plants varied with experiments at 10 (df=6,98; *F*=66.28; *P*<0.0001), 20 (df=6,98; *F*=301.35; *P*<0.0001), and 30 (df=6,98; *F*=188.66; *P*<0.0001) days post thrips release. Similarly, feeding damage indices varied with the repeats of the experiments on Georgia-06G peanut plants at 10 (df=6,98; *F*=41.80; *P*<0.0001), 20 (df=6,98;

F=64.17; P<0.0001), and 30 (df=6,98; F=65.39; P<0.0001) days post thrips release. Feeding damage indices, in general, were lower in all treatments when compared with untreated control. At 10 days post thrips release, all insecticides were as effective as aldicarb and phorate in reducing thrips feeding on Georgia Green. Whereas on Georgia-06G, feeding damage indices from imidacloprid, cyantraniliprole, spinetoram, and aldicarb treated plants were significantly less than thiamethoxam and phorate treated plants at 10 days post thrips release (Fig. 3.7 and Table 3.3). At 20 days, feeding damage indices on imidacloprid, cyantraniliprole, and aldicarb treated plants were significantly lower when compared with thiamethoxam, phorate, and untreated control (Fig. 3.7 and Table 3.3). However, in Georgia-06G, all insecticides had significantly lower feeding damage when compared with the untreated control (Fig. 3.7 and Table 3.3). At 30 days post thrips release, feeding damage indices from imidacloprid, cyantraniliprole, spinetoram, and aldicarb treated Georgia Green plants were lower than thiamethoxam, phorate, and untreated Georgia Green plants (Fig. 3.7 and Table 3.3). In Georgia-06G plants, feeding damage indices from thiamethoxam and untreated plants were significantly higher when compared with plants treated with other insecticides (Fig. 3.7 and Table 3.3).

Evaluation of alternative insecticides on TSWV transmission: The incidence of TSWV infection (%) was evaluated among treatments in Georgia Green and Georgia-06G by DAS-ELISA at four weeks post inoculation. TSWV incidence in untreated plants was greater than in insecticide treated plants in Georgia Green (df=6,98; χ^2=29.00; P<0.0001) and in Georgia-06G (df=6,98; χ^2=58.00; P<0.0001**) (**Fig. 3.8 and Table 3.4**)**. Irrespective of cultivars, incidence of TSWV infection (%) in untreated plants (≥80%) was at least twice as high when compared with treated plants (<35%) (Fig. 3.8 and Table 3.4). All alternative insecticides tested were as effective as aldicarb and phorate in reducing TSWV transmission.

Discussion

Thrips counts over three years (2011-2013) indicated the presence of immatures and adult non-vectors and vectors. Of the adults sampled, <5% were non-vectors. The common non-vectors sampled include adult thrips from the family Phlaeothripidae (suborder Tubulifera) and *F. tritici* (Fitch) adults. This indicated that remaining (95%) adults were competent vectors of TSWV. Within the vectors, more than 95% of the adults sampled were *F. fusca* and less than 5% were *F. occidentalis*. Therefore, it is likely that the majority of the immatures could be *F. fusca* as well. These findings substantiate earlier reports, which indicated that *F. fusca* is the major vector of TSWV in peanut in Georgia (Todd et al. 1994a, 1995). Earlier studies also documented that *F. fusca* could colonize and reproduce efficiently on peanut foliage (Todd et al. 1994a, 1995, 1996). Though *F. occidentalis* is considered to be most important vector in other cropping systems and other places, its role on peanut production seems to be minimal.

The most common management tactic for spotted wilt management in peanut includes planting peanut cultivars with high levels of field resistance to TSWV (Culbreath et al. 2003, Culbreath and Srinivasan 2011). However, these cultivars are not resistant to the vector and produce TSWV-associated symptoms upon infection (Shrestha et al. 2013, Sundaraj et al. 2014). Therefore, management of thrips is critical not only for spotted wilt management but also for improving crop quality. Peanut growers typically use aldicarb (Temik®) and phorate (Thimet®) for thrips management. In-furrow applications of either insecticide were effective in suppressing thrips populations as well as thrips feeding (Todd et al. 1994b, 1995, 1996; Todd and Culbreath 1995, Culbreath et al. 2003, 2008). However, aldicarb and phorate belong to carbamate and organophosphate insecticide groups, respectively. They possess broad-spectrum toxicity and induce non-target effects (AgroNews 2010, Digiuseppe 2010, GFB News 2010). Also, based on

an agreement between the United States Environmental Protection Agency and Bayer CropScience, the usage of aldicarb will be phased out by 2018. Phorate is also quite similar to aldicarb in terms of toxicity and non-target effects. Hence, there is a critical need for alternative insecticides to aldicarb and phorate.

In order to identify alternatives, representatives of neonicotinoids, diamides, spinosyns, pyrethroids, and others were evaluated. Feeding injuries and thrips counts in imidacloprid, cyantraniliprole, and spinetoram treated plots were comparable to that of phorate treated plots. Previous studies also indicate that imidacloprid applications reduced thrips feeding damage and thrips immatures when compared with the applications of aldicarb and phorate (Todd et al. 1994b, 1995, 1996; Todd and Culbreath 1995). Feeding damage indices and thrips populations on Georgia-06G were less than that of Georgia Green. It is not clear why such differences were observed, as Georgia-06G is only believed to be resistant to TSWV and not thrips. Insecticides that showed potential for thrips suppression under field conditions were further examined in the greenhouse. Imidacloprid, thiamethoxam, spinetoram, and cyantraniliprole were evaluated against thrips feeding. Results indicated that all the selected insecticides suppressed thrips feeding as efficiently as aldicarb and phorate.

Prior studies have shown that reduction in thrips feeding injuries did not result in reduction of TSWV incidence in the field (Weeks and Hagan 1991, Todd et al. 1994a, 1994b, 1995, 1996; Todd and Culbreath 1995, Marois and Wright 2003, Culbreath et al. 2008). Todd et al. (1994a) also found that despite a significant reduction of thrips populations and feeding, there was an increase in incidence of spotted wilt in peanut. Viruliferous thrips can transmit the virus within five minutes of feeding (Wijkamp and Peters 1993). In order for insecticides to prevent virus transmission, they should cause rapid feeding cessation or death of thrips. The rapid action

could explain the efficacy of aldicarb and phorate in reducing virus incidence. On the contrary, the study conducted by Baldwin et al. (2001) showed a reduction in TSWV incidence following in-furrow application of phorate. Although, the reduction in TSWV incidence might not be related to prevention of TSWV transmission, phorate applications typically cause phytotoxicity in peanut plants. Such effects could have triggered host defense responses that could have interfered with virus replication. Gene expression in phorate treated plants differed from untreated plants (Gallo-Meagher et al. 2001). In this study, no reduction in spotted wilt incidence was observed among various insecticide treatments in 2011 and 2012 when compared with untreated check. However, in 2013, treatment differences were observed. Spotted wilt incidence in 2013 was also higher than in the previous two years. A number of selected alternative insecticides were as effective as phorate in reducing spotted wilt incidence. Nevertheless, spotted wilt incidences in phorate treated plots were not different from that of untreated plots. In fact, spotted wilt incidences in some insecticide treated plots were more than untreated plots. These findings reiterate that insecticides might not play a significant role in reducing spotted wilt incidence. Spotted wilt incidences did not vary between Georgia -06G plots and Georgia Green plots from 2011 to 2013. TSWV-resistance cultivars are not immune to the virus and could succumb to the virus under a high TSWV pressure, as in the case of 2013. However, greenhouse assays indicated that all the selected insecticides were as effective as phorate in reducing spotted wilt incidence and all insecticides tested reduced spotted wilt incidence by more than half when compared with untreated check. A number of factors could have contributed to the observed differences under field and greenhouse situations. In the greenhouse assays, ten viruliferous thrips were released per plant at once, whereas multiple thrips introductions could have occurred

over time. Also, the number of thrips per plant under field conditions could have also contributed to the observed difference.

Irrespective of the treatments, yields in general, were higher in 2012 than in 2011 and 2013. Yields were lowest in 2011; this might be due to the delayed planting in 2011. Reduction in yields in 2013 could be due to increase in thrips populations as well as increased spotted wilt incidence. Irrespective of the insecticide treatments, yields were not influenced by the cultivars used in 2011. Yields were greater on Georgia-06G plots than on Georgia Green plots in 2012, whereas it was the opposite in 2013. Yields on phorate treated plots were greater than on untreated plots, thiamethoxam (at-cracking), and lambda-cyhalothrin treated plots. However, the yields in phorate treated plots were comparable with plots treated with numerous alternative insecticides such as seed treatments, in-furrow applications and at-crack applications. These findings indicate that the application of phorate or other alternative insecticides might not have a significant impact on yield.

The findings of this study indicate that several alternative insecticides were as effective as aldicarb and phorate in suppressing thrips populations and thrips feeding injuries. On the contrary to in-furrow applications of aldicarb and phorate, the alternative insecticides such as neonicotinoids are amenable as seed treatments, in-furrow or at-crack applications. These provide growers with more flexibility in insecticide use patterns. Besides, the alternative insecticides are less toxic and have fewer non-target effects when compared with aldicarb and phorate. In spite of the reduction in thrips feeding damage, none of the alternatives along with aldicarb and phorate significantly reduced spotted wilt incidence. Even though the insecticides are not effective in reducing spotted wilt incidence, it might still be important in thrips management in peanuts. Thrips feeding damage, especially in the early season, could lead to

compensatory growth and subsequently delay harvest. A delay in crop maturity in late-maturing peanut cultivars (>150 days) could expose the crop to inclement weather and cause significant yield losses. Most of the currently grown cultivars are late maturing. The currently available cultivars possess a reasonable amount TSWV resistance and are generally high yielding. Hence, it might be possible to use alternative insecticides to reduce thrips feeding damage without significantly compromising yields. Even though a number of insecticide classes could be used as alternatives, registration for use in peanut and cost could prohibit use by growers. Incorporation of alternative insecticides such as neonicotinoids in a risk mitigation index designed for growers (Peanut Risk Index) could encourage the usage of alternative insecticides in peanut production and reduce the usage of carbamate and organophosphate insecticides (Culbreath et al. 2003, Culbreath and Srinivasan 2011, Brown et al. 2005).

Literature Cited

Adkins, S. 2000. *Tomato spotted wilt virus* – positive steps towards negative success. Mol. Plant Pathol. 1: 151-157.

AgroNews. 2010. Bayer CropScience to close a plant as Temik phasing out. http://news.agro pages.com/News/NewsDetail---2766.htm.

Ames Herbert, D., S. Malone, S. Aref, R. L. Brandenburc, D. L. Jordan, B. M. Royals, and P. D. Johnson. 2007. Role of insecticides in reducing thrips injury to plants and incidence of *Tomato spotted wilt virus* in Virginia market-type peanut. J. Econ. Entomol. 100: 1241-1247.

Baldwin, J. A., J. W. Todd, J. R. Weeks, D. W. Gorbet, A. K. Culbreath, A. S. Luke-Morgan, S. M. Fletcher, and S. L. Brown. 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. Proc. Ann. Southern Cons. Till. Conf. Sust. Agric. 24: 26-34.

Bertrand, P. F. 1998. 1997 Georgia plant disease loss estimates. Univ. Georgia Coop. Ext. Pub. Path: 98-107.

Brown, S. L., A. K. Culbreath, J. W. Todd, D. W. Gorbert, J. A. Baldwin, and J. P. Beasley. 2005. Development of a method of risk assessment to facilitate integrated management of spotted wilt of peanut. Plant Dis. 89: 348-356.

Clark, M. E., and A. N. Adams. 1977. Characteristics of the microplate method of enzyme linked immunosorbent assay for the detection of plant viruses. J. Gen. Virol. 34: 475-483.

Culbreath, A. K., B. L. Tillman, D. W. Gorbet, C. C. Holbrook, and C. Nischwitz. 2008. Response of new field-resistant peanut cultivars to twin-row pattern or in-furrow applications of phorate for management of spotted wilt. Plant Dis. 92: 1307-1312.

Culbreath, A. K., J. W. Todd, D. W. Gorbet, F. M. Shokes, and H. R. Pappu. 1997. Field response of new peanut cultivar UF 91108 to *Tomato spotted wilt virus*. Plant Dis. 81: 1410-1415.

Culbreath, A. K., J. W. Todd, J. W. Demski, and J. R. Chamberlin. 1992. Disease progress of spotted wilt in peanut cultivars florunner and southern runner. Phytopathol. 82: 766-771.

Culbreath, A. K., J. W. Todd, and S. L. Brown. 2003. Epidemiology and management of tomato spotted wilt in peanut. Annu. Rev. Phytopathol. 41: 53-75.

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

Digiuseppe, G. 2010. Bayer to phase out use of Temik. *In* Crop Production. http://magissues.farmprogress.com/MDS/MS11Nove10/mds024.pdf.

Gallo-Meagher, M., K. Chengalrayan, J. M. Davis, G. E. McDonald. 2001. Phorate induced genes that may condition acquired resistance to tomato spotted wilt (Abstr.). Proc. Am. Peanut Res. Ed. Soc. 33: 29.

German, T. L., D. E. Ullman, and J. W. Moyer. 1992. *Tospoviruses*: diagnosis, molecular biology, phylogeny, and vector relationships. Annu.Rev. Phytopathol. 30: 315-348.

GFB News. 2010. Georgia Farm Bureau: the voice of Georgia farmers. Bayer CropScience to phase out pesticide aldicarb, close Georgia plant. http://www.gfb.org./gfbnews/gfbnews moreinfo.asp?recordid=284.

IRAC. 2012. Insecticide Resistance Action Committee. IRAC MoA classification scheme, issued April 2012, version 7.2. http://www.irac-online.org.

Jones, D. R. 2005. Major review: plant viruses transmitted by thrips. Eur. J. Plant Pathol. 113: 119-157.

Lynch, R. E., J. W. Garner, and L. W. Morgan. 1984. Influence of systemic insecticides on thrips damage and yield of florunner peanuts in Georgia. J. Agric. Entomol. 1: 33-42.

Maris, P. C., N. N. Joosten, D. Peters, and R. W. Goldback. 2003. Thrips resistance in pepper and its consequences for the acquisition and inoculation of *Tomato spotted wilt virus* by the western flower thrips. Phytopathol. 93: 96-101.

Marois, J. J., and D. L. Wright. 2003. Effect of tillage system, phorate, and cultivar on tomato spotted wilt of peanut. Agron. J. 95: 386-389.

Munger, F. 1942. A method of rearing citrus thrips in the laboratory. J. Econ. Entomol. 35: 373-375.

Pappu, H. R., R. A. C. Jones, and R. K. Jain. 2009. Global status of *Tospovirus* epidemics in diverse cropping systems: successes achieved and challenges ahead. Vir. Res. 141: 219-236.

Riley, D. G., S. V. Joseph, R. Srinivasan, and S. Diffie. 2011. Thrips vectors of tospoviruses. J. Integ. Pest Mngmt. 1: 1-10.

Shrestha, A., R. Srinivasan, D. G. Riley, and A. K. Culbreath. 2012. Direct and indirect effects of a thrips-transmitted *Tospovirus* on the preference and fitness of its vector, *Frankliniella fusca*. Entomologia Experimentalis et Applicata 145: 260-271.

Todd, J. W., J. R. Chamberlin, A. K. Culbreath, and J. W. Demski. 1994a. Timing and duration of vector management in relation to spotted wilt disease incidence in peanut. Proc. Am. Peanut Res. Ed. Soc. 25: 86.

Todd, J. W., J. R. Chamberlin, A. K. Culbreath, D. Rogers, and J. W. Demski. 1994b. Contradictions of insecticide use relative to vector control and spotted wilt disease progress in peanut. Proc. Am. Peanut Res. Ed. Soc. 26: 42.

Todd, J. W., and A. K. Culbreath. 1995. Thrips populations and spotted wilt disease progress on resistance/susceptible cultivars treated with various insecticides. Proc. Am. Peanut Res. Ed. Soc. 27: 35.

Todd, J. W., A. K. Culbreath, J. R. Chamberlin, R. J. Beshear, and B. G. Mullinix. 1995. Colonization and population dynamics of thrips in peanuts in the southern United States, pp. 453-460. *In* B. Parker, M. Skinner and T. Lewis (eds.), Thrips Biol. Mgt. Plenum Press, New York.

Todd, J. W., A. K. Culbreath, and S. L. Brown. 1996. Dynamics of vector populations and progress of spotted wilt disease relative to insecticides use in peanuts. Acta Hortic. 431: 483-490.

Todd, J. W., A. K. Culbreath, H. R. Pappu, and S. L. Brown. 1997. Thrips as *Tospovirus* vectors in peanut. Proc. Am. Peanut Res. Ed. Soc. 29: 17.

Triplehorn, C. A., and N. F. Johnson. 2005. Borror and Delong's introduction to the study of insects, 7th ed. Brooks/Cole, Cengage Learning, U.S.A.

Tubbs, R., K. Balkcom, M. Toews, and R. Srinivasan. 2013. Effects of fertilization, tillage, and phorate on thrips and TSWV incidence in early planted peanuts (Abstr.). Southern Cons. Agric. Sys. Conf. http//:www.ars.usda.gov/research/publications/publications.htm.

Ullman, D. E., J. J. Cho, R. F. L. Mau, W. B. Hunter, D. M. Westcot, and D. M. Custer. 1992. Thrips-tomato spotted wilt virus interactions: morphological, behavioral and cellular components influencing thrips transmission. Adv. Dis. Vect. Res. 9: 195-240.

Weeks, J. R., and A. K. Hagan. 1991. The effects of planting date and insecticide applications on thrips populations, *Tomato spotted wilt virus* incidence and yield of peanut in Alabama (Abstr.). Proc. Am. Peanut Res. Ed. Soc. 23: 41.

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

Whitfield, A. E., D. E. Ullman, and T. L. German. 2005. Tospovirus-thrips interactions. Annu. Rev. Phytopathol. 43: 459-C-5.

Tables

Table 3.1. List of selected insecticides for field trials.

Treatment No.	Main Group and Primary Site of Action[y]	Chemical Name/ Sub-group or Exemplifying Active Ingredient	Active Ingredient	Trade Name	Rate per Hectare (Ha)[z]	Price	Type of Application	Manufacturer
1	4 Nicotinic acetylcholine receptor (nAChR) agonists	4A Neonicotinoids	Thiamethoxam	Actara®	0.14 kg	$7.05/kg	At cracking	Syngenta
2			Imidacloprid	Admire® Pro	0.51 l	$52.42/l	In-furrow	Bayer CropScience
3			Imidacloprid	Admire® Pro	0.12 l	$52.42/l	At cracking	Bayer CropScience
4			Acetamiprid	Assail® 30SG	0.28 kg	$201.06/kg	At cracking	United Phosphorous, Inc.
			Thiamethoxam	Cruiser Maxx™	0.21-0.28 kg per 45.36 kg seed	NA	Seed treatment	Syngenta
5	UN	UN	Azadirachtin	Azatin® XL	1.53 l	$199.74/l	At cracking	OHP, Inc.
6	28 Ryanodine receptor modulators	Diamides	Cyantraniliprole	HGW086 10C	1.49 l	NA	At cracking	DuPont

7	3 Sodium channel modulators	3A Pyrethroids	Lambda-cyhalothrin	Karate®	0.26 l	$84.86/l	At cracking	Syngenta
8	23 Inhibitors of acetyl CoA carboxylase/ Lipid biosynthesis inhibitor	Tetronic and Tetramic acid derivatives	Spirotetratmat	Movento™ 2SC	0.37 l	$240.78/l	At cracking	Bayer CropScience
9	5 Nicotinic acetylcholine receptor (nAChR) allosteric activators	Spinosyn	Spinetoram	Radiant™ SC	0.37 l	$196.14/l	At cracking	Dow AgroSciences
10	1 Acetylcholinesterase (AChE) inhibitors	1A Carbamates	Aldicarb	Temik® 15G	5.60 kg	NA	In-furrow	Bayer CropScience
11		1B Orghanophosphates	Phorate	Thimet® 10G	5.60 kg	$7.32/kg	In-furrow	Amvac

[y] Mode of action (MoA); (UN) a compound with an unknown mode of action (IRAC 2012).

[z] Based on the manufacturer's recommended rates.

Table 3.2. List of selected alternative insecticides for greenhouse experiment.

Treatment No.	Active Ingredient	Trade Name	Rate per Acre (A)[x]	Rate per peanut plant[y]	Type of Application	Manufacturer
1	Thiamethoxam	Actara®	0.20 kg	0.0014 g	Foliar Spray	Syngenta
2	Imidacloprid	Admire® Pro	0.12 l	0.00087 ml	Foliar Spray	Bayer CropScience
3	Cyantraniliprole	HGW086 10C	1.49 l	0.018 ml	Foliar Spray	DuPont
4	Spinetoram	Radiant® 1SC	0.37 l	0.0025 ml	Foliar Spray	Dow AgroSciences
5	Aldicarb	Temik® 15G	5.60 kg	0.039 g	In-furrow	Bayer CropScience
6	Phorate	Thimet® 10G	5.60 kg	0.039 g	In-furrow	Amvac

[x] Based on the manufacturer's recommended rates.

[y] Insecticide doses were calculated on a per plant basis at ~143,260 peanut plants per hectare.

Table 3.3. *Frankliniella fusca* feeding on TSWV-susceptible and -resistant peanut cultivars.

Treatments[x]	Mean feeding damage index[y]					
	10 days		20 days		30 days	
	Georgia Green	Georgia-06G	Georgia Green	Georgia-06G	Georgia Green	Georgia-06G
Thiamethoxam	0.07 ± 0.02 (bc)	0.08 ± 0.02 (c)	0.52 ± 0.19 (b)	0.23 ± 0.06 (b)	1.25 ± 0.39 (b)	1.07 ± 0.22 (b)
Imidacloprid	0.02 ± 0.01 (c)	0.02 ± 0.01 (d)	0.02 ± 0.01 (d)	0.02 ± 0.01 (b)	0.03 ± 0.01 (d)	0.02 ± 0.01 (c)
Cyantraniliprole	0.00 ± 0.00 (c)	0.00 ± 0.00 (d)	0.00 ± 0.00 (d)	0.00 ± 0.00 (b)	0.00 ± 0.00 (d)	0.00 ± 0.00 (c)
Spinetoram	0.00 ± 0.00 (c)	0.00 ± 0.00 (d)	0.00 ± 0.00 (d)	0.00 ± 0.00 (b)	0.00 ± 0.00 (d)	0.00 ± 0.00 (c)
Aldicarb	0.00 ± 0.00 (c)	0.00 ± 0.00 (d)	0.00 ± 0.00 (d)	0.00 ± 0.00 (b)	0.00 ± 0.00 (d)	0.00 ± 0.00 (c)
Phorate	0.19 ± 0.05 (bc)	0.17 ± 0.04 (b)	0.23 ± 0.06 (c)	0.19 ± 0.04 (b)	0.44 ± 0.14 (c)	0.26 ± 0.06 (c)
Untreated	0.97 ± 0.11 (a)	0.56 ± 0.13 (a)	3.47 ± 0.35 (a)	2.01 ± 0.35 (a)	4.19 ± 0.22 (a)	3.30 ± 0.41 (a)
Type III analysis[z] (df=6,98)						
F	66.28	41.80	301.35	64.17	186.66	65.39
P > F	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

[x] Selected alternative insecticides to aldicarb and phorate for thrips feeding evaluation.

[y] Statistical differences in feeding damage at three time intervals, 10. 20, and 30 days after thrips release were assessed. Means (±SE) within a column followed by the same letter are not significantly different at P=0.05

[z] Type III analysis was conducted using PROC GLIMMIX in SAS.

Table 3.4. Incidence of *Tomato spotted wilt virus* (TSWV) infection in *Frankliniella fusca*-inoculated peanut cultivars.

Number of plants infected in two peanut cultivars[y]		
	TSWV transmission (%) (mean ± standard error)	
Treatments[x]	**Georgia Green**	**Georgia-06G**
Thiamethoxam	20.00 ± 10.69 (b)	26.67 ± 11.82 (b)
Imidacloprid	26.67 ± 11.82 (b)	0.00 ± 0.00 (d)
Cyantraniliprole	33.33 ± 12.60 (b)	26.67 ± 11.82 (b)
Spinetoram	13.33 ± 9.09 (b)	6.67 ± 6.67 (cd)
Aldicarb	6.67 ± 6.67 (b)	0.00 ± 0.00 (d)
Phorate	26.67 ± 11.82 (b)	26.67 ± 11.82 (b)
Untreated	80.00 ± 10.69 (a)	86.67 ± 9.09 (a)
Type III analysis (df=6,98)[z]		
χ^2	29.00	58.00
$P>\chi^2$	$P<0.0001$	$P<0.0001$

[x] Selected alternative insecticides to aldicarb and phorate for the evaluation of TSWV transmission.

[x] Peanut cultivars, Georgia Green (TSWV-susceptible) and Georgia-06G (TSWV-resistant), were subjected to thrips inoculation. TSWV infection status of inoculated plants was confirmed by DAS-ELISA.

[z] Type III analysis was conducted using PROC GENMOD in SAS.

Figures

Fig. 3.1. Thrips composition in Georgia Green and Georgia-06G peanut cultivars. Samples were counted and identified to species under a dissecting microscope using dichotomous keys. Thrips were grouped as immatures, non-vector adults, and vector adults.

Fig. 3.2. Vector species in Georgia Green and Georgia-06G peanut cultivars. Samples were counted and identified to species under a dissecting microscope using dichotomous keys. Thrips vectors were grouped as *F. fusca* and other thrips species.

Fig. 3.3. Mean (±SE) cumulative counts of thrips across six weeks over four replications in two two peanut cultivars. Treatment means represented by the same letter indicate that the treatments are not significantly different from each other. Sampling for thrips was done for six consecutive weeks at ~three weeks after planting except for 2011. In 2011, sampling was done only for five consecutive weeks. Quadrifoliate peanut terminal leaves were collected on the first three weeks while peanut blooms were collected on the last three weeks. Thrips samples were collected in 70% ethyl alcohol and identified to species under a dissecting microscope.

Fig. 3.4. Mean (±SE) thrips damage over four replications in two peanut cultivars. Treatment means represented by the same letter indicate that the treatments are not significantly different from each other. In 2011, thrips damage was not rated. Thrips damage was assessed using an arbitrary scale of 0 to 10, wherein 0 represented no damage and 10 represented a dead plant. Peanut plants were evaluated from the 2^{nd} and 5^{th} rows of each plot. Assessment was done at five weeks after planting of peanuts.

Fig. 3.5. Mean (±SE) spotted wilt incidence (%) over four replications in two peanut cultivars. Treatment means represented by the same letter indicate that the treatments are not significantly different from each other. The assessment of spotted wilt incidence was obtained from two

center rows (3rd and 4th rows) of each plot using a 30.48-cm hit stick based on standard protocols. Observations were done approximately two weeks prior to harvest.

Fig. 3.6. Mean (±SE) yield over four replications in two peanut cultivars. Treatment means represented by the same letter indicate that the treatments are not significantly different from each other. Peanut plants in the 3rd and 4th rows of each plot were dug, inverted, air-dried, picked, and weighed (in kg) using standard protocols.

Fig. 3.7. Mean (±SE) feeding indices on peanut plants treated with aldicarb and phorate and selected insecticides. Five one-week old peanut plants for each treatment and cultivar were placed separately in insect proof cage. Approximately 0.05 g of pine pollen grains were dusted on each plant. Ten non-viruliferous female *F. fusca* were collected in 0.6 ml microcentrifuge tubes using a paintbrush and then released at the base of each plant. Thrips feeding damage was assessed on each plant every two days for a month after initial thrips release.

Fig. 3.8. Percent (± SE) *Tomato spotted wilt virus* (TSWV) infection in peanut plants treated with aldicarb and phorate and selected insecticides. Five one-week old peanut plants for each treatment and cultivar were used. Approximately 0.05 g of pine pollen grains was dusted on each plant. Incidence of TSWV infection of inoculated plants was confirmed by DAS-ELISA.

Fig. 3.1.

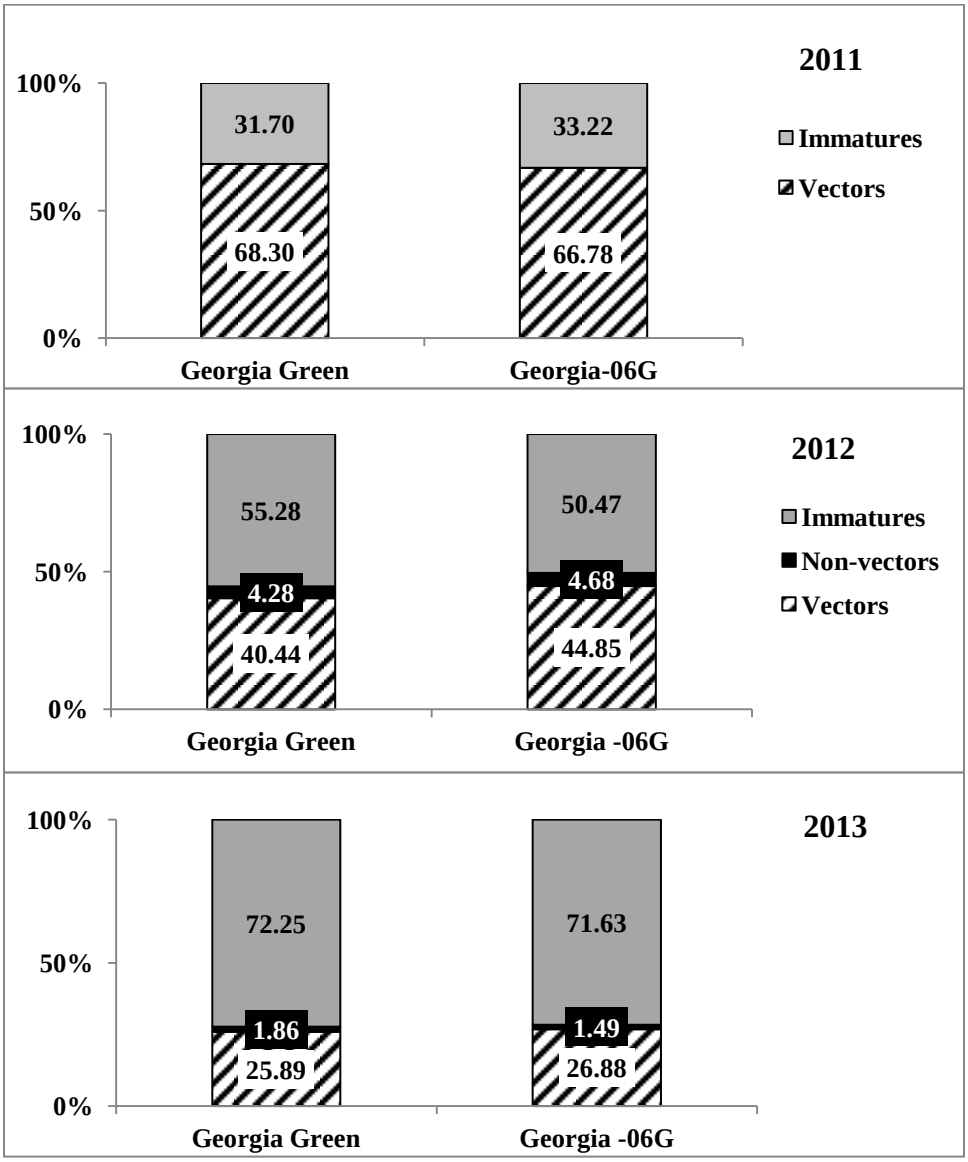

2011
100%
50%
0%
31.70
33.22
68.30
66.78
Immatures
Vectors
Georgia Green
Georgia-06G
2012
100%
50%
0%
55.28
50.47
4.28
4.68
40.44
44.85
Immatures
Non-vectors
Vectors
Georgia Green
Georgia -06G
2013
100%
50%
0%
72.25
71.63
1.86
1.49
25.89
26.88
Georgia Green
Georgia -06G

Fig. 3.2.

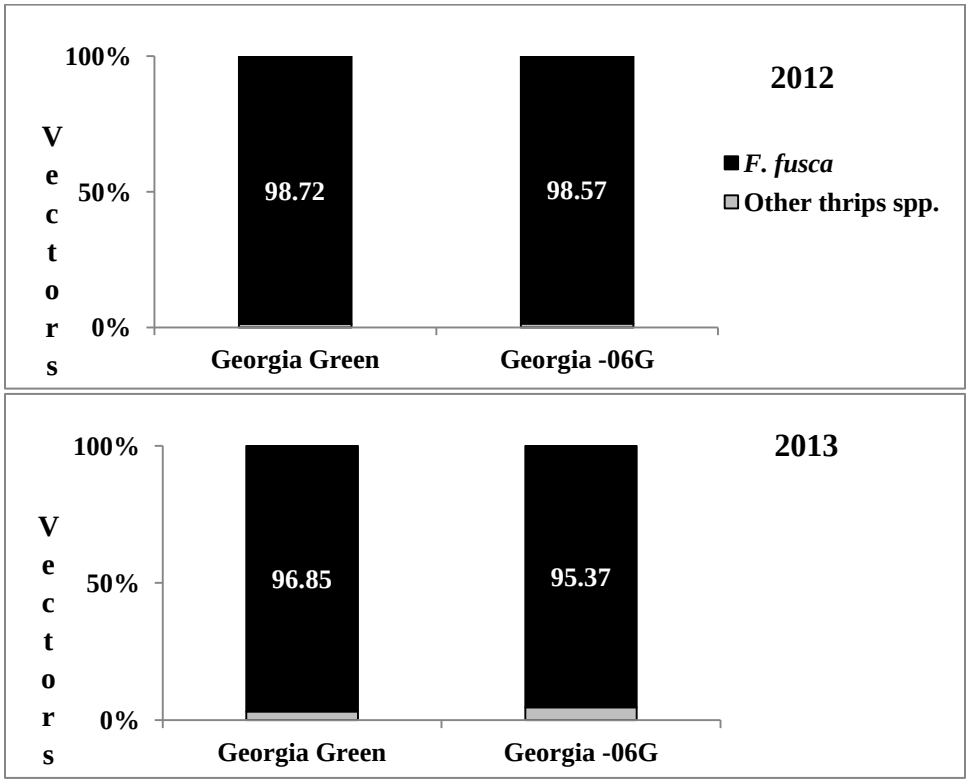

2012
100%
50%
0%
Vectors
98.72
98.57
F. fusca
Other thrips spp.
Georgia Green
Georgia -06G
2013
100%
50%
0%
Vectors
96.85
95.37
Georgia Green
Georgia -06G

Fig. 3.3.

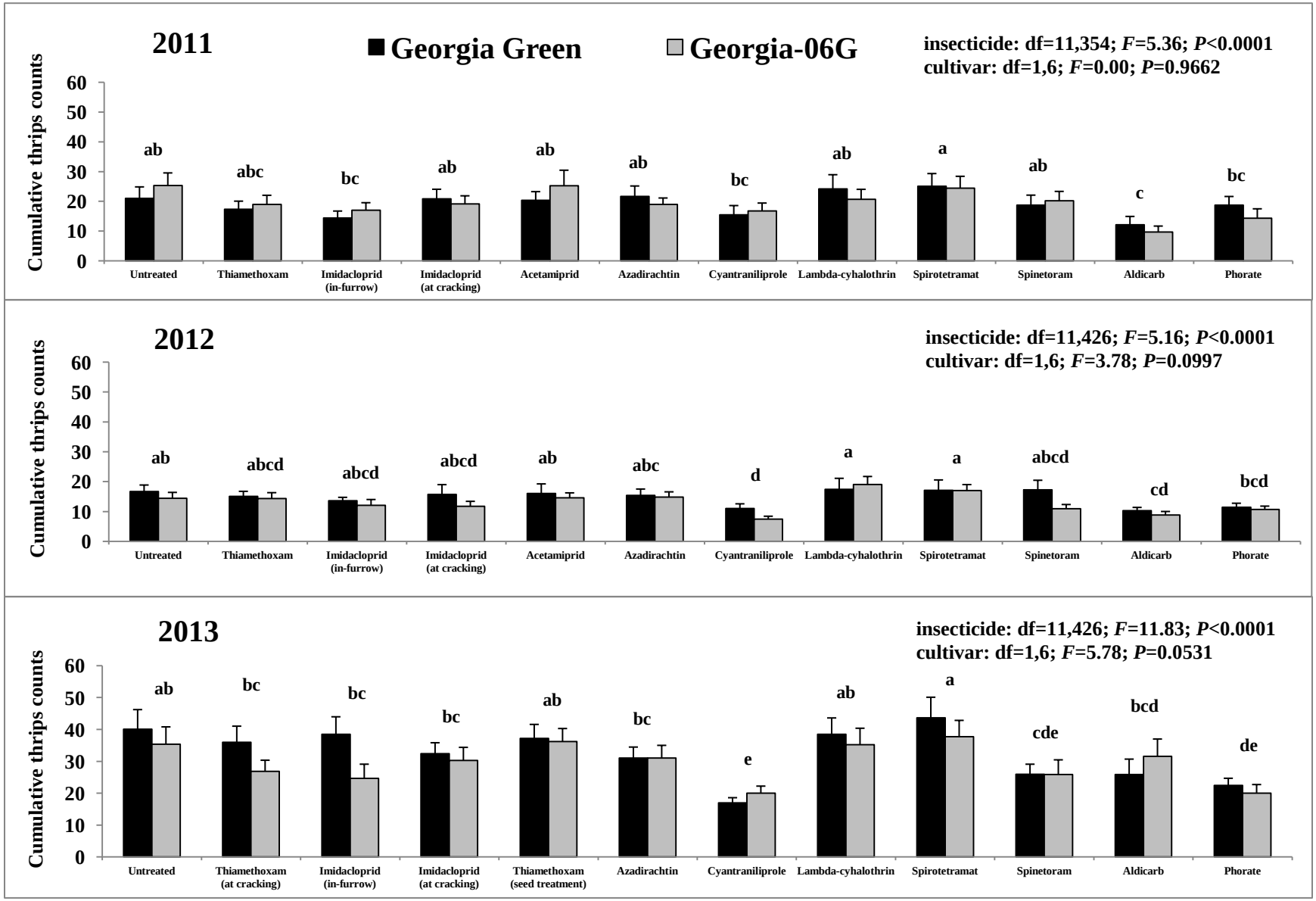

2011
Georgia Green
Georgia-06G
insecticide: df=11,354; F=5.36; P<0.0001
cultivar: df=1,6; F=0.00; P=0.9662
Cumulative thrips counts
60
50
40
30
20
10
0
ab
abc
bc
ab
ab
ab
bc
ab
a
ab
c
bc
Untreated
Thiamethoxam
Imidacloprid (in-furrow)
Imidacloprid (at cracking)
Acetamiprid
Azadirachtin
Cyantraniliprole
Lambda-cyhalothrin
Spirotetramat
Spinetoram
Aldicarb
Phorate
2012
insecticide: df=11,426; F=5.16; P<0.0001
cultivar: df=1,6; F=3.78; P=0.0997
Cumulative thrips counts
ab
abcd
abcd
abcd
ab
abc
d
a
a
abcd
cd
bcd
Untreated
Thiamethoxam
Imidacloprid (in-furrow)
Imidacloprid (at cracking)
Acetamiprid
Azadirachtin
Cyantraniliprole
Lambda-cyhalothrin
Spirotetramat
Spinetoram
Aldicarb
Phorate
2013
insecticide: df=11,426; F=11.83; P<0.0001
cultivar: df=1,6; F=5.78; P=0.0531
Cumulative thrips counts
ab
bc
bc
bc
ab
bc
e
ab
a
cde
bcd
de
Untreated
Thiamethoxam (at cracking)
Imidacloprid (in-furrow)
Imidacloprid (at cracking)
Thiamethoxam (seed treatment)
Azadirachtin
Cyantraniliprole
Lambda-cyhalothrin
Spirotetramat
Spinetoram
Aldicarb
Phorate

Fig. 3.4.

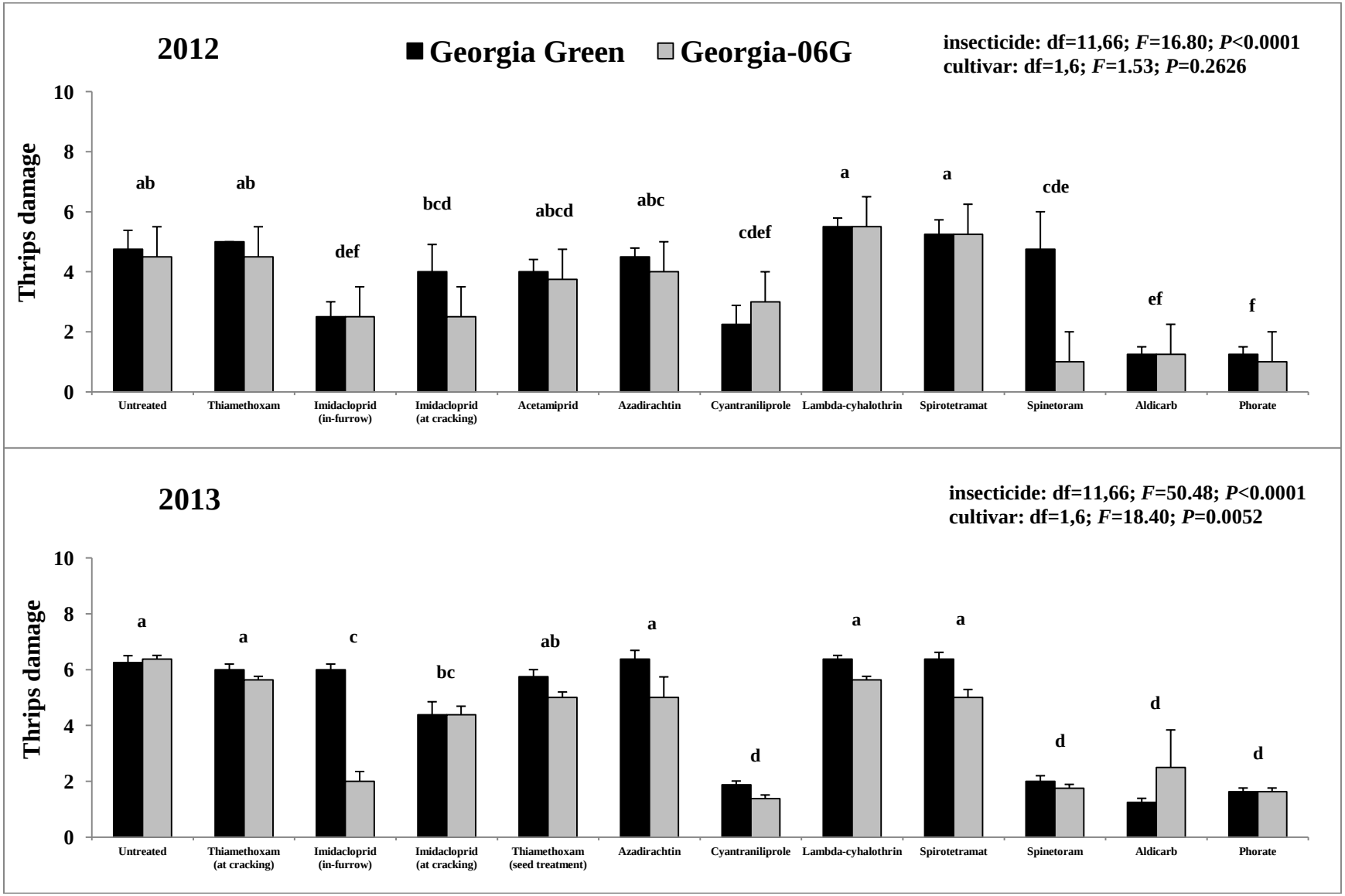

2012
Georgia Green
Georgia-06G
insecticide: df=11,66; F=16.80; P<0.0001
cultivar: df=1,6; F=1.53; P=0.2626
Thrips damage
10
8
6
4
2
0
ab
ab
def
bcd
abcd
abc
cdef
a
a
cde
ef
f
Untreated
Thiamethoxam
Imidacloprid (in-furrow)
Imidacloprid (at cracking)
Acetamiprid
Azadirachtin
Cyantraniliprole
Lambda-cyhalothrin
Spirotetramat
Spinetoram
Aldicarb
Phorate
2013
insecticide: df=11,66; F=50.48; P<0.0001
cultivar: df=1,6; F=18.40; P=0.0052
Thrips damage
10
8
6
4
2
0
a
a
c
bc
ab
a
d
a
a
d
d
d
Untreated
Thiamethoxam (at cracking)
Imidacloprid (in-furrow)
Imidacloprid (at cracking)
Thiamethoxam (seed treatment)
Azadirachtin
Cyantraniliprole
Lambda-cyhalothrin
Spirotetramat
Spinetoram
Aldicarb
Phorate

Fig. 3.5.

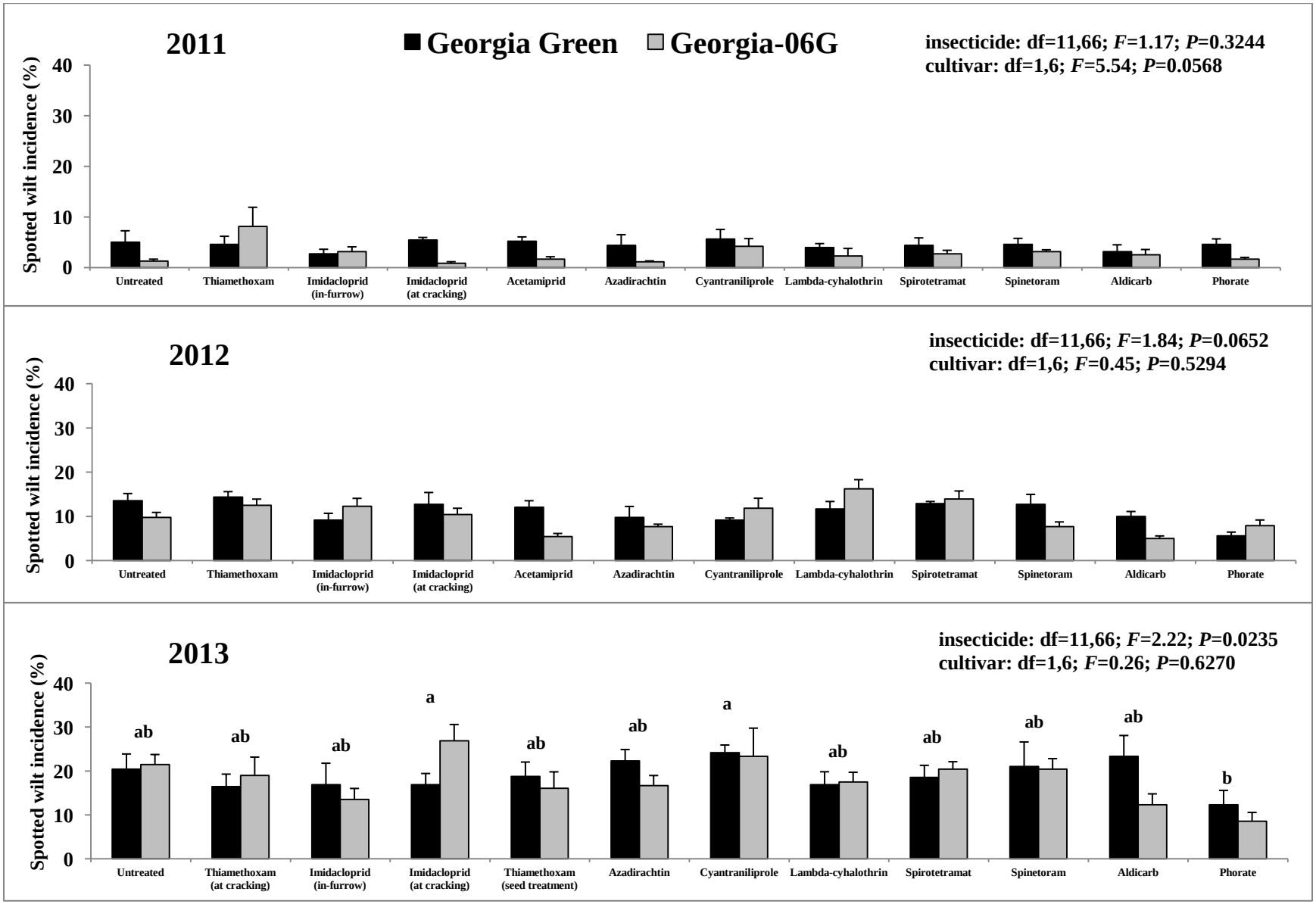

2011
Georgia Green
Georgia-06G
insecticide: df=11,66; F=1.17; P=0.3244
cultivar: df=1,6; F=5.54; P=0.0568
Spotted wilt incidence (%)
0
10
20
30
40
Untreated
Thiamethoxam
Imidacloprid (in-furrow)
Imidacloprid (at cracking)
Acetamiprid
Azadirachtin
Cyantraniliprole
Lambda-cyhalothrin
Spirotetramat
Spinetoram
Aldicarb
Phorate
2012
insecticide: df=11,66; F=1.84; P=0.0652
cultivar: df=1,6; F=0.45; P=0.5294
Spotted wilt incidence (%)
0
10
20
30
40
Untreated
Thiamethoxam
Imidacloprid (in-furrow)
Imidacloprid (at cracking)
Acetamiprid
Azadirachtin
Cyantraniliprole
Lambda-cyhalothrin
Spirotetramat
Spinetoram
Aldicarb
Phorate
2013
insecticide: df=11,66; F=2.22; P=0.0235
cultivar: df=1,6; F=0.26; P=0.6270
Spotted wilt incidence (%)
0
10
20
30
40
ab
ab
ab
a
ab
ab
a
ab
ab
ab
ab
b
Untreated
Thiamethoxam (at cracking)
Imidacloprid (in-furrow)
Imidacloprid (at cracking)
Thiamethoxam (seed treatment)
Azadirachtin
Cyantraniliprole
Lambda-cyhalothrin
Spirotetramat
Spinetoram
Aldicarb
Phorate

Fig. 3.6.

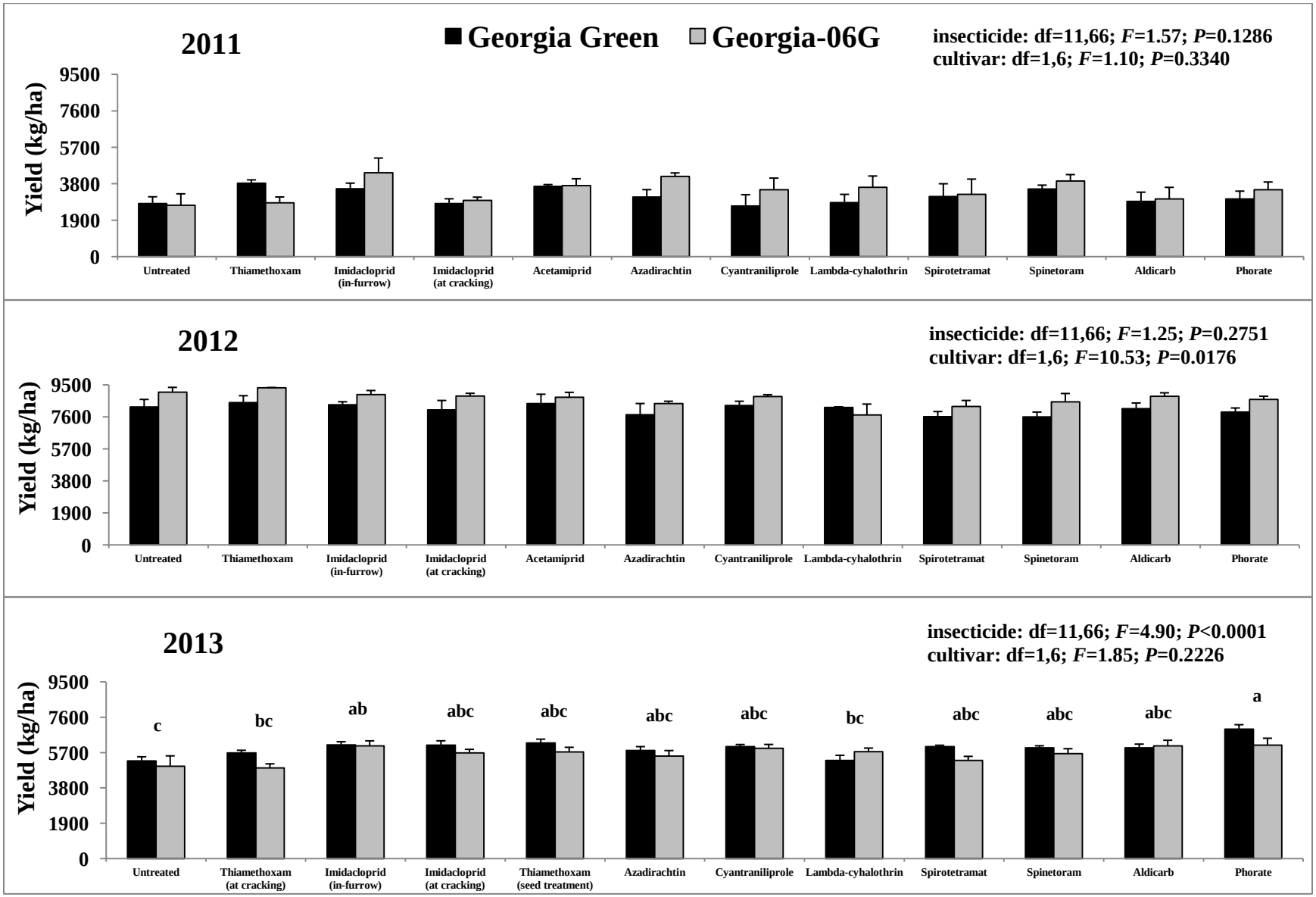

2011
Georgia Green
Georgia-06G
insecticide: df=11,66; F=1.57; P=0.1286
cultivar: df=1,6; F=1.10; P=0.3340
Yield (kg/ha)
9500
7600
5700
3800
1900
0
Untreated
Thiamethoxam
Imidacloprid (in-furrow)
Imidacloprid (at cracking)
Acetamiprid
Azadirachtin
Cyantraniliprole
Lambda-cyhalothrin
Spirotetramat
Spinetoram
Aldicarb
Phorate
2012
insecticide: df=11,66; F=1.25; P=0.2751
cultivar: df=1,6; F=10.53; P=0.0176
Yield (kg/ha)
9500
7600
5700
3800
1900
0
Untreated
Thiamethoxam
Imidacloprid (in-furrow)
Imidacloprid (at cracking)
Acetamiprid
Azadirachtin
Cyantraniliprole
Lambda-cyhalothrin
Spirotetramat
Spinetoram
Aldicarb
Phorate
2013
insecticide: df=11,66; F=4.90; P<0.0001
cultivar: df=1,6; F=1.85; P=0.2226
Yield (kg/ha)
9500
7600
5700
3800
1900
0
c
bc
ab
abc
abc
abc
abc
bc
abc
abc
abc
a
Untreated
Thiamethoxam (at cracking)
Imidacloprid (in-furrow)
Imidacloprid (at cracking)
Thiamethoxam (seed treatment)
Azadirachtin
Cyantraniliprole
Lambda-cyhalothrin
Spirotetramat
Spinetoram
Aldicarb
Phorate

Fig. 3.7.

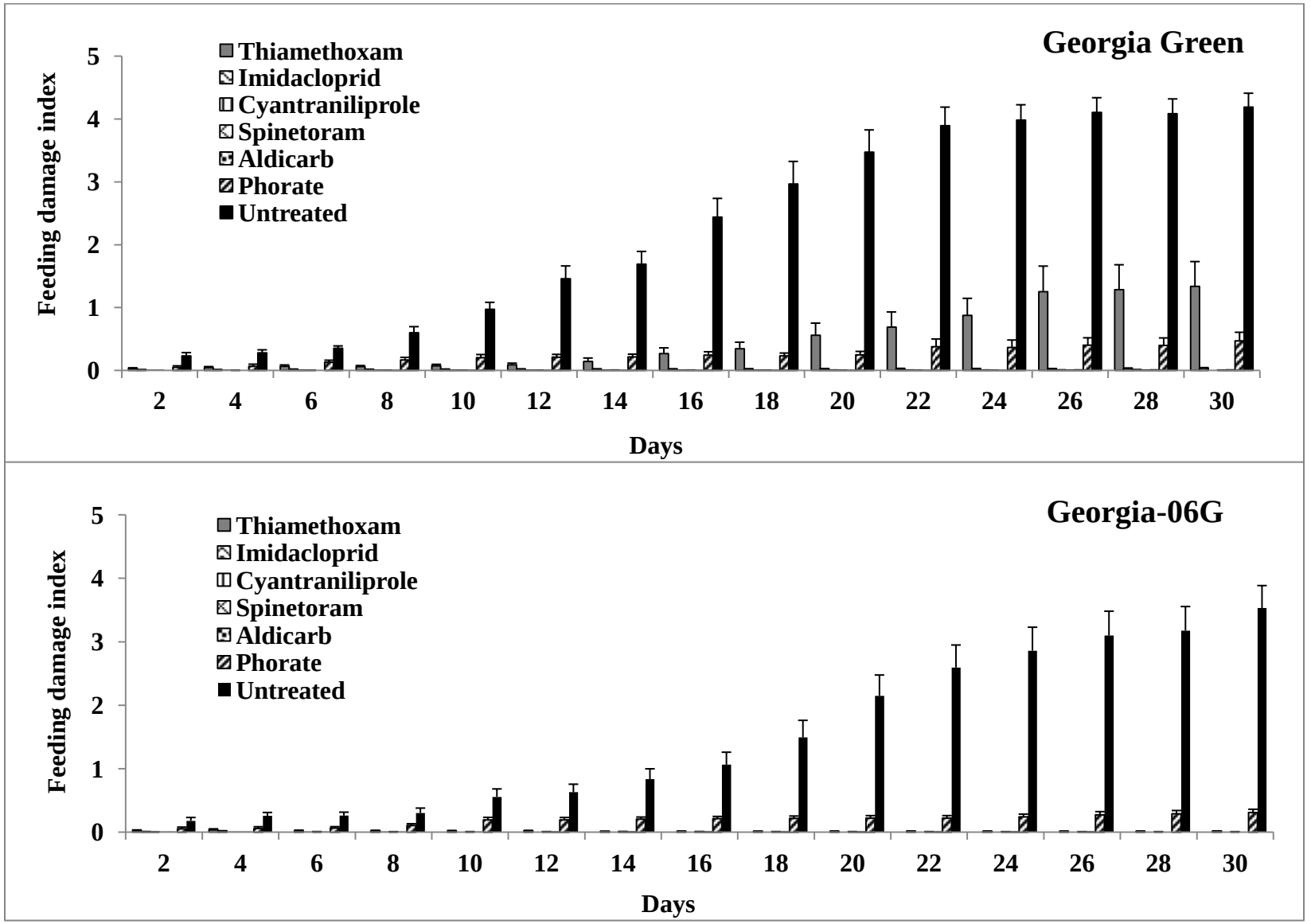
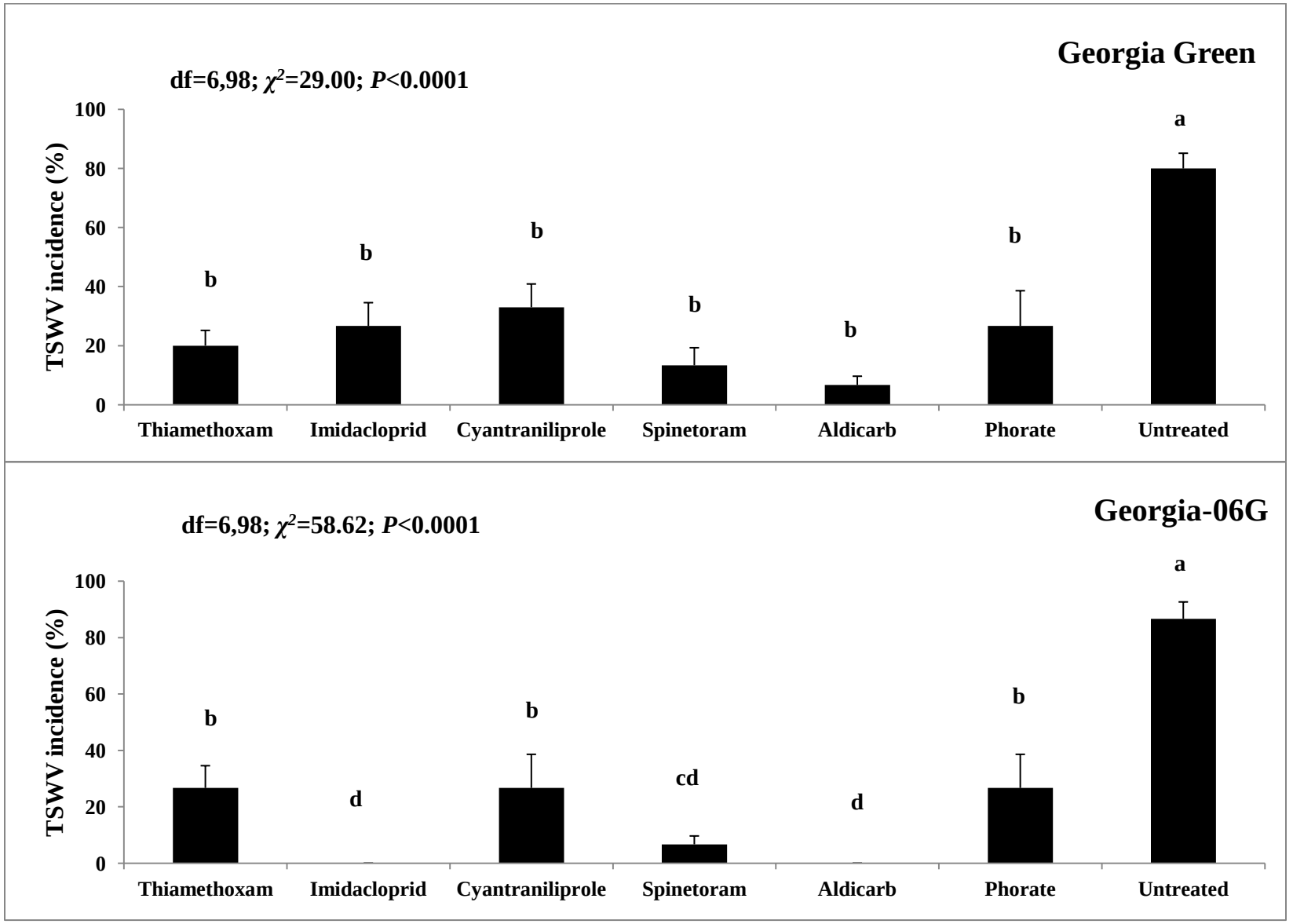

Georgia Green
Thiamethoxam
Imidacloprid
Cyantraniliprole
Spinetoram
Aldicarb
Phorate
Untreated
Feeding damage index
0
1
2
3
4
5
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
Days
Georgia-06G
Thiamethoxam
Imidacloprid
Cyantraniliprole
Spinetoram
Aldicarb
Phorate
Untreated
Feeding damage index
0
1
2
3
4
5
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
Days

Fig. 3.8.


Georgia Green
df=6,98; χ^2=29.00; P<0.0001
TSWV incidence (%)
100
80
60
40
20
0
b
b
b
b
b
b
a
Thiamethoxam
Imidacloprid
Cyantraniliprole
Spinetoram
Aldicarb
Phorate
Untreated
Georgia-06G
df=6,98; χ^2=58.62; P<0.0001
TSWV incidence (%)
100
80
60
40
20
0
b
d
b
cd
d
b
a
Thiamethoxam
Imidacloprid
Cyantraniliprole
Spinetoram
Aldicarb
Phorate
Untreated

CHAPTER 4

EVALUATION OF ALTERNATIVES TO AN ORGANOPHOSPHATE INSECTICIDE WITH SELECTED CULTURAL PRACTICES: EFFECTS ON THRIPS, *FRANKLINIELLA FUSCA* (HINDS), AND SPOTTED WILT INCIDENCE IN PEANUT FARMSCAPES[1]

[1]Marasigan, K., M. Toews, R. Kemerait, Jr., and R. Srinivasan. 2014.

Abstract

Peanut growers use a combination of tactics to manage spotted wilt disease caused by thrips-transmitted *Tomato spotted wilt virus* (TSWV). They include planting TSWV-resistant cultivars, application of insecticides, and various cultural practices. Two of the most commonly used insecticides are aldicarb and phorate. However, both insecticides exhibit broad-spectrum toxicity and cause non-target effects. Aldicarb usage in peanut will be phased out by 2018. Research over the last two years led to the identification of alternatives to aldicarb and phorate. In this study, we evaluated conventional versus strip tillage; single row versus twin rows; and, 4 seed/ft versus 6 seed/ft, with alternative insecticides, respectively. Three field trials were conducted in Georgia in 2012 and 2013. Thrips counts, thrips feeding injuries, and spotted wilt incidences were less under strip tillage than under conventional tillage. Reduced thrips feeding injuries was observed in twin row plots compared with single row plots in 2013 but not in 2012. Thrips counts, thrips feeding injuries, and spotted wilt incidences did not vary on plots with 4 seed/ft when compared with plots with 6 seed/ft. Yields from twin row plots were greater than yields from single row plots only in 2012. Yields were not affected by other cultural practices. Alternative insecticides including imidacloprid and spinetoram were as effective as phorate in suppressing thrips and reducing spotted wilt incidence in conjunction with cultural practices. Results suggest that alternatives to phorate could be used with cultural practices to suppress thrips populations and spotted wilt incidence in peanut.

Additional Key Words: Tobacco thrips, *Tomato spotted wilt virus*, management

Introduction

Spotted wilt, caused by *Tomato spotted wilt virus* (TSWV) in the family *Bunyaviridae* and genus *Tospovirus*, is one of the most economically important diseases affecting peanut production (Culbreath et al. 2003, Culbreath and Srinivasan 2011). In 1971, the disease was first reported in Texas in the United States (Haliwell and Philley 1974). Shortly, it became widespread to nearby southeastern states such as Georgia, Florida, and Alabama during late 80's and 90's (Culbreath et al. 1992, 2003). Serious annual yield losses due to the disease were documented in southeastern states (Bertrand 1998, Culbreath et al. 2003, Culbreath and Srinivasan 2011). An average of 12% (>$40 million) yield loss was recorded in Georgia from 1990 through 1997 (Bertrand 1998, Culbreath et al. 2003, Culbreath and Srinivasan 2011). Thrips in the family Thripidae (Order: Thysanoptera) exclusively transmit TSWV in a persistent and propagative manner (German et al. 1992, Ullman et al. 1992, Whitfield et al. 2005). Out of the ten species of thrips that transmit TSWV, tobacco thrips, *Frankliniella fusca* (Hinds), and western flower thrips, *Frankliniella occidentalis* (Pergande), are primarily responsible for spotted wilt epidemics in southeastern United States (Todd et al. 1994a, 1994b, 1995). Between the two species, the former is known to colonize and reproduce efficiently on peanut foliage especially in the early season than the latter (Todd et al. 1995, 1996, 1997).

Several tactics are available for the management of thrips and TSWV (Culbreath et al. 2003, Culbreath and Srinivasan 2011). The most common tactic is the use of peanut cultivars with high levels of field resistance to TSWV. Three generations of peanut cultivars were released from 90s to 2012 (Culbreath et al. 2003, Culbreath and Srinivasan 2011, Beasley 2013). In addition, peanut growers also rely on application of insecticides to manage thrips (Todd et al. 1994b, 1996; Culbreath et al. 2003, Culbreath and Srinivasan 2011). Aldicarb and phorate

insecticides are currently being used to suppress thrips populations as well as reduce feeding damage in peanut production. However, these insecticides have broad-spectrum toxicity and can cause harmful effects to the environment and mammals. In addition, Bayer CropScience and United States Environmental Protection Agency (US-EPA) agreed to phase out the usage of aldicarb by 2018 (AgroNews 2010, Digiuseppe 2010).

Several cultural practices along with peanut cultivars displaying field resistance to TSWV and insecticides have contributed to the reduction of TSWV. They include tillage system modifications, various row patterns, and increased seeding rates (Gorbet and Shokes 1994, Baldwin et al. 1998, 2001a, 2001b; Branch et al. 2003, Marois and Wright 2003, Cantonwine et al. 2006, Tillman et al. 2006, Culbreath et al. 2008, 2010, 2013). Most of these cultural practices seem to have an impact on TSWV reduction by affecting thrips populations. Tillage systems are known to influence thrips populations and TSWV incidence. Reduced TSWV incidence and increased pod yield were observed under strip tillage conditions compared with conventional tillage conditions (Baldwin et al. 2001a). Other field trials conducted in southeastern states such as Georgia, Florida, and Alabama indicated that plots under minimum or reduced tillage conditions had fewer insect pests, reduced thrips feeding injuries, and less TSWV incidence than plots under conventional tillage conditions (Minton et al. 1991, Brown et al. 1996, Baldwin and Hook 1998, Brandenburg et al. 1998, Hurt et al. 2006).

Another cultural practice that is commonly adopted is to plant peanuts in twin or double rows (Culbreath et al. 2003, Culbreath and Srinivasan 2011). Several studies have shown that planting in twin rows provided more advantages than planting in single rows such as increase in pod yield, and reduction of TSWV incidence and thrips density (Baldwin et al. 1998, 2001a, 2001b; Lanier et al. 2004, Tillman et al. 2006, Culbreath et al. 2008, Tubbs and Beasley 2009,

Tubbs et al. 2011). Twin rows are known to enhance plant stands, reduce intra-row competition and reduce TSWV incidence (Tubbs et al. 2011). All of the mentioned factors could contribute towards increasing yield (Tubbs et al. 2011). Besides row patterns, seeding rates could also be increased to obtain desired plant stands and affect TSWV incidence (Brown et al. 1996, 1999, 2005; Culbreath et al. 2003, 2012, 2013; Tillman et al. 2006, Culbreath and Srinivasan 2011,). A number of studies found an inverse relationship between increase in seeding rate and reduction in spotted wilt incidence (Gorbet and Shokes 1994, Culbreath et al. 2013). Lower spotted wilt incidence in plots with higher seeding rates than plots with lower seeding rates might be due to bare ground effects. Availability of bare ground area in plots with higher seeding rates could be reduced and consequently reduce thrips host finding on peanut plants (Culbreath et al. 2003, Culbreath and Srinivasan 2011).

As previously mentioned, the cultural practices are used in conjunction with insecticide applications as well as with cultivars displaying field resistance to TSWV. The two commonly available insecticides, carbamate (aldicarb) and organophosphate (phorate), have broad-spectrum toxicity. They are also capable of inducing several undesirable non-target effects in the environment and possess high mammalian toxicity. Of the two, aldicarb will be phased out soon. Field research over the last few years has identified a number of potential alternatives. The focus of this study is to evaluate selected alternatives to phorate with cultural practices such as strip tillage, twin rows, and increased seeding rates. Field trials were conducted in Tifton and in Attapulgus, GA from 2012 through 2013. Our hypothesis was that the use of alternative insecticides in conjunction with cultural practices would reduce thrips populations, feeding injuries, and spotted wilt incidence in the field as efficiently as phorate.

Materials and Methods

Field experiments. Three field trials were conducted in total. A trial was conducted at the Belflower Farm, Coastal Plain Experimental Station at Tifton, GA to evaluate the efficacy of alternatives to phorate under strip tillage and conventional tillage conditions in 2012 and 2013, respectively. The tillage experiments were conducted using Georgia-06G (TSWV-resistant). Remaining trials were conducted in the Attapulgus Research and Education Station at Attapulgus, GA in 2012 and in 2013 to evaluate the efficacy of selected alternatives to phorate with cultural practices (row patterns and seeding rates) and with TSWV-resistant and/or susceptible (Georgia Green) cultivars.

Evaluation of selected alternative insecticides and tillage systems on thrips and spotted wilt incidence. Three alternative insecticides were selected for this trial. The insecticides, their mode of application, as well as their dosage/acre are included in Table 4.1. Two tillage conditions conventional tillage and strip tillage were evaluated. Georgia-06G was planted under both tillage conditions. A split plot design was adopted. Tillage systems and insecticides (treatments) were assigned as main plot effects and subplot effects, respectively. Land preparation was done approximately four months prior to the actual year of field trial following standard protocols (Marois and Wright 2003). Each plot was 9.14 m long and 5.49 m wide. There were six rows in each plot. Peanuts were planted between April 25 and April 27. Thrips samples were collected ~three weeks after planting for six consecutive weeks. In the first three weeks, quadrifoliate peanut terminals were collected. In the next three weeks, peanut blooms were collected. Ten terminals or blooms were randomly collected for each plot from the 2^{nd} and 5^{th} rows. The samples were placed in glass vials containing ~10 ml of 70% ethyl alcohol. The samples were brought to the vector biology laboratory in the University of Georgia at the Tifton Campus.

Samples were enumerated under a dissecting microscope (40x) (MEIJI TECHNO, Santa Clara, CA) and were identified to species using dichotomous keys (Triplehorn and Johnson 2005). Thrips feeding injuries were assessed using an arbitrary scale that measured from 0 to 10 (wherein 0 represented no feeding injuries and 10 represented a dead plant) (Lynch et al. 1984, Brandenburg et al. 1998). Feeding injuries were assessed on peanut plants from the 2nd and 5th rows of each plot at five weeks after planting

Spotted wilt incidence was rated visually using a standard procedure (Culbreath et al. 1997). Plants exhibiting spotted wilt symptoms on 3rd and 4th rows of each plot were identified and rated (Culbreath et al. 1997). In every plot, TSWV-infected plants in row cm were obtained using a 30.48-cm hit stick and converted to percentages. Plots were rated for spotted wilt ~two weeks prior to harvest. At harvest, peanut plants in the 3rd and 4th rows of each plot were dug, inverted, air-dried, picked, and weighed (in kg) following standard protocols (Baldwin et al. 1998).

Thrips injury rating and counts, spotted wilt incidence, and yield data were subjected to linear mixed models using PROC GLIMMIX in SAS (SAS Enterprise 4.2, SAS Institute, Cary, NC). Insecticides and tillage systems were considered fixed effects while replications were considered random effects. Interactions between insecticides and tillage systems, if any, were also analyzed. Tukey-Kramer Grouping, as an adjustment for multiple comparisons at P=0.05, was used to test the statistical significance of differences among treatments and between tillage systems.

Evaluation of selected alternative insecticides, row patterns, and cultivar effect on thrips and spotted wilt incidence. Three alternative insecticides were selected for this trial. The insecticides, their mode of application, as well as their dosage/acre are included in Table 4.1.

Two types of row patterns, single and twin, were evaluated on Georgia Green (TSWV-susceptible) and Georgia-06G (TSWV-resistant) peanut cultivars. The single row pattern consisted of two rows planted 91.44 cm apart on a 1.83 m wide bed. The twin row pattern also had a 1.83 m wide bed consisted of four rows in total. Measurement of the outside rows and inside rows was 91.44 cm and 45.72 cm, respectively. A split-split plot design adopted. Row patterns served as main plot effects while peanut cultivars and insecticides (treatments) were assigned as subplot and sub-sub plot effects, respectively. Thrips counts and feeding injuries, incidence, and yields were obtained as as previously described.

Thrips injury rating and counts, spotted wilt incidence, and yield data were subjected to linear mixed models using PROC GLIMMIX in SAS. Insecticides, peanut cultivars, and row patterns were considered fixed effects while replications were considered random effects. Two-way and three-way interactions between insecticides, peanut cultivars, and row patterns, if any, were also analyzed. Tukey-Kramer Grouping, as an adjustment for multiple comparisons at P=0.05, was used to test the statistical significance of differences among treatments, between cultivars, and between row patterns.

Evaluation of selected alternative insecticides and seeding rates on thrips and spotted wilt incidence. Two alternative insecticides were selected for this trial. The insecticides, their mode of application, as well as their dosage/acre are included in Table 4.1. Two seeding rates were evaluated on Georgia-06G peanut cultivar: 4 seed/ft and 6 seed/ft. A split plot design was adopted. Seeding rates and insecticides (treatments) were assigned as main plot effects and subplot effects, respectively. Thrips counts and feeding injuries, spotted wilt incidence, and yields were obtained as as previously described. Stand counts of peanut plants were also recorded within 3.05 m measurement from the two center rows (3rd and 4th rows).

Thrips injury rating and counts, spotted wilt incidence, yield, and stand counts were subjected to linear mixed models using PROC GLIMMIX in SAS. Insecticides and seeding rates were considered fixed effects while replications were considered random effects. Interactions between insecticides and seeding rates, if any, were also analyzed. Tukey-Kramer Grouping, as an adjustment for multiple comparisons at P=0.05, was used to test the statistical significance of differences among treatments and between seeding rates.

Results

Evaluation of selected alternative insecticides and tillage systems on thrips and spotted wilt incidence: Thrips on peanut terminal foliage and blooms were counted and identified. In 2012 and 2013, thrips were further classified as immatures, adult non-vector, or adult vector species. The percentages of immatures ranged from 44.69 ± 3.30 to 72.99 ± 0.96 (mean ± standard error). The percentages of vector adults ranged from 25.30 ± 1.03 to 51.57 ± 3.56. The non-vector adult percentages ranged from 1.56 ± 0.07 to 4.26 ± 0.26. Among vectors, *F. fusca* was the most predominant species. The percentage of *F. fusca* sampled ranged from 95.51 ± 0.28 to 99.28 ± 0.27. In general, irrespective of thrips species, fewer thrips were found in 2012 than in 2013 (Fig. 4.1). The treatment effects as well as tillage effects on thrips populations, thrips feeding injuries, spotted wilt incidence, and yield are described below.

Thrips counts: Irrespective of insecticide treatments, more thrips were found on plots with conventional tillage than on plots with strip tillage in 2012 (df=1,6; F=14.46; P=0.0089) and in 2013 (df=1,6; F=8.43; P=0.0272) (Fig. 4.1). Irrespective of tillage, thrips counts from untreated plots were different from thrips counts from treated plots in 2012 (df=5,210; F=3.13; P=0.0096) and in 2013 (df=5,210; F=6.86; P<0.0001). In both years, imidacloprid (in-furrow and at cracking) and spinetoram were as effective as phorate in suppressing thrips (Fig. 4.1). Thrips

sampled from imidacloprid (in-furrow), spinetoram, and phorate treated plots alone were less than thrips sampled from untreated plots. No interactions between treatment and tillage were found in 2012 (df=5,210; *F*=0.54; *P*=0.7466) and in 2013 (df=5,210; *F*=0.97; *P*=0.4378).

Thrips feeding damage: Intensity of thrips feeding damage was recorded in both years. Thrips feeding damage varied with insecticide treatments in 2012 (df=5,30; *F*=16.49; *P*<0.0001) as well as in 2013 (df=5,30; *F*=89.36; *P*<0.0001) (Fig. 4.2). In 2012, significantly more feeding damage was observed on plots with conventional tillage than on plots with strip tillage (df=1,6; *F*=31.68; *P*=0.0013) but not in 2013 (df=1,6; *F*=2.14; *P*=0.1940) (Fig. 4.2). Irrespective of tillage conditions, imidacloprid (at cracking) and spinetoram were as effective as phorate in reducing thrips feeding damage in 2012 (Fig. 4.2). However, feeding damage on imidacloprid (at cracking) treated plots was not different from imidacloprid (in-furrow) treated plots (Fig. 4.2). Similarly, in 2013, feeding damage on imidacloprid (in-furrow) treated plots and spinetoram treated plots was similar to phorate treated plots (Fig. 4.2). In addition, feeding damage on imidacloprid (at cracking) treated plots was less than the feeding damage on thiamethoxam treated plots and untreated plots. An interaction between treatments and tillage conditions was observed only in 2012 (df=5,30; *F*=8.61; *P*<0.0001) but not in 2013 (df=5,30; *F*=1.39; *P*=0.2561).

Spotted wilt incidence: Irrespective of insecticides and tillage conditions tested, spotted wilt incidence in 2013 was higher than in 2012. Spotted wilt incidence was higher in plots with conventional tillage than in strip tillage in 2012 (df=1,6; *F*=7.69; *P*=0.0323) and in 2013 (df=1,6; *F*=11.38; *P*=0.0150) (Fig. 4.3). Spotted wilt incidence was not affected by insecticide treatments in 2012 (df=5,30; *F*=1.02; *P*=0.4215). However, in 2013, spotted wilt incidences in imidacloprid (in-furrow) treated plots and phorate treated plots were lower when compared with other

insecticide treated plots and untreated plots (df=5,30; *F*=6.34; *P*=0.0004) (Fig. 4.3). No interaction between treatments and tillage conditions was observed in 2012 (df=5,30; *F*=1.02; *P*=0.4215) and in 2013 (df=5,30; *F*=1.64; *P*=0.1788).

Yields: Irrespective of the insecticide treatments, yields did not vary between plots with conventional tillage and plots with strip tillage in 2012 (df=1,6; *F*=0.00; *P*=0.9908) and in 2013 (df=1,6; *F*=2.46; *P*=0.1679). Yields were not influenced by insecticide treatments in 2012 (df=5,30; *F*=0.36; *P*=0.8691) and in 2013 (df=5,30; *F*=2.39; *P*=0.0610) irrespective of tillage conditions (Fig. 4.4). No interaction between treatments and tillage condition was observed in 2012 (df=5,30; *F*=0.97; *P*=0.4509) and in 2013 (df=5,30; *F*=0.35; *P*=0.8800).

Evaluation of selected alternative insecticides, row patterns, and cultivar effect on thrips and spotted wilt incidence: Thrips in peanut terminal foliage and blooms were counted and identified. In 2012 and 2013, thrips were classified as immatures, adult non-vector, or adult vector species. The percentages of immatures ranged from 26.54 ± 0.83 to 64.92 ± 3.03 (mean ± standard error). The percentages of vector adults ranged from 32.14 ± 2.53 to 71.05 ± 0.80. Non-vector adult percentages ranged from 1.51 ± 0.19 to 5.27 ± 0.56. Among vectors, *F. fusca* was the most predominant species. The percentages of *F. fusca* sampled ranged from 90.90 ± 1.09 to 99.07 ± 0.57. In general, irrespective of thrips species, fewer thrips were found in 2012 than in 2013 (Fig. 4.5). The treatment, row pattern, and cultivar effects on thrips populations, thrips feeding injuries, spotted wilt incidence, and yields are described below.

Thrips counts: Irrespective of insecticides treatments, more thrips per plant were found on plots with twin rows than on plots with single rows in 2013 (df=1,12; *F*=10.98; *P*=0.0062) but not in 2012 (df=1,12; *F*=0.12; *P*=0.7311) (Fig. 4.5). Thrips counts were not significantly different between peanut cultivars in 2012 (df=1,12; *F*=1.80; *P*=0.2044) and in 2013 (df=1,12; *F*=0.23;

P=0.6397). On the contrary, thrips counts were influenced by insecticide treatments in 2012 (df=4,348; *F*=30.65; *P*<0.0001) and in 2013 (df=4,348; *F*=10.59; *P*<0.0001). Fewer thrips were found on phorate, imidacloprid (at cracking), and spinetoram treated plots when compared with untreated plots (Fig. 4.5). In addition, thrips counts from thiamethoxam treated plots were not significantly different from thrips counts from spinetoram treated plots and untreated plots in 2012. In 2013, thrips counts for all insecticide treated plots were less when compared with thiamethoxam treated plots. Also, thrips counts from thiamethoxam treated plots were not significantly different from untreated plots (Fig. 4.5). Interactions between peanut cultivars and row patterns were observed in 2012 (df=1,12; *F*=12.11; *P*=0.0045) and in 2013 (df=1,12; *F*=6.55; *P*=0.0250). In both years, no interactions were observed between treatments and cultivars (df=4,348; *F*=1.04; *P*=0.3878 [2012] and df=4,348; *F*=0.66; *P*=0.6218 [2013]); treatments and row patterns (df=4,348; *F*=0.71; *P*=0.5833 [2012] and df=4,348; *F*=1.89; *P*=0.1120 [2013]); and, treatments, cultivars, and row patterns (df=4,348; *F*=1.97; *P*=0.0993 [2012] and df=4,348; *F*=0.45; *P*=0.7740 [2013]).

Thrips feeding damage: Intensity of thrips feeding damage was recorded in both years. Thrips feeding damage varied with insecticide treatments in 2012 (df=4,48; *F*=195.71; *P*<0.0001) as well as in 2013 (df=4,48; *F*=98.89; *P*<0.0001). Only in 2012, significantly more feeding damage was observed on Georgia Green plots than on Georgia-06G plots (df=1,12; *F*=5.39; *P*=0.0387) but not in 2013 (df=1,12; *F*=0.11; *P*=0.7452). More feeding damage was observed on plots with single rows than on plots with twin rows in 2013 (df=1,12; *F*=9.98; *P*=0.0082) but not in 2012 (df=1,12; *F*=0.02; *P*=8937) (Fig. 4.6). Irrespective of row patterns, imidacloprid (at cracking), spinetoram, and phorate were effective in reducing thrips feeding damage when compared with thiamethoxam and untreated plots in 2012 (Fig. 4.6). However, feeding damage on imidacloprid

(at cracking) and spinetoram treated plots were higher when compared with phorate treated plots (Fig. 4.6). Irrespective of peanut cultivars, feeding damage on imidacloprid (at cracking), spinetoram, and phorate treated plots were less when compared with thiamethoxam treated plots and untreated plots in 2012 and in 2013 (Fig. 4.6). To add, phorate, imidacloprid (at cracking), and spinetoram treated plots were significantly different to each other. There were interactions observed between treatments and row patterns in 2012 (df=4,48; *F*=6.16; *P*=0.0004) and in 2013 (df=4,48; *F*=4.78; *P*=0.0025). Interactions were observed between peanut cultivars and row patterns in 2012 (df=1,12; *F*=6.73; *P*=0.0235) but not in 2013 (df=1,12; *F*=0.03; *P*=0.8707). No interactions were observed between treatments and cultivars in 2012 (df=4,48; *F*=0.66; *P*=0.6248) and in 2013 (df=4,48; *F*=0.29; *P*=0.8800); and, between treatments, cultivars, and row patterns (df=4,48; *F*=1.08; *P*=0.3776 [2012] and df=4,48; *F*=0.40; *P*=0.8069 [2013]).

Spotted wilt incidence: Spotted wilt incidence was affected by insecticide treatments in 2012 (df=4,48; *F*=5.62; *P*=0.0009) but not in 2013 (df=4,48; *F*=1.38; *P*=0.2555). Irrespective of row patterns used, spotted wilt incidences in imidacloprid (at cracking) and spinetoram treated plots were similar to phorate treated plots (Fig. 4.7). However, spotted wilt incidences in phorate treated plots alone were less than spotted wilt incidences in untreated plots for spotted wilt incidence in 2012. In 2012, spotted wilt incidences were higher on Georgia Green plots when compared with Georgia-06G plots (df=1,12; *F*=13.66; *P*=0.0031). In contrast, in 2013, spotted wilt incidences on Georgia-06G plots were higher than on Georgia Green plots (df=1,12; *F*=5.04; *P*=0.0444). Spotted wilt incidences did not vary between plots with single rows and plots with twin rows in in 2012 (df=1,12; *F*=0.43; *P*=0.5248) and in 2013 (df=1,12; *F*=3.67; *P*=0.0797) (Fig.4.7). Interactions were observed between peanut cultivars and row patterns in 2012 (df=1,12; *F*=14.52; *P*=0.0025) but not in 2013 (df=1,12; *F*=0.67; *P*=0.4276). In both years,

no interactions were observed between treatments and cultivars (df=4,48; *F*=0.40; *P*=0.8100 [2012] and df=4,48; *F*=1.43; *P*=0.2380 [2013]); treatments and row patterns (df=4,48; *F*=0.96; *P*=0.4400 [2012] and df=4,48; *F*=0.15; *P*=0.9605 [2013]); and, treatments, cultivars, and row patterns (df=4,48; *F*=1.29; *P*=0.2880 [2012] and df=4,48; *F*=1.03; *P*=0.4039 [2013]).

Yields: Yields among insecticide treatments did not vary in 2012 (df=4,48; *F*=0.66; *P*=0.6214) and in 2013 (df=4,48; *F*=0.55; *P*=0.7034). Irrespective of the insecticide treatments, greater yields were obtained from Georgia-06G plots than from Georgia Green plots in 2012 (df=1,12; *F*=25.81; *P*=0.0003) but not in 2013 (df=1,12; *F*=2.45; *P*=0.1433) (Fig. 4.8). Yields were also influenced by row patterns in 2012 (df=1,12; *F*=7.02; *P*=0.0212) but not in 2013 (df=1,12; *F*=0.23; *P*=0.6408). Greater yields were obtained in plots with twin rows than from plots with single rows in 2012 (Fig. 4.8). No interactions between peanut cultivars and row patterns were observed in 2012 (df=1,12; *F*=2.58; *P*=0.1341) and in 2013 (df=1,12; *F*=0.38; *P*=0.5478). In both years, no interactions were observed between treatments and cultivars (df=4,48; *F*=1.73; *P*=0.1583 [2012] and df=4,48; *F*=0.69; *P*=0.6053 [2013]); treatments and row patterns (df=4,48; *F*=0.96; *P*=0.4387 [2012] and df=4,48; *F*=0.27; *P*=0.8938 [2013]); and, treatments, cultivars, and row patterns (df=4,48; *F*=1.47; *P*=0.2253 [2012] and df=4,48; *F*=0.42; *P*=0.7946 [2013]).

Evaluation of selected alternative insecticides and seeding rates on thrips and spotted wilt incidence: Thrips in peanut terminal foliage and blooms were counted and identified. In 2012 and 2013, thrips were further classified as immatures, adult non-vector, or adult vector species. The percentages of immatures ranged from 30.27 ± 0.81 to 65.52 ± 4.18 (mean ± standard error). The percentages of vector adults ranged from 32.06 ± 3.48 to 67.47 ± 0.97. The non-vector adult percentages ranged from 2.25 ± 0.15 to 3.82 ± 0.70. Among vectors, *F. fusca* was the most predominant species. The percentage of *F. fusca* sampled ranged from 94.37 ± 0.78 to 99.16 ±

0.35. In general, irrespective of thrips species, fewer thrips were found in 2012 than in 2013. The treatment effects on thrips populations, thrips feeding injuries, spotted wilt incidence, and yields are described below.

Thrips counts: Irrespective of insecticide treatments, thrips counts per plant did not vary between plots with 4 seed/ft and plots with 6 seed/ft in 2012 (df=1,6; F=3.23; P=0.1223) and in 2013 (df=1,6; F=4.04; P=0.0911) (Fig. 4.9). Thrips counts varied with insecticide treatments in 2012 (df=3,138; F=5.53; P=0.0013) and in 2013 (df=3,138; F=7.25 P=0.0001). Imidacloprid (at cracking) application was as effective as phorate application in suppressing thrips (Fig. 4.9). However, in 2013, thrips counts from thiamethoxam treated plots alone were significantly different than thrips counts from other treated plots and untreated plots (Fig. 4.9). No interaction between seeding rates and insecticide treatments was observed for thrips counts in 2012 (df=3,138; F=0.39 P=0.7617) as well as in 2013 (df=3,138; F=0.87 P=0.4575).

Thrips feeding damage: Intensity of thrips feeding damage was recorded in both years. Thrips feeding damage varied with insecticide treatments in 2012 (df=3,18; F=19.92; P<0.0001) as well as in 2013 (df=3,18; F=38.56; P<0.0001). Feeding damage was not influenced by seeding rates in 2012 (df=1,6; F=0.00; P=1.0000) and in 2013 (df=1,6; F=1.26; P=0.3037). Irrespective of seeding rates, only phorate was effective in reducing thrips feeding damage in 2012 (Fig. 4.10). In 2013, feeding damage on imidacloprid (at cracking) treated plots was significantly different from feeding damage on phorate treated plots. However, feeding damage on imidacloprid (at cracking) treated plots was less than the feeding damage observed on untreated plots (Fig. 4.10). No interactions between insecticide treatments and seeding rates were observed in 2012 (df=3,18; F=0.67; P=0.5819) and in 2013(df=3,18; F=0.87; P=0.4726).

Spotted wilt incidence: Higher spotted wilt incidence was observed in 2013 than in 2012 irrespective of insecticide treatments and seeding rates used (Fig. 4.11). Spotted wilt incidence was not affected by insecticide treatments in 2012 (df=3,18; F=0.97; P=0.4292) and in 2013 (df=3,18; F=0.10; P=0.9584). Spotted wilt incidence was also not affected by seeding rates in 2012 (df=1,6; F=0.73; P=0.4260) and in 2013 (df=1,6; F=0.56; P=0.4823) (Fig. 4.11). No interactions between treatments and seeding rates were observed in 2012 (df=3,18; F=0.63; P=0.6042) and in 2013 (df=3,18; F=1.62; P=0.2204).

Stand counts: Irrespective of insecticide treatments, increased peanut stand counts were found on plots with 6 seed/ft than on plots with 4 seed/ft in 2012 (df=1,6; F=1153.81; P<0.0001) and in 2013 (df=1,6; F=7.38; P=0.0348) (Fig. 4.12). In 2012, differences among treatments were found for stand counts of peanut plants (df=3,18; F=4.65; P=0.0142). In 2013, stand counts on thiamethoxam treated plots were better than stand counts on imidacloprid (at cracking) treated plots and phorate treated plots (df=3,18; F=3.98; P=0.0244) (Fig. 4.12). Whereas stand counts on thiamethoxam treated plots were not significantly different from stand counts on untreated plots. No interactions between treatments and seeding rates were observed in 2012 (df=3,88; F=2.18; P=0.1252) and in 2013 (df=3,18; F=1.34; P=0.2921).

Yields: Irrespective of seeding rates, yields were influenced by insecticide treatments in 2012 (df=3,18; F=3.88; P=0.0266) but not in 2013 (df=3,18; F=1.08; P=0.3832). Yields from imidacloprid (at cracking) treated plots were greater than yields from phorate treated plots in 2012 (Fig. 4.13). However, the yields from phorate treated plots were not different from thiamethoxam treated plots and untreated plots. Yields from thiamethoxam treated plots and untreated plots were not different from yields obtained from phorate treated plots. Yields were not influenced by seeding rates in 2012 (df=1,6; F=1.12; P=0.3308) and in 2013 (df=1,6;

F=0.23; *P*=0.6494). No interactions between insecticide treatments and seeding rates were observed in 2012 (df=3,18; *F*=1.42; *P*=0.2691) and in 2013 (df=3,18; *F*=0.50; *P*=0.6901).

Discussion

In this study the effects of alternatives to an organophosphate insecticide in conjunction with cultural practices were evaluated. The effects of these alternatives on thrips populations, thrips feeding damage, spotted wilt incidence, and yields were assessed in comparison with phorate. Two types of tillage conditions, row patterns, and seeding rates were tested with alternatives: conventional tillage and strip tillage; single rows and twin rows; and, 4 seed/ft and 6 seed ft, respectively. Composition of thrips species across cultural practices was consistent in 2012 and in 2013. Thrips counts from 2012 through 2013 indicated the presence of immatures and adult non-vectors and vectors. Of the adults sampled, only <6% were non-vectors. Adult thrips from the family Phlaeothripidae (suborder Tubulifera) and *F. tritici* (Fitch) were the most common non-vectors. This indicated that the remaining (94%) adults were vectors of TSWV. Within the vectors, more than 90% of the adults sampled were *F. fusca* and less than 10% were *F. occidentalis*. Therefore, it is likely that the majority of the immatures were *F. fusca* as well. These findings substantiate earlier reports, which indicated that *F. fusca* is the major vector of TSWV in peanut in Georgia (Todd et al. 1994a, 1995). This could be attributed to the ability of *F. fusca* to colonize and reproduce efficiently on peanut foliage early in the season than *F. occidentalis* (Todd et al. 1994a, 1995, 1996). *F. occidentalis* is considered to be most important vector in other cropping systems and other places; however, its role on peanut production seems to be minimal.

Several cultural practices along with peanut cultivars displaying field resistance to TSWV and insecticides have contributed to the reduction of thrips and TSWV (Gorbet and

Shokes 1994, Baldwin et al. 1998, 2001a, 2001b; Branch et al. 2003, Marois and Wright 2003, Tillman et al. 2006, Culbreath et al. 2008, 2013). Most of these cultural practices seem to have an impact on thrips populations as well as thrips feeding damage. In this study, plots with strip tillage conditions led to suppression of thrips populations as well as reduction of thrips feeding damage when compared with plots with conventional tillage in 2012 and in 2013. Similar to other studies conducted Florida and Alabama, plots under minimum or reduced tillage conditions had fewer thrips and reduced thrips feeding damage than plots under conventional tillage conditions (Minton et al. 1991, Brown et al. 1996, Baldwin and Hook 1998, Brandenburg et al. 1998, Baldwin et al. 2001a). A recent study by Tubbs et al. (2013) found a two-fold reduction of *F. fusca* under strip tillage conditions than under conventional tillage conditions. The observed differences might be due to plant debris present under conservation or strip tillage conditions. This in turn could interfere to the visual cues used by thrips in locating suitable host plants (Culbreath et al. 2003, Culbreath and Srinivasan 2011).

This study also compared two types of row patterns. More thrips were found on peanut plants with twin rows than on peanut plants with single rows in 2013 but not in 2012. On the contrary, more feeding damage was found on plots with single rows when compared with plots with twin rows in 2013 but not in 2012. Several studies have shown that fewer thrips were found on twin row plots than on single row plots (Baldwin et al. 1998, 2001a, 2001b; Lanier et al. 2004, Tillman et al. 2006, Culbreath et al. 2008, Tubbs and Beasley 2009, Tubbs et al. 2011). It is not clear as to what factors contribute to thrips suppression but it could be due to the visual interference of thrips and their ability to land on host plants. In addition, by using twin rows, the bare ground effects could also be reduced (Culbreath et al. 2003, Culbreath and Srinivasan 2011).

A number of studies found an inverse relationship between increase in seeding rate and reduction in spotted wilt incidence (Gorbet and Shokes 1994, Culbreath et al. 2013). This might be due to availability of bare ground area in plots with higher seeding rates. Bare ground area may be reduced and consequently reduce thrips landing on peanut plants (Culbreath et al. 2003, Culbreath and Srinivasan 2011). However, no differences in thrips counts and thrips feeding damage were observed between 4 seed/ft and 6 seed/ft.

Among the insecticides evaluated, imidacloprid (Admire® Pro) and spinetoram (Radiant®) were as effective as phorate (Thimet®) in reducing thrips populations as well as thrips feeding damage irrespective of cultural practices tested. In other studies, the application of imidacloprid, aldicarb or phorate to peanut plants led to a significant reduction of thrips immatures and/or adults in the field (Weeks and Hagan 1991, Todd et al. 1994a, 1996; Culbreath et al. 2008, Ames Herbert et al. 2007, Tubbs et al. 2013). The results of this study corroborate results from previous field studies when the use of insecticides such as imidacloprid and phorate led to lower thrips feeding damage in peanut plants (Todd et al. 1994b, 1995; Ames Herbert et al. 2007; Culbreath et al. 2008). In addition, the ability to use alternative insecticides as seed treatments, in-furrow treatments, and/or at-crack sprays would provide growers with more flexibility than using aldicarb and phorate. Older insecticides such as phorate and aldicarb do not provide this flexibility and are amenable only as in-furrow treatments.

In general, the use of insecticides in thrips management does not guarantee a direct reduction in TSWV transmission and/or spotted wilt incidence (Weeks and Hagan 1991, Todd et al. 1994b, 1995, 1996; Todd and Culbreath 1995, Marois and Wright 2003, Culbreath et al. 2008). The study conducted by Todd et al. (1994b) showed a significant reduction of thrips immatures and feeding activity when imidacloprid was used, but it led to increase in incidence of

spotted wilt in peanut. In some cases, in-furrow applications of phorate resulted in reduced spotted wilt incidence (Baldwin et al. 2001a; Culbreath et al. 2008). Although, the precise reasons for spotted wilt suppression in phorate treated plots are not clear, it might be due to the up regulation of defense pathways observed in phorate-treated plants than in untreated plants. In a study conducted by Gallo-Meagher et al. (2001), they were able to identify several genes that up and down regulated and they could have influenced TSWV replication in the host. However, the identified genes were not annotated functionally. In this study, lower percent of spotted wilt incidence was observed in plots with strip tillage than in plots with conventional tillage in 2012 and in 2013. Among the insecticides evaluated, imidacloprid (in-furrow) and spinetoram were as effective as phorate in reducing spotted wilt incidence in 2013 but not in 2012. As for field trials involving row patterns, spotted wilt incidences were higher on plots with single rows than on plots with twin rows in 2013 alone. In general, higher spotted wilt incidences were observed in Georgia Green plots when compared with Georgia-06G plots in 2012 and in 2013. This might be due to higher field resistance to TSWV in Georgia-06G than in Georgia Green. Georgia-06G is a second-generation TSWV-resistant cultivar and Georgia Green is a first generation TSWV resistant cultivar. Second generation TSWV-resistant cultivars exhibit a greater degree of field resistance than first-generation TSWV resistant cultivars. However, even the most recently released TSWV-resistant cultivars are not immune to the virus and produce symptoms suggestive of TSWV infection under moderate to high thrips and TSWV pressure (Culbreath et al. 2003, Culbreath and Srinivasan 2011, Shrestha et al. 2013, Sundaraj et al. 2014). Our study also showed that increased in seeding rates did not influence spotted wilt incidence in both years. In peanut production, the normal seeding rate in ‘90s was less than 4 seed/ft. Previous studies

indicated a significant reduction in spotted wilt incidence when seeding rates increased from 3 seed/ft to more than 4 seed/ft (Branch et al. 2003, Culbreath et al. 2013).

Among the cultural practices evaluated, yields from twin row plots were greater than yields from single row plots. However, the yields from conventional tillage plots were greater than in strip tillage plots. Effects of insecticides on yields were minimal, suggesting that alternatives were as effective as phorate in suppressing thrips populations and spotted wilt incidence. These results indicate that replacing phorate with alternatives while using cultural practices might not lead to an increase in thrips populations, spotted wilt incidence, and reduce yields. Additionally, they provide growers with more flexibility in using insecticides. For instance, the alternatives such as imidacloprid and thiamethoxam could be used as seed treatments, in-furrow applications, or as at-crack applications. At-crack applications could be applied in conjunction with fungicides. These alternative insecticides do not have the broad-spectrum toxicity and could induce reduced non-target effects. Therefore, growers could easily transition from using phorate to using alternatives without tremendously modifying other thrips and TSWV management practices.

Literature Cited

Ames Herbert, D., S. Malone, S. Aref, R. L. Brandenburc, D. L. Jordan, B. M. Royals, and P. D. Johnson. 2007. Role of insecticides in reducing thrips injury to plants and incidence of *Tomato spotted wilt virus* in Virginia market-type peanut. J. Econ. Entomol. 100: 1241-1247.

Baldwin, J. A., and J. Hook. 1998. Reduced tillage systems for peanut production in Georgia. Proc. Amer. Peanut Res. Ed. Soc. 30:48.

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

Baldwin, J. A., J. W. Todd, J. R. Weeks, D. W. Gorbet, A. K. Culbreath, A. S. Luke-Morgan, S. M. Fletcher, and S. L. Brown. 2001a. 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. Proc. Ann. Southern Cons. Till. Conf. Sust. Agric. 24: 26-34.

Baldwin, J. A., R. McDaniel, D. E. McGriff, and B. Tankersley. 2001b. Yield, grade and tomato spotted wilt incidence of Georgia Green and AT201 peanut when planted in twin vs. single row pattern. Abstr. Proc. Am. Peanut Res. Ed. Soc. 33: 31.

Beasley, J. P. 2013. Cultivar options in 2013. *In* J.P. Beasley (ed.), 2013 Peanut Production Update. The University of Georgia Cooperative Extension Service, Athens, GA.

Bertrand, P. F. 1998. Georgia plant disease loss estimates. Univ. Georgia Coop. Ext. Pub. Path: 98-107.

Black, M. C., P. F. Lummus, D. H. Smith, and J. W. Demski. 1986. An epidemic of spotted wilt disease in south Texas peanuts in 1985. Proc. Am. Peanut Res. Educ. Soc. 18: 66.

Branch, W. D., J. A. Baldwin, and A. K. Culbreath. 2003. Genotype x seeding rate interaction among TSWV-resistant, runner-type peanut cultivars. Peanut Sci. 30: 108-111.

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

Brown, S. L., A. K. Culbreath, J. W. Todd, D. W. Gorbert, J. A. Baldwin, and J. P. Beasley. 2005. Development of a method of risk assessment to facilitate integrated management of spotted wilt of peanut. Plant Dis. 89: 348-356.

Brown, S. L., J. W. Todd, and A. K. Culbreath. 1996. Effect of selected cultural practices on incidence of *Tomato spotted wilt virus* and populations of thrips vectors in peanuts. Acta Hortic. 431: 491-498.

Brown, S. L., J. W. Todd, A. K. Culbreath, H. Pappu, J. A. Baldwin, and J. P. Beasley. 1999. Tomato spotted wilt of peanut: identifying and avoiding high-risk situations. Univ. GA. Ext. Bull. No.1165.

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

Culbreath, A. K., J. W. Todd, J. W. Demski, and J. R. Chamberlin. 1992. Disease progress of spotted wilt in peanut cultivars florunner and southern runner. Phytopathol. 82: 766-771.

Culbreath, A. K., J. W. Todd, D. W. Gorbet, F. M. Shokes, and H. R. Pappu. 1997. Field response of new peanut cultivar UF 91108 to *Tomato spotted wilt virus*. Plant Dis. 81: 1410-1415.

Culbreath, A. K., J. W. Todd, and S. L. Brown. 2003. Epidemiology and management of tomato spotted wilt in peanut. Annu. Rev. Phytopathol. 41: 53-75.

Culbreath, A. K., B. L. Tillman, D. W. Gorbet, C. C. Holbrook, and C. Nischwitz. 2008. Response of new field-resistant peanut cultivars to twin-row pattern or in-furrow applications of phorate for management of spotted wilt. Plant Dis. 92: 1307-1312.

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

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

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

Gallo-Meagher, M., K. Chengalrayan, J. M. Davis, G. E. McDonald. 2001. Phorate induced genes that may condition acquired resistance to tomato spotted wilt (Abstr.). Proc. Am. Peanut Res. Ed. Soc. 33: 29.

German, T. L., D. E. Ullman, and J. W. Moyer. 1992. *Tospoviruses*: diagnosis, molecular biology, phylogeny, and vector relationships. Annu.Rev. Phytopathol. 30: 315-348.

Gorbet, D. W., and F. M. Shokes. 1994. Plant spacing and *Tomato spotted wilt virus*. Abstr. Proc. Am. Peanut Res. Ed. Soc. 26: 50.

Halliwell, R. S., and G. Philley. 1974. Spotted wilt of peanut in Texas. Plant Dis. Rep. 58: 23-25.

Hurt, C. A., R. L. Brandenburg, D. L. Jordan, B. M. Royals, and P. D. Johnson. 2006. Interactions of tillage with management practices designed to minimize tomato spotted wilt of peanut (*Arachis hypogaea* L.). Peanut Sci. 33: 83-89.

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

Lynch, R. E., J. W. Garner, and L. W. Morgan. 1984. Influence of systemic insecticides on thrips damage and yield of florunner peanuts in Georgia. J. Agric. Entomol. 1: 33-42.

Marois, J. J., and D. L. Wright. 2003. Effect of tillage system, phorate, and cultivar on tomato spotted wilt of peanut. Agron. J. 95: 386-389.

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

Mitchell, F. L. and J. W. Smith. 1991. Epidemiology of *Tomato spotted wilt virus* relative to thrips populations. ARS-US Depmnent of Agriculture, Agri. Research Service 87: 46-52.

Shrestha, A., R. Srinivasan, S. Sundaraj, A. K. Culbreath, and D. G. Riley. 2013. Second generation peanut genotypes resistant to thrips-transmitted *Tomato spotted wilt virus* exhibit tolerance rather than true resistance and differentially affect thrips fitness. J. Eco. Ent. 106: 587-596.

Sundaraj, S., R. Srinivasan, A. K. Culbreath, D. G. Riley, and H. R. Pappu. 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. Phytopathol. 104: 202-210.

Tillman, B. L., D. W. Gorbet, A. K. Culbreath, and J. W. Todd. 2006. Response of peanut cultivars to seeding density and row patterns. Crop Management, doi: 10.1094/CM-2006-0711-01-RS.

Todd, J.W. and A. K. Culbreath. 1995. Thrips populations and spotted wilt disease progress on resistance/susceptible cultivars treated with various insecticides. Proc. Am. Peanut Res. Ed. Soc. 27: 35.

Todd, J.W., J. R. Chamberlin, A. K. Culbreath, and J. W. Demski. 1994a. Timing and duration of vector management in relation to spotted wilt disease incidence in peanut. Proc. Am. Peanut Res. Ed. Soc. 25: 86.

Todd, J.W., J. R. Chamberlin, A. K. Culbreath, D. Rogers, and J. W. Demski. 1994b. Contradictions of insecticide use relative to vector control and spotted wilt disease progress in peanut. Proc. Am. Peanut Res. Ed. Soc. 26: 42.

Todd, J. W., A. K. Culbreath, J. R. Chamberlin, R. J. Beshear, and B. G. Mullinix. 1995. Colonization and population dynamics of thrips in peanuts in the southern United States, pp. 453-460. *In* B. Parker, M. Skinner and T. Lewis (eds.), Thrips Biol. Mgt. Plenum Press, New York.

Todd, J. W., A. K. Culbreath, and S. L. Brown. 1996. Dynamics of vector populations and progress of spotted wilt disease relative to insecticides use in peanuts. Acta Hortic. 431: 483-490.

Todd, J. W., A. K. Culbreath, H. R. Pappu, and S. L. Brown. 1997. Thrips as *Tospovirus* vectors in peanut. Proc. Am. Peanut Res. Ed. Soc. 29: 17.

Triplehorn, C. A., and N. F. Johnson. 2005. Borror and Delong's introduction to the study of insects, 7th ed. Brooks/Cole, Cengage Learning, U.S.A.

Tubbs, R. S., and J. P. Beasley. 2009. Tillage and row pattern update *In* 2009 Peanut Update. The University of Georgia, College of Agriculture and Environmental Science.

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

Tubbs, R., K. Balkcom, M. Toews, and R. Srinivasan. 2013. Effects of fertilization, tillage, and phorate on thrips and TSWV incidence in early planted peanuts (abstract). Southern Cons. Agric. Sys. Conf. http//:www.ars.usda.gov/research/publications/publications.htm

Ullman, D. E., J. J. Cho, R. F. L. Mau, W. B. Hunter, D. M. Westcot, D. M. Custer. 1992. Thrips-tomato spotted wilt virus interactions: morphological, behavioral and cellular components influencing thrips transmission. Adv. Dis. Vect. Res. 9: 195-240.

Weeks, J. R., and A. K. Hagan. 1991. The effects of planting date and insecticide treatments on thrips populations, *Tomato spotted wilt virus* and yield of peanut in Alabama. . Proc. Am. Peanut Res. Ed. Soc. 23: 41.

Whitfield, A. E., D. E. Ullman, and T. L. German. 2005. Tospovirus-thrips interactions. Annu. Rev. Phytopathol. 43: 459-C-5.

Table

Table 4.1. List of selected insecticides in conjunction with cultural practices for field trials.

Cultural Practice	Treatment No.	Chemical Name	Trade Name	Rate per Hectare (Ha)[x]	Price	Type of Application	Manufacturer
Tillage systems: conventional and strip	1	Thiamethoxam	Actara®	0.14 kg	$7.05/kg	At cracking	Syngenta
	2	Imidacloprid	Admire® Pro	0.51 l	$52.42/l	In-furrow	Bayer CropScience
	3	Imidacloprid	Admire® Pro	0.12 l	$52.42/l	At cracking	Bayer CropScience
	4	Spinetoram	Radiant® 1SC	0.37 l	$196.14/l	At cracking	Dow AgroSciences
	5	Phorate	Thimet® 10G	5.60 kg	$7.32/kg	In-furrow	Amvac
Row patterns: single and twin	1	Phorate	Thimet® 10G	5.60 kg	$7.32/kg	In-furrow	Amvac
	2	Imidacloprid	Admire® Pro	0.12 l	$52.42/l	At cracking	Bayer CropScience
	3	Thiamethoxam	Actara®	0.14 kg	$7.05/kg	At cracking	Syngenta
	4	Spinetoram	Radiant® 1SC	0.37 l	$196.14/l	At cracking	Dow AgroSciences
Seeding rates: 4seed/ft and 6seed/ft	1	Phorate	Thimet® 10G	5.60 kg	$7.32/kg	In-furrow	Amvac
	2	Imidacloprid	Admire® Pro	0.12 l	$52.42/l	At cracking	Bayer CropScience
	3	Thiamethoxam	Actara®	0.14 kg	$7.05/kg	At cracking	Syngenta

[x] Based on the manufacturer's recommended rates.

Figures

Fig. 4.1. Mean (±SE) cumulative counts of thrips across six weeks over four replications in two tillage systems. Treatment means represented by the same letter indicate that the treatments are not significantly different from each other. Sampling for thrips was done for six consecutive weeks at ~three weeks after planting. Quadrifoliate peanut terminal leaves were collected on the first three weeks while peanut blooms were collected on the last three weeks. Thrips samples were collected in 70% ethyl alcohol and identified to species under a dissecting microscope.

Fig. 4.2. Mean (±SE) thrips damage over four replications in two tillage systems. Treatment means represented by the same letter indicate that the treatments are not significantly different from each other.Thrips damage was assessed using an arbitrary scale of 0 to 10, wherein 0 represented no damage and 10 represented a dead plant. Peanut plants were evaluated from the 2^{nd} and 5^{th} rows of each plot. Assessment was done at five weeks after planting of peanuts.

Fig. 4.3. Mean (±SE) spotted wilt incidence (%) over four replications in two tillage systems. Treatment means represented by the same letter indicate that the treatments are not significantly different from each other. The assessment of spotted wilt incidence was obtained from two center rows (3^{rd} and 4^{th} rows) of each plot using a 30.48-cm hit stick based on standard protocols. Observations were done approximately two weeks prior to harvest.

Fig. 4.4. Mean (±SE) yield over four replications in two tillage systems. Treatment means represented by the same letter indicate that the treatments are not significantly different from each other. Peanut plants in the 3^{rd} and 4^{th} rows of each plot were dug, inverted, air-dried, picked, and weighed (in kg) using standard protocols.

Fig. 4.5. Mean (±SE) cumulative counts of thrips across six weeks over four replications in two row patterns and two peanut cultivars. Treatment means represented by the same letter indicate

that the treatments are not significantly different from each other.. Sampling for thrips was done for six consecutive weeks at ~three weeks after planting. Quadrifoliate peanut terminal leaves were collected on the first three weeks while peanut blooms were collected on the last three weeks. Thrips samples were collected in 70% ethyl alcohol and identified to species under a dissecting microscope.

Fig. 4.6. Mean (±SE) thrips damage over four replications in two row patterns and two peanut cultivars. Treatment means represented by the same letter indicate that the treatments are not significantly different from each other. Thrips damage was assessed using an arbitrary scale of 0 to 10, wherein 0 represented no damage and 10 represented a dead plant. Peanut plants were evaluated from the 2^{nd} and 5^{th} rows of each plot. Assessment was done at five weeks after planting of peanuts.

Fig. 4.7. Mean (±SE) spotted wilt incidence (%) over four replications in two row patterns and two peanut cultivars. Treatment means represented by the same letter indicate that the treatments are not significantly different from each other. The assessment of spotted wilt incidence was obtained from two center rows (3^{rd} and 4^{th} rows) of each plot using a 30.48-cm hit stick based on standard protocols. Observations were done approximately two weeks prior to harvest.

Fig. 4.8. Mean (±SE) yield over four replications in two row patterns and two peanut cultivars. Treatment means represented by the same letter indicate that the treatments are not significantly different from each other. Peanut plants in the 3^{rd} and 4^{th} rows of each plot were dug, inverted, air-dried, picked, and weighed (in kg) using standard protocols.

Fig. 4.9. Mean (±SE) cumulative counts of thrips across six weeks over four replications in two seeding rates. Treatment means represented by the same letter indicate that the treatments are not significantly different from each other. Sampling for thrips was done for six consecutive weeks

at ~three weeks after planting. Quadrifoliate peanut terminal leaves were collected on the first three weeks while peanut blooms were collected on the last three weeks. Thrips samples were collected in 70% ethyl alcohol and identified to species under a dissecting microscope.

Fig. 4.10. Mean (±SE) thrips damage over four replications in two seeding rates. Treatment means represented by the same letter indicate that the treatments are not significantly different from each other. Thrips damage was assessed using an arbitrary scale of 0 to 10, wherein 0 represented no damage and 10 represented a dead plant. Peanut plants were evaluated from the 2^{nd} and 5^{th} rows of each plot. Assessment was done at five weeks after planting of peanuts.

Fig. 4.11. Mean (±SE) spotted wilt incidence (%) over four replications in two seeding rates. Treatment means represented by the same letter indicate that the treatments are not significantly different from each other. The assessment of spotted wilt incidence was obtained from two center rows (3^{rd} and 4^{th} rows) of each plot using a 30.48-cm hit stick based on standard protocols. Observations were done approximately two weeks prior to harvest.

Fig. 4.12. Mean (±SE) stand counts over four replications in two seeding rates. Treatment means represented by the same letter indicate that the treatments are not significantly different from each other. Stand counts of peanut plants were recorded within 3.05 m measurement from the two center rows (3^{rd} and 4^{th} rows).

Fig. 4.13. Mean (±SE) yield over four replications in two seeding rates. Treatment means represented by the same letter indicate that the treatments are not significantly different from each other. Peanut plants in the 3^{rd} and 4^{th} rows of each plot were dug, inverted, air-dried, picked, and weighed (in kg) using standard protocols.

Fig. 4.1.

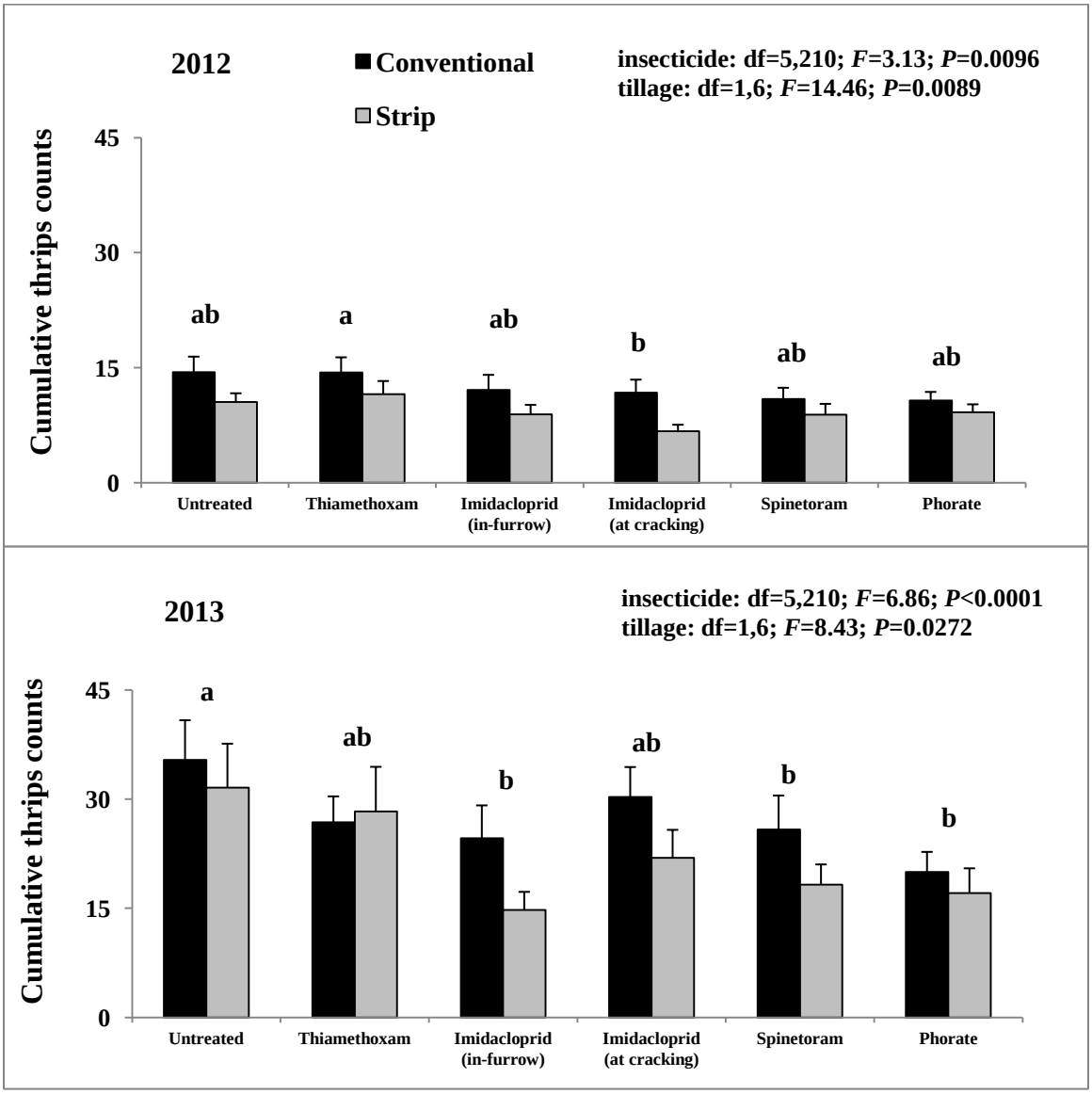

2012
Conventional
Strip
insecticide: df=5,210; F=3.13; P=0.0096
tillage: df=1,6; F=14.46; P=0.0089
Cumulative thrips counts
45
30
15
0
ab
a
ab
b
ab
ab
Untreated
Thiamethoxam
Imidacloprid (in-furrow)
Imidacloprid (at cracking)
Spinetoram
Phorate
2013
insecticide: df=5,210; F=6.86; P<0.0001
tillage: df=1,6; F=8.43; P=0.0272
Cumulative thrips counts
45
30
15
0
a
ab
b
ab
b
b
Untreated
Thiamethoxam
Imidacloprid (in-furrow)
Imidacloprid (at cracking)
Spinetoram
Phorate

Fig. 4.2.

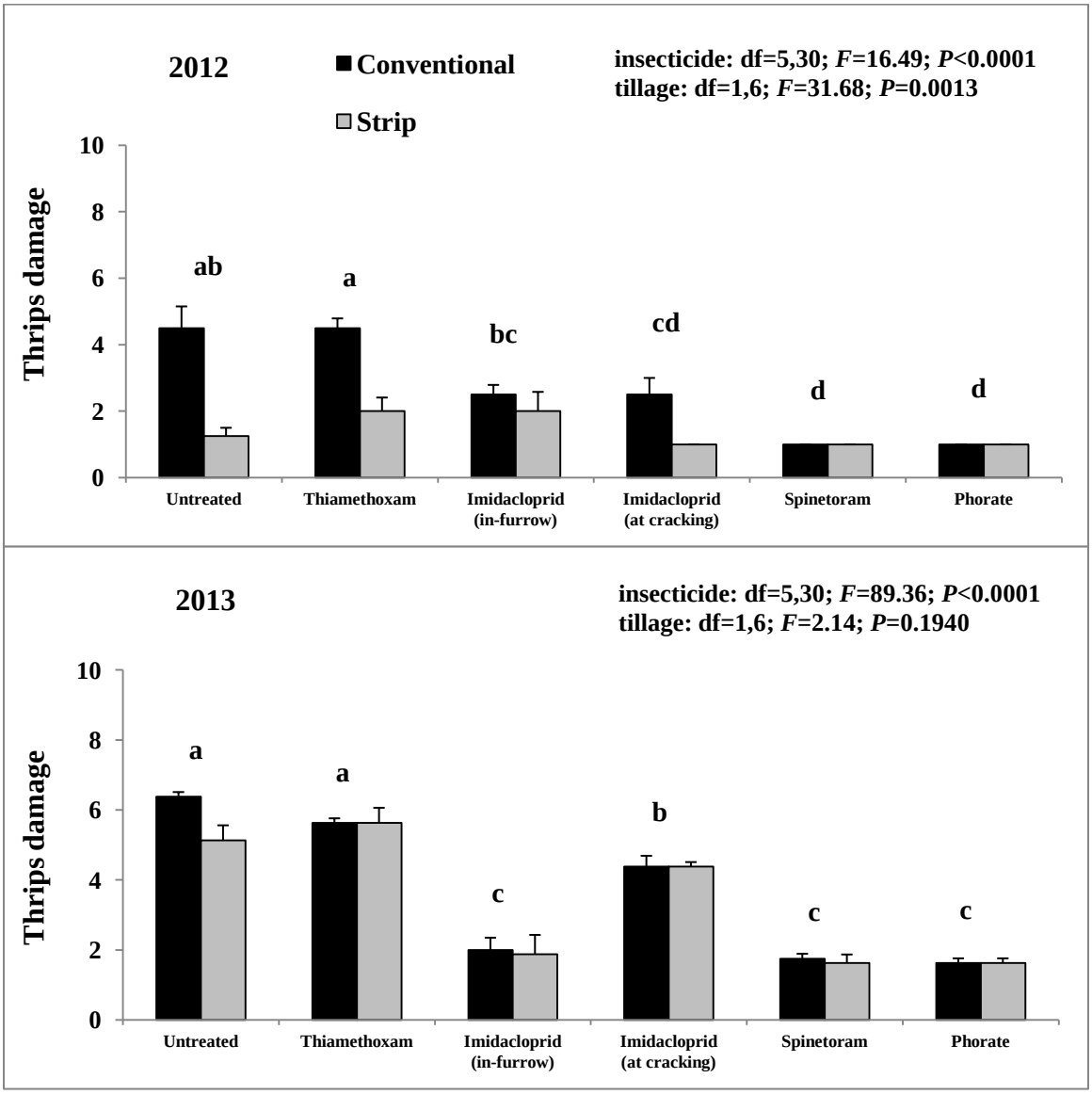

2012
Conventional
Strip
insecticide: df=5,30; F=16.49; P<0.0001
tillage: df=1,6; F=31.68; P=0.0013
Thrips damage
10
8
6
4
2
0
ab
a
bc
cd
d
d
Untreated
Thiamethoxam
Imidacloprid (in-furrow)
Imidacloprid (at cracking)
Spinetoram
Phorate
2013
insecticide: df=5,30; F=89.36; P<0.0001
tillage: df=1,6; F=2.14; P=0.1940
Thrips damage
10
8
6
4
2
0
a
a
c
b
c
c
Untreated
Thiamethoxam
Imidacloprid (in-furrow)
Imidacloprid (at cracking)
Spinetoram
Phorate

Fig. 4.3.

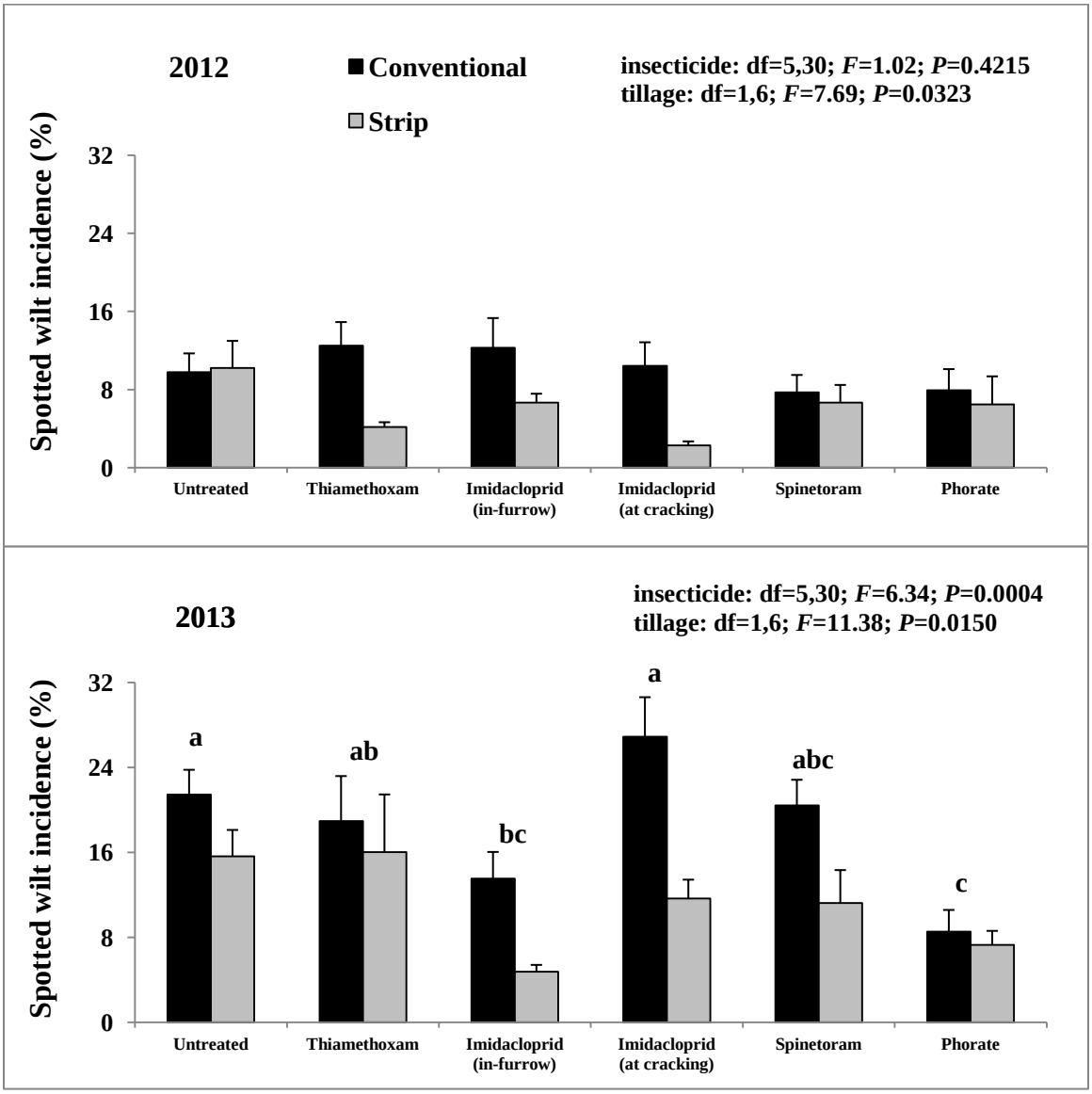

2012
Conventional
Strip
insecticide: df=5,30; F=1.02; P=0.4215
tillage: df=1,6; F=7.69; P=0.0323
Spotted wilt incidence (%)
32
24
16
8
0
Untreated
Thiamethoxam
Imidacloprid (in-furrow)
Imidacloprid (at cracking)
Spinetoram
Phorate
2013
insecticide: df=5,30; F=6.34; P=0.0004
tillage: df=1,6; F=11.38; P=0.0150
a
ab
bc
a
abc
c

Fig. 4.4.

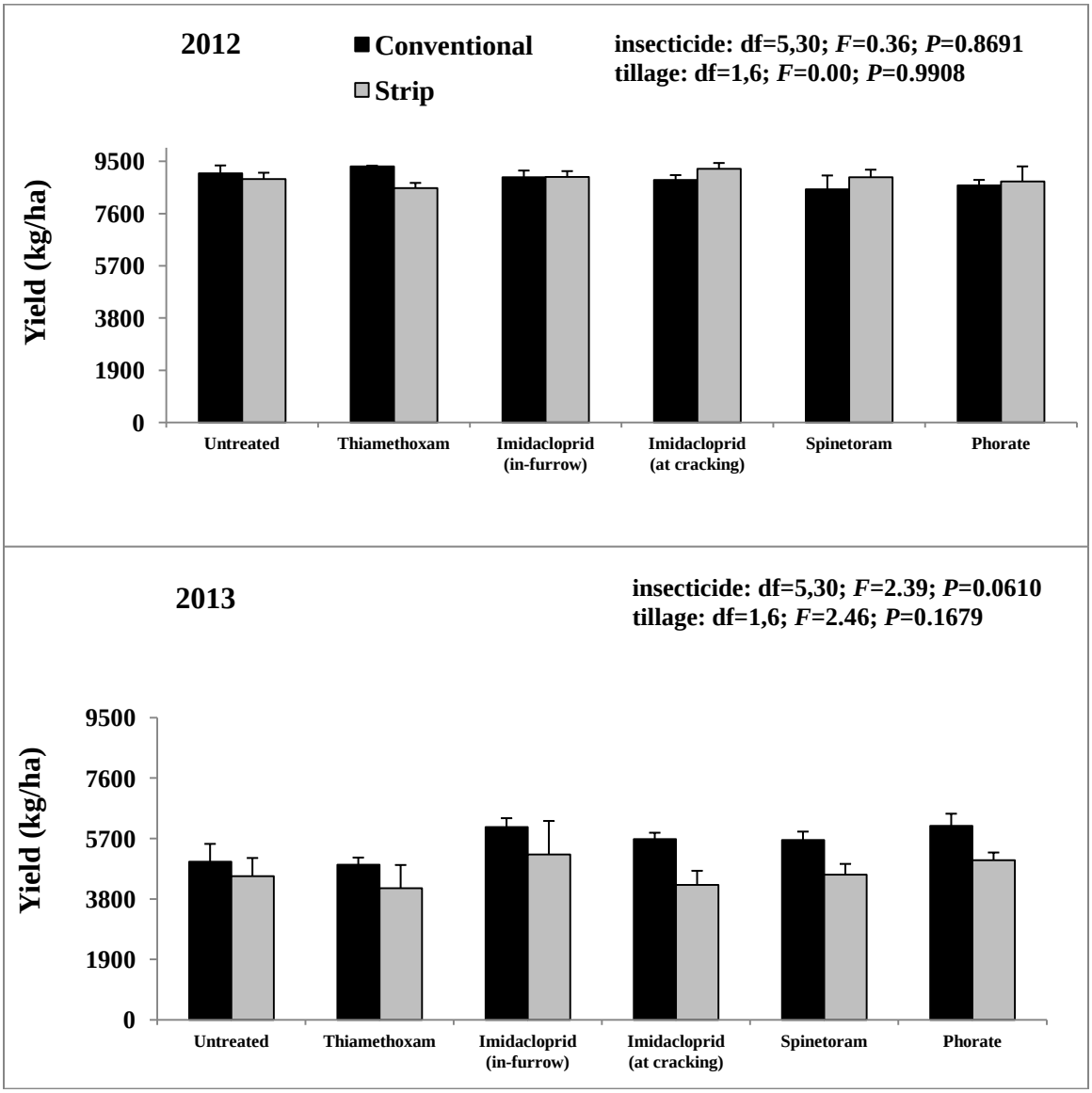

2012
Conventional
Strip
insecticide: df=5,30; F=0.36; P=0.8691
tillage: df=1,6; F=0.00; P=0.9908
Yield (kg/ha)
9500
7600
5700
3800
1900
0
Untreated
Thiamethoxam
Imidacloprid (in-furrow)
Imidacloprid (at cracking)
Spinetoram
Phorate
2013
insecticide: df=5,30; F=2.39; P=0.0610
tillage: df=1,6; F=2.46; P=0.1679
Yield (kg/ha)
9500
7600
5700
3800
1900
0
Untreated
Thiamethoxam
Imidacloprid (in-furrow)
Imidacloprid (at cracking)
Spinetoram
Phorate

Fig. 4.5.

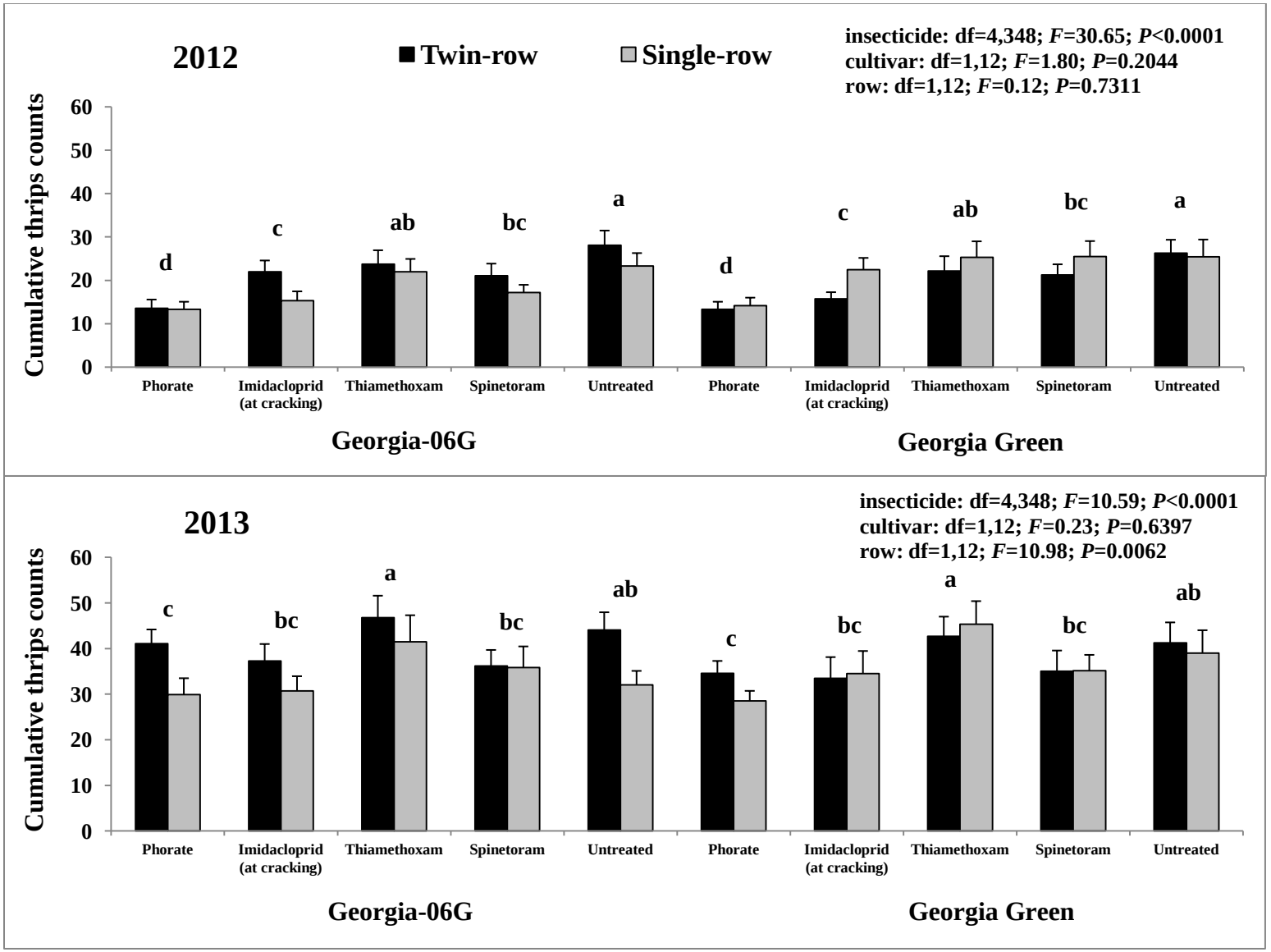

2012
Twin-row
Single-row
insecticide: df=4,348; F=30.65; P<0.0001
cultivar: df=1,12; F=1.80; P=0.2044
row: df=1,12; F=0.12; P=0.7311
Cumulative thrips counts
60
50
40
30
20
10
0
d
c
ab
bc
a
d
c
ab
bc
a
Phorate
Imidacloprid (at cracking)
Thiamethoxam
Spinetoram
Untreated
Phorate
Imidacloprid (at cracking)
Thiamethoxam
Spinetoram
Untreated
Georgia-06G
Georgia Green
2013
insecticide: df=4,348; F=10.59; P<0.0001
cultivar: df=1,12; F=0.23; P=0.6397
row: df=1,12; F=10.98; P=0.0062
Cumulative thrips counts
60
50
40
30
20
10
0
c
bc
a
bc
ab
c
bc
a
bc
ab
Phorate
Imidacloprid (at cracking)
Thiamethoxam
Spinetoram
Untreated
Phorate
Imidacloprid (at cracking)
Thiamethoxam
Spinetoram
Untreated
Georgia-06G
Georgia Green

Fig. 4.6.

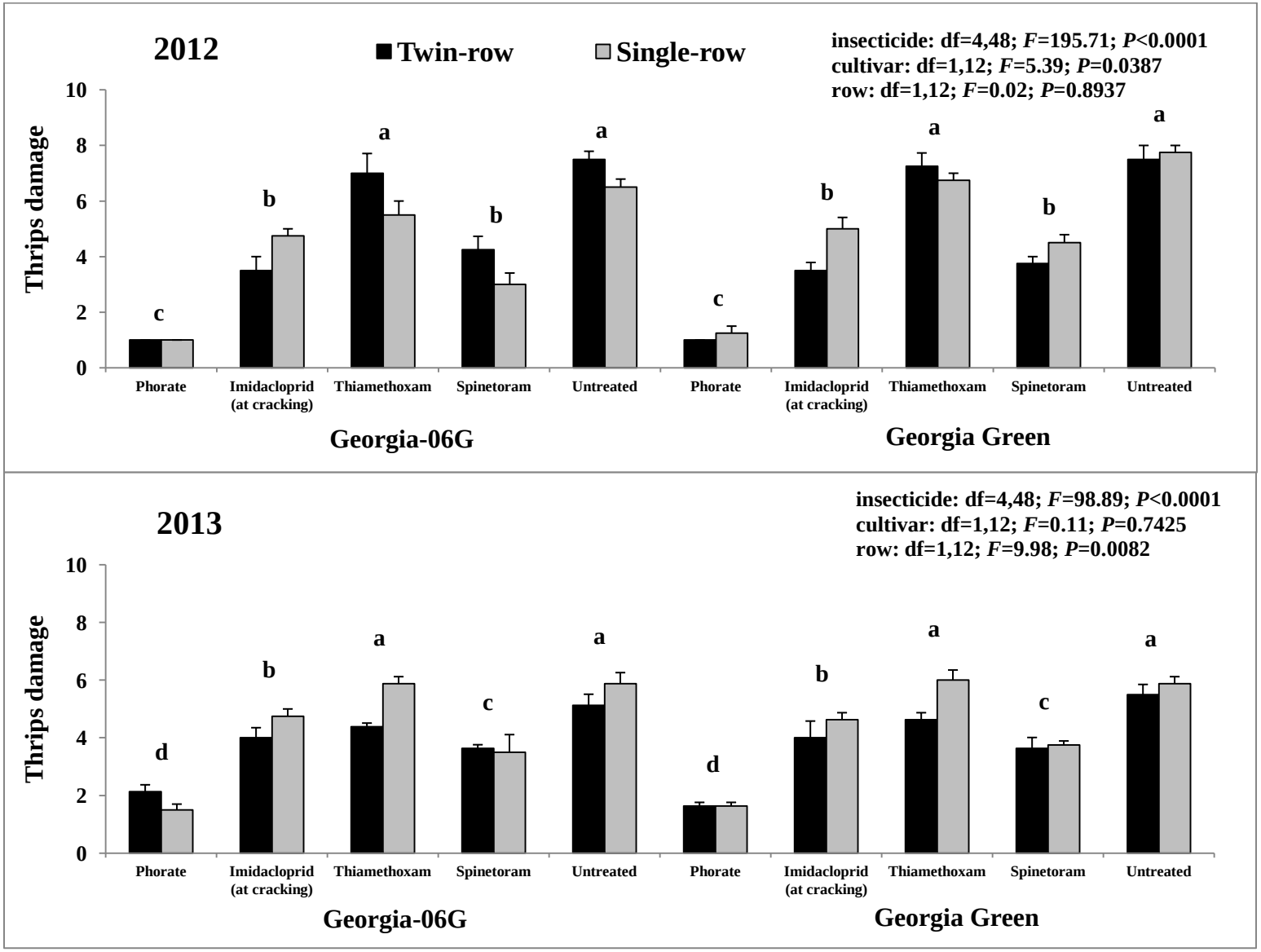

2012
Twin-row
Single-row
insecticide: df=4,48; F=195.71; P<0.0001
cultivar: df=1,12; F=5.39; P=0.0387
row: df=1,12; F=0.02; P=0.8937
Thrips damage
10
8
6
4
2
0
c
b
a
b
a
c
b
a
b
a
Phorate
Imidacloprid (at cracking)
Thiamethoxam
Spinetoram
Untreated
Phorate
Imidacloprid (at cracking)
Thiamethoxam
Spinetoram
Untreated
Georgia-06G
Georgia Green
2013
insecticide: df=4,48; F=98.89; P<0.0001
cultivar: df=1,12; F=0.11; P=0.7425
row: df=1,12; F=9.98; P=0.0082
Thrips damage
10
8
6
4
2
0
d
b
a
c
a
d
b
a
c
a
Phorate
Imidacloprid (at cracking)
Thiamethoxam
Spinetoram
Untreated
Phorate
Imidacloprid (at cracking)
Thiamethoxam
Spinetoram
Untreated
Georgia-06G
Georgia Green

Fig. 4.7.

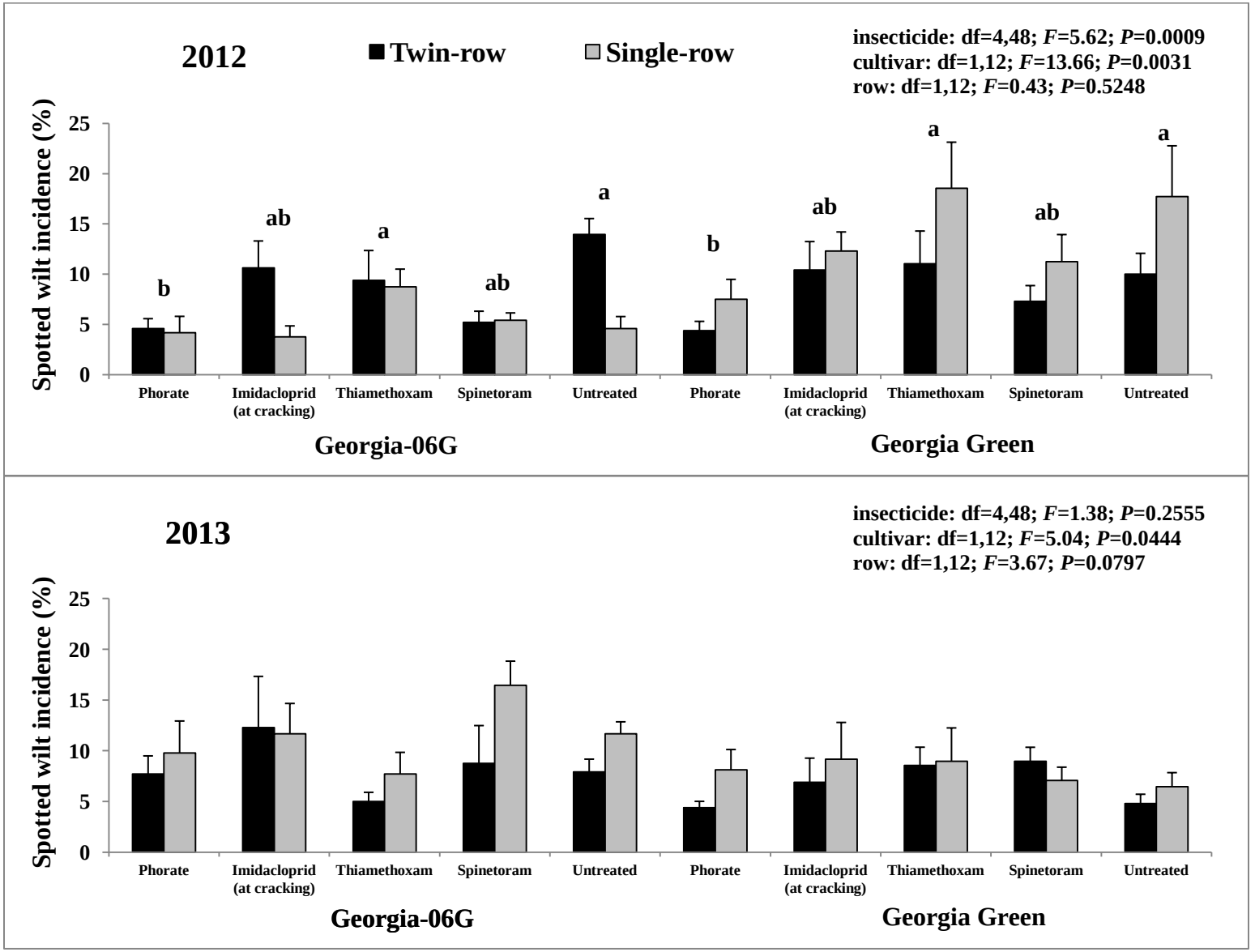

2012
Twin-row
Single-row
insecticide: df=4,48; F=5.62; P=0.0009
cultivar: df=1,12; F=13.66; P=0.0031
row: df=1,12; F=0.43; P=0.5248
Spotted wilt incidence (%)
0
5
10
15
20
25
b
ab
a
ab
a
b
ab
a
ab
a
Phorate
Imidacloprid (at cracking)
Thiamethoxam
Spinetoram
Untreated
Phorate
Imidacloprid (at cracking)
Thiamethoxam
Spinetoram
Untreated
Georgia-06G
Georgia Green
2013
insecticide: df=4,48; F=1.38; P=0.2555
cultivar: df=1,12; F=5.04; P=0.0444
row: df=1,12; F=3.67; P=0.0797
Spotted wilt incidence (%)
0
5
10
15
20
25
Phorate
Imidacloprid (at cracking)
Thiamethoxam
Spinetoram
Untreated
Phorate
Imidacloprid (at cracking)
Thiamethoxam
Spinetoram
Untreated
Georgia-06G
Georgia Green

Fig. 4.8.

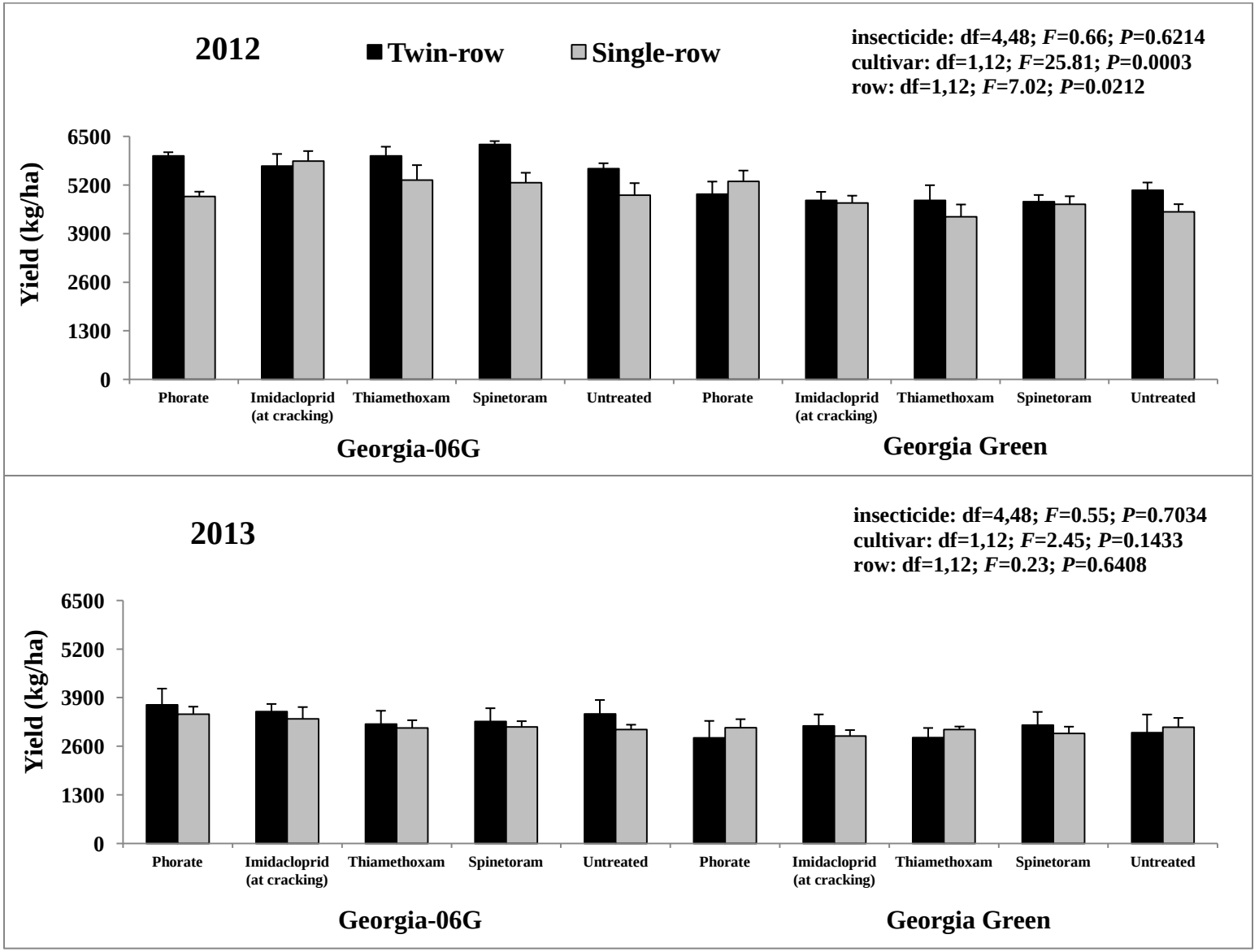

2012
Twin-row
Single-row
insecticide: df=4,48; F=0.66; P=0.6214
cultivar: df=1,12; F=25.81; P=0.0003
row: df=1,12; F=7.02; P=0.0212
Yield (kg/ha)
6500
5200
3900
2600
1300
0
Phorate
Imidacloprid (at cracking)
Thiamethoxam
Spinetoram
Untreated
Phorate
Imidacloprid (at cracking)
Thiamethoxam
Spinetoram
Untreated
Georgia-06G
Georgia Green
2013
insecticide: df=4,48; F=0.55; P=0.7034
cultivar: df=1,12; F=2.45; P=0.1433
row: df=1,12; F=0.23; P=0.6408
Yield (kg/ha)
6500
5200
3900
2600
1300
0
Phorate
Imidacloprid (at cracking)
Thiamethoxam
Spinetoram
Untreated
Phorate
Imidacloprid (at cracking)
Thiamethoxam
Spinetoram
Untreated
Georgia-06G
Georgia Green

Fig. 4.9.

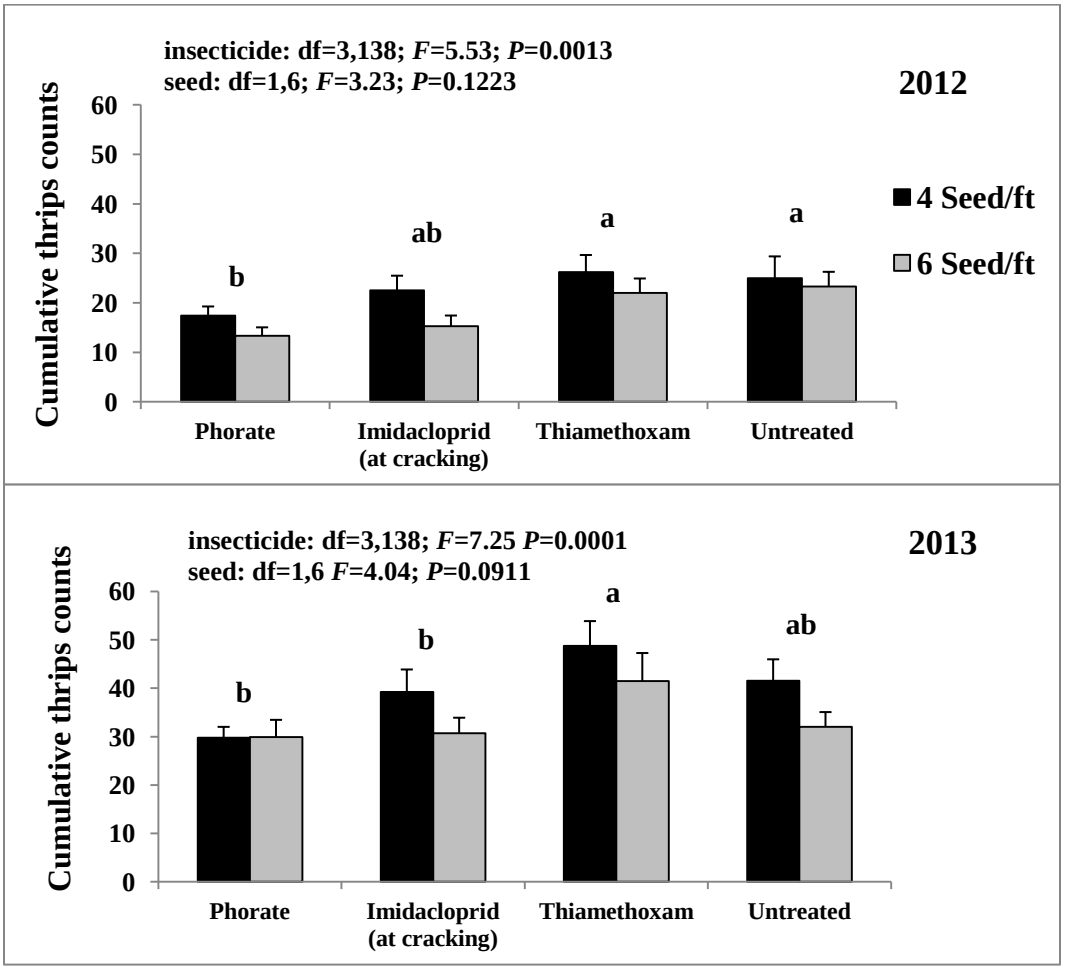

insecticide: df=3,138; F=5.53; P=0.0013
seed: df=1,6; F=3.23; P=0.1223
2012
Cumulative thrips counts
60
50
40
30
20
10
0
b
ab
a
a
4 Seed/ft
6 Seed/ft
Phorate
Imidacloprid (at cracking)
Thiamethoxam
Untreated
insecticide: df=3,138; F=7.25 P=0.0001
seed: df=1,6 F=4.04; P=0.0911
2013
Cumulative thrips counts
60
50
40
30
20
10
0
b
b
a
ab
Phorate
Imidacloprid (at cracking)
Thiamethoxam
Untreated

Fig. 4.10.

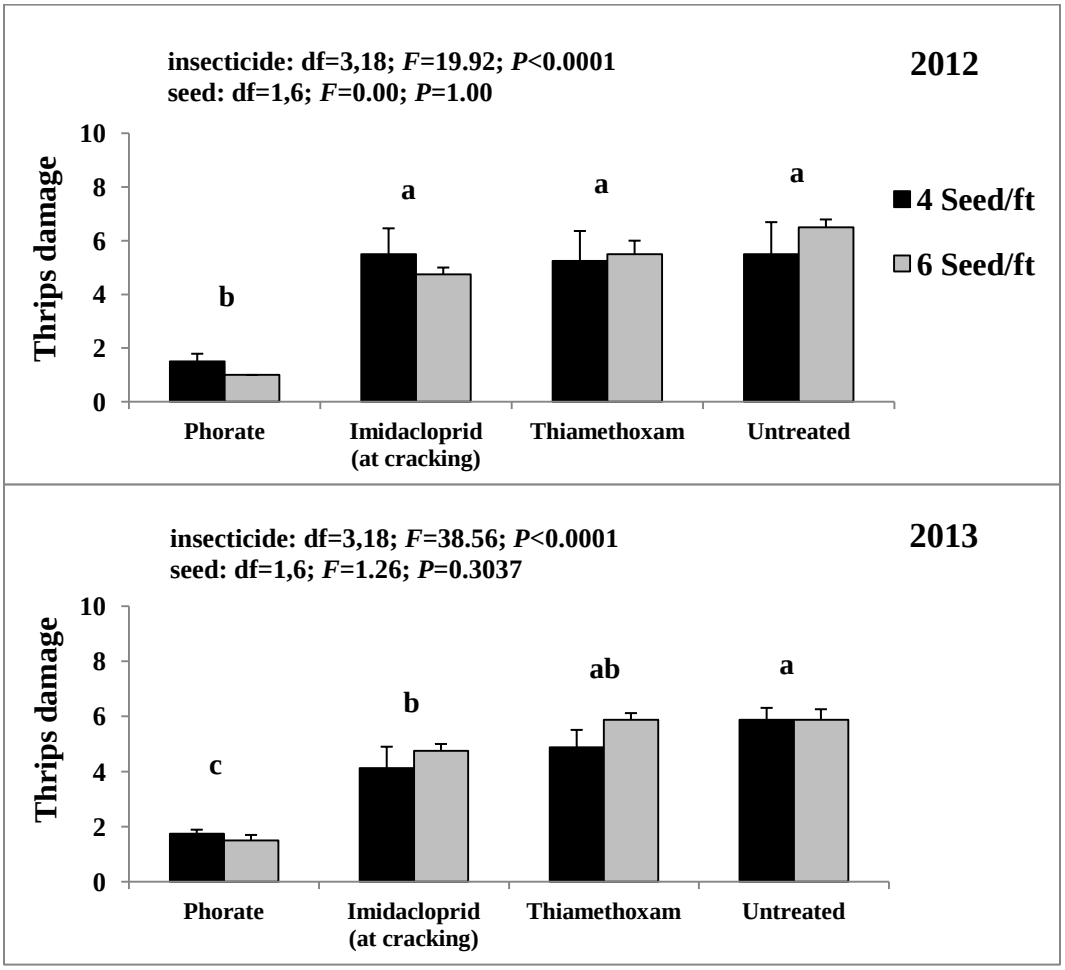

insecticide: df=3,18; F=19.92; P<0.0001
seed: df=1,6; F=0.00; P=1.00
2012
Thrips damage
10
8
6
4
2
0
b
a
a
a
4 Seed/ft
6 Seed/ft
Phorate
Imidacloprid (at cracking)
Thiamethoxam
Untreated
insecticide: df=3,18; F=38.56; P<0.0001
seed: df=1,6; F=1.26; P=0.3037
2013
Thrips damage
c
b
ab
a
Phorate
Imidacloprid (at cracking)
Thiamethoxam
Untreated

Fig. 4.11.

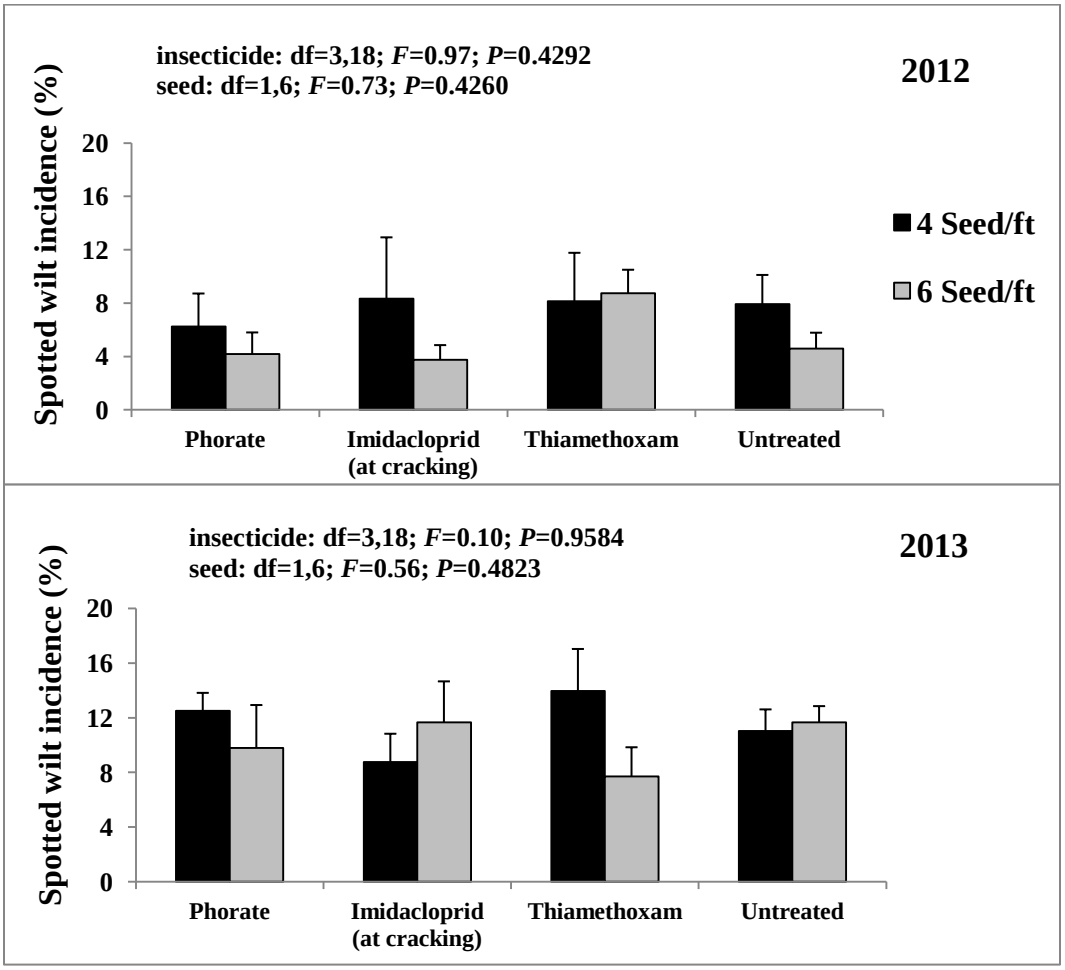

insecticide: df=3,18; F=0.97; P=0.4292
seed: df=1,6; F=0.73; P=0.4260
2012
Spotted wilt incidence (%)
20
16
12
8
4
0
4 Seed/ft
6 Seed/ft
Phorate
Imidacloprid (at cracking)
Thiamethoxam
Untreated
insecticide: df=3,18; F=0.10; P=0.9584
seed: df=1,6; F=0.56; P=0.4823
2013
Spotted wilt incidence (%)
20
16
12
8
4
0
Phorate
Imidacloprid (at cracking)
Thiamethoxam
Untreated

Fig. 4.12.

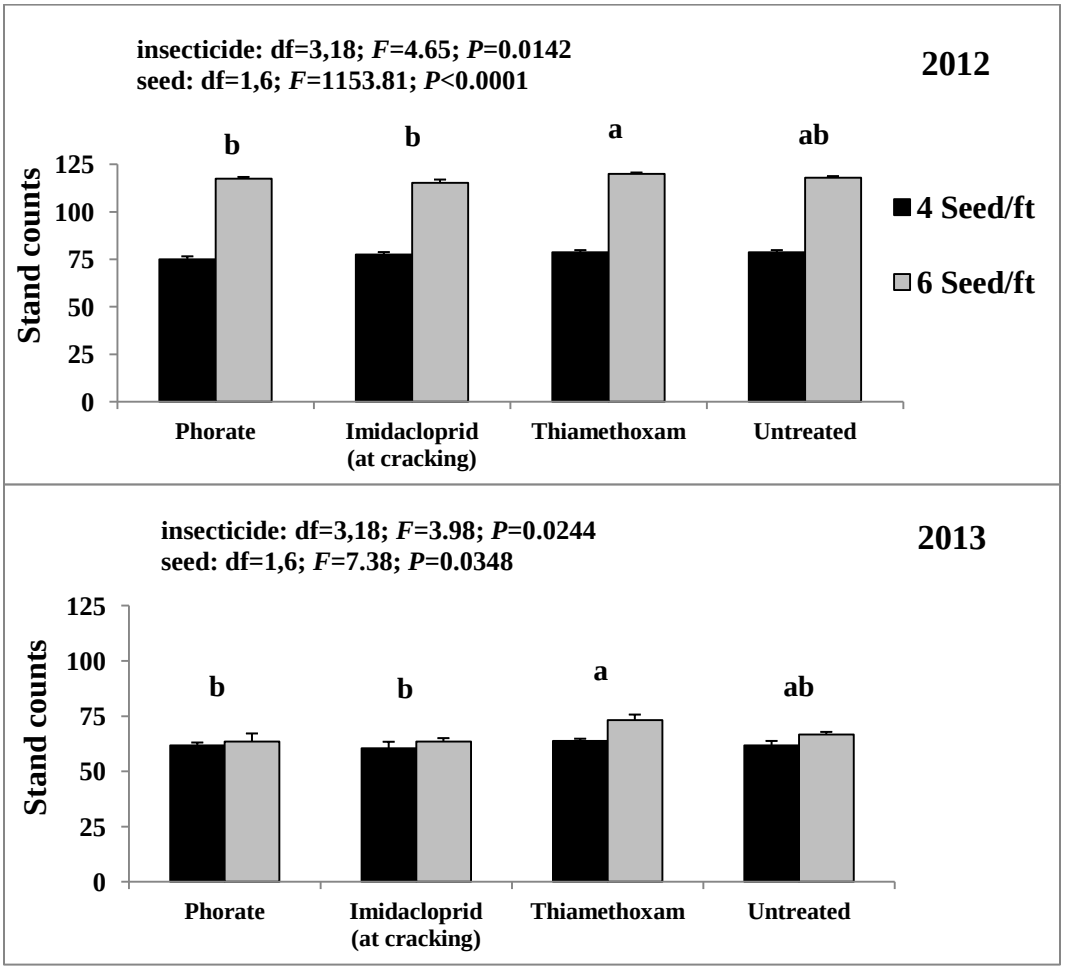
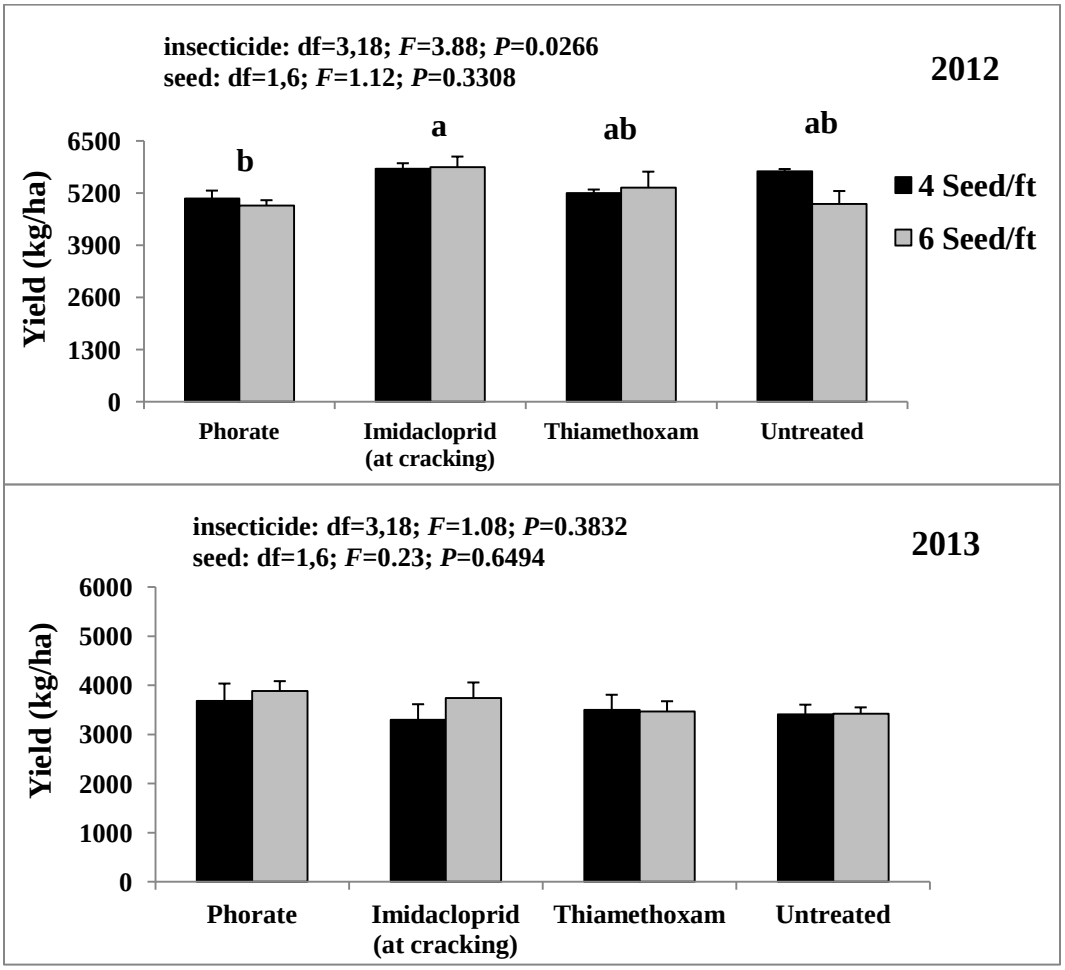

insecticide: df=3,18; F=4.65; P=0.0142
seed: df=1,6; F=1153.81; P<0.0001
2012
Stand counts
125
100
75
50
25
0
b
b
a
ab
4 Seed/ft
6 Seed/ft
Phorate
Imidacloprid (at cracking)
Thiamethoxam
Untreated
insecticide: df=3,18; F=3.98; P=0.0244
seed: df=1,6; F=7.38; P=0.0348
2013
Stand counts
125
100
75
50
25
0
b
b
a
ab
Phorate
Imidacloprid (at cracking)
Thiamethoxam
Untreated

Fig. 4.13.


insecticide: df=3,18; F=3.88; P=0.0266
seed: df=1,6; F=1.12; P=0.3308
2012
Yield (kg/ha)
6500
5200
3900
2600
1300
0
b
a
ab
ab
4 Seed/ft
6 Seed/ft
Phorate
Imidacloprid (at cracking)
Thiamethoxam
Untreated
insecticide: df=3,18; F=1.08; P=0.3832
seed: df=1,6; F=0.23; P=0.6494
2013
Yield (kg/ha)
6000
5000
4000
3000
2000
1000
0
Phorate
Imidacloprid (at cracking)
Thiamethoxam
Untreated

CHAPTER 5

EFFECTS OF PINE (*PINUS TAEDA* L.) POLLEN ON THRIPS, *FRANKLINIELLA FUSCA* (HINDS), HOST PREFERENCE, FEEDING BEHAVIOR, FITNESS, AND TRANSMISSION OF *TOMATO SPOTTED WILT VIRUS* WITH REFERENCE TO PEANUT (*ARACHIS HYPOGEAE* L.)[1]

[1]Marasigan, K., M. Toews, R. Kemerait, Jr., and R. Srinivasan. 2014.

Abstract

Tobacco thrips, *Frankliniella fusca* (Hinds), is a major pest of peanut, *Arachis hypogeae* L., in Southeastern United States. Besides causing direct feeding injuries, they also indirectly transmit *Tomato spotted wilt virus* (TSWV). In general, plant foliage is poor in protein content and thrips supplement their dietary requirements through feeding on protein rich sources such as pollen. Pollen from predominant plant hosts in the landscape such as pine (*Pinus* sp.) trees in the southeastern United States could serve as a good nutritional source. Prior studies have documented the effects of pine pollen on thrips biology. In this study we examined the impact of loblolly pine (*P. taeda* L.) pollen on thrips feeding behavior, host preference, thrips fitness, and transmission of TSWV using peanut as a host. Olfactometer assays indicated that addition of pine pollen positively influenced thrips host preference. Greenhouse assays revealed that topical addition of pollen grains on peanut foliage enhanced thrips feeding. Microcosm experiments conducted in the laboratory showed that the addition of pine pollen on peanut leaflets increased thrips oviposition but did not affect other thrips fitness parameters. Perhaps, the presence of substantial amounts of free amino acids could have positively influenced oviposition. Even though pollen addition resulted in increased thrips feeding, it did not translate to increased TSWV transmission by thrips. These results indicate that impact of pine pollen on a host such as peanut might only have a marginal effect on the fitness of polyphagous thrips species such as *F. fusca*.

Additional Key Words: Tobacco thrips, nutrition, dehiscence, free amino acids

Introduction

Thrips (Thysanoptera: Thripidae) are major pests of agricultural crops (Culbreath et al. 2003, Culbreath and Srinivasan 2011). Thrips typically feed on leaf tissue and pollen (Kirk 1984, 1985, 1997). The nutrient contents in vegetative plant parts such as leaves and stems are often limiting when compared with reproductive parts such as pollens in flowers (Stanley and Linskins 1974, Riley et al. 2011a). Thrips tends to compensate for the lack of nutrients in the foliage by feeding on nutrient-rich food sources such as pollen. Phenotypic homogeneity of crops is typical of agricultural cropping systems, which implies that the availability of nutrient-rich tissues such as pollen is influenced by time. Even during the pollen-free period, thrips tend to be successful in exploiting nutrient-limiting agricultural crop foliage by compensating their diet with nutrient-rich resources such as pollen from other crops simultaneously available in the landscape. For instance, tobacco thrips, *Frankliniella fusca* (Hinds) typically colonizes peanut and cotton seedlings soon after emergence in southeastern United States (Todd et al. 1994a, 1995, 1996). Thrips colonization on these seedlings is very closely correlated to pine (*Pinus* spp.) pollen dehiscence in the southeast (Riley and Pappu 2000, 2004; Riley et al. 2007, 2011a). Therefore, the availability of pine pollen in the landscape seems to aid *F. fusca* population buildup and utilization of nutrient-limiting food resources such as peanut and cotton foliage.

In general, pollen grains in general are good sources of nutrients. Based on the quantity of pollen dry weight, it is estimated that the nutritional value of protein, lipids, and carbohydrates measures approximately 12-61%, 1.5-18.9%, and 15%, respectively, depending on the plant species (Lundgren 2009). Pollen grains typically have substantial amounts of free amino acids. Among the amino acids present in pollen, proline is often the most abundant (3% of pollen dry weight) (Erhardt and Baker 1990, Lundgren 2009). Besides amino acids, other nutrients present

in pollen include simple sugars such as fructose, glucose, and sucrose; fatty acids, such as linoleic, stearic, palmitic, and palmitoleic acid; vitamins such as thiamine, riboflavin, niacin, biotin, folic acid, and minerals such as potassium, sulfur, phosphorus, boron, calcium, and manganese (Stanley and Linskins 1974, Lundgren 2009). Nutrients such as free amino acids and soluble carbohydrates, though limiting in plant tissues, are very essential for the growth and development of insects. Therefore, pollen feeding by thrips seems to play major part in their fitness.

Free amino acids in pollen play a major role in egg production and maturation of thrips (Wheeler 1996, Rojas et al. 1998, Klowden 2007, Lundgren 2009). Pine pollen feeding significantly improved egg production of western flower thrips, *Frankliniella occidentalis*, and tobacco thrips, *F. fusca* (Hulshof and and Vänninen 2001, Chitturi et al. 2006, Riley et al. 2007, 2011a; Angelella and Riley 2010). Hulshof and Vänninen (2001) also showed that the addition downy birch (*Betula pubescens* Ehrh.) pollen and scots pine (*P. sylvestris* L.) pollen to cucumber (*Cucumis sativus* L.), resulted in an increase in the fecundity (68-90%) of *F. occidentalis* when compared with cucumber leaf alone. Addition of pollen grains from tea (*Camellia sinensis* (L.) O. Kuntze), pear (*Pyrus* spp.), strawberry (*Fragaria* x *ananassa* Duchesne), tulip (*Tulipa* spp.), or pine (*Pinus thunbergii* Parl.) to an artificial diet increased the fecundity of two flower thrips species *F. intosa* (Trybom) and *Thrips hawaiiensis* (Morgan) (Murai and Ishii 1982). Besides influencing oviposition addition of pollen is also known to improve host utilization. For example, pollen was essential for *F. occidentalis* oviposition on peanut leaves (Riley et al. 2007). Similarly, *F. occidentalis* was able to reproduce on poinsettia (*Euphorbia pulcherrima* Wild ex. Klotzsch) foliage only in the presence of pine pollen (Hulshof and Vänninen 2001). Addition of

pollen to thrips diet also improved adult emergence rates of *F. intosa* by up to 85% when compared with the rearing on strawberry foliage or tomato fruit alone (Murai and Ishii 1982).

The effects of pine pollen on thrips are well documented. However, the effects on thrips host preference, settling, and feeding patterns are not known. The effects on thrips behavior and fitness could be critical from an agricultural perspective. In the process of feeding, thrips are also known to transmit viruses, such as tospoviruses (Sakimura 1962, 1963; Cho et al. 1987, Ullman et al. 1992, 1997). These viruses are of grave concern in agricultural crop production. Tospoviruses are exclusively transmitted by thrips in a persistent and propagative and stage-specific manner (German et al. 1992, Whitfield et al. 2005, Pappu et al. 2009, Riley et al. 2011b). Only early instar larvae can acquire the virus and later instar larvae and adults transmit the virus (German et al. 1992, Whitfield et al. 2005). Enhanced oviposition, better host utilization, and increased adult emergence, facilitated by the addition of pollen to thrips diet, could also increase the opportunities for virus acquisition and inoculation, and ultimately influence viral epidemics.

In this study, we attempt to address a number of questions on the effects of pollen addition on thrips fitness as well as its behavior using *F. fusca*. Besides assessing the effects of pollen on *F. fusca* fitness, the influence of pollen on thrips host settling and feeding was evaluated using peanut as the host plant. Additionally, transmission assays were conducted using *F. fusca* and *Tomato spotted wilt virus* (TSWV) in the presence and absence of pollen on peanut. Our hypothesis was that besides improving thrips fitness, pollen addition would also improve thrips settling and feeding on peanut foliage and ultimately enhance *F. fusca*'s ability to transmit TSWV.

Materials and Methods

Non-infected peanut plants. Georgia-06G peanut seeds were pre-germinated on moistened paper towels and incubated in a growth chamber with a temperature of 25 to 27^{o}C for one week. Germinated peanut seeds were transplanted into 10.16-cm diameter plastic pots (Hummert International, St. Louis, MO) containing commercial potting mix (LT5 Sunshine mix, Sun Gro Horticulture Industries, Bellevue, WA). Peanut plants were placed in 47.5-cm^{3} insect proof cages (Megaview Science Co., Taichung, Taiwan) and maintained in a greenhouse at 25 to 30^{o}C with 80 to 90% relative humidity (RH) and 14:10 (L:D) h photoperiod.

Collection of pine pollen grains. Pine (*Pinus taeda* L.) needles with pollen grains were placed inside a brown paper bag collected in February 2012 at Tifton, GA. The brown paper bag was shook vigorously followed by the removal of pine needles. Pine pollen grains were collected in a glass vial using a paintbrush (2 Silver 5300 S Round, India) after which, pine pollen grains were stored at 4^{o}C.

Maintenance of non-viruliferous *F. fusca*. In 2012, a colony of *F. fusca* was established by collecting *F. fusca* from peanut blooms from Belflower Farm, Coastal Plain Experimental Station, Tifton, GA. Thrips were transferred and maintained in Munger cages (11.43 x 8.89 x 1.77 cm^{3}) (Munger 1942) containing healthy non-infected peanut leaflets dusted with pine (*Pinus taeda* L.) pollen grains. The Munger cages were placed in a growth chamber (Thermo scientific, Dubuque, IA) at 25 to 27^{o}C with 14:10 (L:D) h photoperiod. New foliage was added to the Munger cages every two to three days.

Maintenance of potentially viruliferous *F. fusca.* A colony of potentially viruliferous thrips was initiated and maintained on TSWV-infected peanut foliage in Munger cages as described for non-viruliferous thrips. TSWV-infected peanut foliage was initially obtained from the Belflower

Farm, Coastal Plain Experimental Station, Tifton, GA. The cages were routinely replaced with infected foliage obtained either from the Belflower farm or from the greenhouse. Infection status of peanut foliage was confirmed by double antibody sandwich enzyme linked immunosorbent assay (DAS-ELISA). Thrips reared for an entire generation (adult to adult) on TSWV-infected leaflets were alone considered potentially viruliferous.

***F. fusca* settling with and without pine pollen grains.** Two one week-old Georgia-06G peanut plants were used for each set-up. Two peanut leaflets from separate plants were inserted into the two arms of a Y-shaped glass tube olfactometer (Analytical Research systems, Gainesville, FL). Pine pollen grains weighing 0.02 g were added to one leaf and then one leaflet was introduced into one arm of the olfactometer. Both leaflets were held in position using Parafilm® (American National Can Company, Greenwich, CT). A tripod (Fisher Scientific, Pittsburgh, PA) was used as a support in holding the Y-tube in horizontal position (90 degree between Y-tube and tripod) (Kogel et al. 1999). Twenty five non-viruliferous *F. fusca* (up to two days old) were collected in a 0.6 ml microcentrifuge tubes and released at the base of the tube (N=25 non-viruliferous thrips for each set-up). Thrips were given 24 h to settle on either of the leaflets and after which, the number of thrips settled on each side was recorded. Percent feeding damage was also recorded for comparison between the treatments. The whole experiment was repeated 24 times. Prior to each new set-up, the Y-tube was cleaned with acetone (J.T. Baker, Mallinckrodt Baker, Inc., Phillipsburg, NJ) and allowed to air dry in a fumehood. The Y-tube was rotated 90 degrees to eliminate directional effects after each new set-up. The position of plants was also exchanged to avoid positional effects. New set of plants was used for each set-up.

Statistical analysis was conducted to compare the settling preference of non-viruliferous thrips on Georgia-06G leaflets with and without pine pollen grains. Treatments were considered

as fixed effects while replications were considered as random effects. PROC GLIMMIX in SAS (SAS Enterprise 4.2, SAS Institute, Cary, NC) was used for the analysis. Least square means at *P*=0.05 was used to compare the statistical significance of differences between treatments.

***F. fusca* feeding damage on peanut plants with and without pine pollen grains.** Peanut plants with and without pine pollen grains were evaluated for thrips feeding. Ten one-week old peanut plants for each treatment were placed separately in 47.5-cm^3 insect proof cage (Megaview Science Co., Taichung, Taiwan). Approximately 0.05g of pine pollen grains were dusted on each treated plant. Ten non-viruliferous female adult *F. fusca* (up to two days old) were collected in 0.6 ml microcentrifuge tubes using a paintbrush (2 Silver 5300 S Round, India) and then released at the base of each plant. Thrips feeding damage was assessed on each plant every two days for a month after initial thrips release. The feeding damage index (FDI) was calculated based on the formula deduced by Maris et al. (2003) with minor adjustments.

$$\text{FDI} = \frac{\text{No.of leaflets with feeding damage}}{\text{Total no.of leaflets in a plant}} \text{ x Intensity of feeding scar}$$

The intensity of feeding scars was based on an arbitrary scale (0.5 = 0-10%, 1 = 10-20%, 1.5 = 20-30% 2 = 30-40%, 2.5 = 40-50%, 3 = 50-60%, 3.5 = 60-70%, 4 = 70-80%, 4.5 = 80-90% and 5 = 90-100%). The experiment was repeated twice (N=30 plants for each treatment).

A completely randomized design (CRD) was used as a treatment structure. Treatments were considered fixed effects while replications were considered random effects. Statistical differences in feeding damage at three time intervals, 10, 20, and 30 days after thrips release were assessed. PROC GLIMMIX in SAS was used for the analysis. The same plant population was observed for the entire sampling period and the observations were considered as repeated measures. Least square means at *P*=0.05 was used to compare the statistical significance of differences between treatments.

***F. fusca* oviposition with and without pine pollen grains.** Peanut leaflets with and without pine pollen grains were evaluated for oviposition efficiency. Two leaflets of same size from two to three week-old plants were placed in each Munger cage. Each cage constituted a replication and ten cages for each treatment were used in the experiment. The whole experiment was repeated once (N=20 cages for each treatment). For the treatment that requires pollen grains, approximately 0.02 g pine pollen grains were dusted on two leaflets in each Munger cage. Ten non-viruliferous female adult *F. fusca* (up to two days old) were released on each cage and then removed after three days. Munger cages were maintained in a growth chamber as previously described. The leaflets were then stained. Leaflets were boiled for 20 to 40 min at 60^{o}C in a 1:1:2 (by volume) solution of glacial acetic acid (Fisher Scientific, Fair Lawn, NJ), 10% lactic acid aqueous solution (Ricca Chemical Company, Arlington, TX), and 95% ethanol (Decon Laboratories Inc., Baltimore, MD) until they turned pale. A thermometer was used to continuously monitor the temperature of the solution. Leaflets were allowed to cool. Acid-fuchsin solution was used to stain the eggs in each leaflet for two to four minutes. Fixed leaflets were placed in a lacto phenol acid solution consisting of 1:2:1:1:1 solution of 10% lactic acid, 50% glycerin aqueous solution (Ricca Chemical Company, Arlington, TX), distilled water, saturated phenol buffered at pH 4.3 (Fisher Scientific, Fair Lawn, NJ), and 1 g/l of acid fuchsin high purity biological stain (Acros Organics, Morris Plains, NJ). Warm water was used to remove excess stain on the leaflets. Leaflets were laid on a paper towel to absorb excess liquid. Eggs were counted afterwards under the dissecting microscope (MEIJI TECHNO, Santa Clara, CA).

Statistical analysis was conducted to compare the number of eggs oviposited by thrips in peanut leaflets with and without pine pollen grains. Treatments were considered as fixed effects

while replications were considered as random effects. PROC GLIMMIX in SAS was used for the analysis. Least square means at *P*=0.05 was used to compare the statistical significance of differences between treatments.

***F. fusca* development with and without pine pollen grains.** Peanut leaflets with and without pine pollen grains were evaluated for thrips fitness. Two leaflets of same size from two to three week-old plants were placed in each Munger cage. Each cage constituted a replication and ten cages for each treatment were used in the experiment. The whole experiment was repeated once (N=20 cages for each treatment). For the treatment that requires pollen grains, approximately 0.02 g pine pollen grains were dusted on two leaflets in each Munger cage. Ten non-viruliferous female adult *F. fusca* (up to two days old) were released in each cage and then removed after five days. Munger cages were maintained in growth chamber as previously described. The cages were observed at 24 h interval under a compound microscope (MEIJI TECHNO, Santa Clara, CA). Number of newly emerged adults were recorded and removed daily. The set-up was maintained until all larvae turned into adult for each cage.

Statistical analysis was conducted to compare the differences in the number of adults produced by 10 initial adult thrips for one complete generation (adult to adult). PROC GLIMMIX in SAS was used for the analysis. Least square means at *P*=0.05 was used to compare the statistical significance of differences between treatments. The developmental time required for the first adult emergence for each treatment was also analyzed using PROC NPAR1WAY in SAS. Wilcoxon Rank Sum test, with a continuity correction factor of 0.5, was used to compare statistical significance of differences between treatments.

Free amino acid concentrations peanut leaf tissue with and without pine pollen grains. Five two to three week old non-infected peanut plants were used for each treatment. Leaf tissue

(~0.15 g) was collected from top one-third portion of each plant and subjected to free amino acid analysis (Hacham et al. 2002). Approximately 0.05 g pine pollen grains were added to each treated leaf tissue. First, samples were frozen in liquid nitrogen (Airgas south, Tifton, GA) and ground with 600 µl of water consisting of 3:5:12 by volume of water, chloroform (J.T. Baker, Phillipsburg, NJ), and methanol (Fisher Scientific, Fair Lawn, NJ). Next, ground samples were transferred to 1.5 ml microcentrifuge tubes (Fisher Scientific, Pittsburgh, PA) and centrifuged (Eppendorf, Hamburg, Germany) at full speed (14,000 rpm) for two min. Collected supernatants were placed in 2 ml microcentrifuge tubes (Fisher Scientific, Pittsburgh, PA) and the remaining residues were re-extracted with another 600 µl of water:chloroform:methanol extraction buffer. Samples were centrifuged at full speed for 2 min. Supernatants were collected again to combine with the previously collected supernatants in a 2 ml microcentrifuge tubes. Then, 300 µl of chloroform and 450 µl of water were added to the combined supernatants. Samples were centrifuged at full speed for 2 min. The observed upper water:methanol phase in the tubes were transferred to a new 2 ml tube. These tubes were subjected and placed to speed vac concentrator (Thermo Fisher Scientific, Asheville, NC) at 45^{o}C for 1 h. Samples were stored at -20^{o}C until they were shipped to the Molecular Structure Facility at the University of California, Davis, at CA for free amino acid analysis. The samples were then subjected to dry extraction in a vacuum concentrator. Extracted samples were diluted in a 200 µl AE-Cys dilution buffer with a dilution factor of 1:5. Fifty microliters of each sample (note: each 50 µl injection=5.0 nmol AE-Cys) was passed through an L-8900 Hitachi amino acid analyzer for the quantification of free amino acids.

Statistical analysis was conducted to compare the free amino acids in peanut leaf tissues with and without pine pollen grains. Treatments were considered as fixed effects while replications were considered as random effects. PROC GLIMMIX in SAS was used for the

analysis. Least square means test at P=0.05 was used to compare the statistical significance of differences between treatments.

Thrips-mediated TSWV transmission with and without pine pollen grains. Peanut plants with and without pine pollen grains were evaluated for TSWV susceptibility. Ten one-week old peanut plants for each treatment were used in the experiment, and the whole experiment was repeated twice (N=30 peanut plants for each treatment). Each plant was individually 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). Each treated plant was dusted with approximately 0.05 g. of pine pollen grains. Ten potentially viruliferous thrips placed in a 0.6 ml microcentrifuge tube (Fisher Scientific, Pittsburgh, PA) using a paintbrush (2 Silver 5300 S Round, India) and then released at the base of each peanut plant. TSWV infection status of plants was confirmed by DAS-ELISA using antibodies specific to the nucleocapsid protein and following manufacturer's recommendations (Clark and Adams 1977, Shrestha et al. 2012).

TSWV detection using DAS-ELISA. Leaf tissue (approximately 0.1 g) was used for DAS-ELISA. The assay was performed in a 96 well microtiter plate (Maxisorp, Nunc, Rochester, NY) with suitable positive and negative controls. 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) also was 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 microplate reader 1 h after substrate addition (Model Elx 800, Bio-Tek®, Kocherwaldstr, Germany). An average absorbance value of negative control samples plus four standard deviations was considered positive.

A CRD was used as treatment structure. Treatments were considered fixed effects while replications were considered random effects. Incidence of TSWV infection was compared between treatments. TSWV incidence was treated as binomial response (positive or negative) and data were analyzed using PROC GENMOD in SAS. Pairwise contrasts at P=0.05 were used to test the statistical significance of differences between treatments.

Results

***F. fusca* settling with and without pine pollen grains:** Thrips settling preference was tested between peanut leaflet dusted with and without pine pollen grains. Significant differences in thrips settling preference were observed between treatments (df=1,48; F=203.30; P<0.0001). More thrips settled on the peanut leaflet dusted with pine pollen grains (64.48 ± 1.43%) (mean ± standard error) when compared with peanut leaflet without pine pollen grains (35.56 ± 1.44%).

***F. fusca* feeding damage on peanut plants with and without pine pollen grains:** Feeding damage indices were observed for each treatment separately. Significant differences in thrips feeding damage indices at 10, 20, and 30 days were observed on both treatments (Fig. 5.1 and Table 5.1). Feeding damage indices did not vary between repeats of the experiment at 10 (df=2,57; F=1.72; P=0.1899) and 30 (df=2,57; F=2.76; P=0.0743) days post thrips release but not with 20 (df=2,57; F=12.08; P<0.0001) days post thrips release. Feeding damage indices on peanut plants without pine pollen grains were significantly lower than on peanut plants dusted with pine pollen grains at 10 (df=1,58; F=42.02; P<0.0001), 20 (df=1,58; F=80.73; P<0.0001), and 30 (df=1,58; F=79.19; P<0.0001) days post thrips release (Fig. 5.1 and Table 5.1). Thrips feeding injuries on peanut plants without pine pollen grains (Fig. 5.2a) was less severe when compared with peanut plants dusted with pine pollen grains (Fig. 5.2b). This indicated that the addition of pine pollen grains on peanut plants enhanced thrips feeding.

***F. fusca* oviposition with and without pine pollen grains:** Oviposition rates of non-viruliferous *F. fusca* were tested on peanut leaflets with and without pine pollen grains. Significant differences in the number of eggs produced were observed between treatments (df=1,38; F=17.30; P=0.0002) but not with the repeats of the experiment (df=1,38; F=0.00; P=0.9523) (Table 5.2). *F. fusca* produced significantly more eggs on peanut leaflets dusted with pine pollen grains (96.55 ± 5.29) (mean ± standard error) than on peanut leaflets without pine pollen grains (72.40 ± 2.95) (Fig. 5.3 and Table 5.2).

***F. fusca* development with and without pine pollen grains:** Thrips were monitored for an entire generation (adult to adult) in Munger cages on peanut leaflets with and without pine pollen grains. The number of adults developed per adult released did not vary between repeats of the experiment (df=1,38; F=0.49; P=0.4888) and between treatments (df=1,38; F=0.01; P=0.9091) (Table 5.3). These results indicated that pine pollen grains had no influence on the reproductive potential of *F. fusca*. The median developmental time required to complete one generation (adult to adult) was not statistically significant among treatments (Table 5.4). Developmental time of *F. fusca* was not influenced by the addition of pine pollen grains (df=1,38; χ^2=0.08; P=0.7819).

Free amino acid concentrations peanut leaf tissue with and without pine pollen grains: The levels of free amino acids were quantified and compared between treatments. The titers of seven essential free amino acids (histidine, isoleucine, leucine, lysine, phenylalanine, threonine, and valine) were significantly greater on leaf tissue samples with pine pollen grains than on leaf tissue samples without pine pollen grains (Fig. 5.4a and Table 5.5). The titers of nine non-essential free amino acids (alanine, arginine, asparagine, aspartic acid, glycine, proline, serine, and tyrosine) were significantly greater on leaf tissue samples with pine pollen when compared with leaf tissue samples without pine pollen (Fig. 5.4b and Table 5.5). The titers of four other

free amino acids (citrulline, γ-aminobutyric acid, hydroxyproline, and ornithine) were also significantly greater on leaf tissue samples with pine pollen grains than on leaf tissue without pine pollen grains (Fig. 5.4c and Table 5.5). Except for γ-aminobutyric acid, the other three amino acids were only found in leaf tissue samples with pine pollen grains (Fig. 5.4c). On the contrary, γ-aminobutyric acid was present in leaf tissue samples with and without pine pollen grains.

Thrips-mediated TSWV transmission with and without pine pollen grains: The incidence of TSWV infection (%) was evaluated in peanut plants dusted with and without pine pollen grains with DAS-ELISA at four weeks post inoculation. TSWV incidence did not vary between repeats of the experiment (df=2,57; χ^2=0.22; *P*=0.8940). TSWV incidence in peanut plants dusted with pine pollen grains (90.00 ± 10.00%) (mean ± standard error) was not different from TSWV incidence in peanut plants without pine pollen grains (86.67 ± 11.11%) (df=1,58; χ^2=0.11; *P*=0.7439) (Table 5.6). Addition of pollen grains on peanut foliage did not influence TSWV transmission by thrips.

Discussion

The 24 h settling assay using a Y-tube olfactometer clearly showed that significantly more *F. fusca* settled on peanut leaflets dusted with pine pollen grains than on peanut leaflets without pine pollen grains. Using another experimental set up, Chitturi et al. (2006) also showed an increase in *F. fusca* and *F.occidentalis* settling following the addition of slash pine pollen (*P. elliottii* Engelm) on tomato and peanut foliage. These results together suggested that the addition of pine pollen on peanut foliage could influence the settling behavior of thrips. Thrips settling counts were taken at 24-h only in the current study, whereas thrips settling counts were taken daily for eight days in the study conducted by Chitturi et al (2006). Their findings indicated that

thrips settled on pollen treated leaflets only for 3 days post pollen addition and no settling differences were detected thereafter. They attributed the lack of settling differences after 3 days due to deterioration of pine pollen quality (Chitturi et al. 2006). The feeding assay described in this manuscript indicated that the addition of pine pollen increased *F. fusca* feeding on peanut plants. On the contrary to differences in settling observed by Chitturi et al. (2006), feeding damage indices on plants treated with pollen in our study was consistently higher than on the untreated plants for up to 30 days. This indicated that in spite of deterioration in pine pollen quality, if any, pollen addition facilitated increased thrips feeding. Thrips used in these assays were reared on peanut leaflets supplemented with pollen. This could have also affected the outcomes of the settling and feeding assays. Nevertheless, these results reiterate that addition of pollen makes the host more suitable to thrips and aids in improved host utilization in what would be otherwise unsuitable or unacceptable hosts.

In addition to affecting *F. fusca* settling and feeding patterns, addition of pollen grains also influenced the fitness of *F. fusca*. The oviposition test conducted in this study revealed that addition of pollen resulted in increased *F. fusca* oviposition on peanut foliage. These results are in concurrence with a number of other studies. *Haplothrips brevitubus* (Karny) produced eggs in the presence of pollen from strawberry, eggplant (*Solanum melongena* L.), pine, and pepper (*Capsicum annum* L.) (Morita et al. 2008). Riley et al. (2007) also indicated that addition of slash pine pollen resulted in 1.6- and 2.9-fold increase in egg production by *F. fusca* and *F. occidentalis*, respectively. However, effects of pollen on thrips oviposition seem to vary with thrips species as well as the type of pollen. For instance, Murai and Loomans (2001) demonstrated that egg production by *F. intosa* increased with the addition of tea (*Camellia sinensis* (L.) O. Kuntze) pollen but pollen addition did not affect egg production by *T. palmi*.

Enhanced egg production with the addition of pollen might be due to the increased availability of nutrients (Erhardt and Baker 1990, Lundgren 2009). Pollen grains typically have substantial amounts of nutrients such as amino acids. Among the amino acids present in pollen, proline is often the most abundant (3% of pollen dry weight) (Erhardt and Baker 1990, Lundgren 2009). Amino acids such as proline are also associated with vitellogenesis, oogenesis, and egg maturation (Wheeler 1996, Rojas et al. 1998, Carter et al. 2006, Klowden 2007, Lundgren 2009). Results from this study revealed that the addition of pollen grains on peanut leaflets increased the availability of 20 of the 22 free amino acids identified. Concentrations of some amino acids increased up to 14 folds. Therefore, the increase in *F. fusca* egg production on peanut leaflets dusted with pine pollen could be due, in part, to the elevated concentrations of free amino acids in pine pollen.

In addition to increase in egg production addition of pollen is known to enhance other fitness parameters (Tsai et al. 1996, Hulshof et al. 2003, Angelella and Riley 2010). Hulshof et al. (2003) showed that the addition of scots pine (*P. sylvestris* L.) pollen to cucumber (*Cucumis sativus* L.) and downy birch (*Betula pubescens* Ehrh.) led to 68 to 90% increase in the larvae produced by of *F. occidentalis*. Angelella and Riley (2010), observed a 3.4- to 8.0- fold increase on adult emergence rates of *F. fusca* with the addition of slash pine pollen to onion foliage. Addition of pollen to cucumber and cotton foliage resulted in reduced developmental time of *F. occidentalis* (Trichilo and Leigh 1988, Hulshof and Vänninen et al. 2001, Hulshof et al. 2003). Besides directly improving the fitness, pollen supplementation is known to improve utilization of otherwise unsuitable hosts. For instance, *F. occidentalis* was able to reproduce in poinsettia (*Euphorbia pulcherrima* Wild ex. Klotzsch) only in the presence of pine pollen (Hulshof and Vänninen 2001). Despite the increase in *F. fusca* oviposition following pine pollen addition in

this study, other fitness parameters such as adult emergence and developmental time of *F. fusca* remained unaffected by pollen supplementation. It is not precisely clear as to why increase in certain *F. fusca* fitness parameters were not observed following pollen addition. Nevertheless, the lack of certain fitness benefits could be in part, due to, the host species on which the insect was reared as well as host suitability to the insect. For instance, even though *F. fusca* prefers flowers than vegetative parts of the peanut plant, it still reproduces efficiently on foliage especially on younger plants (Todd et al. 1994, 1995). Additionally, some leguminous hosts such as peanut could have higher nitrogen or amino acid contents in their foliage than in non-leguminous hosts (McNiel and Southwood 1978, Scheublin et al. 2004). Together, these factors could have aided in efficient host utilization even in the absence of pollen grains. Morita et al. (2008) also observed that, despite enhancing oviposition, addition of pollen grains did not affect development and adult emergence of *H. brevitubus* on strawberry, eggplant, pepper, and pine. It is also possible that nutrients critical for egg production and maturation are more abundantly found in pollen grains than the nutrients required for growth and development.

Pollen supplementation, in general, is known to increase at least some fitness parameters of several thrips species. Besides the fitness parameters, it is also known to modify thrips host utilization behaviors such as settling and feeding. These effects together could in turn facilitate pathogen acquisition and inoculation in the case of thrips that serve as vectors. *F. fusca* is an important vector of TSWV, and it transmits the virus in a persistent and propagative manner (German et al. 1992, Whitfield et al. 2005, Pappu et al. 2009). Increased fecundity and feeding in *F. fusca* on TSWV-infected peanut plants could therefore aid in increased virus acquisition. Further, increased settling of potentially viruliferous thrips on plants with pollen dusting could result in increased virus inoculation. Our results showed that the addition of pine pollen did not

improve the incidence of TSWV infection in plants that were inoculated by potentially viruliferous thrips. In both treatments more than 80% of the inoculated plants were infected. Even though the addition of pine pollen enhanced feeding by *F. fusca*, there was still substantial feeding on peanut plants without pine pollen, which might be sufficient to facilitate virus inoculation. Peanut foliage seems to be suitable hosts to *F. fusca*. In the case of non-suitable hosts, the effects however could be different. Such effects could also be important from an epidemic standpoint. For example, a number of winter weeds in a farmscape, which are hosts of TSWV and possess differential abilities to support thrips populations, could support increased populations of thrips following pollen dehiscence (Groves et al. 2001, 2002, Morsello and Kennedy 2009, Srinivasan et al. 2014). Such an increase in thrips populations could then facilitate virus spread among weeds as well as between weeds and crops. Conducting the transmission assay on a sub-optimal or an unacceptable host with pine pollen supplementation could help address this issue better.

In conclusion, addition of pine pollen to host foliage seems to improve the fitness benefits of *F. fusca*. However, the effects seem to be rather marginal than substantial as observed with other thrips species such as *F. occidentalis* (Riley et al. 2007). The ability of *F. fusca* to feed on host foliage than other species such as *F. occidentalis* (Riley et al. 2007) as well as the host nutrient contents could have affected the observed outcomes. These results indicate that *F. fusca* has more flexibility in host utilization than other thrips species. The pollen-induced effects on thrips with such flexibility in host utilization is probably not as effective on thrips species that are exclusive flower feeders or those species that have less flexibility in host utilization .

Literature Cited

Angelella, G. M. and D. G. Riley. 2010. Effects of pine pollen supplementation in an onion diet on *Frankliniella fusca* reproduction. Environ. Entomol. 39: 505-512.

Carter, C., S. Shafir, L. Yehonatan, R. G. Palmer, and R. Thornburg. 2006. A novel role for proline in plant floral nectars. Naturwissenschafter 93: 72-79.

Chitturi, A. D. G. Riley, and P. Houston Joost. 2006. Effect of pine pollen on settling behavior of *Frankliniella occidentalis* and *Frankliniella fusca* (Thysanoptera: Thripidae) on tomato and peanut. Environ. Entomol. 35: 1396-1403.

Cho, J. J., R. F. L. Mau, W. C. Mitchell, D. Gonsalves, and L. S. Yudin. 1987. Host list of plants susceptible to *Tomato spotted wilt virus* (TSWV). Research Extension Series 078, Hawaii Institute of Tropical Agriculture and Human Resources, Honolulu, Hawaii. 1-10.

Clark, M. E., and A.N. Adams. 1977. Characteristics of the microplate method of enzyme linked immunosorbent assay for the detection of plant viruses. J. Gen. Virol. 34: 475-483.

Culbreath, A. K., J. W. Todd, and S. L. Brown 2003. Epidemiology and management of tomato spotted wilt in peanut. Annu. Rev. Phytopathol. 41: 53-75.

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

Erhardt, A. and I. Baker. 1990. Pollen amino acids – an additional diet for a nectar feeding butterfly? Plant Syst. Evol. 169: 111-121.

German, T. L., D. E. Ullman, and J. W. Moyer. 1992. *Tospoviruses*: diagnosis, molecular biology, phylogeny, and vector relationships. Annu.Rev. Phytopathol. 30: 315-348.

Groves, R. L., J. F. Walgenbach, J. W. Moyer, and G. G. Kennedy. 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. Phytopathol. 91: 891-899.

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

Hacham, Y., T. Avraham, and R. Amir. 2002. The N-terminal region of *Arabidopsis* cystathionine gamma-synthase plays an important regulatory role in methionine metabolism. Plant Physiol. 128: 454-462.

Hulshof, J., and I. Vänninen. 2001. Western flower thrips feeding on pollen, and its implications for control, pp. 173-179. *In* R. Marullo and L. Mound (eds.), Thrips and Tospoviruses: Proceedings of the 7th International Symposium on Thysanoptera.

Hulshof, J., E. Ketoja, and I. Vaenninen. 2003. Life history characteristics of *Frankliniella occidentalis* on cucumber leaves with and without supplemental food. Entomologia Experimentalis et Applicata 108: 19-32.

Kirk, W. D. J. 1984. Pollen feeding in thrips (Insecta: Thysanoptera). J. Zoo. 204: 107-117.

Kirk, W. D. J. 1985. Pollen-feeding and the host specificity and fecundityof flower thrips (Thysanoptera). Ecol. Entomol. 10: 281-289.

Kirk, W. D. J. 1997. Feeding. *In* T. L. Lewis (ed.). Thrips as crop pests. CAB International, London, UK.

Klowden, M. J. 2007. Physiological Systems in Insects (2nd ed.).Academic Press, SanDiego, CA, USA.

Kogel, W.J., E.H. Koschier, and J.H. Visser. 1999. Y-tube olfactometer to determine the attractiveness of plant volatiles to western flower thrips. Proc. Exper. and Appl. Entomol.

N.E.V. Amsterdam 10.

Lundgren, J. G. 2009. Relationships of natural enemies and non-prey foods. Springer, USA.

Maris, P.C., N. N. Joosten, D. Peters, and R.W. Goldbach. 2003. Thrips resistance in pepper and its consequences for the acquisition and inoculation of Tomato spotted wilt virus by the western flower thrips. Phytopathol. 93: 96-101.

McNiel, S., and T. R. E. Southwood. 1978. The role of nitrogen in the development of insect/plant relationships. *In* Harbone, J. B. (ed.). Biochemical Aspects of Plants and Animal Coevolution. Academic Press, London.

Morita, S., T. Kashio, H. Inoue, and K. Kakimoto. 2008. Development and fecundity of *Haplothrips brevitubus* (Karny) (Thysanoptera: Phlaeothripinae) on the pollen of some fruit vegetables. Kyushu Pl. Prot. Res. 54: 78-84.

Morsello, S. C., and G. G. Kennedy. 2009. Spring temperature and precipitation affect tobacco thrips, *Frankliniella fusca*, population growth and *Tomato spotted wilt virus* spread within patches of the winter annual weed *Stellaria media.* Entomol. Exp. Appl. 130: 138-148.

Munger, F. 1942. A method of rearing citrus thrips in the laboratory. J. Econ. Entomol. 35: 373-375.

Murai, T., and T. Ishii. 1982. Simple rearing method for flower thrips (Thysanoptera: Thripidae) on pollens. Jap. J. Appl. Ent. Zool. 26: 149-154.

Murai, T., and A. J. M. Loomans. 2001. Evaluation of an improved method for mass-rearing of thrips and a thrips parasitoid. Entomologia Experimentalis et Applicata 101: 281-289.

Pappu, H. R., R. A. C. Jones, and R. K. Jain. 2009. Global status of tospovirus epidemics in diverse cropping systems: successes achieved and challenges ahead. Virus Res. 141: 219-236.

Riley, D. G., and H. R. Pappu. 2000. Evaluation of tactics for management of thrips-vectored *Tomato spotted wilt tospovirus* in tomato. Plant Dis. 84: 847-852.

Riley, D. G., and H. R. Pappu. 2004. Tactics for management of thrips (Thysanoptera, Thripidae) and *Tomato spotted wilt tospovirus* in tomato. J. Econ. Entomol. 97: 1648-1658.

Riley, D. G., A. Chitturi, and A. N. Sparks. 2007. Does natural deposition of pine pollen affect the ovipositional behavior of *Frankliniella occidentalis* and *Frankliniella fusca*? Entomologia Experimentalis et Applicata 124: 133-141.

Riley, D. G., G. M. Angelella, and R. M. McPherson. 2011a. Pine pollen dehiscence relative to thrips population dynamics. Entomologia Experimentalis et Applicata 138: 223-233.

Riley, D. G., S. V. Joseph, R. Srinivasan, and S. Diffie. 2011b. Thrips vectors of tospoviruses. J. Integ. Pest Mngmt. 1: 1-10.

Rojas, M. G., J. A. Morales-Ramos, E. G. King, G. Saldana, and S. M. Greenberg. 1998. Use of a factitious host and supplemented adult diet to rear and induce oogenesis in *Catolaccus grandis* (Hymenoptera:Pteromalida). Environ. Entomol. 27: 499–507.

Sakimura, K. 1962. *Frankliniella occidentalis* (Thysanoptera: Thripidae), a vector of the *Tomato spotted wilt virus*, with special references to the color forms. Annu. Entomol. Soc. Am. 55: 387-389.

Sakimura, K. 1963. *Frankliniella fusca*, an additional vector for the *Tomato spotted wilt virus*, with notes on *Thrips tabaci*, another vector. Phytopathol. 53.

Scheublin, T. R., K. P. Ridgway, J. P. W. Young, and M. G. A. van der Heijden. 2004. Nonlegumes, legumes, and root nodules harbor different arbuscular mycorrhizal fungal communities. App. Environ. Micro. 70: 6240-6246.

Shrestha, A., R. Srinivasan, D. G. Riley, and A. K. Culbreath. 2012. Direct and indirect effects of a thrips-transmitted *Tospovirus* on the preference and fitness of its vector, *Frankliniella fusca*. Entomologia Experimentalis et Applicata 145: 260-271.

Srinivasan, R., D. Riley, S. Diffie, A. Shrestha, and A. Culbreath. 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. Environ. Entomol. (in press)

Stanley, R. G. and H. F. Linskins. 1974. Pollen: biology, biochemistry, management. Springer, New York.

Todd, J. W., A. K. Culbreath, J. R. Chamberlin, R. J. Beshear, and B. G. Mullinix. 1995. Colonization and population dynamics of thrips in peanuts in the southern United States, pp. 453-460. *In* B. Parker, M. Skinner and T. Lewis (eds.), Thrips Biol. Mgt. Plenum Press, New York.

Todd, J. W., A. K. Culbreath, and S. L. Brown. 1996. Dynamics of vector populations and progress of spotted wilt disease relative to insecticides use in peanuts. Acta Hortic. 431: 483-490.

Todd, J.W., J. R. Chamberlin, A. K. Culbreath, and J. W. Demski. 1994a. Timing and duration of vector management in relation to spotted wilt disease incidence in peanut. Proc. Am. Peanut Res. Ed. Soc. 25: 86.

Trichilo, P. J., and T. F. Leigh. 1988. Influence of resource quality on the reproductive fitness of flower thrips (Thysanoptera, Thripidae). Annals Entomol. Soc. America 81: 64-70.

Tsai, H. J., B.S. Yue, J. E. Funderburk, and S. E. Webb. 1996. Effect of plant pollen on growth and reproduction of *Frankliniella bispinosa*. Acta Horticulturae 431: 535–541.

Ullman, D. E., J. J. Cho, R. F. L. Mau, W. B. Hunter, D. M. Westcot, D. M. Custer. 1992. Thrips-*Tomato spotted wilt virus* interactions: morphological, behavioral and cellular components influencing thrips transmission. Adv. Dis. Vect. Res. 9: 195-240.

Ullman, D. E., J. L. Sherwood, and T. L. German. 1997. Thrips as vectors of plant pathogens, pp. 539–565. *In* T. Lewis (ed), Thrips as Crop Pests. CAB International, New York.

Wheeler, D. 1996. The role of nourishment in oogenesis. Annual Review of Entomology 41: 407-431.

Whitfield, A. E., D. E. Ullman, and T. L. German. 2005. Tospovirus-thrips interactions. Annu. Rev. Phytopathol. 43: 459-C-5.

Tables

Table 5.1. *Frankliniella fusca* feeding on peanut plants dusted with and without pine pollen.

Treatments[x]	Mean feeding damage index[y]		
	10 days	20 days	30 days
With pollen	1.42 ± 0.09 (a)	3.90 ± 0.08 (a)	4.83 ± 0.04 (a)
Without pollen (untreated)	0.84 ± 0.05 (b)	3.11 ± 0.07 (b)	4.12 ± 0.07 (b)
Type III analysis[z] (df=1,58)			
***F* value**	42.02	80.73	79.19
P* > *F	<0.0001	<0.0001	<0.0001

[x] Peanut plants dusted with and without pine pollen grains for thrips feeding evaluation.

[y] Statistical differences on feeding damage at three time intervals, 10, 20, and 30 days post thrips release were assessed. Means (±SE) within a column followed by the same letter are not significantly different at P=0.05.

[z] Type III analysis was conducted using PROC GLIMMIX in SAS.

Table 5.2. Oviposition efficiency of tobacco thrips, *Frankliniella fusca*, on peanut leaflets with and without pine pollen.

Treatments[w]	Number of eggs (mean ± standard error)[x]		
	Repeats of the experiment[y]		
	I	II	Number of eggs (mean ± standard error)
With pollen	91.60 ± 7.86 (a)	101.50 ± 7.11 (a)	96.55 ± 5.28 (a)
Without pollen (untreated)	77.70 ± 3.86 (a)	67.10 ± 3.94 (b)	72.40 ± 2.95 (b)
Type III analysis[z]	**(df=1,18)**		**(df=1,38)**
***F* value**	2.52	22.12	17.30
P>F	0.1310	0.0002	0.0002

[w] Thrips oviposition on peanut plants with and without pine pollen grains

[x] Number of eggs found in peanut leaflets (mean ± standard error).

[y] Roman numerals I and II refer to the repeats of the experiment.

[z] Type III analysis was conducted using PROC GLIMMIX in SAS.

Table 5.3. Mean number of *Frankliniella fusca* on peanut leaflets with and without pine pollen.

	Repeats of the experiment[y]		
Treatments[x]	**I**	**II**	**Number of adults**
With pollen	9.26 ± 1.18	9.05 ± 1.23	9.16 ± 0.83
Without pollen (untreated)	8.20 ± 0.85	9.87 ± 0.86	9.04 ± 0.62
Type III analysis[z]	**(df=1,18)**		**(df=1,38)**
F value	0.54	0.30	0.01
P>F	0.4726	0.5921	0.9091

[x] Non-viruliferous female thrips were released on Georgia-06G peanut leaflets with and without pine pollen grains in Munger cages, separately. Mean (± SE) of newly emerged adults were recorded at 24 h intervals are included.

[y] Roman numerals I and II refer to the repeats of the experiment.

[z] Type III analysis was conducted using PROC GLIMMIX in SAS.

Table 5.4. Developmental time required to complete one generation (adult to adult) of *Frankliniella fusca* on peanut leaflets with and without pine pollen.

	Repeats of the experiment[y]		
Treatments[x]	**I**	**II**	**Median development time (days)**
With pollen	14 (13–15)	13 (10–14)	14 (10–15)
Without pollen (untreated)	14 (13–16)	13 (11–14)	14 (11–16)
Wilcoxon rank sum test[z]		**S**	**P**
Peanut leaflet with pollen vs. peanut leaflet without pollen		400.50	0.7932

[x] Non-viruliferous female thrips were released on Georgia-06G peanut leaflets dusted with and without pine pollen grains in Munger cages, separately. Median developmental times and their ranges in parentheses are included.

[y] Roman numerals I and II refer to the repeats of the experiment.

[z] Wilcoxon Rank Sum test was conducted using PROC NPAR1WAY in SAS.

Table 5.5. Free amino acids on peanut leaf tissue samples with and without pine pollen.

Amino acid	*F* value$_{(1,8)}$	*P*>*F*[x]	Significance[y]
Essential			
Histidine	68.41	<0.0001	***
Isoleucine	107.12	<0.0001	***
Leucine	31.56	0.0008	***
Lysine	185.14	<0.0001	***
Methionine	0.32	0.5882	NS
Phenylalanine	117.42	<0.0001	***
Threonine	20.26	0.0028	**
Valine	108.39	<0.0001	***
Non-essential			
Alanine	9.31	0.0185	*
Arginine	217.34	<0.0001	***
Asparagine	186.70	<0.0001	***
Aspartic acid	64.52	<0.0001	***
Glutamic acid	0.63	0.4547	NS
Glutamine	65.85	<0.0001	***
Glycine	188.02	<0.0001	***
Proline	197.06	<0.0001	***
Serine	15.57	0.0056	**
Tyrosine	137.26	<0.0001	***
Others			
Citrulline	231.18	<0.0001	***
γ -aminobutyric acid	113.02	<0.0001	***
Hydroxyproline	184.89	<0.0001	***
Ornithine	244.05	<0.0001	***

[x] Differences in the levels of free amino acids between peanut leaflets with and without pine pollen grains were calculated using PROC GLIMMIX in SAS. Samples from five peanut plants with and without pine pollen grains, respectively, were tested. Approximately 0.15 g of leaf tissue was collected from the top one-third portion of the plant for amino acid analysis.

[y] $P<0.05$ (*); $P<0.01$ (**); $P<0.001$ (***); and not significant (NS).

Table 5.6. Incidence of *Tomato spotted wilt virus* infection on *Frankliniella fusca*-inoculated peanut plants.

Treatments[w]	Number of plants infected[x]			
	Repeats of the experiment[y]			
	I	II	III	TSWV transmission (%) (mean ± standard error)
With pollen	9/10	9/10	9/10	90.00 ± 10.00
Without pollen (untreated)	9/10	8/10	9/10	86.67 ± 11.11
Type III analysis ($P>\chi^2$)[z]				
df=1,58; Chi-Square (χ^2)=0.11; P=0.7439				

[w] Peanut plants with and without pine pollen grains were used for the TSWV transmission assay.

[x] Peanut plants were subjected to thrips inoculation. TSWV infection was confirmed using double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA).

[y] Roman numerals I and II refer to the repeats of the experiment.

[z] Type III analysis was conducted using PROC GENMOD in SAS.

Figures

Fig. 5.1. Mean (±SE) feeding indices on peanut plants with and without pine pollen. Ten one-week old peanut plants for each treatment were placed separately in insect proof cage. Approximately 0.05 g of pine pollen grains were dusted on each treated plant. Ten non-viruliferous female *F. fusca* were collected in 0.6 ml microcentrifuge tubes using a paintbrush and then released at the base of each plant. Thrips feeding damage was assessed on each plant every two days for a month after initial thrips release.

Fig. 5.2. Feeding injuries (silvery patches) on peanut plants with and without pine pollen. One-week old peanut plants were inoculated with ten non-viruliferous adult *F. fusca*: without pine pollen (a) and with pine pollen (b). Photos were taken at the final week within 30 days observation.

Fig. 5.3. Mean (±SE) cumulative counts of *Frankliniella fusca* eggs on peanut leaflets with and without pine pollen under dissecting microscope at 100x magnification. Acid-fuchsin was used to stain the eggs in each leaflet for two to four minutes: eggs on leaflet without pine pollen (a) and eggs on leaflet with pine pollen (b).

Fig. 5.4. Mean (± SE) free amino acids in peanut leaf tissue samples with and without pine pollen: essential (a), non-essential (b), and other amino acids (c). Leaf tissue (~0.15 g) was excised from the top one-third portion of plants and subjected to free amino acid analysis. Approximately 0.05 g pine pollen grains were added to each treated leaf tissue.

Fig. 5.1.

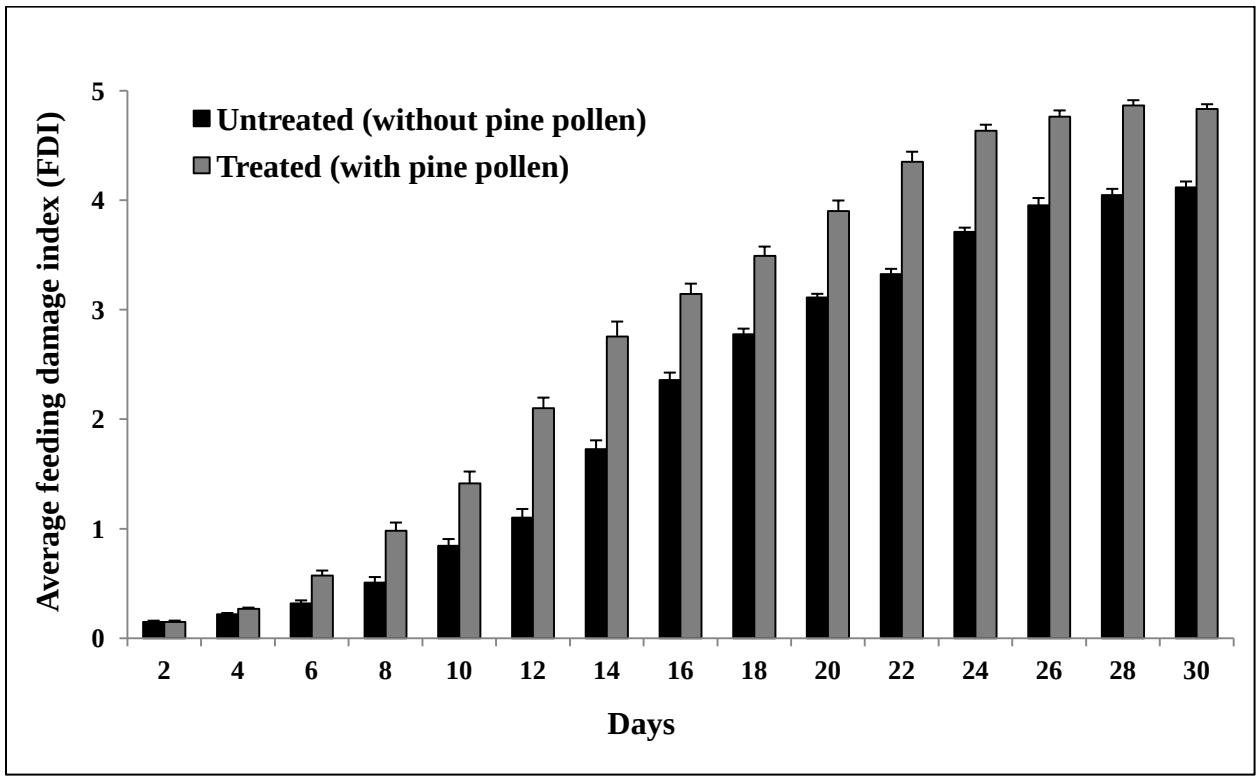

Untreated (without pine pollen)
Treated (with pine pollen)
Average feeding damage index (FDI)
0
1
2
3
4
5
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
Days

Fig. 5.2.

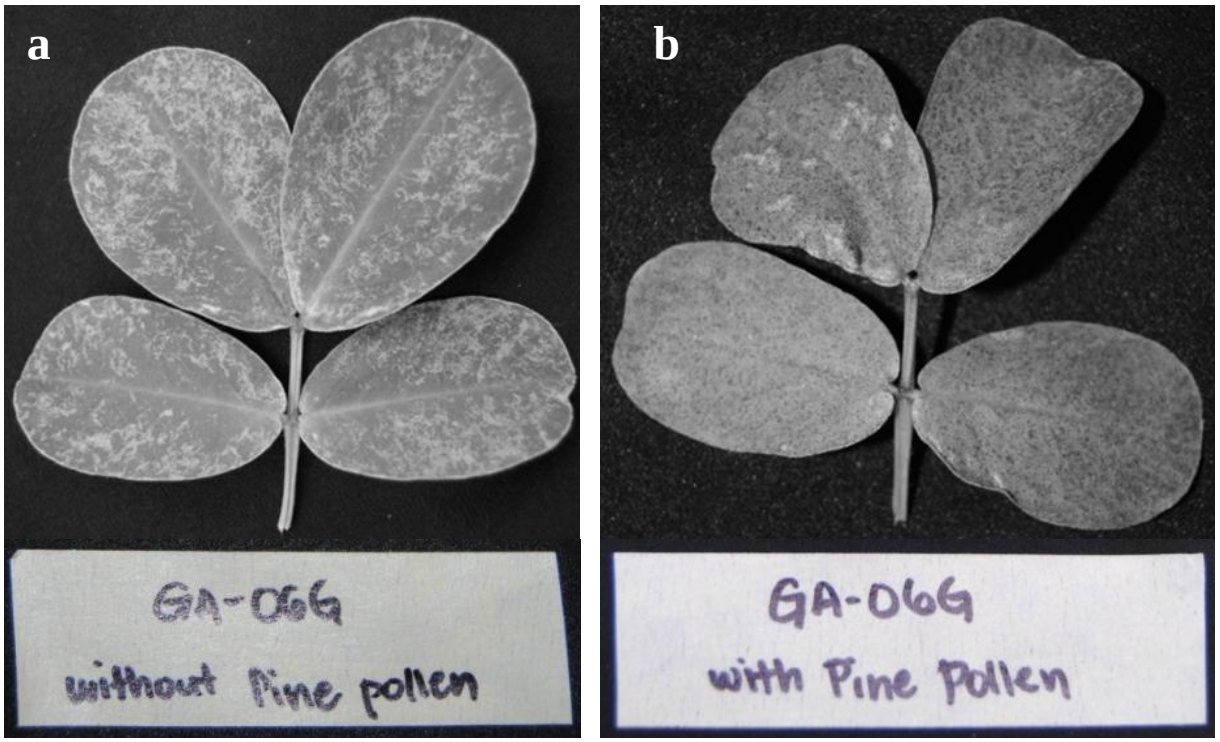

a
GA-06G
without Pine pollen
b
GA-06G
with Pine Pollen

Fig. 5.3.

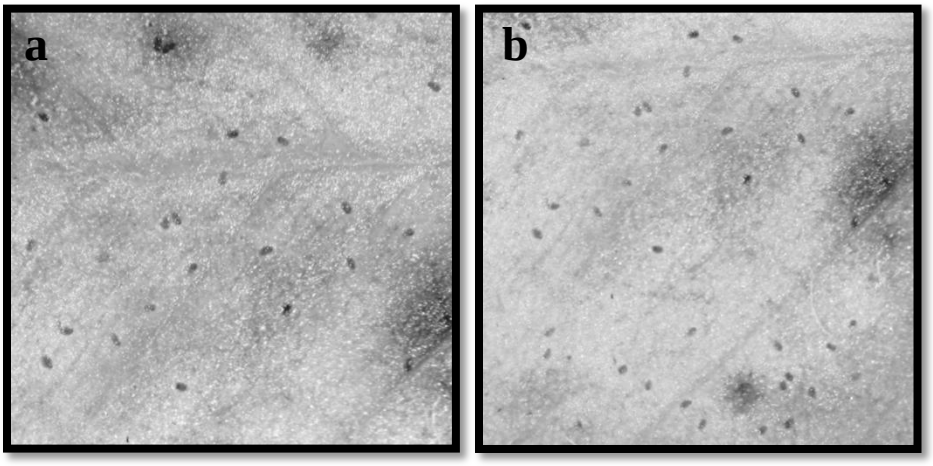


Fig. 5.4a.

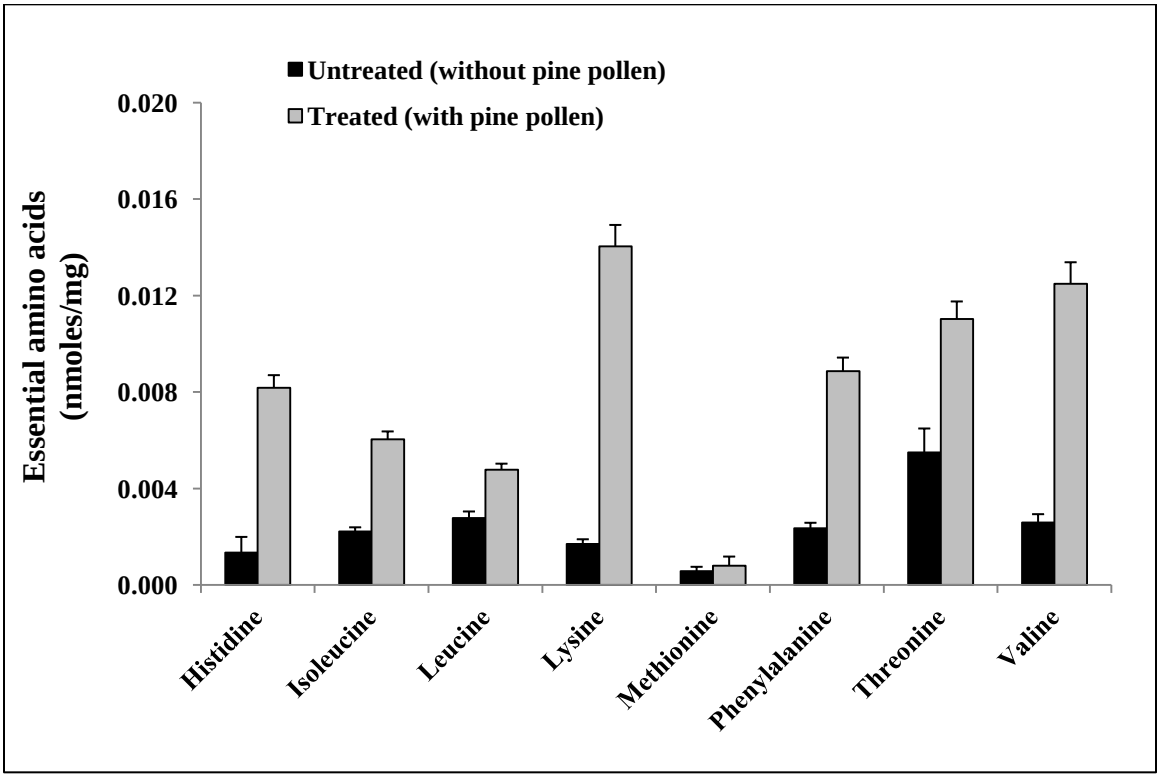


Fig. 5.4b.

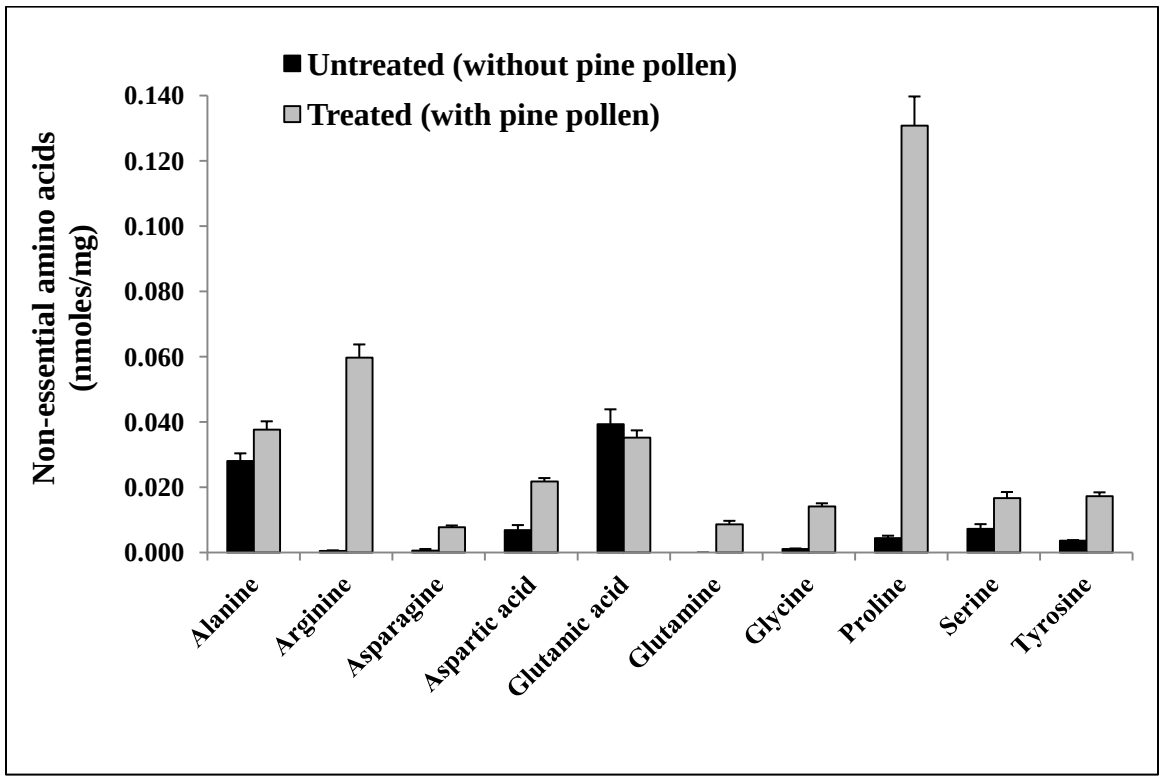
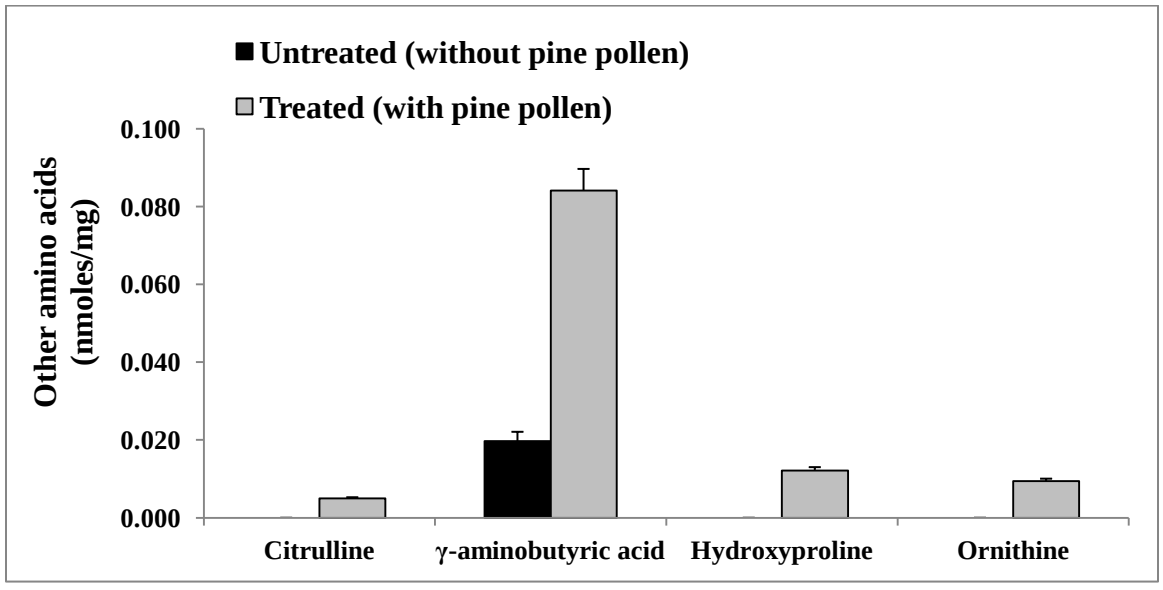


Fig. 5.4c.



CHAPTER 6

ROLE OF COTTON CROP AS A THRIPS RESERVOIR AND AS A *TOMATO SPOTTED WILT VIRUS* (TSWV) INOCULUM SOURCE: EFFECTS ON THRIPS FITNESS AND TRANSMISSION OF TSWV[1]

[1]Marasigan, K., M. Toews, R. Kemerait, Jr., and R. Srinivasan. 2014.

Abstract

Cotton is grown in proximity to peanut in southeastern United States. Cotton is also a reservoir of *Frankliniella fusca* (Hinds). *F. fusca* is the most important vector of *Tomato spotted wilt virus* (TSWV), which severely affects peanut production. Earlier studies indicated that cotton is a symptomless host of TSWV. However, the role of cotton in TSWV epidemics in peanut is unknown. To assess cotton as a TSWV inoculum source, a transmission assay was conducted using *F. fusca* mediated inoculations. Other TSWV hosts such as peanut, tobacco, and tomato were included as controls. TSWV infection in inoculated plants was tested by DAS-ELISA, immunostrips, and RT-PCR. DAS-ELISA results indicated that TSWV incidence was ≥85% in all the hosts except cotton. TSWV infection in cotton was ≤30%. When DAS-ELISA positive cotton plants were retested by immunostrips and RT-PCR, they tested negative. On the contrary, the congruency between DAS-ELISA and other detection techniques was 100% for peanut, tobacco, and tomato. This indicated that DAS-ELISA positive cotton plants could be false positives. To attest, a back transmission assay was conducted using *F. fusca* mediated transmission. Peanut plants inoculated with potentially viruliferous thrips from TSWV positive cotton foliage were not infected with TSWV. Results suggested that cotton was not susceptible to TSWV. Further, microcosm studies indicated that thrips fitness improved when they were transferred from cotton to peanut. Together, these results suggested that cotton might not influence TSWV incidence in peanut directly as an inoculum source but might do so indirectly as a thrips reservoir.

Additional Key Words: Non-symptomatic host, susceptibility, epidemics

Introduction

Tomato spotted wilt virus (TSWV) is a member of the plant-infecting genus *Tospovirus* in the family *Bunyaviridae* (German et al. 1992, Adkins 2000, Pappu et al. 2009). More than 1000 host-plant species (crops and non-crops) are confirmed as hosts of TSWV (Cho et al. 1987, Parrella et al. 2003, Groves et al. 2001, 2002; Mullis and Martinez 2009, Srinivasan et al. 2014). TSWV is exclusively transmitted by thrips in the family Thripidae (Order: Thysanoptera) in a persistent and propagative manner (German et al. 1992, Ullman et al. 1992, Jones 2005, Whitfield et al. 2005, Pappu et al. 2009). Thrips demonstrate stage specific acquisition and inoculation of TSWV. For instance, thrips ought to acquire TSWV at the first instar larval stage in order to transmit the virus at late instar stages or as adults. In other words, if adult thrips acquire the virus for the first time they will not be able to transmit the virus (Whitfield et al. 2005). Also, TSWV is non-seed transmitted (Costa 1941, Pappu et al. 2009). Therefore, in order to influence TSWV epidemics, a host should support thrips populations at least for one generation as well as serve as a host for TSWV (Whitfield et al. 2005, Pappu et al. 2009).

In southeastern United States, besides alternate hosts, numerous crop hosts including cotton, peanut, pepper, tobacco, and tomato serve as hosts of thrips capable of transmitting TSWV as well as the virus itself (Cho et al. 1986, 1987; Riley et al. 2011, Schuster and Haliwell 1994, Groves et al. 1998). A number of these hosts produce a suite of symptoms upon TSWV infection and suffer yield losses whereas most other hosts are symptomless carriers of the virus (Culbreath et al. 2003, Culbreath and Srinivasan 2011). Also, a number of these hosts are reservoirs of thrips. Hence, it is possible that symptomless carriers of the virus that are abundantly present in the farmscape, by serving as thrips reservoirs and as virus hosts, could influence TSWV incidence in crop hosts and inflict yield losses. Cotton is one such host; it is

believed to be symptomless host for TSWV (Schuster and Haliwell 1994 and Groves et al. 1998). Cotton is also a good reservoir of thrips including *F. fusca,* and *F. occidentalis,* and *F. tritici* (Toews et al. 2010). Of the three, only *F. fusca* and *F. occidentalis* are capable of transmitting TSWV (Riley et al. 2011). Most often, *F. fusca* populations on cotton outnumber *F. occidentalis*.

Millions of acres of cotton are gown annually in the southeastern United States. Georgia alone had a cotton production area of 1.36 million acres in 2013 (NASS 2014). It is also most commonly rotated with peanuts (Johnson et al. 2001). Incidentally, more than 1 million acres are under peanut production in the southeastern United States as well (NASS 2014). Therefore, both cotton and peanut are more often than not grown in proximity to each other. Unlike cotton, peanut is highly susceptible to TSWV and TSWV infection in peanut leads to serious yield losses (Bertrand 1998, Culbreath et al. 2003, Culbreath and Srinivasan 2011). Cotton and peanut are documented as hosts of thrips such as *F. fusca* and *F. occidentalis*. Thrips infestation in cotton normally occurs at early stages of the plant (Toews et al. 2010). The planting windows for cotton and peanut extend from April 20th to end of May. However, as part of TSWV risk management recommendation, a majority of peanut acreage is only planted from the second week of May. Therefore, the cropping scenario presents ample opportunities for viruliferous thrips to move from the cotton crop to peanut crop and consequently colonize peanut plants and transmit TSWV.

Even though cotton is believed to be a host for TSWV (Schuster and Haliwell 1994, Groves et al. 1998), its role in TSWV epidemics is not all that clear. Thrips-mediated transmission assay by Groves et al. (1998) indicated that that cotton plants were infected by TSWV following thrips inoculation. Even though TSWV infection in cotton was evaluated on an

indicator host, the infection status of cotton plants suspected to be infected with TSWV in this study and all the other studies were predominantly evaluated by DAS-ELISA. It is not uncharacteristic for DAS-ELISA to produce high background absorbance values in microtiter plates for numerous hosts leading to identification of false positives (Timmerman et al. 1985, Smith et al 2006). This could be a critical issue especially for non-symptomatic hosts. Furthermore, there is also no indication of higher incidences of TSWV in peanut fields that are in proximity to cotton fields.

In order to understand the role of cotton in TSWV epidemics better, we conducted single plant transmission assays using *F. fusca* with cotton and peanut plants. The incidence of TSWV infection was assessed by at least three detection techniques, they include: assays with immunostrips; double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA); and reverse transcriptase polymerase chain reaction (RT-PCR). Further we also evaluated if cotton could serve as an inoculum source of TSWV by performing a back transmission assay using viruliferous thrips that developed on DAS-ELISA positive cotton and peanut seedlings as recipients. TSWV transmission from cotton to peanut, facilitated by movement of viruliferous thrips from cotton to peanut, could be influenced if peanuts substantially affected the fitness of dispersing thrips. To examine the effects of such dispersal on fitness of thrips, microcosm fitness experiments were also conducted in the laboratory.

Materials and Methods

Non-infected peanut, tobacco, tomato, and cotton plants. Georgia-06G peanut seeds were pre-germinated on moistened paper towels and incubated in a growth chamber with a temperature of 25 to 27^{o}C for one week. Germinated peanut seeds were transplanted into 10.16-cm diameter plastic pots (Hummert International, St. Louis, MO) containing commercial potting

mix (LT5 Sunshine mix, Sun Gro Horticulture Industries, Bellevue, WA). Peanut plants were placed in 47.5-cm^3 insect proof cages (Megaview Science Co., Taichung, Taiwan) and maintained in a greenhouse at 25 to 30^oC with 80 to 90% relative humidity (RH) and 14:10 (L:D) h photoperiod.

Tomato (Florida 47), tobacco (K 326), and Deltapine® (DP) 1050B2RF cotton seeds were planted in seedling trays (Lewis Taylor Farms, Inc. Supplied by Rantway) at one host per tray. Trays were placed in insect proof cages and maintained in a greenhouse at 25 to 30^oC with 80 to 90% relative humidity (RH) and 14:10 (L:D) h photoperiod.

Collection of pine pollen grains. Pine (*Pinus taeda* L.) needles with pollen grains were placed inside a brown paper bag collected in February 2012 at Tifton, GA. The brown paper bag was shook vigorously followed by the removal of pine needles. Pine pollen grains were collected in a glass vial using a paintbrush (2 Silver 5300 S Round, India) after which, pine pollen grains were stored at 4^oC.

Maintenance of non-viruliferous *F. fusca* on peanut and cotton foliage. In 2013, a colony of *F. fusca* was established by collecting *F. fusca* from peanut blooms from Belflower Farm, Coastal Plain Experimental Station, Tifton, GA. Thrips were transferred and maintained in Munger cages (11.43 x 8.89 x 1.77 cm^3) (Munger 1942) containing healthy non-infected peanut leaflets and cotton leaves dusted with pine (*Pinus taeda* L.) pollen grains, separately. The Munger cages were placed in a growth chamber (Thermo scientific, Dubuque, IA) at 25 to 27^oC with 14:10 (L:D) h photoperiod. New foliage was added to the Munger cages every two to three days.

Maintenance of potentially viruliferous *F. fusca* on peanut and cotton foliage. A colony of potentially viruliferous thrips was initiated and maintained on TSWV-infected peanut foliage in

Munger cages as described for non-viruliferous thrips. TSWV-infected peanut foliage was initially obtained from the Belflower Farm, Coastal Plain Experimental Station, Tifton, GA. The cages were routinely replaced with infected foliage obtained either from the Belflower farm or from the greenhouse. Infection status of peanut foliage was confirmed by double antibody sandwich enzyme linked immunosorbent assay (DAS-ELISA) (Clark and Adams 1977). Thrips reared for an entire generation (adult to adult) on TSWV-infected leaflets were alone considered potentially viruliferous. Similarly, potentially viruliferous thrips were also maintained on cotton leaves that were DAS-ELISA positive. These thrips were also reared for an entire generation (adult to adult) before they were used for transmission experiments.

TSWV detection using DAS-ELISA. Leaf tissue (approximately 0.1 g) was used for DAS-ELISA. The assay was performed in a 96 well microtiter plate (Maxisorp, Nunc, Rochester, NY) with suitable positive and negative controls. 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) also was 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 microplate reader 1 h after substrate addition (Model Elx 800, Bio-Tek®, Kocherwaldstr, Germany). An average absorbance value of negative control samples plus four standard deviations was considered positive.

Susceptibility of various host plants to TSWV following thrips-mediated transmission. Peanut (one-week), tomato (two to three weeks), tobacco (three to four weeks), and cotton seedlings (one-week; cotyledon stage and two weeks; true leaf stage) were used for transmission assays. Two stages of cotton were used based on the transmission experiment conducted by

Groves et al. (1998). Each plant species/stage was considered a treatment. Ten plants for each treatment were used in the experiment, and the whole experiment was repeated once (N=20 plants for each treatment). Each plant was individually 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). Each treated plant was dusted with approximately 0.05 g. of pine pollen grains. Ten potentially viruliferous adult *F. fusca* placed in a 0.6 ml microcentrifuge tube (Fisher Scientific, Pittsburgh, PA) using a paintbrush (2 Silver 5300 S Round, India) and released at the base of each peanut plant. TSWV infection status of plants was confirmed by DAS-ELISA as described previously. Fifty percent of the samples from each repeat (five samples) of the experiment were retested using immunostrips and by RT-PCR (Sundaraj et al. 2014). Foliar samples that had the highest absorbance readings from each repeat of the experiment for each treatment were selected for testing by immunostrips and by PCR.

TSWV detection using Immunostrips. Immunostrips (Agdia®, Elkhart, IN) containing the monoclonal antibodies for the nucleocapsid protein were used for testing. The samples were tested following the manufacturer's recommendations. Leaf tissue sample was placed in the mesh bag and macerated with an extraction buffer. The immunostrip was submerged into the buffer for 30 minutes. Presence of bands specific to TSWV infection indicated the presence of TSWV in those samples. A control band always indicated the validity of the test.

TSWV detection using reverse transcriptase polymerase chain reaction (RT-PCR). Total RNA was extracted from ELISA-positive foliar samples of host plant species as described previously. RT-PCR was performed by a OneStep RT-PCR kit (Qiagen). The reaction volume was 25 µl, which included 0.3 µM each forward (5'-ATGTCTAAGGTTAAGCTC-3') and reverse primer (5'-TTAAGCAAGTTCTGTGAG-3') (Jain et al. 1998), 1 µl of one-step RT-PCR

enzyme mix, 5 µl of RT-PCR buffer, 5 µl of Q solution, and 200 µM each dNTP. Reverse transcription was performed in an automated thermal cycler (Eppendorf) programmed at 50°C for 30 min. Initial PCR activation was conducted at 95°C for 15 min; followed by 35 cycles at 94°C for 1 min, 52°C for 1 min, and 72°C for 1 min; and a final extension step at 72°C for 10 min. The amplicons were analyzed by electrophoresis on 1% agarose gel.

Statistical analyses to assess differences in TSWV infection was conducted for each detection method separately. A completely randomized design (CRD) was used as a treatment structure. Treatments were considered fixed effects while replications were considered random effects. Incidence of TSWV infection was compared among treatments. TSWV incidence was treated as binomial response (positive or negative) and data were analyzed using PROC GENMOD in SAS (SAS Enterprise 4.2, SAS Institute, Cary, NC). Pairwise contrasts at P=0.05 were used to test the statistical significance of differences between treatments.

Back transmission of TSWV from cotton to peanut. One-week old peanut seedlings served as recipients. Each plant was individually enclosed in a cylindrical Mylar film cage with a copper mesh top. Each plant was dusted with approximately 0.05 g. of pine pollen grains. Potentially viruliferous thrips reared on DAS-ELISA positive cotton foliage were used to inoculate peanut seedlings. Ten potentially viruliferous adult *F. fusca* were placed in a 0.6 ml microcentrifuge tube using a paintbrush and released at the base of each peanut plant. DAS-ELISA was conducted as described previously. Peanut seedlings inoculated with thrips reared on TSWV-infected peanut foliage served as controls.

A CRD was used as a treatment structure. Treatments were considered fixed effects while replications were considered random effects. Incidence of TSWV infection was compared among treatments. TSWV incidence was treated as binomial response (positive or negative) and data

were analyzed using PROC GENMOD in SAS. Pairwise contrasts at *P*=0.05 were used to test the statistical significance of differences between treatments. TSWV infection status of all the inoculated plants was initially evaluated by DAS-ELISA as described above.

***F. fusca* development on peanut and cotton following host switching.** Non-viruliferous *F. fusca* from peanut and cotton colonies were used for this experiment. Thrips from cotton colony was reared on cotton and peanut foliage, respectively. For comparison purposes thrips from the peanut colony was reared for an entire generation on peanut foliage. One- to two-week old foliage from cotton and peanut were used were placed in individual Munger cages. Each cage constituted a replication and ten cages for each host species were used in the experiment, and the whole experiment was repeated once (N=20 cages for each treatment). Approximately 0.02 g pine pollen grains were dusted on two peanut leaflets or cotyledon leaves in each Munger cage. Ten non-viruliferous female adult *F. fusca* (up to two days old) from thrips colony in peanut or cotton were released in each cage and then removed after five days. Munger cages were maintained in growth chamber as previously described. The cages were observed at 24 h intervals under a compound microscope (MEIJI TECHNO, Santa Clara, CA). Number of newly emerged adults were recorded and removed daily. The set-up was maintained until all larvae turned into adult for each cage.

Statistical analysis was conducted to compare the differences in the number of adults produced among treatments. PROC GLIMMIX in SAS was used for the analysis. Least square means at *P*=0.05 was used to compare the statistical significance of differences among treatments. The developmental time required for the first adult emergence for each treatment was also analyzed using PROC NPAR1WAY in SAS. Wilcoxon Rank Sum test, with a continuity

correction factor of 0.5, was used to compare statistical significance of differences among treatments.

Results

Susceptibility of various host plants to TSWV following thrips-mediated transmission. The incidences of TSWV infection (%) were evaluated in peanut, tobacco, tomato, and two stages of cotton plants with DAS-ELISA at four weeks post inoculation. Significant differences were observed among repeats of the experiment (df=1,98; χ^2=10.76; P=0.0010). The incidences of TSWV infection varied with host plants (df=4,95; χ^2=82.31; P<0.0001). TSWV incidences in peanut and tobacco plants were not different (Fig. 6.1a). However, TSWV incidences in peanut and tobacco plants were significantly greater than TSWV incidences in tomato and cotton (Fig. 6.1a). TSWV incidence in tomato was greater than TSWV incidences in cotton (Fig. 6.1a). TSWV incidence in cotton at cotyledon stage was greater than TSWV incidence in cotton at first true leaf stage of cotton (Fig. 6.1a).

The incidences of TSWV infection (%) were evaluated in peanut, tobacco, tomato, and two stages of cotton plants with immunostrips at four weeks post inoculation. No variation was found among the repeats of the experiment (df=1,48; χ^2=0.00; P=1.000). The incidences of TSWV infection varied with host plants (df=4,45; χ^2=67.30; P<0.0001). TSWV incidences in peanut, tobacco, and tomato plants were not different (Fig. 6.1b). However, TSWV incidences in peanut, tobacco, and tomato plants were significantly greater than TSWV incidences in two stages of cotton (Fig. 6.1b). No incidences of TSWV infection (%) were found in cotton at cotyledon and first true leaf stages (Fig. 6.1b).

Similarly, the incidences of TSWV infection (%) were evaluated in peanut, tobacco, tomato, and two stages of cotton plants with RT-PCR at four weeks post inoculation. No

variation was found among repeats of the experiment (df=1,48; χ^2=0.00; *P*=1.000). The incidences of TSWV infection varied with host plants (df=4,45; χ^2=67.30; *P*<0.0001). TSWV incidences in peanut, tobacco, and tomato plants were not different (Fig. 6.1c). However, TSWV incidences of peanut, tobacco, and tomato plants were significantly greater than TSWV incidences in two stages of cotton (Fig. 6.1c). No incidences of TSWV infection (%) were found in cotton at cotyledon and first true leaf stages (Fig. 6.1c and Fig. 6.2).

Back transmission of TSWV from cotton to peanut. The incidences of TSWV infection (%) between treatments (df=1,38; χ^2=37.94; *P*<0.0001) varied significantly but not with repeats of the experiment (df=1,38; χ^2=0.40; *P*=0.5278). No TSWV incidence was observed in peanut plants when potentially viruliferous thrips reared on DAS-ELISA positive cotton foliage were used for inoculation. On the contrary, the incidence of TSWV infection on peanut plants was 85.00 ± 1.58% (mean ± standard error) when potentially viruliferous thrips reared on TSWV-infected peanut plants were used.

***F. fusca* development on peanut and cotton following host switching** Adult emergence rates varied with treatments (df=2,57; *F*=14.48; *P*<0.0001) but not with repeats of the experiments (df=1,58; *F*=2.26; *P*=0.1384). In general, host switching from cotton to peanut increased thrips fitness (Fig. 6.3). Thrips from cotton colony produced 2.90 ± 0.31 (mean ± standard error) and 6.83 ± 1.04 adults per adult released when reared on cotton and peanut, respectively (Fig. 6.3). The adult emergence rate from peanut to peanut (8.41 ± 0.98) was greater than the adult emergence rate from cotton to cotton. These results clearly indicate that switching from cotton to peanut could increase the fitness of *F. fusca*. Developmental time of *F. fusca* reared on cotton and peanut varied from each other (df=2,57; χ^2=27.23; *P*<0.0001). Median developmental time from peanut to peanut was 14d (11-15) (median and range in parenthesis), from cotton to peanut

was 13d (11-13), and from cotton to cotton was 12d (10-13). Developmental time, in general was longer on peanut irrespective of the rearing host than on cotton.

Discussion

In this study the susceptibility of cotton plants at two stages (cotyledon and first true-leaf stage) to TSWV was evaluated using *F. fusca* mediated transmission assay. Other hosts such as peanut, tobacco, and tomato were included as controls. DAS-ELISA results indicated that both stages of cotton were infected with TSWV. Results suggested that the cotton plants were more susceptible at the cotyledon stage than first true leaf stage. Groves et al. (1998) conducted a *F. fusca*-mediated transmission assay using four cotton varieties (DP 20, DP 52, DP 5409, and HS 46) at three different stages (cotyledon, first true leaf, and four leaf stages). Using mechanical inoculation on an indicator host and DAS-ELISA, they observed 10, 63, and 5% TSWV infection for the three developmental stages of cotton, respectively. This indicated that the first true leaf was the most susceptible to TSWV among the tested developmental stages of cotton. On the contrary, our results showed that the cotyledon stage was more susceptible to TSWV than the first true leaf stage of cotton. It is not clear why such differences were observed; the different cotton variety (DP 1050B2RF) used in this study as well as the thrips rearing protocol and the virus isolate used in this study could have influenced the outcome. In general, the incidence of TSWV infection in cotton seedlings were at least 2.8 times less than the incidences of TSWV infection in peanut, tobacco, and tomato. Additionally, cotton did not exhibit any symptoms suggestive of TSWV infection post inoculation, whereas typical TSWV symptoms were observed in other hosts (Krishna Kumar et al. 1993, Groves et al. 1998, Mandal et al. 2006, Shrestha et al. 2013). Together, these results indicated that cotton is susceptible to TSWV but to

a lesser degree when compared with the other predominant TSWV susceptible crop hosts in the southeast.

Another earlier study also reported TSWV incidence in cotton using the same serological technique (DAS-ELISA) from Texas (Schuster and Haliwell 1994). To examine the susceptibility of cotton to TSWV further, we retested DAS-ELISA positive samples using another technique that utilizes the same monoclonal antibodies in immunostrips and also with a nucleic acid-based detection technique (RT-PCR). It is widely believed that DAS-ELISA is more sensitive than immunostrips and that RT-PCR is more sensitive than both the serological techniques (Dang et al. 2009, Liebenberg et al. 2009). Results revealed that DAS-ELISA positive cotton plants did not test positive by the other two techniques. On the contrary, there was one hundred percent congruency in the incidences of TSWV infection between DAS-ELISA and the other two techniques for peanut, tomato, and tobacco. These results suggested that the observed DAS-ELISA positive cotton samples could indeed be false positives. It is not uncommon for DAS-ELISA to generate false positives when unintended hosts are tested (Timmerman et al. 1985, Smith et al. 2006). False positives could be generated through non-specific binding leading to development of high background absorbance in microtiter plates. Though there are options to ameliorate this high background absorbance issue with DAS-ELISA (Towbin and Gordon 1984, Smith et al. 2006), it is generally recommended that DAS-ELISA results be confirmed with other detection methods and by conducting inoculation and/or transmission assays (Timmerman et al. 1985).

Since the DAS-ELISA positive cotton samples did not test positive by the other two techniques used in this study, to further examine the susceptibility of cotton to TSWV, a *F. fusca* mediated back transmission assay was conducted with cotton foliage as the inoculum source and

one-week old peanut plants as recipients. Another treatment in the assay included inoculation of peanut seedlings with potentially viruliferous thrips that were reared on TSWV-infected peanut foliage. None of the peanut plants inoculated with potentially viruliferous thrips from TSWV-infected cotton foliage were infected with TSWV, whereas ~85% of peanut plants inoculated with potentially viruliferous thrips were infected with TSWV. These results suggest that cotton might not be a suitable host for TSWV. These results should be carefully interpreted as one cotton cultivar alone as evaluated in this study. Nevertheless, these results cast a serious doubt on the ability of cotton to serve as a TSWV inoculum source and influence TSWV epidemics in peanut.

Though it seems unlikely that cotton could contribute to TSWV epidemics in peanut by serving as an inoculum source, it could indirectly do so by serving as a thrips reservoir. In this study we evaluated the suitability of cotton as a thrips host as well as the effects of host switching from cotton to peanut. Thrips reared on cotton and peanut were used for this study. When thrips from cotton colony were reared on cotton and peanut for an entire generation (adult to adult) results indicated that the adult emergence rate in peanut was twice as that of cotton. The adult emergence rates were on peanut from peanut colony was three times that of thrips from cotton to cotton and switching hosts from cotton to peanut increased the fitness of *F. fusca*. These results suggest that peanut in general is a better host for *F. fusca*. This could be due to the fact that peanut is a leguminous plant and typically possesses more nitrogen than non-leguminous plants (Scheublin et al. 2004). Increased nitrogen content could translate to increased availability of amino acids and/or protein. Availability of increased amino acids could profoundly affect thrips development as they are often limiting in most plants that phytophagous thrips colonize and reproduce (Wheeler 1996, Rojas et al. 1998, Klowden 2007, Lundgren 2009).

Besides nutrients, secondary metabolites in cotton could also limit thrips development when compared with secondary metabolites in peanut (de Jager et al. 1996).

Therefore, cotton might not significantly contribute to TSWV epidemics in peanut by serving as an inoculum source. Though cotton is a poor thrips host when compared with peanuts, considering the acreage of cotton in the southeastern United States and the proximity of cotton fields to peanut fields, it is very likely that a substantial amount of thrips disperse from cotton to peanut. Such dispersal could indirectly aid in hastening TSWV spread in peanut fields.

Literature Cited

Adkins, S. 2000. *Tomato spotted wilt virus* – positive steps towards negative success. Mol. Plant Pathol. 1: 151-157.

Cho, J. J., R. F. L. Mau, D. Gonsalves, and W. C. Mitchell. 1986. Reservoir weed hosts of *Tomato spotted wilt virus*. Plant Dis. 70:1014-1017.

Cho, J. J., R. F. L. Mau, W. C. Mitchell, D. Gonsalves, and L. S. Yudin. 1987. Host list of plants susceptible to *Tomato spotted wilt virus* (TSWV). Research Extension Series 078, Hawaii Institute of Tropical Agriculture and Human Resources, Honolulu, Hawaii. 1-10.

Clark, M. E., and A. N. Adams. 1977. Characteristics of the microplate method of enzyme linked immunosorbent assay for the detection of plant viruses. J. Gen. Virol. 34: 475-483.

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

Culbreath, A. K., J. W. Todd, and S. L. Brown 2003. Epidemiology and management of tomato spotted wilt in peanut. Annu. Rev. Phytopathol. 41: 53-75

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

de Jager, C. M., R. P. T. Butôt, E. van der Meijden, R. Verpoorte. 1996. The role of primary and secondary metabolites in chrysanthemum resistance to *Frankliniella occidentalis.* J. Chem. Ecol. 22: 1987-1999.

Dang, P. M., D. L. Rowland, and W. H. Faircloth. 2009. Comparison of ELISA and RT-PCR assays for the detection of *Tomato spotted wilt virus* in peanut. Peanut Sci. 36: 133-137.

German, T. L., D. E. Ullman, and J. W. Moyer. 1992. *Tospoviruses*: diagnosis, molecular biology, phylogeny, and vector relationships. Annu.Rev. Phytopathol. 30: 315-348.

Groves, R. L., J. F. Walgenbach, J. W. Moyer, and G. G. Kennedy. 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. Phytopathol. 91: 891-899.

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

Groves, R. L., G. G. Kennedy, and J. F. Walgenbach. 1998. Inoculation of *Tomato spotted wilt virus* into cotton. Disease notes in Plant Dis. 82: 959.

Halliwell, R. S., and G. Philley. 1974. Spotted wilt of peanut in Texas. Plant Dis. Rep. 58: 23-25.

Jain, R. K., S. S. Pappu, H. R. Pappu, A. K. Culbreath, and J. W. Todd. 1998. Molecular diagnosis of *Tomato spotted wilt tospovirus* infection of peanut and other field and greenhouse crops. Plant Dis. 82: 900-904.

Johnson, W. C., T. B. Brenneman, S. H. Baker. A. W. Johnson, D. R. Summer, and B. G. Mullinix. 2001. Tillage and pest management consideration in a peanut-cotton rotation in the southeastern coastal plain. Agron. J. 93: 570-576.

Jones, D. R. 2005. Major review: plant viruses transmitted by thrips. Eur. J. Plant Pathol. 113: 119-157.

Klowden, M. J. 2007. Physiological Systems in Insects (2nd ed.).Academic Press, SanDiego, CA, USA.

Krishna Kumar, N. K., D. E. Ullman, J.J. Cho. 1993. Evaluation of *Lycopersicon* germplasm for tomato spotted wilt tospovirus resistance by mechanical and thrips transmission. Plant Dis. 77:938-941.

Liebenberg, A., M-J. Freeborough, C. J. Visser, D. U. Bellstedt, and J. T. Burger. 2009. Genetic variability within the coat protein gene of *Grapevine fanleaf virus* isolates from South Africa and the evaluation of RT-PCR, DAS-ELISA, and immunostrips as virus diagnostic assays. Virus Res. 142: 28-35.

Lundgren, J. G. 2009. Relationships of natural enemies and non-prey foods. Springer, USA.

Mandal, B., H. R. Pappu, A. S. Csinos, and A. K. Culbreath. 2006. Response of peanut, pepper, tobacco, and tomato cultivars to two biologically distinct isolates of *Tomato spotted wilt virus.* Plant Dis. 90: 1150-1155.

Mullis, S. and N. Martinez-Ochoa. 2009. Non-crop hosts of TSWV, pp. 20-21. *In* S. W. Mullis (ed.), Tospoviruses in solanaceae and other crops in the coastal plain of Georgia. University of Georgia, CAES Bulletin 1354, p. 51.

Munger, F. 1942. A method of rearing citrus thrips in the laboratory. J. Econ. Entomol. 35: 373-375.

NASS. 2014. National Agricultural Statistics Service. Crop production 2013 summary: updated January 2014. United States Department of Agriculture.

Pappu, H. R., R. A. C. Jones, and R. K. Jain. 2009. Global status of tospovirus epidemics in diverse cropping systems: successes achieved and challenges ahead. Virus Res. 141: 219-236.

Parrella, G., P. Gognalons, K. Gebre-Selassie, C. Vovlas, and G. Marchoux. 2003. An update of the host range of *Tomato spotted wilt virus*. J. Plant Pathol. 85: 227-264.

Riley, D. G., S. V. Joseph, R. Srinivasan, and S. Diffie. 2011. Thrips vectors of tospoviruses. J. Integ. Pest Mngmt. 1: 1-10.

Rojas, M.G., J. A. Morales-Ramos, E. G. King, G. Saldana, and S. M. Greenberg. 1998. Use of a factitious host and supplemented adult diet to rear and induce oogenesis in *Catolaccus grandis* (Hymenoptera:Pteromalida). Environ. Entomol. 27: 499-507.

Scheublin, T. R., K. P. Ridgway, J. P. W. Young, and M. G. A. van der Heijden. 2004. Nonlegumes, legumes, and root nodules harbor different arbuscular mycorrhizal fungal communities. App. Environ. Micro. 70: 6240-6246.

Schuster, G. L., and R. S. Haliwell. 1994. Six new hosts of *Tomato spotted wilt virus* in Texas. Disease Notes in Plant Dis. 78:100.

Shrestha, A., R. Srinivasan, S. Sundaraj, A. K. Culbreath, and D. G. Riley. 2013. Second generation peanut genotypes resistant to thrips-transmitted *Tomato spotted wilt virus* exhibit tolerance rather than true resistance and differentially affect thrips fitness. J. Eco. Ent. 106: 587-596.

Smith, T. N., S. J. Wylie, B. A. Coutts, and R.A.C. Jones. 2006. Localized distribution of *Iris yellow spot virus* within leeks and its reliable large-scale detection. Plant Dis. 90: 729-733.

Sundaraj, S., R. Srinivasan, A. K. Culbreath, D. G. Riley, and H. R. Pappu. 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. Phytopathol. 104: 202-210.

Srinivasan, R., D. Riley, S. Diffie, A. Shrestha, and A. Culbreath. 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. Environ. Entomol. (in press).

Timmerman, E. L., C. J. D. Arcy, and W. E. Splittstoesser. 1985. *Beet western yellows virus* in Illinois vegetable crops and weeds. Plant Dis. 69: 933-936.

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

Towbin, H., and J. Gordon. 1984. Immunoblotting and dot immunobindingÑcurrent status and outlook. J. Immunol. Methods 72: 313-340.

Ullman, D. E., J. J. Cho, R. F. L. Mau, W. B. Hunter, D. M. Westcot, D. M. Custer. 1992. Thrips-*Tomato spotted wilt virus* interactions: morphological, behavioral and cellular components influencing thrips transmission. Adv. Dis. Vect. Res. 9: 195-240.

Wheeler, D. 1996. The role of nourishment in oogenesis. Ann. Rev. Entomol. 41: 407-431.

Whitfield, A. E., D. E. Ullman, and T. L. German. 2005. Tospovirus-thrips interactions. Annu. Rev. Phytopathol. 43: 459-489.

Figures

Fig. 6.1. Percent (± SE) *Tomato spotted wilt virus* (TSWV) infection in peanut, tobacco, tomato, and cotton plants. Two stages of cotton were selected: cotyledon and first true leaf. Ten plants were used for each treatment. Approximately 0.05 g of pine pollen grains were dusted on each plant. Incidence of TSWV infection of plants was confirmed by DAS-ELISA (Fig.6.1a), immunostrip (Fig.6.1b), and RT-PCR (Fig.6.1c).

Fig. 6.2. A representative photograph of an electrophoresis gel. The ≈ 700 bp bands is an indication of *Tomato spotted wilt virus* infection in foliar samples from different host plant species. The lanes in the gel photograph represent: (A0 and B0) 1 kb ladder, (A1-A5) inoculated tobacco, (A6-A10) inoculated cotton at first true leaf stage, (A11) TSWV-infected tobacco, (A12) non-infected tobacco, (A13) no-template check, (B1-B5) inoculated tomato, and (B6-B10) inoculated peanut.

Fig. 6.3. Mean (± SE) cumulative counts of *Frankliniella fusca* adults per adult released on peanut and cotton foliage. Two leaflets of same size and age were placed in each Munger cage. Ten non-viruliferous female adult *F. fusca* were released on each cage and then removed after five days. Munger cages were maintained in growth chamber as previously described. Number of newly emerged adults were recorded and removed daily. The set-up was maintained until all larvae turned into adult for each cage.

Fig. 6.1a.

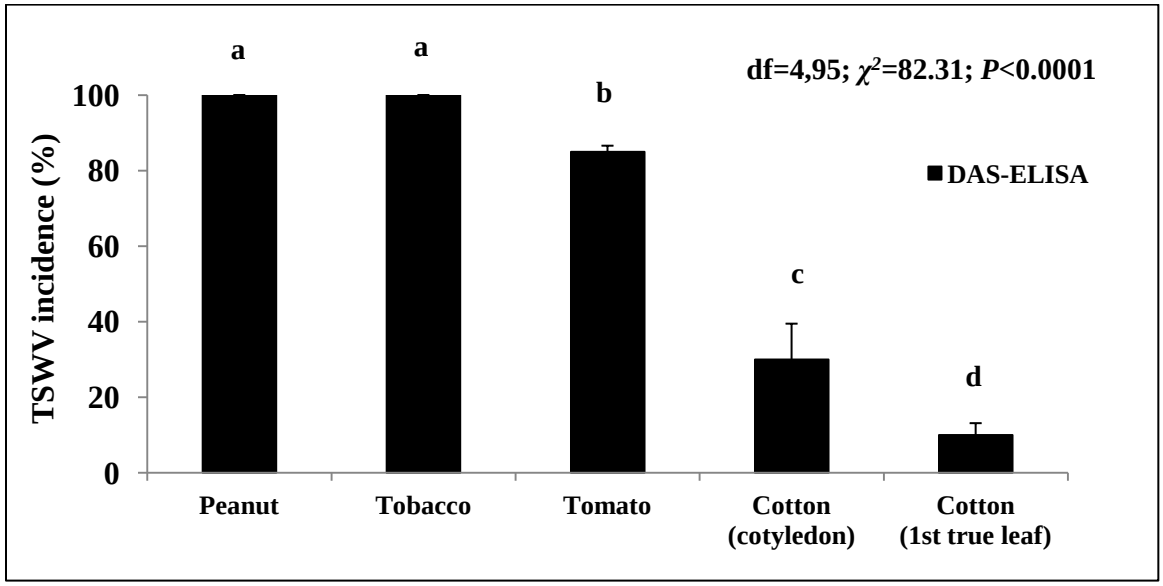
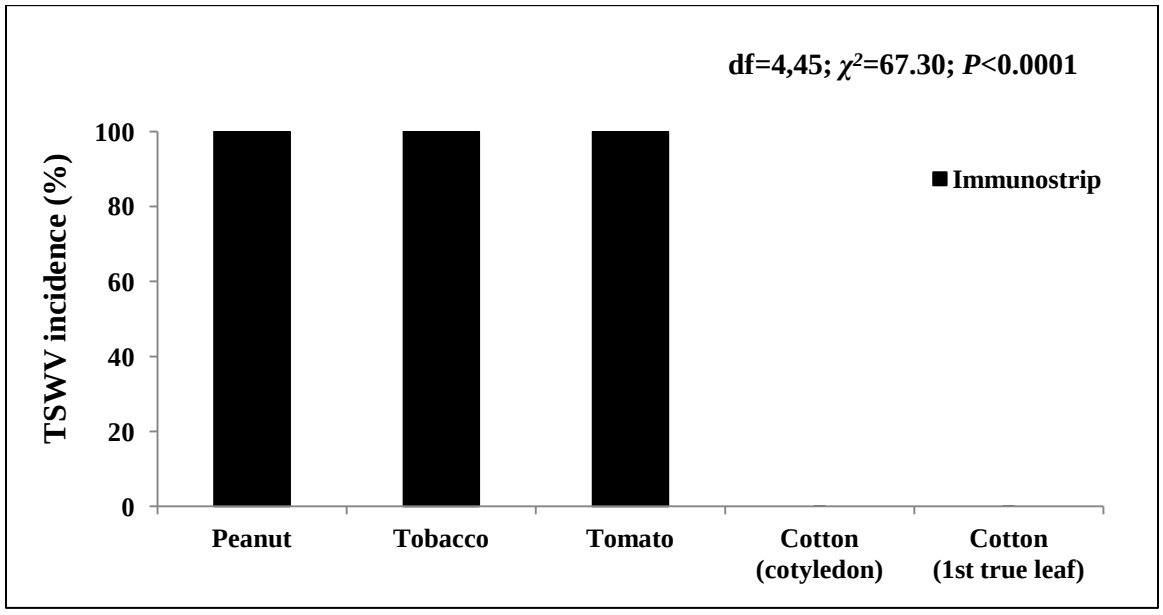


Fig. 6.1b.



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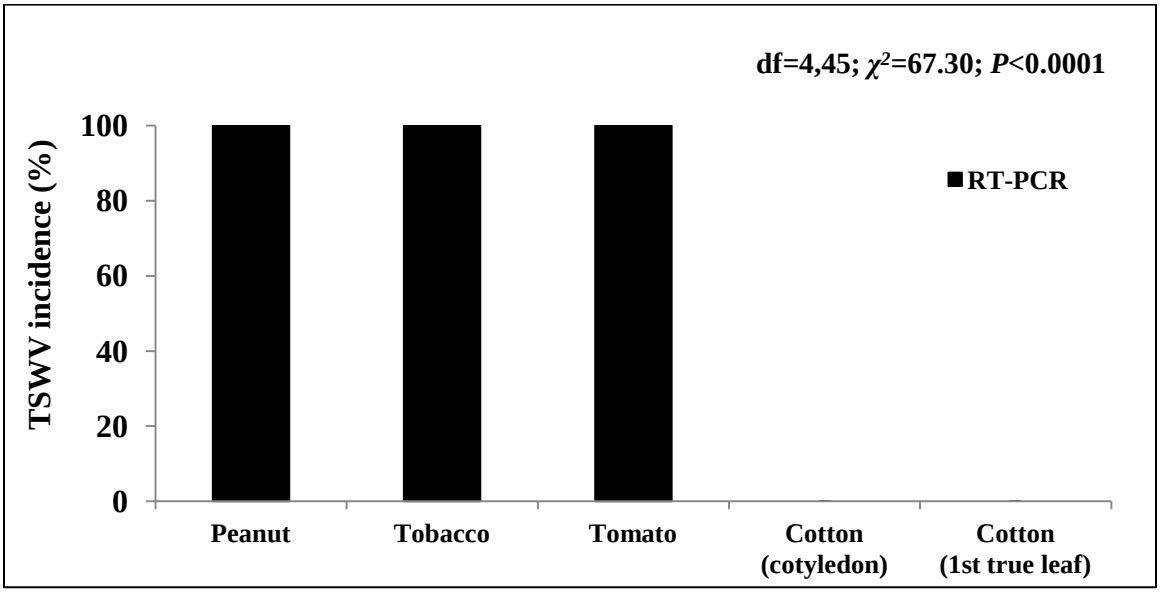


Fig. 6.2.

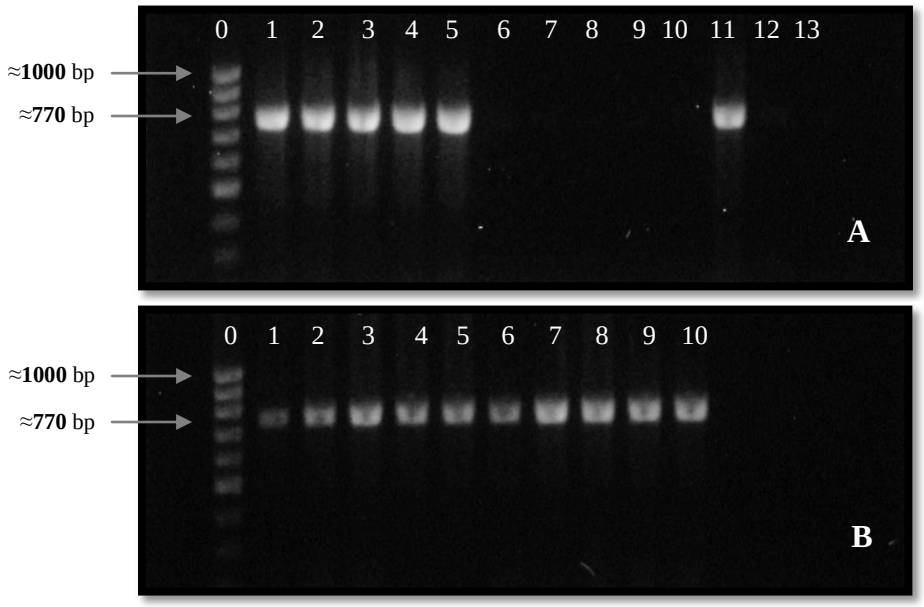
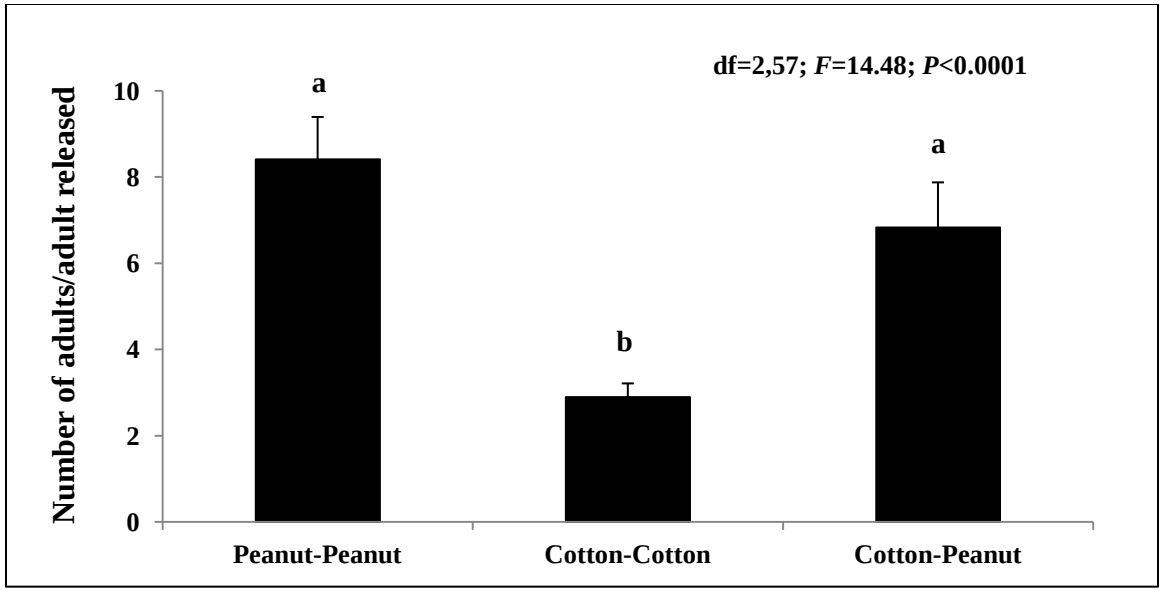


Fig. 6.3.


df=2,57; F=14.48; P<0.0001
Number of adults/adult released
10
8
6
4
2
0
a
b
a
Peanut-Peanut
Cotton-Cotton
Cotton-Peanut

CHAPTER 7

SUMMARY

The goal of this research was to evaluate a number of management tactics, including insecticides and cultural practices, against tobacco thrips, *Frankliniella fusca* (Hinds), and *Tomato spotted wilt virus* (TSWV) in peanut production in Georgia. In addition, effects of ecological factors in peanut farmscapes of Georgia, such as pine pollen dehiscence, were assessed on thrips and TSWV transmission. Furthermore, the role of other crops grown in proximity to peanut (e.g. cotton) as a thrips reservoir and a TSWV inoculum source was evaluated. There were four main objectives. The first objective focused on evaluating alternative insecticides to carbamate (aldicarb) and organophosphate (phorate) usage in peanut production. The effects of eight insecticides on thrips populations and spotted wilt incidence were assessed and compared with aldicarb and phorate. Efficacy of alternative insecticides was assessed based on four parameters: thrips population and feeding injury, spotted wilt incidence, and yield. Among the insecticides tested, imidacloprid (Admire® Pro), thiamethoxam (Actara®), spinetoram (Radiant®), and cyantraniliprole were as effective as aldicarb and phorate in suppressing thrips, thrips feeding injury, and reducing spotted wilt incidence without affecting the yields significantly. Greenhouse results also showed that selected alternative insecticides were as effective as aldicarb and phorate in reducing thrips feeding indices as well as TSWV infection. These results together suggested that aldicarb and phorate usage in peanut could be replaced with other effective insecticides without compromising yields.

The second objective investigated the integration of selected alternative insecticides with different cultural practices such as tillage systems, row patterns, and seeding rates. In general, our results indicated alternative insecticides in conjunction with cultural practices were as effective as aldicarb and phorate with cultural practices. Using alternative insecticides with cultural practices did not lead to an increase in thrips populations, spotted wilt incidence, and yield reduction. Additionally, the ability to use alternative insecticides as seed treatments, in-furrow treatments, and/or at-crack sprays would provide growers with more flexibility than using aldicarb and phorate. Both the older insecticides do not provide this flexibility and could only amenable as in-furrow treatments.

The goal of the third objective was to identify the effects of pine pollen, as supplemental source of protein, on thrips populations and their ability to transmit TSWV. The impact of loblolly pine (*Pinus taeda* L.) pollen on thrips feeding behavior, host preference, thrips fitness, and transmission of TSWV was examined. Olfactometer assays indicated that the addition of pine pollen positively influenced thrips host preference. Greenhouse assays also revealed that topical addition of pollen grains on peanut foliage enhanced thrips feeding. Microcosm experiments conducted in the laboratory showed that the addition of pine pollen on peanut leaflets increased thrips oviposition but did not affect other thrips fitness parameters. Perhaps, the presence of substantial amounts of free amino acids could have positively influenced oviposition. Even though pollen addition resulted in increased thrips feeding, it did not translate to increase TSWV transmission by thrips. These results indicated that impact of pine pollen on a host such as peanut might only have a marginal effect on the fitness of a polyphagous thrips species such as *F. fusca*. The reason being that peanut is a legume plant and could innately

possess more nitrogen content than other non-leguminous hosts and that *F. fusca* is more adapted to feeding on peanut foliage than other thrips species

The fourth objective assessed the role of cotton as thrips reservoir and as inoculum source for TSWV. It was long believed that cotton is a host of TSWV. It is also well known that cotton is a good thrips reservoir. However, the influence of cotton to TSWV epidemics in peanut is not quite clear. To evaluate cotton as a TSWV inoculum source, a transmission assay was conducted with cotton and three other TSWV hosts using potentially viurliferous *F. fusca*. TSWV infection in inoculated plants was tested by three detection techniques. DAS-ELISA results indicated greater incidences of TSWV infection in peanut, tobacco, and tomato than cotton. However, the use of other two detection techniques (immunostrips and RT-PCR) contradicted DAS ELISA results; none of the DAS-ELISA positive cotton plants were positive for TSVW infection with immunostrips and RT-PCR. These results questioned the ability of cotton to serve as a TSWV host. To further examine this issue, a back transmission assay was conducted with DAS-ELISA positive cotton foliage as an inoculum source and peanut plants as recipients. Peanut plants inoculated with potentially viruliferous thrips from TSWV positive cotton foliage were not infected with TSWV. These results cast a serious doubt on the ability of cotton plants to serve as TSWV inoculum sources and directly influence TSWV epidemics in peanut. However, fitness studies indicated that cotton could indirectly contribute to TSWV epidemics in peanut by serving as a thrips reservoir.