ECOLOGICAL INTERACTIONS OF *FRANKLINIELLA FUSCA* (HINDS) AND AN ORTHOTOSPOVIRUS IN THE PEANUT PATHOSYSTEM

by

PIN-CHU LAI

(Under the Direction of Rajagopalbabu Srinivasan and Mark R Abney)

ABSTRACT

Tomato spotted wilt orthotospovirus (TSWV) is an orthothospovirus causing spotted wilt disease in peanut, which is a major yield-limiting factor in peanut production in the United States. The predominant vector in the Southeast is *Frankliniella fusca* (Hinds), which is also a direct pest of peanut causing severe feeding injury on seedling foliage. While TSWV field-resistant cultivars have provided sufficient suppression of TSWV, insecticides were routinely applied solely for managing thrips population and feeding injury without economic justifications. A beat-cup sampling method was evaluated for thrips in peanut, and economic injury levels for *F. fusca* were established in TSWV resistant cultivars based on the relationships between thrips abundance, feeding injury, and yield. Thrips density exceeded the economic injury levels especially when thrips infestation occurred at early plant ages. Although TSWV resistant cultivars are planted on almost all Southeast US peanut acres, TSWV spread in those cultivars and the impact(s) of virus spread on yield were unclear. Analysis of temporal and spatial TSWV spread in resistant cultivars indicated that substantial secondary spread occurred after mid-season in addition to significant primary spread in early season. Early infection resulted in more severe symptoms than late infection, and significant yield losses to TSWV were observed in plants

showing symptoms before 91 days after planting. TSWV infection in peanut leaf and root tissue was commonly confirmed by DAS-ELISA. However, the effect of tissue type on the reliability of DAS-ELISA for TSWV detection has never been evaluated. Using RT-PCR and qRT-PCR as checks, DAS-ELISA overestimated TSWV infection when peanut root tissue was tested. Higher TSWV loads occur in leaf than root tissue and indicate that leaf tissue is a better sink for TSWV and a better tissue type for TSWV detection in peanut. The intensive use of TSWV-resistant cultivars raised concerns about the durability of resistance and changes in TSWV virulence. Evidence was not found to support exertion of selection pressure from resistance in peanut on the five TSWV genes. Some TSWV resistant cultivars differentially affected thrips fitness, which could help maintain the effectiveness of resistance in peanut.

INDEX WORDS: *Frankliniella fusca*, Tomato spotted wilt orthotospovirus, *Arachis hypogaea*, host plant resistance, economic injury levels, sampling method, virus spread, virus detection methods, population genetics

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DEDICATION

To my parents:

thank you for believing in me and always telling me that I can accomplish anything.

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Introduction

Cultivated peanut (*Arachis hypogaea* L.) is produced in the southern and southeastern United States, and the state of Georgia produces around 50% of domestic peanut annually (USDA-NASS 2020a). Tomato spotted wilt orthotospovirus (TSWV) causes spotted wilt in peanut, which is a serious disease accounting for losses of over $20 million US dollars annually in GA alone in recent years (Little 2017, 2019a, 2019b, 2020). Typical spotted wilt symptoms include concentric ringspots and chlorosis on leaflets, stunting of the above-ground plant parts, and small and irregular shaped pods and kernels (Culbreath and Srinivasan 2011).

TSWV is exclusively transmitted by nine species of thrips (Riley et al. 2011; Rotenberg et al. 2015). Tobacco thrips, *Frankliniella fusca* (Hinds), colonizes peanut soon after plant emergence and is the most abundance thrips species throughout the growing season (Todd et al. 1995). In addition to transmitting TSWV, *F. fusca* is also a direct pest of peanut causing severe feeding injury on peanut seedlings (Shear and Miller 1941; Wightman and Rao 1994; Young et al. 1972). Phorate, an organophosphate insecticide, is the only insecticide proven to be effective in suppressing TSWV incidence in addition to thrips control and is one of the major components in integrated management programs for TSWV in peanut (Brown et al. 2005; Culbreath et al. 2003; Marasigan et al. 2016; Srinivasan et al. 2017).

Thrips transmit TSWV in a persistent and propagative manner (Whitfield et al. 2005). Thrips can transmit TSWV only when they acquire TSWV in their first or early second instar stages (Pappu 2008; Van De Wetering et al. 1996; Whitfield et al. 2005). TSWV epidemics in peanut start with immigrating viruliferous thrips inoculating plants in peanut fields. Primary infection is important, as it is the only way to start an epidemic (Culbreath and Srinivasan 2011). As a result, almost all management tactics for TSWV are prophylactic and designed to reduce primary infection (Brown et al. 2005). Secondary spread can occur as in-field inoculum and *F. fusca* populations are available to spread TSWV from plant to plant in the field. However, limited studies have focused on secondary spread of TSWV in peanut. More importantly, effects of TSWV spread on disease severity and yield loss are largely unknown especially in TSWV resistant cultivars. Detecting TSWV in peanut is often achieved by serological or molecular techniques, such as DAS-ELISA and RT-PCR. However, the reliability of these detection methods can be affected by factors such as tissue type and age and virus distribution in infected plants (Lacroix et al. 2016; Sutula et al. 1986; Van Schadewijk et al. 2011). Peanut leaf and root tissues have commonly been used for TSWV detection. However, the reliability of DAS-ELISA and RT-PCR for detecting TSWV in different peanut tissue types has not been quantified.

The use of TSWV resistant cultivars is the most important management tactic available to peanut producers. While resistant cultivars are not immune to TSWV, they generally express less severe symptoms with reduced TSWV incidence and increased yield when compared to susceptible cultivars (Culbreath et al. 2003; Srinivasan et al. 2017). While newer resistant cultivars generally provide greater suppression of TSWV and increased yield when compared to older cultivars, the use of phorate for TSWV and thrips management has been decreasing. Nevertheless, growers routinely applied at-plant insecticides other than phorate only for

managing thrips and reducing feeding injury while the economic injury level of thrips on peanut was unknown. The stability of resistance in peanut is another concern that has been raised due to the intensive use of resistant cultivars for over twenty years. The underlying mechanisms of resistance in peanut are not fully understood. Population genetics of TSWV isolates among resistant cultivars could likely provide evidence for selection pressure exerted by resistance on TSWV. In addition, evaluating the impact of TSWV resistance on thrips fitness could further elucidate the role of resistant cultivars in three-way interactions of the pathosystem. In this study, we attempted to fill some of the important knowledge gaps that exist concerning the use of peanut resistant cultivars within the TSWV pathosystem.

Literature Review

Peanut production in the United States. Originating from South America, cultivated peanut, *Arachis hypogaea* L., is a tetraploid species in the family Fabaceae (Pattee and Stalker 1995). Peanut is also known as groundnuts, which came from the special biology of its reproduction process. After self-pollination, the above ground blooms wither with the formation of peanut ovaries, which is called pegs. The pegs grow down toward the ground forming a stem that extends to the soil where the pods form (Pattee and Stalker 1995). Peanut has high nutritional value and is a good source of plant-based protein, unsaturated fat, and fiber (Arya et al. 2016). In the United States, peanut is planted in the mid-Atlantic, southeastern, and southwestern regions. Georgia, Florida, Alabama, Texas, North Carolina, and South Carolina are the six major peanut producing states that together produce more than 90 % of the U.S. crop (USDA-NASS 2020a). Georgia is the top peanut producer and produces around 50% of the U.S. peanut crop annually. In 2019, planted area of peanut in the U.S. was estimated at 1.43 million

acres with 5.5 billion pounds of production valued at $1.1 billion US dollars (USDA-NASS 2020b). Runner, Virginia, Spanish, and Valencia are the four types of peanut commercially grown in the U.S. The Runner-type peanut, used primarily for peanut butter, makes up more than 80 % of U.S. production; while the Virginia-type accounts for 15 % of production, and is used mainly for snacks (Schnepf 2016).

Thrips in peanut. Thrips are tiny insects in the order Thysanoptera. Most of the thrips species considered serious crop pests are in the suborder Terebrantia and family Thripidae (Mound 1997; Riley et al. 2011). Thrips have a dorsoventrally flattened and slender body in yellow/orange to brown/black color, and the body length is less than 2 mm long (Lewis 1973; Moritz 1997). Adult thrips have two pair of fringed wings, which are the most distinct morphological character of thrips. The thrips life cycle includes six stages: egg, two larval stages, prepupal and pupal stages, and adult stage (Lewis 1973, Riley et al. 2011). The first and second instars of thrips larvae are active feeders, while the prepupal and pupal stages are immobile and non-feeding. Pupation may occur on any part of the plant or in some cases in the soil or litter (Ananthakrishnan 1993; Broadbent et al. 2003). The complete life cycle of thrips typically lasts for 10 to 30 days, depending on the thrips and host plant species, temperature, and relative humidity (Ananthakrishnan 1993; Lewis 1973; McDonald et al. 1998).

The damage caused by thrips to crops is usually the result of feeding on leaves, fruits, or flowers (Childers 1997; Morse and Hoddle 2006; Ullman et al. 1997). Mouthparts, located on the underside of their head, are asymmetrical. The feeding process can be described as piercing-sucking: a needle-like single mandible is protracted to pierce a hole through the cell walls, and a tube structure formed by a pair of maxillary stylets is used to suck up liquids in the cells (Kirk 1997; Mound 1971). As crop pests, thrips can injure plants directly by feeding and can also serve

as vectors transmitting viruses to the host plant. Tobacco thrips, *Frankliniella fusca* (Hinds), is considered as a major pest of peanut with its dual pest status: as a direct pest causing foliar damage and as an indirect pest transmitting tomato spotted wilt orthotospovirus (TSWV).

Tobacco thrips is the predominant thrips species colonizing peanut in the southern and southeastern United States (Tappan and Gorbet 1979, 1981; Todd et al. 1995). The first report of thrips attacking peanut was by Watson (1922) in Florida and stated that peanut plants can be permanently dwarfed upon severe thrips injury. Thrips are commonly found on peanut foliage soon after plant emergence, and severe foliage damage by thrips often occurs in the early season during seedling stages (Todd et al. 1995). Thrips use their sucking mouthparts to feed on newly emerging quadrifoliate buds that are folded at the terminal of the plants. Thrips cause feeding scars and lesions that result in distorted leaflets that in severe cases never expand. Damage to foliage adversely affects seedling growth by reducing photosynthetic area that can lead to stunting of the plant (Shear and Miller 1941; Wightman and Rao 1994; Young et al. 1972). Todd et al. (1995) found peak immature thrips numbers and severe foliar injury co-occurred at 28 to 35 days after planting, which suggested that immature thrips are responsible for most of the leaf injury in the early growing season.

The economic status of *F. fusca* in peanut has been debated since the early 1970s (Tappan and Gorbet 1979, 1981; Lynch et al. 1984, Smith and Sams 1977). Reducing thrips pressure in peanut by insecticides does not always correspond to higher yield. Insecticide applications resulted in inconsistent yield response in a number of studies across peanut producing areas in the United States (Lynch et al. 1984; Funderburk et al. 1998; Ames Herbert et al. 2007; Knight et al. 2015; Culbreath et al. 2016; Marasigan et al. 2016; Jordan et al. 2017; Mahoney et al. 2018; Marasigan et al. 2018; Brandenburg et al. 2019; Smith and Sams 1977).

Severe thrips injury on foliage often occurs when environmental conditions are not favorable for rapid growth of peanut seedlings, such as low temperature in the early spring (Wightman and Rao 1994). Significant yield reduction was more likely to occur when peanut plants were under other abiotic stress such as herbicide injury and water stress in addition to thrips injury (Herbert et al. 1991; Funderburk et al. 1998). Yield loss can be greater if the growing season is not long enough for peanut to compensate for the slow growth caused by thrips injury combined with other stress; this situation has been observed in peanut planted in Mid-Atlantic states (Herbert et al. 1991).

The economic status of *F. fusca* in peanut is unclear, but peanut growers routinely use at-plant insecticides to reduce thrips injury. Decision-making thresholds, economic injury levels, and thrips sampling methods are crucial components of integrated pest management, and they are currently lacking for thrips in peanut.

TSWV in peanut. Tomato spotted wilt orthotospovirus (TSWV) is the causal agent of spotted wilt disease, which can significantly reduce peanut yield in the United States (Culbreath et al. 2003). Since the first report of peanut spotted wilt disease in Texas in 1971, TSWV has become ubiquitous across all the major peanut producing states (Srinivasan et al. 2017). Spotted wilt is known to cause tremendous economic impact in peanut. For example, peanut yield losses to spotted wilt were estimated at over $20 million U.S. dollars annually in Georgia state in recent years (Little 2017, 2019a, 2019b).

Tomato spotted wilt orthotospovirus is the type species of the genus *Orthotospovirus* in the family *Tospoviridae* and the order *Bunyavirales* (Walker et al. 2020). The virus particles are spherical and are 80-120 nm in diameter. TSWV possesses a single-stranded, tripartite RNA genome, which consists of the large (L), medium (M), and small (S) RNAs. These RNA

segments are encapsidated by nucleocapsid protein (N) and enclosed in a host-derived, bilayer membrane with two virus-encoded glycoproteins projecting at the surface (Whitfield et al. 2005). The L RNA encodes the RNA-dependent RNA polymerase (RdRp) in the negative sense. RdRp of TSWV is suggested to play important roles in the genome replication (Adkins et al. 1995; De Haan et al. 1991). The M RNA encodes a nonstructural protein (NSm) in the positive sense and the Gn/Gc glycoprotein precursor in the negative sense (Kormelink et al. 1992). NSm is involved in cell-to-cell movements in plant hosts (Kormelink et al. 1994; Soellick et al. 2000; Storms et al. 1995). Gn/Gc glycoproteins participated in maturation and assembly of virions as well as thrips transmission (Bandla et al. 1998; Whitfield et al. 2005). The S RNA encodes another nonstructural protein (NSs) in the positive sense and the nucleocapsid protein (N) in the negative sense (De Haan et al. 1990). NSs was identified as an RNA silencing suppressor during plant infections (Takeda et al. 2002). N protein encapsidates the RNA segments to form ribonucleoprotein, which serves as the template for replication and transcription (Richmond et al. 1998). TSWV can infect at least 1090 plant species in 69 dicotyledonous and 15 monocotylenous families (Parrella et al. 2003). More importantly, a number of crop species, such as tomato, pepper, lettuce, potato, and tobacco are also TSWV hosts and suffer from severe yield reduction upon TSWV infections (Pappu et al. 2009).

TSWV induces an array of symptoms on peanut upon infection. Typical symptoms include concentric ringspots, patterns of chlorosis on leaflets, and stunting of the above ground plant parts with small and distorted foliage. Symptoms found in below-ground plant parts include small and misshaped pegs, pods, and kernels with reddish discoloration of seed coats (Halliwell and Philley 1974; Culbreath and Srinivasan 2011). TSWV infections can be quickly assessed visually by symptoms expression. However, TSWV symptoms can be confounded by other

biotic and/or abiotic factors, such as infection of other pathogens, injury from insect pests, chemical injury, temperature/water stress, and nutrition deficiency. Oftentimes, laboratory techniques are used to detect the presence of TSWV in plant tissue samples and confirm diagnoses based on observed symptoms. Assessment of TSWV infection in peanut tissues is most commonly aided by serology-based double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) (Culbreath et al. 1991; Dang et al. 2009; Kresta et al. 1995; Murakami et al. 2006; Pappu et al. 1999; Rowland et al. 2005; Wang et al. 2007). Relatively low cost and scalability for larger sample sizes have made DAS-ELISA the standard method for TSWV detection (Boonham et al. 2014; Torrance and Jones 1981; Ward et al. 2004). Nucleic acid-based reverse transcription polymerase chain reaction (RT-PCR) is also available but less frequently used for TSWV detection partly due to the requirement of RNA extraction from plant tissues, specialized equipment, and advanced operational skills with relatively higher cost than DAS-ELISA (Boonham et al. 2014; Dang et al. 2009; Jain et al. 1998; Pappu et al. 1999; Putnam 1995). Asymptomatic infection has been reported and considered to be fairly common based on DAS-ELISA of root tissues from field-grown peanut (Culbreath et al. 1991; Culbreath and Srinivasan 2011; Murakami et al. 2006); however, limited studies have focused on this topic, and the importance of asymptomatic infection in the TSWV pathosystem is unknown. TSWV detection is essential for studying TSWV in all aspects. When asymptomatic infection occurs, detecting TSWV in plant tissues is more crucial and often more challenging as symptoms were not available aiding selection of samples for detection. The reliability of detection methods can be affected by tissue type, virus distribution in infected plants, and plant age when a sample is collected (Lacroix et al. 2016; Sutula et al. 1986; Van Schadewijk et al. 2011).

TSWV transmission. TSWV is exclusively transmitted by thrips vectors (Whitfield et al. 2005). Among the nine species of thrips that are known vectors of TSWV, *F. fusca* and *Frankliniella occidentalis* (Pergande) are commonly found in most of the peanut-producing areas in the United States (Culbreath et al. 2003; Riley et al. 2011; Rotenberg et al. 2015). In peanut farmscapes, *F. fusca* is the predominant thrips species on peanut plants (>80%), whereas *F. occidentalis* is predominant in vegetation around cultivated fields (Lowry et al., 1992). While *F. occidentalis* primarily feed on the floral parts of the plant and mostly are found in peanut blooms, *F. fusca* are flower and foliage feeders and better colonizers of peanut. In addition, *F. fusca* is the most abundant TSWV vector species that can be found throughout the growing season (Riley et al. 2011; Todd et al. 1995). Hence, *F. fusca* is considered the primary vector of TSWV in peanut in the southeastern United States (Culbreath et al. 2003; Lowry et al. 1992).

Epidemics of TSWV can only occur when the three key components of the pathosystem: thrips vectors, TSWV, and host plants of both TSWV and thrips vectors, coincide in a suitable environment (German et al. 1992). Thrips transmit TSWV in a circulative and propagative manner. TSWV enters the midgut cells through binding to TSWV-specific receptors, and the virus multiplies and spreads within the thrips' body (Bragard et al. 2013; Casteel and Falk 2016; Pappu 2008; Whitfield et al. 2005). Transmission of TSWV by thrips vectors involves three distinct events: acquisition, latent period, and inoculation. Thrips acquire TSWV through feeding on TSWV infected plants, but acquisition of TSWV by thrips is stage specific. To become a transmitter, thrips have to acquire TSWV in their first and/or early second instar stages, and the acquisition efficiency decreases as thrips larvae age (Pappu 2008; Van De Wetering et al. 1996; Whitfield et al. 2005). Once TSWV enters the thrips body at the midgut, the virus replicates and spreads within the body where it is retained through molting, pupation, and emergence to the

adult stage (German et al. 1992; Kritzman et al. 2002; Ullman et al. 1993). While acquisition can happen in as little as 30 minutes, acquisition feeding often occurs throughout the larval development in nature (German et al. 1992; Ullman et al. 1992). There is no evidence of transovarial transmission, thus TSWV must be acquired through larval feeding (Wijkamp et al. 1996). Latent period is the time between thrips larvae acquiring TSWV and when they can subsequently transmit TSWV to healthy plants. During this time period, TSWV passes across several membrane barriers in the thrips body from midgut to surrounding muscle cells to the salivary glands. Once in the salivary glands, TSWV can be transferred to host plants through feeding and salivation (Casteel and Falk 2016; Ng and Falk 2006; Whitfield et al. 2005, 2015). The latent period of TSWV in thrips vectors ranges from 3 to10 days depending on temperature (German et al. 1992; Wijkamp and Peters 1993). Viruliferous thrips can transmit TSWV to non-infected plant hosts in an inoculation access period as short as 5 minutes (Allen and Broadbent 1986; Amin et al. 1981; German et al. 1992). Because thrips larvae lack wings and tend to have small range of movement, it has been suggested that adult thrips are the major transmitters of TSWV (German et al. 1992; Pappu 2008; Whitfield et al. 2015).

TSWV epidemiology. TSWV transmission by viruliferous thrips is the only important means of inoculation known to occur in natural epidemics in peanut (Culbreath et al. 2003; Culbreath and Srinivasan 2011; Ullman et al. 2002). TSWV has been detected in the pods and the testa of peanut kernels, but there is no evidence of TSWV seed transmission (Culbreath and Srinivasan 2011; Pappu et al. 1999). A source of viruliferous thrips and TSWV inoculum is the key factor for initiating TSWV epidemics in peanut. Several wild plant species occurring in peanut farmscapes during crop-free seasons (winter and early spring) have been identified as hosts of TSWV and thrips vectors (Groves et al. 2002). Furthermore, Srinivasan et al. (2014)

demonstrated that thrips can efficiently transmit TSWV from peanut to winter weed hosts and from infected weed hosts to peanut, which indicated the important contribution of winter weeds to the year-round persistence of TSWV in the farmscapes. Thrips larvae and adults have been found in post-season peanut fields on weeds and volunteer peanut plants. While the majority of *F. fusca* adults found in late fall and early spring were brachypterous (having nonfunctional, reduced wings), it is suggested that some *F. fusca* populations overwintered in post-season peanut fields without leaving the areas (Chamberlin et al. 1992). In addition, TSWV was detected in up to 10 % of macropterous adults (having fully developed wings) overwintering in post-season peanut fields, indicating the potential of those viruliferous thrips inoculating TSWV in peanut and initiating epidemics in the following new season (Chamberlin, Culbreath, et al. 1993). The two wing morphs of *F. fusca* adults might have different roles in TSWV epidemics in peanut; brachypterous adults were predominant during fall and winter, suggesting a role of harboring TSWV in the population over the winter, whereas macropterous adults were more common in the population in spring and summer suggesting a role of colonizing and transmitting TSWV to newly emerged peanut plants (Wells et al. 2002). However, destruction of winter annual weeds and volunteer peanut plants through tillage in winter and spring did not decrease the abundance of brachypterous *F. fusca* or TSWV incidence in the subsequent peanut crops (Chamberlin, Todd, et al. 1993). Moreover, even when insecticide application significantly reduced *F. fusca* abundance on volunteer peanut plants in early spring, incidence of TSWV was not decreased in the following peanut cropping season (Chamberlin, Todd, et al. 1993).

During TSWV epidemics in peanut fields, new TSWV infections can be found throughout the course of the growing season (Culbreath et al. 1996; Culbreath, Todd, Demski, et al. 1992; Camann et al. 1995; Culbreath et al. 1997). Studies of TSWV spread in peanut are

limited, though Camann et al. (1995) observed significant aggregation of TSWV-infected plants randomly distributed in peanut fields and hypothesized that a continuous immigration of viruliferous thrips led to the aggregated spatial patterns of TSWV-infected plants. The temporal progression of TSWV incidence in peanut cultivars used in the1990s was best described by the monomolecular model, which suggested a monocyclic epidemic. The authors concluded that most TSWV infections were a result of primary spread of TSWV with limited in-field (secondary) spread (Camann et al. 1995). Besides primary spread of TSWV driven by immigrating thrips, the availability of thrips vectors and inoculum in the field enable secondary spread to occur. While thrips populations normally peak before 35 days after peanut planting, TSWV thrips vectors can be found throughout the whole season in terminal buds and blooms (Todd et al. 1995). Lowry et al. (1995) found 2% of thrips collected from terminals and blooms of peanut plants from flowering to harvest were infected with TSWV, and most were *F. fusca*. *Frankliniella fusca* is able to colonize peanut plants, and secondary spread of TSWV could occur if the progeny of immigrating *F. fusca* acquire TSWV from peanut and transmit it to nearby virus-free plants in the same field (Lowry et al. 1995). Secondary spread of TSWV in peanut fields was also indicated by the positive correlation between abundance of in-field inoculum and TSWV incidence. Black et al. (1993) found higher TSWV incidence in plants surrounded by more in-field inoculum with TSWV susceptible cultivars when compared with those surrounded by TSWV resistant cultivars with less inoculum.

Spotted wilt disease severity greatly varies between individual infected plants within the same epidemic; disease severity in terms of symptoms ranges from only a few spots on very few leaflets to serious stunting that leads to plant death (Culbreath and Srinivasan 2011). The timing of TSWV symptom expression is a critical factor related to disease intensity and subsequent

yield losses in TSWV-infected plants. Most severe yield losses occur in infected plants with early symptom appearance (Culbreath, Todd, and Demski 1992; Narendrappa and Siddaramaiah 1986; Saharan et al. 1983).

Understanding TSWV spread in peanut can be beneficial for developing better TSWV management practices. The importance of primary spread of TSWV in peanut has been acknowledged by studies focused on finding the inoculum source that accounted for the initiation of the epidemic. However, the contribution of secondary spread of TSWV and mid- to late season infection has rarely been investigated. In addition, impacts of TSWV spread on disease severity and yield loss remain largely unknown, especially in modern TSWV resistant cultivars.

TSWV management. Managing TSWV in peanut relies on a combination of management tactics as none of the available tools provide sufficient suppression of TSWV alone (Culbreath et al. 2003). A number of management practices have been developed to reduce the risk of severe TSWV outbreaks (Brown et al. 2005). Several cultural practices are designed to reducethe exposure of peanut to thrips vectors. Adjusting planting dates to avoid the synchronization of peak thrips movement and peanut emergence has been shown to reduce TSWV incidence; the recommended planting window generally falls between 10 May and end of May in the Southeast for reducing TSWV despite agronomically, the ideal planting window begins earlier in mid- to late April (Culbreath et al. 2010; Mahoney et al. 2018; Tillman et al. 2007). Planting peanut in twin-row pattern and using reduced tillage methods are associated with reduced thrips feeding injury and TSWV incidence (Culbreath et al. 2008; Marasigan et al. 2018; Tubbs et al. 2011); it is suggested that the rapid ground coverage of twin-row pattern and more ground cover in reduced tillage may interfere with the ability of thrips to locate host plants (Brown et al. 2005). While several insecticides reduce thrips number and feeding injury on

peanut, phorate, an organophosphate insecticide, is the only insecticide that has been found to suppress TSWV incidence (Srinivasan et al. 2017). In addition to thrips control, phorate has been shown to induce plant defense responses; several genes related to signaling pathways, pathogenesis, and defense proteins were found to be upregulated in peanut treated with phorate (Jain et al. 2015).

Host plant resistance is the most important management tactic for TSWV in peanut. When TSWV first appeared in the United States and infected peanut, cultivars in use were highly susceptible to the virus. Intensive screening of peanut cultivars and breeding lines has identified several sources of TSWV resistance, and a number of resistant cultivars have been released from breeding programs over the years with newer cultivars possessing higher levels of resistance (Srinivasan et al. 2017). While complete immunity to TSWV has not been found in peanut, resistant cultivars expressed less severe symptoms and produced higher yield than susceptible cultivars upon TSWV infection. Hence, TSWV resistance in peanut is considered tolerance and is often referred to as field-resistance (Culbreath et al. 2016; Shrestha et al. 2013; Srinivasan et al. 2017). The underlying mechanisms of field-resistance in peanut have not been completely revealed. While such hypersensitive reaction has not been observed in peanut resistant cultivars, TSWV resistance in peanut likely governed by quantitative traits. Several quantitative trait loci (QTLs) have been linked to resistance in peanut (Agarwal et al. 2018; Khera et al. 2016; Pandey et al. 2017; Tseng et al. 2016). TSWV resistant cultivars have been widely adopted by peanut producers in the Southeast, and it is estimated that over 95% of peanut acreage is planted with resistant cultivars each year (Srinivasan et al. 2017). Although a number of TSWV resistant cultivars are available, only two resistant cultivars have been planted in the majority acreage of peanut fields in Georgia for the past twenty years. Since its release in 1995, Georgia Green was

the predominant cultivar for over ten years and later replaced by Georgia-06G in the late 2000s. The intensive use of monotonous resistant cultivars raised concerns about the durability of resistance in peanut. However, the effect of resistance in peanut on TSWV evolution has received little research attention. TSWV resistant cultivars have been found to differentially affect thrips preference and fitness (Shrestha et al. 2013; Sundaraj et al. 2014). The interaction between resistant cultivars and thrips could affect the degree of resistance to TSWV.

Research Objectives

TSWV in peanut has been studied for more than three decades, but the virus continues to be a major threat for the peanut industry in the United States. Managing TSWV in peanut is heavily dependent on the use of field-resistant cultivars. Several important research questions also revolve around the use of field-resistant cultivars within the complex of three-way interactions between TSWV, thrips vectors, and resistant peanut. The goal of this research was to investigate ecological interactions of TSWV and thrips with resistant peanut cultivars to fill the knowledge gaps in the TSWV pathosystem. In the first objective, we elucidated the relationships between thrips abundance, feeding injury, and peanut yield to develop economic injury levels for thrips in TSWV resistant cultivars while an effective sampling method for thrips in peanut was also evaluated. In the second objective, we examined temporal and spatial spread of TSWV in resistant cultivars and estimated yield losses in relation to primary and secondary spread of TSWV. In the third objective, we evaluated the reliability of serological and molecular detection methods for TSWV in leaf and root tissues of resistant peanut plants with or without symptom appearance. Lastly, in the fourth objective, we studied the population genetics of TSWV isolates from peanut cultivars with varying level of TSWV susceptibility to see if resistance in peanut

exerts selection pressure on TSWV, and we also examined the effect of several resistant cultivars on thrips fitness.

Literature Cited

Adkins, S., Quadt, R., Choi, T. J., Ahlquist, P., and German, T. 1995. An RNA-dependent RNA polymerase activity associated with virions of tomato spotted wilt virus, a plant- and insect-infecting bunyavirus. Virology. 207:308–311.

Agarwal, G., Clevenger, J., Pandey, M. K., Wang, H., Shasidhar, Y., Chu, Y., et al. 2018. High-density genetic map using whole-genome resequencing for fine mapping and candidate gene discovery for disease resistance in peanut. Plant Biotechnol. J. 16:1954–1967.

Allen, W. R., and Broadbent, A. B. 1986. Transmission of tomato spotted wilt virus in ontario greenhouses by frankliniella occidentalis. Can. J. Plant Pathol. 8:33–38.

Ames Herbert, D., Malone, S., Aref, S., Brandenburg, R. L., Jordan, D. L., Royals, B. M., et al. 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.

Amin, P. W., Reddy, D. V. R., Ghanekar, A. M., and Reddy, M. S. 1981. Transmission of Tomato Spotted Wilt Virus, the Causal Agent of Bud Necrosis of Peanut, by Scirtothrips dorsalis and Frankliniella schultzei. Plant Dis. :663–665.

Ananthakrishnan, T. N. 1993. Bionomics of thrips. Annu. Rev. Entomol. 38:71–92.

Arya, S. S., Salve, A. R., and Chauhan, S. 2016. Peanuts as functional food: a review. J. Food Sci. Technol. 53:31–41.

Bandla, M. D., Campbell, L. R., Ullman, D. E., and Sherwood, J. L. 1998. Interaction of tomato spotted wilt tospovirus (TSWV) glycoproteins with a thrips midgut protein, a potential

cellular receptor for TSWV. Phytopathology. 88:98–104.

Black, M. C., Andrews, T. D., and Smith, O. D. 1993. Interplot interference in field experiments with spotted wilt disease of peanut. In *Proc. Am. Peanut Res. Ed.*, , p. 65.

Boiteux, L. S., and de Ávila, A. C. 1994. Inheritance of a resistance specific to tomato spotted wilt tospovirus in Capsicum chinense "PI 159236." Euphytica. 75:139–142.

Boonham, N., Kreuze, J., Winter, S., van der Vlugt, R., Bergervoet, J., Tomlinson, J., et al. 2014. Methods in virus diagnostics: From ELISA to next generation sequencing. Virus Res. 186:20–31.

Bragard, C., Caciagli, P., Lemaire, O., Lopez-Moya, J. J., Macfarlane, S., Peters, D., et al. 2013. Status and prospects of plant virus control through interference with vector transmission. Annu. Rev. Phytopathol. 51:177–201.

Brandenburg, R. L., Jordan, D. L., Royals, B. R., Mahoney, D. J., and Johnson, P. D. 2019. Utilization of Imidacloprid to Control Thrips in Peanut in North Carolina. Peanut Sci. 46:8–13.

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

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

Camann, M. A., Culbreath, A. K., Pickering, J., Todd, J. W., and Demski, J. W. 1995. Spatial and temporal patterns of spotted wilt epidemics in peanut. Phytopathology. 85:879–885.

Casteel, C. L., and Falk, B. W. 2016. Plant virus-vector interactions: More than just for virus

transmission. In *Current Research Topics in Plant Virology*, Springer International Publishing, p. 217–240.

Chamberlin, J. R., Culbreath, A. K., Todd, J. W., and Demskj, J. W. 1993. Detection of Tomato Spotted Wilt Virus in Tobacco Thrips (Thysanoptera: Thripidae) Overwintering in Harvested Peanut Fields. J. Econ. Entomol. 86:40–45.

Chamberlin, J. R., Todd, J. W., Beshear, R. J., Culbreath, A. K., and Demski, J. W. 1992. Overwintering Hosts and Wingform of Thrips, Frankliniella spp., in Georgia (Thysanoptera: Thripidae): Implications for Management of Spotted Wilt Disease. Environ. Entomol. 21:121–128.

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

Childers, C. C. 1997. Feeding and oviposition injuries to plants. In *Thrips as crop pests*, ed. T. Lewis. Wallingford, UK.: CAB INTERNATIONAL, p. 505–538.

Culbreath, A. K., Csinos, A. S., Brenneman, T. B., Demski, J. W., and Todd, J. W. 1991. Association of Tomato Spotted Wilt Virus with Foliar Chlorosis of Peanut in Georgia. Plant Dis. 75:863C.

Culbreath, A. K., Selph, A. C., Williams, B. W., Kemerait, R. C., Srinivasan, R., Abney, M. R., et al. 2016. Effects of new field resistant cultivars and in-furrow applications of phorate insecticide on tomato spotted wilt of peanut. Crop Prot. 81:70–75.

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

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

Culbreath, A. K., Todd, J. W., and Brown, S. L. 2003. Epidemiology and Management of Tomato Spotted Wilt in Peanut. Annu. Rev. Phytopathol. 41:53–75.

Culbreath, A. K., Todd, J. W., and Demski, J. W. 1992. Productivity of Florunner Peanut Infected with Tomato Spotted Wilt Virus. Peanut Sci. 19:11–14.

Culbreath, A. K., Todd, J. W., Demski, J. W., and Chamberlin, J. R. 1992. Disease Progress of Spotted Wilt in Peanut Cultivars Florunner and Southern Runner. Phytopathology. 82:766.

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

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

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

Funderburk, J. E., Gorbet, D. W., Teare, I. D., and Stavisky, J. 1998. Thrips injury can reduce peanut yield and quality under conditions of multiple stress. Agron. J. 90:563–566.

German, T. L., Ullman, D. E., and Moyer, J. W. 1992. Tospoviruses: Diagnosis, Molecular Biology, Phylogeny, and Vector Relationships. Annu. Rev. Phytopathol. 30:315–348.

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

De Haan, P., Kormelink, R., De Oliveira Resende, R., Van Poelwijk, F., and Peters Goldbach, D. R. 1991. Tomato spotted wilt virus L RNA encodes a putative RNA polymerase. J. Gen. Virol. 72:2207–2216.

De Haan, P., Wagemakers, L., Peters, D., and Goldbach, R. 1990. The S RNA segment of tomato spotted wilt virus has an ambisense character. J. Gen. Virol. 71:1001–1007.

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

Herbert, D. A., Wilcut, J. W., and Swann, C. W. 1991. Effects of Various Postemergence Herbicide Treatments and Tobacco Thrips (Frankliniella fusca) Injury on Peanut Yields in Virginia. Peanut Sci. 18:91–94.

Jagger, M., and Richards, K. 2007. Temporal Analysis I: Quantifying and Comparing Epidemics. In *The Study of Plant Disease Epidemics*, eds. Laurence V. Madden, Gareth Hughes, and Frank van den Bosch. The American Phytopathological Society, p. 63–116.

Jain, M., Gallo, M., Chengalrayan, K., Shaikh, N. P., Macdonald, G. E., and Davis, J. M. 2015. Phorate-induced Host Defence Responses Condition Acquired Resistance to Tomato Spotted Wilt in Cultivated Peanut (Arachis hypogaea L.). J. Phytopathol. 163:853–866.

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

Jordan, D. L., Shew, B. B., and Brandenburg, R. L. 2017. Peanut Yield and Injury from Thrips

with Combinations of Acephate, Bradyrhizobium Inoculant, and Prothioconazole Applied in the Seed Furrow at Planting. Crop. Forage Turfgrass Manag. 3:cftm2016.11.0075.

Khera, P., Pandey, M. K., Wang, H., Feng, S., Qiao, L., Culbreath, A. K., et al. 2016. Mapping quantitative trait loci of resistance to tomato spotted wilt virus and leaf spots in a recombinant inbred line population of peanut (Arachis hypogaea L.) from SunOleic 97R and NC94022 ed. Jinfa Zhang. PLoS One. 11:e0158452.

Kirk, W. D. J. 1997. Feeding. In *Thrips as crop pests*, ed. T. Lewis. Wallingford, UK.: CAB INTERNATIONAL, p. 119–164.

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

Kormelink, R., De Haan, P., Meurs, C., Peters, D., and Goldbach, R. 1992. The nucleotide sequence of the M RNA segment of tomato spotted wilt virus, a bunyavirus with two ambisense RNA segments. J. Gen. Virol. 73:2795–2804.

Kormelink, R., Storms, M., Van Lent, J., Peters, D., and Goldbach, R. 1994. Expression and Subcellular Location of the NSM Protein of Tomato Spotted Wilt Virus (TSWV), a Putative Viral Movement Protein. Virology. 200:56–65.

Kresta, K. K., Mitchell, F. L., and Smith, J. W. 1995. Survey by ELISA of Thrips (Thysanoptera: Thripidae) Vectored Tomato Spotted Wilt Virus Distribution in Foliage and Flowers of Field-Infected Peanut. Peanut Sci. 22:141–149.

Kritzman, A., Gera, A., Raccah, B., Van Lent, J. W. M., and Peters, D. 2002. The route of tomato spotted wilt virus inside the thrips body in relation to transmission efficiency. Arch. Virol. 147:2143–2156.

Lacroix, C., Renner, K., Cole, E., Seabloom, E. W., Borer, E. T., and Malmstrom, C. M. 2016. Methodological guidelines for accurate detection of viruses in wild plant species. Appl. Environ. Microbiol. 82:1966–1975.

Lewis, T. 1973. *Thrips, their biology, ecology and economic importance.*

Little, E. L. 2017. *2015 Georgia Plant Disease Loss Estimates*.

Little, E. L. 2019a. *2016 Georgia Plant Disease Loss Estimates*.

Little, E. L. 2019b. *2017 Georgia Plant Disease Loss Estimates*.

Little, E. L. 2020. *2018 Georgia Plant Disease Loss Estimates*.

Lowry, V. K., Smith, J. W., and Mitchell, F. L. 1992. Life-Fertility Tables for Frankliniella fusca (Hinds) and F. occidentals (Pergande) (Thysanoptera: Thripidae) on Peanut. Ann. Entomol. Soc. Am. 85:744–754.

Lowry, V. K., Smith, J. W., Mitchell, F. L., and Crumley, C. R. 1995. Thrips Vectors Responsible for the Secondary Spread of Tomato Spotted Wilt Virus in South Texas Peanut. In *Thrips Biology and Management*, Springer US, p. 167–170.

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

Mahoney, D. J., Jordan, D. L., Brandenburg, R. L., Royals, B. R., Inman, M. D., Hare, A. T., et al. 2018. Influence of Planting Date and Insecticide on Injury Caused by Tobacco Thrips and Peanut Yield in North Carolina. Peanut Sci. 45:70–74.

Marasigan, K., Toews, M., Kemerait, R., Abney, M. R., Culbreath, A., and Srinivasan, R. 2018. Evaluation of alternatives to an organophosphate insecticide with selected cultural practices: Effects on thrips, frankliniella fusca, and incidence of spotted wilt in peanut farmscapes. J. Econ. Entomol. 111:1030–1041.

Marasigan, K., Toews, M., Kemerait, R., Abney, M. R., Culbreath, A., and Srinivasan, R. 2016. Evaluation of alternatives to carbamate and organophosphate insecticides against thrips and tomato spotted wilt virus in peanut production. J. Econ. Entomol. 109:544–557.

McDonald, J. R., Bale, J. S., and Walters, K. F. A. 1998. Effect of temperature on development of the Western Flower Thrips, Frankliniella occidentalis (Thysanoptera: Thripidae). Eur. J. Entomol. 95:301–306.

Moritz, G. 1997. Structure, growth and development. In *Thrips as crop pests*, ed. T. Lewis. Wallingford, UK.15-63: CAB INTERNATIONAL, p. 15–63.

Morse, J. G., and Hoddle, M. S. 2006. Invasion biology of thrips. Annu. Rev. Entomol. 51:67–89.

Mound, L. A. 1997. Biological diversity. In *Thrips as crop pests*, ed. T. Lewis. Wallingford, UK.: CAB International, p. 197–215.

Mound, L. A. 1971. The feeding apparatus of thrips. Bull. Entomol. Res. 60:547–548.

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

Murakami, M., Gallo-Meagher, M., Gorbet, D. W., and Meagher, R. L. 2006. Utilizing immunoassays to determine systemic tomato spotted wilt virus infection for elucidating field resistance in peanut. Crop Prot. 25:235–243.

Narendrappa, T., and Siddaramaiah, A. L. 1986. Estimation of loss in yield due to bud necrosis of groundnut. Plant Pathol. Newsl. 4:14–15.

Ng, J. C. K., and Falk, B. W. 2006. Virus-Vector Interactions Mediating Nonpersistent and Semipersistent Transmission of Plant Viruses. Annu. Rev. Phytopathol. 44:183–212.

Pandey, M. K., Wang, H., Khera, P., Vishwakarma, M. K., Kale, S. M., Culbreath, A. K., et al. 2017. Genetic dissection of novel QTLs for resistance to leaf spots and tomato spotted wilt virus in peanut (Arachis hypogaea L.). Front. Plant Sci. 8:25.

Pappu, H. R. 2008. Tomato Spotted Wilt Virus. In *Encyclopedia of Virology*, Elsevier Ltd, p. 133–138.

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

Pappu, S. S., Pappu, H. R., Culbreath, A. K., and Todd, J. W. 1999. Localization of Tomato Spotted Wilt Virus (Genus Tospovirus , Family Bunyaviridae) in Peanut Pods. Peanut Sci. 26:98–100.

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

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

Putnam, M. L. 1995. Evaluation of selected methods of plant disease diagnosis. Crop Prot. 14:517–525.

Richmond, K. E., Chenault, K., Sherwood, J. L., and German, T. L. 1998. Characterization of the nucleic acid binding properties of tomato spotted wilt virus nucleocapsid protein. Virology. 248:6–11.

Riley, D. G., Joseph, S. V., Srinivasan, R., and Diffie, S. 2011. Thrips vectors of Tospoviruses. J. Integr. Pest Manag. 1:1–10.

Roselló, S., Díez, M. J., and Nuez, F. 1998. Genetics of tomato spotted wilt virus resistance coming from Lycopersicon peruvianum. Eur. J. Plant Pathol. 104:499–509.

Rotenberg, D., Jacobson, A. L., Schneweis, D. J., and Whitfield, A. E. 2015. Thrips transmission of tospoviruses. Curr. Opin. Virol. 15:80–89.

Rowland, D., Dorner, J., Sorensen, R., Beasley, J. P., and Todd, J. 2005. Tomato spotted wilt virus in peanut tissue types and physiological effects related to disease incidence and severity. Plant Pathol. 54:431–440.

Saharan, G. S., Kaushik, C. D., Kaushik, J. C., and Singh, H. V. 1983. Occurrence effect on yield and yield components and vectors of bud necrosis virus of groundnut. Proc. Natl. Semin. Manag. Dis. oilseed Crop. :7–9.

Van Schadewijk, A. R., Meekes, E. T. M., Verbeek, M., and Verhoeven, J. T. J. 2011. Validation of plant virus detection. In *Acta Horticulturae*, International Society for Horticultural Science, p. 81–86.

Schnepf, R. 2016. *U . S . Peanut Program and Issues*.

Shear, G. M., and Miller, L. I. 1941. Thrips injury of peanut seedlings. Plant Dis. Report.

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

Smith, J. W., and Sams, R. L. 1977. Economics of thrips control on peanuts in Texas. Southwest. Entomol. 2:149–154.

Soellick, T. R., Uhrig, J. F., Bucher, G. L., Kellmann, J. W., and Schreier, P. H. 2000. The movement protein NSm of tomato spotted wilt tospovirus (TSWV): RNA binding, interaction with the TSWV N protein, and identification of interacting plant proteins. Proc. Natl. Acad. Sci. U. S. A. 97:2373–2378.

Srinivasan, R., Abney, M. R., Culbreath, A. K., Kemerait, R. C., Tubbs, R. S., Monfort, W. S., et al. 2017. Three decades of managing Tomato spotted wilt virus in peanut in southeastern United States. Virus Res. 241:203–212.

Srinivasan, R., Riley, D., Diffie, S., Shrestha, A., and Culbreath, A. 2014. Winter Weeds as Inoculum Sources of Tomato Spotted Wilt Virus and as Reservoirs for Its Vector, Frankliniella fusca (Thysanoptera: Thripidae) in Farmscapes of Georgia. Environ. Entomol. 43:410–420.

Stevens, M. R., Scott, S. J., and Gergerich, R. C. 1991. Inheritance of a gene for resistance to tomato spotted wilt virus (TSWV) from Lycopersicon peruvianum Mill. Euphytica. 59:9–17.

Storms, M. M. H., Kormelink, R., Peters, D., Van Lent, J. W. M., and Goldbach, R. W. 1995. The nonstructural NSm protein of tomato spotted wilt virus induces tubular structures in plant and insect cells. Virology. 214:485–493.

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

Sutula, C. L., Gillett, J. M., Morrissey, S. M., and Ramsdell, D. C. 1986. Interpreting ELISA Data and Establishing the Positive-Negative Threshold. Plant Dis. 70:722.

Takeda, A., Sugiyama, K., Nagano, H., Mori, M., Kaido, M., Mise, K., et al. 2002. Identification of a novel RNA silencing suppressor, NSs protein of Tomato spotted wilt virus. FEBS Lett. 532:75–79.

Tappan, W. B., and Gorbet, D. W. 1981. Economics of Tobacco Thrips Control with Systemic Pesticides on Florunner Peanuts in Florida12. J. Econ. Entomol. 74:283–286.

Tappan, W. B., and Gorbet, D. W. 1979. Relationship of Seasonal Thrips Populations to Economics of Control on Florunner Peanuts in Florida. J. Econ. Entomol. 72:772–776.

Thomas-Carroll, M. L., and Jones, R. A. C. 2003. Selection, biological properties and fitness of resistance-breaking strains of Tomato spotted wilt virus in pepper. Ann. Appl. Biol. 142:235–243.

Tillman, B. L., Gorbet, D. W., and Andersen, P. C. 2007. Influence of Planting Date on Yield and Spotted Wilt of Runner Market Type Peanut.

Todd, J. W., Culbreath, A. K., Chamberlin, J. R., Beshear, R. J., and Mullinix, B. G. 1995. Colonization and Population Dynamics of Thrips in Peanuts in the Southern United States. In *Thrips Biology and Management*, Springer US, p. 453–460.

Torrance, L., and Jones, R. A. C. 1981. Recent developments in serological methods suited for use in routine testing for plant viruses. Plant Pathol. 30:1–24.

Tseng, Y. C., Tillman, B. L., Peng, Z., and Wang, J. 2016. Identification of major QTLs underlying tomato spotted wilt virus resistance in peanut cultivar Florida-EPTM “113.” BMC Genet. 17:128.

Tubbs, R. S., Beasley, J. P., Culbreath, A. K., Kemerait, R. C., Smith, N. B., and Smith, A. R. 2011. Row Pattern and Seeding Rate Effects on Agronomic, Disease, and Economic Factors in Large-Seeded Runner Peanut. Peanut Sci. 38:93–100.

Ullman, D. E., Cho, J. J., Mau, R. F. L., Hunter, W. B., Westcot, D. M., and Custer, D. M. 1992. Thrips-Tomato Spotted Wilt Virus Interactions: Morphological, Behavioral and Cellular Components Influencing Thrips Transmission. In Springer, New York, NY, p. 195–240.

Ullman, D. E., German, T. L., Sherwood, J. L., Westcot, D. M., and Cantone, F. A. 1993. Tospovirus replication in insect vector cells: immunocytological evidence that the

nonstructural protein encoded by the S-RNA of Tomato spotted wilt tospovirus is present in thrips vector cells. Phytopathology. :900–905.

Ullman, D. E., Meideros, R., Campbell, L. R., Whitfield, A. E., Sherwood, J. L., and German, T. L. 2002. Thrips as vectors of tospoviruses. Adv. Bot. Res. 36:113–140.

Ullman, D. E., Sherwood, J. L., and German, T. L. 1997. Thrips as vectors of plant pathogens. In *Thrips as crop pests*, CAB INTERNATIONAL, p. 539–565.

USDA-NASS. 2020a. *Crop Production 2019 Summary*.

USDA-NASS. 2020b. *Crop Values 2019 Summary*.

Walker, P. J., Siddell, S. G., Lefkowitz, E. J., Mushegian, A. R., Adriaenssens, E. M., Dempsey, D. M., et al. 2020. Changes to virus taxonomy and the Statutes ratified by the International Committee on Taxonomy of Viruses (2020). Arch. Virol. 165:2737–2748.

Wang, M. L., Pinnow, D. L., and Pittman, R. N. 2007. Preliminary screening of peanut germplasm in the US collection for TSWV resistance and high flavonoid content. Plant Pathol. J. 6:219–226.

Ward, E., Foster, S. J., Fraaije, B. A., and McCartney, H. A. 2004. Plant pathogen diagnostics: Immunological and nucleic acid-based approaches. Ann. Appl. Biol. 145:1–16.

Watson, J. R. 1922. *The flower thrips*.

Wells, M. L., Culbreath, A. K., Todd, J. W., Csinos, A. S., Mandal, B., and McPherson, R. M. 2002. Dynamics of spring tobacco thrips (Thysanoptera: Thripidae) populations: Implications for tomato spotted wilt virus management. Environ. Entomol. 31:1282–1290.

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

Whitfield, A. E., Falk, B. W., and Rotenberg, D. 2015. Insect vector-mediated transmission of plant viruses. Virology. 479–480:278–289.

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

Wightman, J. A., and Rao, G. V. R. 1994. Groundnut pests. In *The Groundnut Crop*, Springer Netherlands, p. 395–479.

Wijkamp, I., Goldbach, R., and Peters, D. 1996. Propagation of tomato spotted wilt virus in Frankliniella occidentalis does neither result in pathological effects nor in transovarial passage of the virus. Entomol. Exp. Appl. 81:285–292.

Wijkamp, I., and Peters, D. 1993. Determination of the Median Latent Period of Two Tospoviruses in Frankliniella occidentalis, Using a Novel Leaf Disk Assay. Phytopathology. 83:986.

Young, S., Kinzer, R. E., Walton, R. R., and Matlock, R. S. 1972. Field Screening for Tobacco Thrips Resistance in Peanuts. J. Econ. Entomol. 65:828–832.

CHAPTER 2

DEVELOPING ECONOMIC INJURY LEVELS FOR *FRANKLINIELLA FUSCA* (HINDS) (THYSANOPTERA: THRIPIDAE) ON PEANUT IN THE SOUTHEASTERN UNITED STATE[1]

[1]Lai, P.-C., Buntin, G. D., Bag, S., Srinivasan, R., and Abney, M. R. To be submitted to *Journal of Economic Entomology*

Abstract

Tobacco thrips, *Frankliniella fusca* (Hinds), is a major pest of peanut, which causes feeding injury on seedling foliage and transmit tomato spotted wilt orthotospovirus (TSWV) in the southeastern United States. While TSWV-resistant cultivars provided sufficient suppression of TSWV incidence, insecticides were used not for the purpose of TSWV management but only to reduce thrips populations and feeding injury when the actual economic impact of *F. fusca* on peanut is unknown. The beat cup sampling method was developed for thrips in peanut to aid in pest management. The effect of thrips density and plant age at thrips infestation on peanut was evaluated using thrips-proof field cages with laboratory-reared thrips. In addition, relationship among thrips density, feeding injury, and yield was determined in small plot field experiments using insecticides to manipulate thrips populations, and economic injury levels (EILs) were calculated based on these relationships analyzed by multilinear regression. The beat cup sampling method was more accurate and precise to estimate adult thrips numbers at two to four weeks after planting (WAP). Thrips feeding injury had negative effects on plant growth and yield, and the effects were more prominent when the infestation occurred before 30 DAP. The strength of negative correlations between cumulated thrips-days (CTDs) and plant biomass varies with time depending on peanut market types planted in different geographic regions. EILs for *F. fusca* were established for two prominent TSWV resistant cultivars Georgia-06G (runner type) and Bailey (Virginia type). EILs were much lower for Bailey planted in NC than Georgia-06G planted in GA, which indicated a higher susceptibility of Bailey to *F. fusca* planted in NC. In GA, EILs were lower for an early planting date than a later planting date peanut. EILs for *F. fusca* in Georgia-06G planted in GA was 1.29-2.45 adult thrips per beat cup sample at 2 WAP.

Our data suggested that thrips density likely exceeds EILs easily, which would justify treatment action. The applications of EILs with practical considerations are discussed.

Introduction

Thrips (order Thysanoptera) are often found in cultivated peanut (*Arachis hypogea* L.) and are most abundant in the early growing season during seedling stages in the United States (Garcia et al. 2000; Marasigan et al. 2016, 2018; Todd et al. 1995). Watson (1922) first documented thrips injury to peanut in 1919 and reported that severe thrips injury may lead to permanently dwarfed plants. Thrips injury to peanut foliage is a result of the insect's feeding and ovipositing activities. Thrips have sucking mouthparts (Chisholm and Lewis 1984; Hunter and Ullman 1989; Mound 1971), and they feed on quadrifoliate buds at the tips of peanut plants. As the terminal buds grow, cells with feeding lesions do not grow or expand as the other uninjured cells, which results in distorted leaflets that never expand in severe cases. Damage to foliage reduces photosynthesis overall and leads to stunted seedling plants (Shear and Miller 1941; Wightman and Rao 1994; Young et al. 1972). Severe foliar injury generally occurs when immature thrips are present in large numbers, and thrips injury to peanut foliage is believed to be caused mainly by immature thrips feeding (Todd et al. 1995). Tobacco thrips, *Frankliniella fusca* (Hinds), and western flower thrips, *Frankliniella occidentalis* (Pergande), are the thrips species most commonly found in peanut in the Southeast. While adult thrips of both species can be found in peanut, *F. fusca* accounted for more than 95% of adult thrips in a multi-year study in Georgia (Marasigan et al. 2016, 2018). *Frankliniella fusca* is a better colonizer of peanut than *F. occidentalis* with higher reproduction and faster development (Lowry et al. 1992). Over 99% of the immature thrips collected from peanut terminal buds and flowers was *F. fusca* when they

were reared to adults in a study conducted in Tifton, GA in 1989 (Todd et al. 1995). Therefore, *F. fusca* is considered the predominant pest thrips species in peanut in the southern and southeastern United States (Tappan and Gorbet 1979, 1981; Todd et al. 1995).

The economic impact of thrips feeding injury on peanut has been a topic of debate. Studies in the late 1970s suggested that thrips injury was often more of a cosmetic issue than a yield-limiting factor (Tappan and Gorbet 1979, 1981; Lynch et al. 1984; Morgan et al. 1970). This belief was based on the observation that severe thrips injury occurring in the early vegetative stages did not cause significant adverse effects at pegging and pod-filling stages that are critical for yield production, and yield was not increased with the use of insecticides in most cases (Tappan and Gorbet 1979, 1981; Lynch et al. 1984; Morgan et al. 1970). Insecticides can reduce thrips number and feeding injury on peanut; however, suppressing thrips population does not always lead to higher yield. Inconsistent yield results from thrips management studies using insecticides have been reported (Lynch et al. 1984; Funderburk et al. 1998; Ames Herbert et al. 2007; Knight et al. 2015; Culbreath et al. 2016; Marasigan et al. 2016; Jordan et al. 2017; Mahoney et al. 2018; Marasigan et al. 2018; Brandenburg et al. 2019). Smith and Sams (1977) reviewed the literature from 1945 to 1977 and found that significant yield increases due to thrips control occurred in only 2 of 14 studies. More recently, several studies have found that the impact of thrips injury on peanut yield is affected by growing conditions. Severe thrips injury was often seen in young seedlings when spring temperatures were not high enough to promote rapid growth (Wightman and Rao 1994). When environmental conditions are not favorable for peanut growth and/or when there are abiotic stresses such as herbicide injury, yield losses due to thrips injury can be worsened (Herbert et al. 1991; Funderburk et al. 1998). In Virginia and North Carolina, the effect of multiple stress factors on yield is more prominent than other

growing areas in the U.S. at lower latitudes. The shorter growing season in the Mid-Atlantic states reduces the time peanut has to compensate for slow growth caused by early season stress (Herbert et al. 1991). Inherent differences in response of runner and Virginia peanut market types to thrips feeding injury could also contribute to greater observed yield losses in the Mid-Atlantic region where Virginia peanut types are grown.

The advent of tomato spotted wilt orthotospovirus (TSWV) in peanut in the United States elevated the pest status of *F. fusca* in peanut. TSWV is transmitted by several species of thrips including *F. fusca* and *F. occidentalis* (Todd et al. 1995; Culbreath et al. 2003; Riley et al. 2011). *Frankliniella fusca* became a serious pest mainly due to its role as a TSWV vector in peanut. Tremendous yield losses were attributed to spotted wilt disease caused by TSWV in peanut in the 1990s, and insecticide use to suppress thrips was an important TSWV management tactic (Culbreath et al. 2003). While a number of insecticides effectively reduce thrips populations and feeding injury on peanut, almost all of them fail to reduce TSWV incidence (Culbreath et al. 2003). Phorate, an organophosphate insecticide applied in-furrow at planting, is the only insecticide provides good efficacy against thrips and suppresses TSWV incidence in peanut. The underlying mechanism of TSWV incidence reduction is not a function of thrips population suppression but is due to induction of plant defense responses (Srinivasan et al. 2017; Jain et al. 2015). Hence, the prophylactic use of phorate became a key component of spotted wilt disease management in peanut (Brown et al. 2005). In addition to phorate use, several cultural practices that affect thrips abundance in peanut have also been demonstrated to reduce TSWV incidence; these include delaying planting until after10 May, using twin-row pattern and reduced tillage, and establishing higher plant populations. Combined with TSWV-resistant cultivars, these practices were integrated into a management program for TSVW in peanut.

With the success of integrated management programs for TSWV and especially the development of peanut cultivars with elevated resistance to TSWV since the late 2000s, phorate use has declined among peanut growers. Reasons for the reduction in phorate use are not completely known, but reduction in losses to TSWV due to resistant cultivars, the high cost of phorate relative to other insecticides, phytotoxicity associated with phorate, and the challenges associated with granular insecticide application likely contributed. Though phorate use has declined, insecticide use at planting has not. In a survey of peanut growers conducted by UGA Extension in 20XX, XX% of respondents said they used imidacloprid at planting or acephate after emergence for thrips management even though these products do not reduce the risk of TSWV (Monfort 20XX, unpublished data). These insecticides are applied in spite of the fact that no economic assessment of thrips impact on commonly used TSWV-resistant peanut cultivars is available. Without valid economic injury levels (EILs) for thrips on peanut, growers often choose to limit their risk of losses by applying insecticides. Nevertheless, insecticide use comes with a cost; quantifying the economic impact of thrips on peanut is required to evaluate the need for insecticide application. The lack of EILs for thrips in peanut represents a crucial knowledge gap preventing growers from making economically justified management decisions. Using insecticides without EILs likely leads to detrimental effects on the environment. In addition, without EILs, losses could come from thrips injury when thrips are not treated and losses could have been avoided by insecticide treatments; losses could also come from cost of application when treatments are not needed.

The objective of this study was to calculate EILs for *F. fusca* in TSWV-resistant peanut cultivars. The foundation of an EIL is the relationship between yield loss and pest abundance/level of pest injury. We assessed the impact of thrips feeding injury on peanut growth

and yield in a runner and a Virginia type peanut cultivar. We evaluated the effect of thrips density and plant age at thrips infestation on peanut yield. The relationships among thrips abundance, feeding injury, and peanut yield were assessed and utilized for calculating EILs. EILs could be based on a measure of pest density or damage caused by the pest. Pest density based EILs would be more effective and preferable because the measure is taken before or when the damage is caused, so management action can be taken in time. To implement thrips density based EILs, an efficient and reliable sampling method is required to monitor thrips density; however, such sampling methods for thrips are lacking in peanut. In this study, a dislodging-based sampling method for thrips were evaluated in peanut. EILs for *F. fusca* in a runner and a Virginia type TSWV-resistant peanut cultivar were calculated based on cumulative thrips-days and thrips density obtained from beat-cup sampling (runner type only).

Materials and Methods

Beat cup sampling method for thrips in peanut. Beat cup sampling, which involves dislodging thrips from plants, was evaluated as a method to measure thrips density in peanut. Thrips were sampled by tapping a single plant four times with a hand at a range of 5-10 cm vertically down from the top of the plant toward a plastic drinking cup (473 ml) with white interior. Immediately after tapping, 15 ml of 70% ethyl alcohol was dispensed from a 250 ml polypropylene wash bottle to rinse all the thrips to the bottom of the cup. The contents of the cup were transferred to a 20 ml glass sampling vial using a polyethylene transfer pipet (Fisherbrand, Thermo Fisher Scientific, Waltham, MA). To obtain the total thrips number, the sampled plant was then cut at the soil surface and placed in a resealable plastic bag for further thrips extraction. The above ground parts of a second whole plant 1 m away in the same row as the beat cup

sampled plant were collected without tapping. This plant served as a reference plant to validate the total thrips number of the beat cup sampled plant. Each plant was taken to the laboratory and placed in a Berlese funnel for 48 hr. Thrips extracted in Berlese funnels were collected in a sampling cup filled with 30 ml 70% ethyl alcohol at the bottom of each funnel. Thrips collected from beat cups and Berlese funnels were counted under a dissecting microscope (40X) (MEIJI TECHNO, Santa Clara, CA).

Beat cup samples were taken from peanut fields planted to runner type peanut cultivar Georgia-06G (Branch 2007) on 24 April 2019 at the Lang Farm of the University of Georgia (UGA) Tifton Campus and at the USDA ARS Jones Farm, and on 25 April 2019 on a commercial farm in Tift County, GA. Five beat cup samples were taken every week from two to six weeks after planting (WAP) at each location. The Virginia type peanut cultivar Bailey (Isleib et al. 2011), was planted on 2 May, 17 May, and 19 May 2016 at the North Carolina State University (NCSU) Peanut Belt Research Station in Lewiston, Border Belt Research Station in Whiteville, and Upper Costal Research Station in Rocky Mount, NC, respectively and on 9 May and 10 May 2017 in Lewiston and Whiteville, respectively. Twelve beat cup samples were taken once a week for two to four consecutive weeks starting at 2 or 3 WAP at each location.

Reference plants were only collected in Tifton, GA in 2019. Thrips collected in the beat cup and the Berlese funnel from individual plants were pooled as the total thrips sample and compared with thrips collected from the Berlese funnel of the respective reference plant with a paired t-test using PROC TTEST procedure in SAS (SAS Institute Inc., Cary, NC). The effect of plant age (fixed) and location (random) on total thrips number from beat cup sampled plants and the reference samples was evaluated by generalized linear mixed model analysis using PROC GLIMMIX procedure in SAS (SAS Institute Inc., Cary, NC).

The beat cup sampling method was evaluated for accuracy and precision at different plant ages and thrips life stages (adult thrips, immature thrips, and all thrips life stages (adult + immature)). Accuracy was defined as the proportion of the total thrips number that was obtained by beat cup sampling and was calculated as the thrips number collected in the beat cup divided by the total thrips number (beat cup + Berlese sample). In addition, the correlation between beat cup thrips number and the total thrips number was evaluated. Higher proportions of beat cup thrips number to total thrips number and higher positive correlation coefficients of the correlation between beat cup thrips number and total thrips number were considered more accurate. The effect of plant age and thrips life stage on the proportion of beat cup thrips number to total thrips number was analyzed by generalized linear mixed model analysis using PROC GLIMMIX procedure in SAS. Plant age and thrips life stage were assigned as fixed factors, while location was a random factor. Least square means (LS-means) of the proportions were used for multiple comparisons among plant age and thrips life stage at $\alpha = 0.05$ significance level with Tukey-Kramer adjustment. The correlation between beat cup thrips number and total thrips number was analyzed by Pearson's correlation analysis using PROC CORR procedure in SAS.

Precision was defined as the degree of error among thrips numbers by beat cup sampling. The precision of the beat cup sampling method was determined by calculating relative variance (RV): RV (%) = (SEM / X) 100, where X = the sample mean (thrips density), and SEM = the standard error of the sample mean. Smaller RV values represent higher precision. A set of samples from a sampling method with a RV value of less than 25% is considered sufficiently precise for the purpose of pest management (Pedigo and Rice 2009; Joost and Riley 2004; De

Paula Silva et al. 2019; Sowthwood 1978). Beat cup sampling data from Georgia and North Carolina were analyzed independently by market type.

Effect of thrips density and plant age at thrips infestation on peanut growth and yield in field cages. *Experimental design.* The effect of thrips density and plant age at thrips infestation on peanut growth and pod yield of individual plants was evaluated in a field experiment. The experiment was arranged in a split-plot, randomized complete block design with six replications. Plant age at thrips infestation was the main-plot factor and thrips density served as the sub-plot factor. Varying plant age at thrips infestation was achieved by releasing thrips on the same day onto plants of different ages, which were created by two to three different planting dates. Cages (1 m^3) constructed of thrips-proof screen were placed over a peanut row immediately after planting. Plant stands were thinned to three plants per cage after emergence. Thrips density treatments of 0, 5, 10, and 20 adult thrips per plant were randomly assigned to cages within each main plot (i.e., plant age at thrips infestation). *Frankliniella fusca* (female; up to five days old) from laboratory colonies maintained at UGA and NCSU were released onto the plants in cages at three to five weeks (depending on trial) after emergence of the earliest planted peanut.

Field trials. Peanut cultivar Georgia-06G was planted using a single-row garden planter on 12 May, 23 May, 2 June 2016 at the Lang Farm on the UGA Tifton Campus. *Frankliniella fusca* from a UGA laboratory colony established in 2009 were used (Marasigan et al. 2016; Shrestha et al. 2012, 2013, 2015, Srinivasan et al. 2017); *F. fusca* were reared on greenhouse-grown Georgia Green leaflets and maintained in small Petri dishes (35 mm diameter) with wet cotton rounds. The colony was maintained in a growth chamber (Thermo Fisher Scientific, Waltham, MA) at 25~30 ℃ and L14:D10 photoperiod. At 39 days after the first planting, thrips

from the colony were placed in microcentrifuge tubes (1.5 ml; Fisherbrand, Thermo Fisher Scientific, Waltham, WA) using a fine paintbrush in the laboratory; the tubes with thrips were then taken to the field and placed with lid open at the base of the plants in the field cages. In 2017, trials were conducted at the Lang and Ponder Farms on the UGA Tifton Campus. Due to limited field space at the Ponder Farm, only five replications were included. Peanut was planted on 16 May, 25 May, and 1 June at the Lang Farm, and on 18 May, 30 May, and 6 June at the Ponder Farm. To reduce possible interference of naturally occurring thrips from the environment, cages were placed in the field after sunset on the same day of planting. Thrips were released in cages at 41 and 43 days after the first planting at the Lang Farm and the Ponder Farm, respectively. Plant ages at thrips infestation were 39, 28, 18 days after planting (DAP) in the 2016 trial; 41, 32, 25 DAP in the 2017 Lang Farm trial; and 43, 31, 24 DAP in the 2017 Ponder Farm trial.

A Virginia-type peanut cultivar Bailey was planted on 2 May and 17 May 2016, and on 9 May and 18 May 2017 at the NCSU Peanut Belt Research Station in Lewiston, NC. *Frankliniella fusca* from a NCSU laboratory colony were placed in vials and released on caged plants when plants from the first planting reached 35 and 21 days after planting in 2016 and 2017, respectively. Plant ages at thrips infestation were 35, 20 DAP in the 2016 trial and 21, 12 DAP in the 2017 trial.

Data collection. Thrips feeding injury was rated weekly for two to four consecutive weeks starting at 7 days after thrips release. Thrips injury was rated on an arbitrary 0-10 scale where 0 = no injury, 1 = 10% of leaves injured, 2 = 20% of leaves injured, 3 = 30% of leaves injured, 4 = 40% of leaves injured, 5 = 50% of leaves injured and < 5% of terminals injured, 6 = 50% of leaves injured with <25% of terminals injured, 7 = 50% of leaves injured with <50% of

terminals injured, 8 = 50% of leaves injured with <75% of terminals injured, 9 = 50% of leaves injured with <90% of terminals injured, and 10 = dead plants (Brandenburg et al. 1998; Marasigan et al. 2016). One whole plant, including above and below ground parts, was removed from each cage at 14 days after thrips release and placed in a resealable plastic bag. Whole plant samples were transported to the laboratory and placed in Berlese funnels for 48 hr to extract thrips. Thrips were collected in a sampling cup filled with 30 ml 70% ethyl alcohol at the bottom of the Berlese funnel. All thrips collected from the Berlese funnel were counted under a dissecting microscope (40X) (MEIJI TECHNO, Santa Clara, CA). After thrips were removed, dry biomass of the whole plant was measured.

Two plants remained in each cage until harvest. In Georgia, above and below ground parts of caged peanut plants were manually harvested on 13 and 10 October at the Lang Farm in 2016 and 2017, respectively, and on 11 October at the Ponder Farm in 2017. All pods on each plant were removed, counted, and weighed. After pods were removed, the remaining plant tissues was dried and weighed. In North Carolina, caged plants were manually harvested on 14 and 18 September in 2016 and 2017, respectively. All pods on each plant were removed, dried, and weighed.

Statistical analyses. The relationships between number of thrips released and subsequent thrips density, thrips feeding injury, plant biomass, and pod weight were analyzed by correlation analyses using PROC CORR procedure in SAS. Thrips injury ratings were combined across sampling date to obtain cumulative thrips injury rating (CTIR). Pearson correlation coefficients were obtained for correlation between number of thrips released and thrips density, plant biomass, and pod weight, while Spearman correlation coefficients were obtained for correlation between number of thrips released and CTIR. Data were analyzed by plant age at thrips

infestation in each trial, and data were also pooled across plant age at thrips infestation in each trial. Simple linear regression analysis was conducted when a significant negative correlation was observed between number of thrips released and pod weight using PROC REG procedure in SAS. Yield reduction per thrips released was quantified by using the absolute slope value divided by the intercept of the regression models.

Effect of thrips density and planting date on peanut growth and yield in small plot field trials. *Field experiments and data collection.* Thrips density treatments were created by applying insecticides in the furrow at planting to manipulate naturally occurring thrips populations in peanut fields. Insecticide treatments were arranged in a randomized complete block design with four replications. Peanut was planted on two dates in each field season to increase the chance of high thrips pressure coinciding with peanut plants at growth stages susceptible to thrips feeding injury.

Runner type peanut cultivar Georgia-06G was planted in 6-row plots that were 9.14 m long with 0.91 m between row spacing at the Lang Farm at the UGA Tifton Campus on 12 May and 23 May in 2016, and 25 April and 3 May in 2017. Four and six levels of thrips pressure were created in 2016 and 2017, respectively, by insecticide treatments as listed in Table 2.1. Plant biomass, plant height and width, and thrips density were measured weekly from 2-6 WAP in all trials. Thrips density was estimated by randomly collecting 10 folded peanut terminals per plot. Terminals were placed in 20 ml sampling vials filled with 15 ml 70% ethyl alcohol. Thrips were also sampled from each plot using the beat cup sampling method as described previously. Thrips were counted under a dissecting microscope (40X) (MEIJI TECHNO, Santa Clara, CA) in the laboratory. One whole plant from each plot was removed each week and placed in a resealable bag, which was then dried, and weighed in the laboratory. Height and width of three randomly

selected plants in each plot were measured and recorded; the product of height and width was used as "plant size" variable for analyses. Thrips feeding injury was rated weekly from 3-6 WAP in the center two rows of each plot using the 0-10 arbitrary scale as previously described. The center two rows of each plot were harvested at 165 DAP for the 1st planting trial and 154 DAP for 2nd planting trial in 2016; in 2017, peanuts were harvested at 146 and 145 DAP for the 1st and 2nd planting trial, respectively.

Virginia type peanut cultivar Bailey was planted at the NCSU Peanut Belt Research Station in Lewiston, NC on 2 May and 17 May 2016 in 2-row plots that were 18.28 m long and on 9 May and 18 May 2017 in 4-row plots that were 9.14 m long. Three levels of thrips pressure were created by insecticide treatments as listed in Table 2.1. Plant biomass, plant height and width (i.e., plant size), thrips density, and thrips injury rating were obtained weekly from 3-7 WAP in 2016 and from 3-6 WAP in 2017. Data collection methods were the same as described previously for trials conducted in GA except that plant biomass was measured from three whole plants per plot on each sampling date and no beat cup samples were collected in NC. Peanut were harvested at xxx, xxx DAP for 1st and 2nd planting trial, respectively, in 2016, and at xxx, xxx DAP for 1st and 2nd planting trial, respectively, in 2017.

Statistical analyses. The effect of planting date on thrips density, thrips feeding injury, plant biomass, plant size, and yield within each year were evaluated by generalized linear mixed model analysis using PROC GLIMMIX procedure in SAS. Multiple comparisons were performed using LS-means with Tukey-Kramer adjustments when treatment effects were significant. Cumulative thrips injury rating (CTIR) was calculated by adding up feeding injury ratings from week to week as growing season progressed. The final CTIR was the sum of injury ratings from 3-6 WAP. Cumulative thrips-days (CTDs) was calculated based on the equation in

Ruppel (1983) using weekly thrips density from 10 terminals per plot. Cumulative thrips-days, the area under the curve of thrips density plotted over time, combined the magnitude and duration of thrips infestation into a single parameter. The final CTDs represented the cumulative thrips-days per 10 terminals from 3-6 WAP. Relationships between thrips and plant growth parameters were evaluated by Pearson's correlation analysis using the PROC CORR procedure in SAS; the relationship between CTDs/CTIR and plant biomass/ plant size (i.e., the product of height and width) were examined weekly from 2-6 WAP.

Extrapolation of economic injury levels for thrips in peanut. For the purpose of calculating EILs, the relationship between yield and final CTIR and between final CTIR and final CTDs were evaluated by linear regression analysis using PROC REG procedure in SAS. Outliers were identified by examining studentized residuals. Studentized residuals were calculated by dividing the residual by its standard deviation. If the studentized residual was greater than "3" (in absolute value), the data point was considered an outlier. Regression analyses were run using both original datasets and datasets with outliers removed. The significant regression model ($p<0.05$) with the highest R^2 value was selected. EILs were calculated based on the general EIL model from Pedigo et al. (1986): EIL = C/VIDK, where C is the cost of the management activity (insecticide application) per unit of production; V is the market value per unit of the produce; I is injury units per insect per production unit; D is damage per unit injury, and K is the proportionate reduction of insect attack with control. EILs were calculated based on cost of control ranging from US$40-60 per hectare and peanut price ranging from US$0.3-0.5 per kg. Damage per unit thrips injury (D) was obtained from the slope (in absolute value) of regression models between yield and final CTIR. Injury unit per thrips (I) was obtained from the slope (in absolute value) of regression models between final CTIR and final

CTDs. For simplicity, the effectiveness of control (K) was set to be 100%. EILs were calculated in the unit of CTDs per hectare. Data were analyzed by trial, and data combining all trials were analyzed as a separate dataset. EILs were calculated only when regression models between yield and final CTIR and between final CTIR and final CTDs were significant within the same dataset.

EILs were transformed from units of CTDs to units of adult thrips number at 2 WAP in beat cup samples for GA trials where beat cup sampling data were available. The relationship between adult thrips number per beat cup samples at 2 WAP and final CTDs was evaluated by regression analyses, and the regression model was used to obtain EILs with the unit of adult thrips number per beat cup sample at 2 WAP.

Results

Beat cup sampling method for thrips in peanut. *Runner type peanut Georgia-06G. Frankliniella fusca* was the predominant thrips species and accounted for 85% of adult thrips collected. The differences in total thrips number (beat cup + Berlese funnel) between the beat cup sampled plant and the reference plant was not affected by plant age ($F_{4, 56} = 0.17$; $p = 0.9523$); hence, data were pooled across plant age. The total thrips number of the beat cup sampled plants was not significantly different from the respective reference plants (df = 74; t = 1.10; $p = 0.2763$). The proportion of beat cup thrips number to total thrips number (beat cup + Berlese funnel) was significantly affected by thrips life stage ($F_{2,196} = 62.85$; $p < 0.0001$) and plant age ($F_{4,196} = 56.14$; $p < 0.0001$). A significant plant age by thrips life stage interaction was observed for the proportion of beat cup thrips number to total thrips number (beat cup + Berlese funnel) ($F_{8,196} = 5.86$; $p < 0.0001$). The proportion of adult thrips was significantly higher from plants at 2 and 3 WAP than at 4 and 6 WAP, and the proportion of immature and all thrips life

stages (adults + immature) collected in beat cups at 2 WAP was significantly higher than older plants (Table 2.2a). When data were pooled across sampling interval, the proportion of beat cup thrips number to total thrips number (beat cup + Berlese funnel) was higher for adult thrips than immature and all life stages combined (Table 2.2a). A positive correlation between beat cup thrips number and total thrips number (beat cup + Berlese funnel) across plant age was found for all thrips life stages, and the correlation coefficient for adult thrips was the highest (Table 2.2a). For adult thrips, positive correlation between beat cup thrips number and total thrips number was found at 2 and 3 WAP with high correlation coefficients (> 0.9). Positive correlations were found at 2 and 4 WAP for all thrips life stages combined and at 4 WAP for immature thrips. Precision of beat cup sampling, indicated by RV values, varied by thrips life stage, plant age, and location (Table 2.2b). Sufficient sampling precision (RV < 25%) was often achieved when samples were taken at 2 WAP (Table 2.2b).

Virginia type peanut Bailey. Thrips life stage and plant age significantly affected the proportion of beat cup thrips number to total thrips number (beat cup + Berlese funnel) (thrips life stage: $F_{2, 397} = 34.96$; $p < 0.0001$; plant age: $F_{3, 397} = 14.80$; $p < 0.0001$), and there was no interaction between plant age and thrips life stage ($F_{6, 397} = 1.16$; $p = 0.3290$). The proportion of beat cup thrips number to total thrips number (beat cup + Berlese funnel) was higher at 3 and 6 WAP than 4 and 5 WAP across thrips life stage, and the proportion of beat cup thrips number to total thrips number (beat cup + Berlese funnel) was higher for adult thrips than immature and all thrips life stages across plant age (Table 2.3a). A positive correlation between beat cup thrips number sample and total thrips number (beat cup + Berlese funnel) across plant age was found for all thrips life stages, and the highest correlation coefficient was found for adult thrips (Table 2.3a). For adult thrips, positive correlation between beat cup thrips number and total thrips

number (beat cup + Berlese funnel) were found at all plant ages, while positive correlation was found at 3 to 5 WAP for immature and all thrips life stages (Table 2.3a). Precision of the beat cup sampling method varied with thrips life stage, plant age, and trial (Table 2.3b). Sufficient sampling precision (RV < 25%) was more often found when samples were taken at 3 to 4 WAP (Table 2.3b).

Overall, using the beat cup method to sample adult thrips was more accurate than to sample immature and all thrips life stages, and the precision was best when beat cup sampling was conducted at 2 to 4 WAP. Regardless of thrips life stage, greater accuracy and precision were achieved with beat cups when younger plants were sampled. Overall, a more accurate estimate of thrips abundance was obtained when beat cup samples were taken at 2 to 4 WAP with adult thrips being counted.

Effect of thrips density and plant age on peanut growth and yield in field cages. The relationship between number of thrips released and thrips density at 14 days after thrips release, thrips feeding injury (i.e., CTIR), plant biomass, and pod weight were analyzed by Pearson's correlation analysis (Table 2.4).

Thrips density. Significant correlation between number of thrips released and subsequent thrips density per plant at 14 days after thrips release was only found in a few cases. There was a positive correlation between thrips density and the number of thrips released in plants with thrips released at 31 and 24 DAP in the 2017 Ponder Farm trial in GA; however, a negative correlation was observed between thrips density and the number of thrips released in plants with thrips released at 12 DAP in the 2017 trial in NC. When data were pooled across plant age at thrips infestation within each trial, a positive correlation was found between thrips density in field

cages and number of thrips released in the 2017 Ponder Farm trial in GA, but a negative correlation was found in the 2017 trial in NC.

Thrips feeding injury. A positive correlation between the number of thrips released and cumulative thrips injury rating (CTIR) was found in plants with thrips released at 32 DAP in the 2017 Land Farm trial in GA, at 35 and 20 DAP in 2016 trial in NC, and at 21 DAP in the 2017 trial in NC. When data were pooled across plant age at thrips infestation, a positive correlation was found between number of thrips released and CTIR in all the trails except the 2017 trail in NC.

Plant biomass. There was no significant correlation between number of thrips released and plant biomass measured at 14 days after thrips release. However, a negative correlation between number of thrips released and plant biomass at harvest was found in plants with thrips released at 28, 18 DAP in the 2016 trial and at 31, 24 DAP in the 2017 Ponder Farm trial in GA. When data were pooled across plant age at thrips infestation, a positive correlation was found between number of thrips released and plant biomass at harvest in the same two trials.

Pod weight. Negative correlation between number of thrips released and pod weight were found in plants with thrips released at 28 DAP in the 2016 trial in GA, at 31, 24 DAP in the 2017 Ponder Farm trial in GA, and at 12 DAP in the 2017 trial in NC; when data were pooled across planting dates, a negative correlation was observed between number of thrips released and pod weight in the three trials mentioned above.

Linear regression models were obtained when a significant negative correlation was found between number of thrips released and pod weight (Table 2.5). The slope of the regression models represented the reduction in pod weight of individual plants for each thrips released. Pod weight was reduced by 1.58-3.87% and 1.22-1.89% for each thrips released in Georgia-06G in

GA and Bailey in NC, respectively. The effect of plant age at thrips infestation was compared in the 2017 GA Ponder Farm trial; pod weight reduction was higher in plants with thrips released at 24 DAP than 31 DAP.

Effect of thrips density and planting date on peanut growth and yield in small plot field trials. *Runner type peanut cultivar Georgia-06G.* Different thrips densities were established in four small plot field trials as indicated by different final CTDs in GA (Fig. 2.1). Thrips density and plant biomass significantly varied by planting date in 2016 and 2017, while plant size varied by planting date in 2016 but not in 2017 (Table 2.6). Thrips density (beat cup sampling and terminal thrips samples) was higher in the 1st planting date than the 2nd planting date. Accordingly, plant biomass and plant size were lower in the 1st planting date than the 2nd planting date, but the effect of planting date on plant size was not significant in 2017 (Table 2.6). The effect of planting date on thrips feeding injury (i.e., CTIR) and yield was not significant in either year.

Significant correlation was only found in a few instances between CTDs/CTIR and plant biomass/size at 2-6 WAP. To increase the power of the analysis, data were pooled across trials to assess the relationship between thrips and plant growth. CTDs were negatively correlated with plant biomass at 3 to 6 WAP, but not at 2 WAP; while CTDs were negatively correlated with plant size at 2 to 5 WAP, but not at 6 WAP (Table 2.7). CTIR were negatively correlated with plant biomass at 3 to 6 WAP, and significant negative correlations between CTIR and plant size were found at 4 and 5 WAP but not at 3 and 6 WAP (Table 2.7). Overall, the strength of correlation between CTDs/CTIR and plant biomass/size, indicated by the correlation coefficients, increased with time up to 5 WAP and decreased thereafter (Table 2.7).

Virginia type peanut cultivar Bailey. Different thrips densities were established in all trials in NC (Fig. 2.2). The effect of planting date was mostly consistent across years, and thrips density, thrips feeding injury (i.e., CTIR), plant biomass, plant size, and yield varied significantly by planting date (Table 2.8). Thrips density and CTIR were higher in the 1st planting date than the 2nd planting date in both years. Plant biomass was lower in the 1st planting date than the 2nd planting date in both years, while plant size and yield was significantly lower in the 1st planting date than 2nd planting date in 2016 but not in 2017 (Table 2.8).

When data were analyzed by trial, significant correlation was only found in a few instances between CTDs/CTIR and plant biomass/size. Data were pooled across trials to increase the power of the analysis. CTDs were negatively correlated with plant biomass at 4 to 7 WAP and negatively correlated with plant size at 5 to 7 WAP, while CTIR negatively correlated with plant biomass at 5 to 7 WAP and negatively correlated with plant size at 4 and 7 WAP (Table 2.9). Overall, the strength of correlation between CTDs/CTIR and plant biomass/size increased from 4 to 7 WAP (Table 2.9).

Economic injury levels (EILs) for *F. fusca* in peanut. *Runner type peanut cultivar Georgia-06G.* Regression models using yield as a function of final CTIR were significant for both planting dates in 2017 and when data were pooled across trials; negative slopes of regression models indicated a negative correlation between peanut yield and final CTIR (Table 2.10). Regression models using final CTIR as a function of final CTDs were significant for all trials and when the data were pooled across trials; positive slopes indicated a positive correlation between final CTIR and final CTDs (Table 2.10). Datasets of 2017 1st planting trial, 2017 2nd planting trial, and the overall data pooled across trials in GA had significant regression models for yield and final CTIR and between final CTIR and final CTDs; hence, those datasets were

used to calculate EILs and was assigned as Model I, Model II, and Model III in the order as mentioned above (Table 2.10). EILs for *F. fusca* in Georgia-06G ranged from 113-283 CTDs based on Model I with data from 2017 1st planting trial; EILs ranged from 219-547 CTDs based on Model II with data from 2017 2nd planting trial; while using data pooled from all trials in GA, Model III provided EILs ranging from 298~746 CTDs (Table 2.11a). Overall, EILs were lower for the 1st planting trial (Model I) than the 2nd planting trial (Model II) in 2017, while EILs with data pooled across trials (Model III) were higher than EILs from Model I and Model II (Table 2.11a). Regression model using thrips number per beat cup sample at 2 WAP as a function of CTDs was significant in Model III but not in Model I or II (Table 2.10). EILs for *F. fusca* in cultivar Georgia-06G ranged from 1.29-2.45 adult thrips per beat cup at 2 WAP based on Model III (Table 2.11b).

Virginia type peanut cultivar Bailey. Regression models using yield as a function of final CTIR were significant in the 2nd planting trial of both years and when data were pooled across all trials; negative slopes of regression models indicated a negative correlation between peanut yield and final CTIR (Table 2.12). Regression models using final CTIR as a function of final CTDs were significant in all trials and when the data were pooled across planting date and year; positive slopes indicated a positive correlation between final CTIR and final CTDs (Table 2.12). Datasets of 2016 2nd planting trial, 2017 2nd planting trial, and the overall data pooled across planting date and year in NC had significant regression models between yield and final CTIR and between final CTIR and final CTDs; therefore, those datasets were used to calculate EILs and were assigned as Model IV, Model V, and Model VI in the order as mentioned above (Table 2.12). EILs for *F. fusca* in cultivar Bailey ranged from 20-50 CTDs based on Model IV with data from 2016 2nd planting trial; EILs ranged from 9-23 CTDs based on Model V with data from

2017 2nd planting trial (Table 2.13). Using data pooled from all NC trials, Model VI provided EILs ranging from 46-114 CTDs (Table 2.13). Overall, EILs for *F. fusca* in Bailey planted in NC were much lower than in Georgia-06G planted in GA.

Discussion

Thrips are the most commonly found early season insect species on peanut in the southeastern United States. Thrips infestation and injury on peanut seedlings were well-documented before 1990s (Lynch et al. 1984; Morgan et al. 1970; Tappan and Gorbet 1979, 1981). Nevertheless, thrips were not considered economically important until the advent of TSWV and the serious yield losses related to the disease caused by the virus since 1990s (Culbreath and Srinivasan 2011). *Frankliniella fusca* is the predominant thrips species that colonizes peanut plants in the Southeast. After TSWV arrived in the Southeast, the pest status of *F. fusca* increased substantially due to its ability to transmit the virus. Managing thrips in peanut was essential to reduce TSWV incidence (Todd et al. 1994, 1995, 1996). The development of TSWV-resistant cultivars and a number of cultural practices to suppress thrips transmission has greatly reduced the risk of severe yield losses in peanut. The use of field-resistant cultivars is the most important management tactic and has made other tactics including phorate application for TSWV management less critical for many peanut growers (Culbreath et al. 2016; Srinivasan et al. 2017). Peanut growers commonly target thrips with insecticides that have no impact on the incidence of TSWV. This practice suggests that growers assume thrips injury would reduce peanut yield even though no yield by thrips injury relationships have ever been published. In this study, the beat cup sampling method has been developed to quickly assess thrips density in the field for making management decision. The relationships among thrips density, feeding

injury, and yield have been evaluated, and EILs for thrips in peanut have been established based on those relationships.

While EIL is pivotal/essential for integrated pest management, monitoring pest populations is also a key component (Ehler 2006). Thrips population monitoring in peanut is not common because growers treated thrips with insecticides assuming thrips injury would reduce yield and insecticides would work effectively to manage thrips. Sampling methods such as peanut terminal and bloom sampling have been used for researchers (Lynch et al. 1984; Marasigan et al. 2016; Todd et al. 1995). However, thrips in terminal and bloom samples are hard to count in the field and usually are counted under microscopes in laboratory. Sampling method for thrips in peanut that can be readily used and quickly provide inferences on thrips density is not yet available. A sampling method that is useful for IPM decision making should be accurate, precise and simple enough to be completed quickly in the field. Beat cup sampling can be conducted with minimal equipment (i.e., a cup), and the beat cup sampling method is accurate and precise for sampling adult thrips at 2-4 WAP. Peak thrips injury in peanut usually occur at 4-5 WAP (Todd et al. 1995). Sampling thrips before 4 WAP would be ideal to assess thrips density in a timely manner for making inferences on whether insecticide treatment is needed. The accuracy and precision of the beat cup sampling method decreased as the plant grew in size, so this method is not as suitable for thrips sampling in peanut after 4 WAP as before 4 WAP.

The effect of thrips feeding on individual peanut plants was investigated by releasing laboratory-reared thrips in an in-field cage experiment. Thrips infestation negatively affected plant growth as indicated by negative correlation between the number of thrips released and plant biomass. The negative correlation was significant in 50% of the analyses, which indicated the impact of thrips on plant growth could likely be detected half of the time as factors other than

thrips affecting plant growth might also exist. In addition, TSWV infection had been found in less than 10% of the field cages, which could also potentially affect peanut growth. When plant biomass was negatively affected by thrips infestation, the pod weight was also reduced suggesting that thrips infestation can have negative impacts on plant growth and more importantly can affect yield. Significant negative impact of thrips on plant biomass and pod weight were more often found on plants infested by thrips before 30 DAP, which indicated that the susceptibility of peanut plants to *F. fusca* infestation decreased with increasing plant age. In general, *F. fusca* population is high in peanut seedlings and decline rapidly at 40~50 DAP, and the population remains low thereafter (Marasigan et al. 2016, 2018; Todd et al. 1995). Peanut plants enter reproductive stages at around 30 days after planting when growth rate increases and plants start branching and flowering (Boote 1982; Kingra and Kaur 2013); visible thrips injury is usually rapidly diluted by loads of new grown leaves. Peanut phenology could likely affect fitness of *F. fusca* because high thrips reproduction and feeding injury was only observed on peanut seedlings in the early season.

The impact of thrips feeding on peanut was also assessed by small plot experiments with a range of thrips populations established by insecticide applications. TSWV infection was the major external concern of this experiment. In GA trials, insecticides chosen for this experiment have little or no known impact on TSWV incidence and transmission. TSWV incidence was evaluated in GA trials after 90 DAP in 2016 and 2017, and incidence did not vary by insecticide treatments (data not shown). Thrips negatively impacted peanut growth as demonstrated by the negative correlation between CTDs/CTIR and plant biomass/plant size. In addition, the strength of the negative correlation between thrips density/injury and plant growth changed over time, and the changes in correlation strength were different for runner type peanut planted in GA and

Virginia type peanut planted in NC. Our results indicated that the two peanut cultivars planted in different states reacted to thrips infestation and feeding injury differently; Georgia-06G planted in GA likely grew out of thrips feeding injury when it reached to 40~50 DAP, while Bailey planted in NC did not seem to grow out of thrips feeding injury at 50 DAP. The slower growth of Virginia type peanut planted in NC than runner type peanut planted in GA due to lower temperature in NC than GA likely caused the difference in reaction to thrips infestation. Thrips density was higher in the early planting than the late planting trial, which was likely related to the time of peak thrips flights and plant emergence. Peak dispersal and thrips flight activities were observed at early to mid-May in Georgia and mid-Atlantic states (Morsello et al. 2008, M. R. Abney, unpublished data). An important tactic used by peanut producers to reduce the risk of thrips injury and TSWV incidence is to delay planting until after 10 May in order to avoid the peak period of thrips movement (Brown et al. 1996, 2005; Mahoney et al. 2018).

For individual peanut plants, peanut yield of Georgia-06G was reduced by 1.58 to 3.87% for every thrips released in the cage study, and the reduction was higher when plants were infested by thrips at 24 DAP than at 31 DAP. Whether yield reduction justifies management action can be assessed by comparing losses with the cost of control. For example, the cost of a phorate application equals 2.76% of net earnings of peanut production based on the average yield (4668 kg /ha) and market price ($0.44/kg) of runner type peanut in 2019 in Georgia (USDA-NASS 2020). Therefore, phorate application is justified if yield is reduced by 2.76% or more, which is within the range of calculated yield reduction based on results from field cages. Yield reduction based on the cage study indicates that thrips management is economically justified, especially under conditions that could increase the impact of thrips feeding injury on peanut yield, such as infestations occurring on plants before 30 DAP.

EIL calculations for *F. fusca* in cultivar Georgia-06G varied by dataset in the small plot field experiment; EILs were lower when calculations were based on an early planting dataset than based on a later planting dataset. The variation in EILs suggested that early planting peanuts might be more susceptible to thrips feeding injury than late planting peanuts, which could be related to the synchronization of peak thrips flights and plant emergence. EILs for *F. fusca* in cultivar Bailey planted in NC were much lower than EILs in cultivar Georgia-06G planted in GA, which indicated that Bailey planted in NC could be more susceptible to thrips feeding injury than Georgia-06G planted in GA. The difference in susceptibility to *F. fusca* could be due to the cultivar/market type itself or the respective environments. Further studies are required to elucidate the interaction of peanut market type and planting environment on the susceptibility to *F. fusca*. EILs were higher when the data were pooled across trials, which likely because more variations of relationships between thrips feeding injury and yield were included. EILs calculations based on a wider range of variation in thrips density and planting conditions increased EILs. Higher EILs would likely increase the risk of failing to treat thrips when needed in cases under certain conditions that are inducive for severe thrips injury.

EILs based on a measure of thrips density are more useful than EILs based on other equivalent measure of thrips or injury such as CTDs. EILs for *F. fusca* in Georgia-06G in GA ranged from 1.29 to 2.45 adult thrips per beat cup sample at 2 WAP based on the EIL model developed from pooled data in GA. The relationship between thrips numbers from beat cup sampling and CTDs were used to extrapolate EILs to a unit of thrips density because this sampling method is suitable for quick assessments of thrips density in the field. Two weeks after planting was chosen as the critical time for thrips density assessment because the decision for taking action to manage thrips needs to be made as early as possible. The frequency of thrips

density exceeding 2.45 adult thrips per beat cup sample at 2 WAP was 67% in cultivar Georgia-06G without insecticide applications (data not shown), which suggested that insecticide application would be warranted 67% of the time with thrips density exceeding EILs. In the ideal IPM paradigm, insecticides are only applied when economic thresholds are reached, which is lower than EILs. However, not all pest/cropping system interactions lend themselves to reactive treatment based on regular pest monitoring. High crop value, low tolerance for injury/contamination, and/or pest populations that are highly predictable can lead to prophylactic insecticide use. The most commonly used management practice for thrips after planting is foliar spray insecticides. However, in reality, applying foliar spray insecticides has some limitations that would increase the risk of not being able to take action on time as wanted; for example, growers may not be able to apply foliar spray insecticides if the weather conditions are unfavorable or the application cannot fit in a busy farming schedule. Considering the unpredictable condition after the growing season starts and a fairly high possibility of thrips density exceeding EILs, prophylactic insecticides might still be a practical and reasonable decision for thrips management in peanut.

EILs based on CTDs should be more accurate than EILs based on thrips number per beat cup sample at 2 WAP. We acknowledged that the EILs based on adult thrips number per beat-cup sample at 2 WAP might underestimate the actual impact of thrips feeding injury on yield due to the systemic characteristic of the insecticides used in the study. While insecticides provided a better control of immature thrips than adult thrips, the same number of adult thrips per beat cup sample at 2 WAP would likely result in different degree of thrips injury caused by immature thrips feeding afterward (Todd et al. 1996). Nevertheless, EILs calculated in our study should be conserved given the effect of systemic insecticides on immature thrips. In addition, EILs from

our study would likely be valid only under the same or similar conditions as in our experiments. When the condition changes, EIL should be calculated based on data from research that addresses factors affecting relationships between thrips feeding injury and yield, such as cultivar, planting date, geographic region, environmental conditions, cultural practices, insecticide efficacy, other chemical inputs, pathogen infections, and other pest infestation. If the growing condition is worsened by any of the biotic and/or abiotic stressors, the detrimental effect of thrips feeding injury on peanut would likely increase, and EILs would be expected to decrease (Funderburk et al. 1998; Herbert et al. 1991).

The current study demonstrated that beat cup sampling is suitable for monitoring thrips density in peanut at 2-4 WAP, which is also the critical time period for making management decision based on thrips density. Thrips feeding injury negatively affects peanut growth and yield, which can result in losses that exceed the cost of control. The impact of thrips feeding injury on peanut growth and yield is likely more severe when thrips infestations occur before 30 WAP. EILs for thrips in peanut cultivar Georgia-06G are 1.29 to 2.45 adult thrips per beat cup sample at 2 WAP under the growing condition in GA. In our experiments over two years and four locations in GA, calculated EILs suggested that using prophylactic insecticide would be economically justified 67% of the time. Therefore, using prophylactic insecticide for thrips management would be appropriate given the high possibility of thrips density exceeding EIL and the risks of not being able to treat thrips when EIL is reached.

Literature Cited

Ames Herbert, D., Malone, S., Aref, S., Brandenburg, R. L., Jordan, D. L., Royals, B. M., et al. 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.

Boote, K. J. 1982. Growth Stages of Peanut (Arachis hypogaea L.). Peanut Sci. 9:35–40.

Branch, W. D. 2007. Registration of 'Georgia-06G' Peanut. J. Plant Regist. 1:120–120.

Brandenburg, R. L., Herbert, D. A., Sullivan, G. A., Naderman, G. C., and Wright, S. F. 1998. The Impact of Tillage Practices on Thrips Injury of Peanut in North Carolina and Virginia1. Peanut Sci. 25:27–31.

Brandenburg, R. L., Jordan, D. L., Royals, B. R., Mahoney, D. J., and Johnson, P. D. 2019. Utilization of Imidacloprid to Control Thrips in Peanut in North Carolina. Peanut Sci. 46:8–13.

Brown, S. L., Culbreath, A. K., Todd, J. W., Gorbet, D. W., Baldwin, J. A., and Beasley, J. P. 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., Todd, J. W., and Culbreath, A. K. 1996. Effect of selected cultural practices on incidence of tomato spotted wilt virus and populations of thrips vectors in peanuts. In *Acta Horticulturae*, International Society for Horticultural Science, p. 491–498.

Chisholm, I. F., and Lewis, T. 1984. A new look at thrips (Thysanoptera) mouthparts, their action and effects of feeding on plant tissue. Bull. Entomol. Res. 74:663–675.

Culbreath, A. K., Selph, A. C., Williams, B. W., Kemerait, R. C., Srinivasan, R., Abney, M. R., et al. 2016. Effects of new field resistant cultivars and in-furrow applications of phorate insecticide on tomato spotted wilt of peanut. Crop Prot. 81:70–75.

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

Culbreath, A. K., Todd, J. W., and Brown, S. L. 2003. Epidemiology and Management of Tomato Spotted Wilt in Peanut. Annu. Rev. Phytopathol. 41:53–75.

Ehler, L. E. 2006. Integrated pest management (IPM): Definition, historical development and implementation, and the other IPM. Pest Manag. Sci. 62:787–789.

Funderburk, J. E., Gorbet, D. W., Teare, I. D., and Stavisky, J. 1998. Thrips injury can reduce peanut yield and quality under conditions of multiple stress. Agron. J. 90:563–566.

Garcia, L. E., Brandenburg, R. L., and Bailey, J. E. 2000. Incidence of Tomato spotted wilt virus (Bunyaviridae) and tobacco thrips in Virginia-type peanuts in North Carolina. Plant Dis. 84:459–464.

Herbert, D. A., Wilcut, J. W., and Swann, C. W. 1991. Effects of Various Postemergence Herbicide Treatments and Tobacco Thrips (Frankliniella fusca) Injury on Peanut Yields in Virginia. Peanut Sci. 18:91–94.

Hunter, W. B., and Ullman, D. E. 1989. Analysis of mouthpart movements during feeding of Frankliniella occidentalis (pergande) and F. schultzei trybom (Thysanoptera : Thripidae). Int. J. Insect Morphol. Embryol. 18:161–171.

Isleib, T. G., Milla-Lewis, S. R., Pattee, H. E., Copeland, S. C., Zuleta, M. C., Shew, B. B., et al. 2011. Registration of 'Bailey' Peanut. J. Plant Regist. 5:27–39.

Jain, M., Gallo, M., Chengalrayan, K., Shaikh, N. P., Macdonald, G. E., and Davis, J. M. 2015. Phorate-induced Host Defence Responses Condition Acquired Resistance to Tomato Spotted Wilt in Cultivated Peanut (Arachis hypogaea L.). J. Phytopathol. 163:853–866.

Joost, P. H., and Riley, D. G. 2004. Sampling techniques for thrips (Thysanoptera: Thripidae) in preflowering tomato. J. Econ. Entomol. 97:1450–1454.

Jordan, D. L., Shew, B. B., and Brandenburg, R. L. 2017. Peanut Yield and Injury from Thrips with Combinations of Acephate, Bradyrhizobium Inoculant, and Prothioconazole Applied in the Seed Furrow at Planting. Crop. Forage Turfgrass Manag. 3:cftm2016.11.0075.

Kingra, P., and Kaur, P. 2013. Phenology and growth dynamics of groundnut (Arachis hypogaea L.) cultivars under different dates of sowing. J Res Punjab agric Univ. 50:24–31.

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

Lowry, V. K., Smith, J. W., and Mitchell, F. L. 1992. Life-Fertility Tables for Frankliniella fusca (Hinds) and F. occidentals (Pergande) (Thysanoptera: Thripidae) on Peanut. Ann. Entomol. Soc. Am. 85:744–754.

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

Mahoney, D. J., Jordan, D. L., Brandenburg, R. L., Royals, B. R., Inman, M. D., Hare, A. T., et al. 2018. Influence of Planting Date and Insecticide on Injury Caused by Tobacco Thrips and Peanut Yield in North Carolina. Peanut Sci. 45:70–74.

Marasigan, K., Toews, M., Kemerait, R., Abney, M. R., Culbreath, A., and Srinivasan, R. 2018. Evaluation of alternatives to an organophosphate insecticide with selected cultural practices: Effects on thrips, frankliniella fusca, and incidence of spotted wilt in peanut farmscapes. J. Econ. Entomol. 111:1030–1041.

Marasigan, K., Toews, M., Kemerait, R., Abney, M. R., Culbreath, A., and Srinivasan, R. 2016. Evaluation of alternatives to carbamate and organophosphate insecticides against thrips and tomato spotted wilt virus in peanut production. J. Econ. Entomol. 109:544–557.

Morgan, L. W., Snow, W. J., and Peach, J. M. 1970. Chemical Thrips Control; Effects on Growth and Yield of Peanuts in Georgia. J. Econ. Entomol. 63:1253–1255.

Morsello, S. C., Groves, R. L., Nault, B. A., and Kennedy, G. G. 2008. Temperature and

precipitation affect seasonal patterns of dispersing tobacco thrips, Frankliniella fusca, and onion thrips, Thrips tabaci (Thysanoptera: Thripidae) caught on sticky traps. Environ. Entomol. 37:79–86.

Mound, L. A. 1971. The feeding apparatus of thrips. Bull. Entomol. Res. 60:547–548.

De Paula Silva, E. M., De Araújo, T. A., Ramos, R. S., De Paulo Arcanjo, L., Das Graças Do Carmo, D., Cavalleri, A., et al. 2019. Conventional Sampling Plan for Common Blossom Thrips, Frankliniella schultzei (Thysanoptera: Thripidae), in Bell Pepper. J. Econ. Entomol. 112:1447–1453.

Pedigo, L. P., Hutchins, S. H., and Higley, L. G. 1986. ECONOMIC INJURY LEVELS IN THEORY AND PRACTICE PERSPECTIVES AND OVERVIEW Further ANNUAL REVIEWS. Annu. Rev. Entomol. 31:341–368.

Pedigo, L. P., and Rice, M. E. 2009. Surveillance and sampling. In *Entomology and pest management*, Pearson Prentice Hall, p. 213–254.

Riley, D. G., Joseph, S. V., Srinivasan, R., and Diffie, S. 2011. Thrips vectors of Tospoviruses. J. Integr. Pest Manag. 1:1–10.

Ruppel, R. F. 1983. *Cumulative Insect-Days as an Index of Crop Protection*.

Shear, G. M., and Miller, L. I. 1941. Thrips injury of peanut seedlings. Plant Dis. Report.

Shrestha, A., Srinivasan, R., Riley, D. G., and Culbreath, A. K. 2012. Direct and indirect effects of a thrips-transmitted tospovirus on the preference and fitness of its vector, frankliniella fusca. Entomol. Exp. Appl. 145:260–271.

Shrestha, A., Srinivasan, R., Sundaraj, S., Culbreath, A. K., and Riley, D. G. 2013. Second generation peanut genotypes resistant to thrips-transmitted tomato spotted wilt virus exhibit tolerance rather than true resistance and differentially affect thrips fitness. J. Econ. Entomol.

106:587–596.

Shrestha, A., Sundaraj, S., Culbreath, A. K., Riley, D. G., Abney, M. R., and Srinivasan, R. 2015. Effects of Thrips Density, Mode of Inoculation, and Plant Age on Tomato Spotted Wilt Virus Transmission in Peanut Plants. Environ. Entomol. 44:136–143.

Smith, J. W., and Sams, R. L. 1977. Economics of thrips control on peanuts in Texas. Southwest. Entomol. 2:149–154.

Sowthwood, T. R. E. 1978. *Ecological methods with particular reference to the study of insect populations*. 2nd ed. Chapman and Hall, London, United Kingdom.

Srinivasan, R., Abney, M. R., Culbreath, A. K., Kemerait, R. C., Tubbs, R. S., Monfort, W. S., et al. 2017. Three decades of managing Tomato spotted wilt virus in peanut in southeastern United States. Virus Res. 241:203–212.

Tappan, W. B., and Gorbet, D. W. 1981. Economics of Tobacco Thrips Control with Systemic Pesticides on Florunner Peanuts in Florida12. J. Econ. Entomol. 74:283–286.

Tappan, W. B., and Gorbet, D. W. 1979. Relationship of Seasonal Thrips Populations to Economics of Control on Florunner Peanuts in Florida. J. Econ. Entomol. 72:772–776.

Todd, J., Culbreath, A., Rogers, D., and Demski, J. 1994. Contraindications of insecticide use relative to vector control of spotted wilt disease in peanut. Proc. Amer. Peanut Res. Educ. 26:42.

Todd, J. W., Culbreath, A. K., and Brown, S. L. 1996. Dynamics of vector populations and progress of spotted wilt disease relative to insecticide use in peanuts. In *Acta Horticulturae*, International Society for Horticultural Science, p. 483–490.

Todd, J. W., Culbreath, A. K., Chamberlin, J. R., Beshear, R. J., and Mullinix, B. G. 1995. Colonization and Population Dynamics of Thrips in Peanuts in the Southern United States. In

Thrips Biology and Management, Springer US, p. 453–460.

USDA-NASS. 2020. *2019 State Agriculture Overview for Georgia*.

Watson, J. R. 1922. *The flower thrips*.

Wightman, J. A., and Rao, G. V. R. 1994. Groundnut pests. In *The Groundnut Crop*, Springer Netherlands, p. 395–479.

Young, S., Kinzer, R. E., Walton, R. R., and Matlock, R. S. 1972. Field Screening for Tobacco Thrips Resistance in Peanuts. J. Econ. Entomol. 65:828–832.

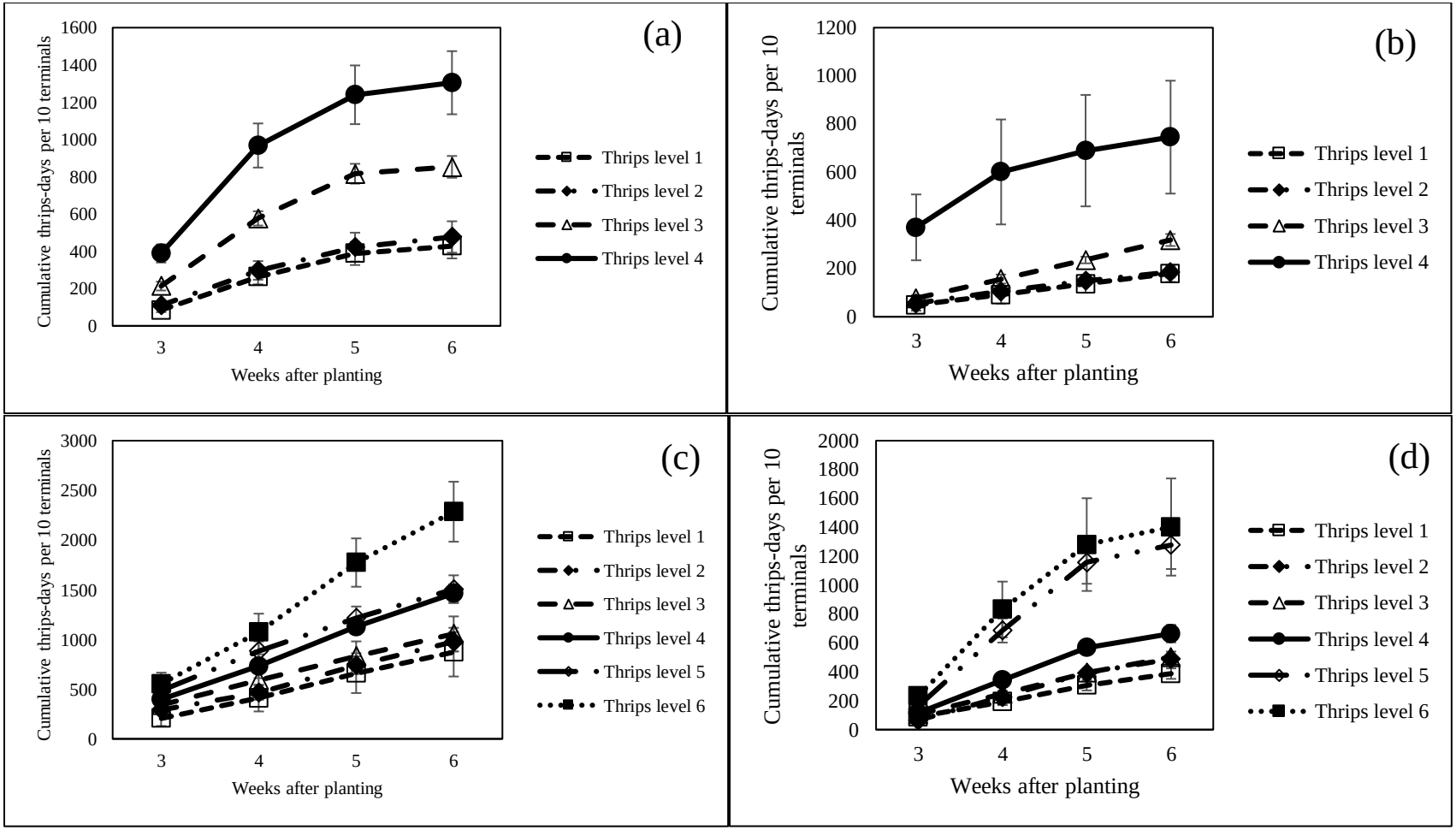


Fig. 2.1. Mean (±SE) cumulative thrips-days (CTDs) over time (a) 1st planting date and (b) 2nd planting date in 2016 and (c) 1st planting date and (d) 2nd planting date in 2017 on a runner type peanut cultivar Georgia-06G in GA.

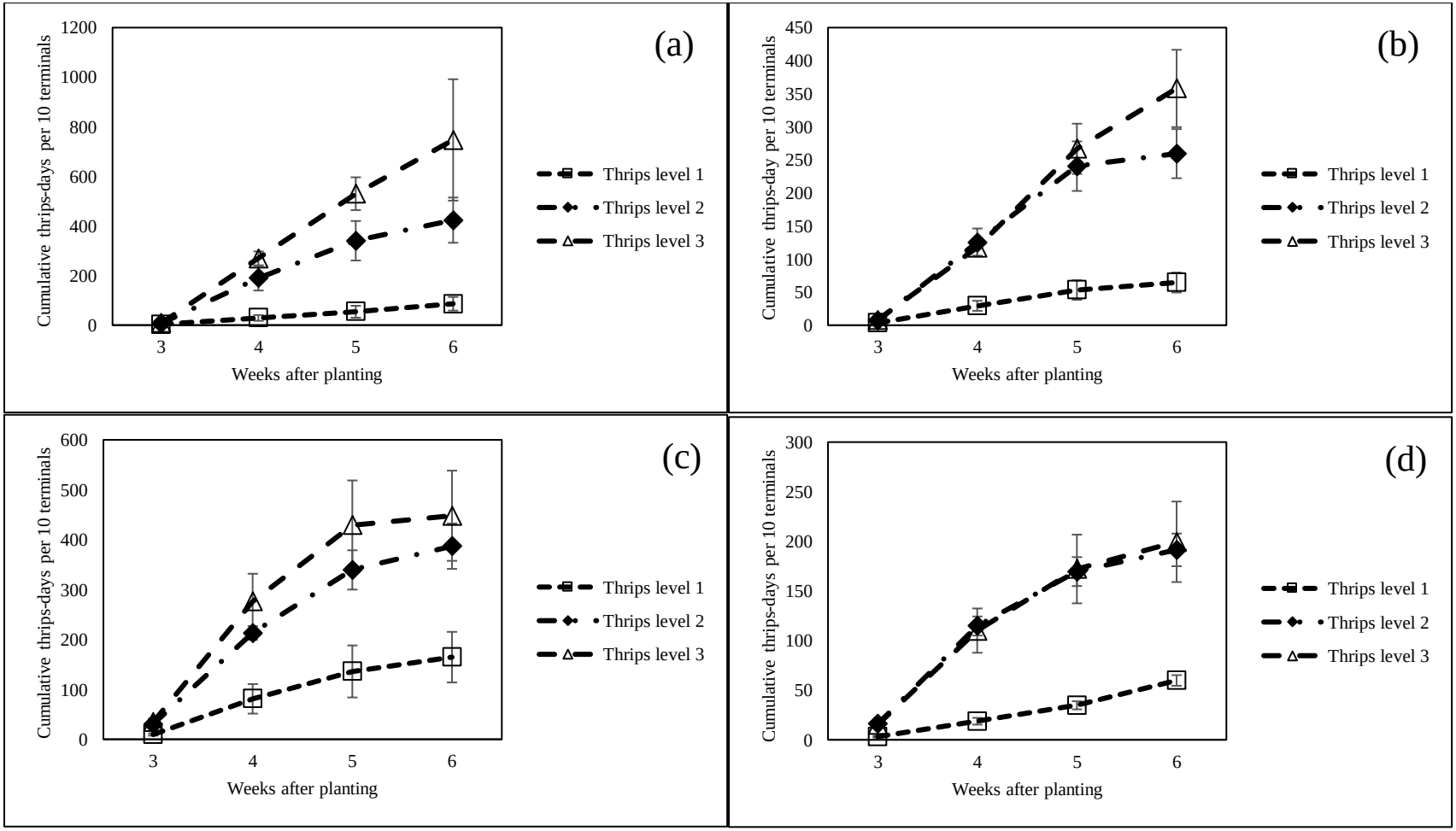


Fig. 2.2. Mean (±SE) cumulative thrips-days (CTDs) over time of (a) 1st planting date and (b) 2nd planting date in 2016 and (c) 1st planting date and (d) 2nd planting date in 2017 on a Virginia type peanut cultivar Bailey in NC.

Table 2.1. List of insecticide treatments for creating levels of thrips pressure in small plot field trials

State	Year	Thrips level[y]	1st planting		2nd planting	
			Insecticide (trade name)	Rate	Insecticide (trade name)	Rate
GA	2016	4	Non-treated control	-	Non-treated control	-
		3	Imidacloprid (Admire Pro)	0.37 l/ha	Aldicarb (AG LOGIC)	1.95 kg/ha
		2	Imidacloprid (Admire Pro)	0.73 l/ha	Aldicarb (AG LOGIC)	3.89 kg/ha
		1	Aldicarb (AG LOGIC)	7.78 kg/ha	Aldicarb (AG LOGIC)	7.78 kg/ha
	2017	6	Non-treated control	-	Non-treated control	-
		5	Imidacloprid (Admire Pro)	0.37 l/ha	Imidacloprid (Admire Pro)	0.37 l/ha
		4	Imidacloprid (Admire Pro)	0.51 l/ha	Imidacloprid (Admire Pro)	0.73 l/ha
		3	Imidacloprid (Admire Pro)	1.10 l/ha	Imidacloprid (Admire Pro)	1.46 l/ha
		2	Cyantraniliprole (Verimark)	0.95 l/ha	Cyantraniliprole (Verimark)	0.95 l/ha
		1	Aldicarb (AG LOGIC)	7.78 kg/ha	Aldicarb (AG LOGIC)	7.78 kg/ha
NC	2016 & 2017	3	Non-treated control	-	Non-treated control	-
		2	Phorate (Thimet 20G) +	5.56 kg/ha	Phorate (Thimet 20G)	5.56 kg/ha
			Acephate (Orthene) when injury threshold reached[z]	0.42 kg/ha	Acephate (Orthene) when injury threshold reached[z]	0.42 kg/ha
		1	Phorate (Thimet 20G) + acephate (Orthene) at 21DAP	5.56 kg/ha	Phorate (Thimet 20G)	5.56 kg/ha
			Acephate (Orthene) at 21 DAP of 1st planting	0.42 kg/ha	Acephate (Orthene) at 21 DAP of 1st planting	0.42 kg/ha

[y] Thrips pressure increased as thrips level number increased.

[z] An arbitrary thrips injury threshold was set at injury rating of "5"; the threshold was never reached in 2016.

Table 2.2. Measurements of (a) accuracy and (b) precision of the beat cup method for sampling adult, immature, and all thrips life stages at different dates in runner type cultivar Georgia 06G in GA

(a)

WAP[v]	N	Mean ± SE proportion to total thrips[w]			Pearson's correlation coefficients[y]		
		Adults	Immatures	All thrips	Adults	Immatures	All thrips
2	15	0.81 ± 0.05 ab	0.80 ± 0.11 a	0.79 ± 0.05 a	0.92 *	0.20	0.89 *
3	15	0.84 ± 0.05 a	0.14 ± 0.05 b	0.22 ± 0.05 b	0.96 *	0.12	0.32
4	15	0.52 ± 0.10 cd	0.12 ± 0.01 b	0.13 ± 0.01 b	0.45	0.80 *	0.80 *
5	15	0.59 ± 0.10 bc	0.08 ± 0.03 b	0.17 ± 0.04 b	0.43	0.38	0.44
6	15	0.33 ± 0.10 d	0.06 ± 0.02 b	0.07 ± 0.01 b	0.44	0.40	0.43
Pooled[x]	75	0.62 ± 0.04 A	0.24 ± 0.04 B	0.28 ± 0.03 B	0.81 *	0.65 *	0.49 *

(b)

WAP[v]	N	Relative variance (RV %)[z]								
		Lang Farm			Jones Farm			Private farm		
		Adults	Immatures	All thrips	Adults	Immatures	All thrips	Adults	Immatures	All thrips
2	5	**21.61**	-	**21.61**	28.45	-	28.45	**12.15**	100.00	**14.14**
3	5	28.78	31.85	26.08	27.30	48.59	**24.74**	37.68	40.98	37.47
4	5	44.72	31.86	32.78	63.25	17.17	**13.39**	41.83	**23.86**	25.00
5	5	51.64	36.42	37.76	**19.81**	50.78	25.22	31.87	30.15	28.10
6	5	61.24	40.82	31.87	53.45	25.51	24.01	46.77	61.91	57.43
Average		41.60	35.24	30.02	38.45	35.51	**23.16**	34.06	51.38	32.43

[v] Weeks after planting.

[w] Values followed by different lower-case letters indicated the mean proportions were significantly different at $p = 0.05$.

[x] Data were pooled across sampling dates for comparison among thrips samples by life stage; values followed by different upper-case letters indicated the mean proportions were significantly different at $p = 0.05$.

[y] Values followed by "*" indicated the correlation was significant at $p < 0.0001$.

[z] RV values less than 25 are bold, a threshold for sufficient precision for pest management purpose.

Table 2.3. Measurements of (a) accuracy and (b) precision of the beat cup method for sampling adult, immature, and all thrips life stages at different dates in Virginia type cultivar Bailey in NC

(a)

WAP[v]	N	Proportion to total thrips[w]			Pearson's correlation coefficients[y]		
		Adults	Immatures	All thrips	Adults	Immatures	All thrips
3	48	0.76 ± 0.04 a	0.49 ± 0.06 a	0.52 ± 0.04 a	0.88 *	0.78 *	0.56 *
4	60	0.69 ± 0.05 b	0.26 ± 0.03 b	0.29 ± 0.03 b	0.90 *	0.77 *	0.74 *
5	36	0.56 ± 0.08 b	0.28 ± 0.04 b	0.26 ± 0.03 b	0.50 *	0.77 *	0.76 *
6	12	0.79 ± 0.11 a	0.51 ± 0.11 a	0.61 ± 0.10 a	0.96 *	0.56	0.52
Pooled[x]	156	0.69 ± 0.03 A	0.35 ± 0.03 B	0.38 ± 0.02 B	0.89 *	0.80 *	0.73 *

(b)

		Relative variance (RV%)[z]														
WAP[v]	N	2016 Lewiston			2016 Rocky Mountain			2016 Whiteville			2017 Lewiston			2017 Whiteville		
		Adults	Immatures	All thrips	Adults	Immatures	All thrips	Adults	Immatures	All thrips	Adults	Immatures	All thrips	Adults	Immatures	All thrips
3	12	**16.42**	42.64	**14.27**	37.80	42.64	31.76	33.71	**20.01**	**18.25**	**19.08**	67.42	**19.20**	NA	NA	NA
4	12	43.81	**15.91**	**15.12**	29.68	**23.99**	**22.38**	**24.99**	**19.33**	**18.15**	100.00	**11.06**	**10.58**	43.90	**18.91**	**15.77**
5	12	28.92	**20.00**	**18.70**	NA[e]	NA	NA	NA	NA	NA	46.06	27.27	**23.39**	52.22	31.76	30.21
6	12	37.80	28.62	**20.79**	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Average		31.74	26.79	**17.22**	33.74	33.32	27.07	29.35	**19.67**	**18.20**	55.05	35.25	**17.72**	55.05	35.25	**17.72**

[v] Weeks after planting.

[w] Values followed by different lower-case letters indicated the mean proportions were significantly different at $p = 0.05$.

[x] Data were pooled across sampling dates for comparison among thrips samples by life stage; values followed by different upper-case letters indicated the mean proportions were significantly different at $p = 0.05$.

[y] Values followed by "*" indicated the correlation was significant at $p < 0.0001$.

[z] RV values less than 25 are bold, a threshold for sufficient precision for pest management purpose.

Table 2.4. Correlation coefficients (r) of Pearson's correlation analyses between number of thrips released and thrips density, cumulative thrips injury ratings (CTIR), plant biomass, and pod weight from peanut plants in field cages in trials conducted in GA with Georgia 06G and NC with Bailey in 2016 and 2017

Trial	Plant age at thrips infestation (DAP)	Thrips density		CTIR[v]		Plant biomass 1[w]		Plant biomass 2[x]		Pod weight	
		r[y]	p[z]	r[y]	p[z]	r[y]	p[z]	r[y]	p[z]	r[y]	p[z]
2016 GA	39	-0.09	0.7215	0.26	0.2178	-0.11	0.6494	-0.24	0.2684	-0.36	0.0880
	28	0.18	0.5064	0.12	0.5607	-0.42	0.0832	-0.43	0.0341*	-0.44	0.0333*
	18	0.36	0.1391	0.36	0.0844	0.07	0.7721	-0.49	0.0157*	-0.25	0.2395
	Pooled	0.06	0.6775	0.26	0.0278*	0.03	0.8040	-0.40	0.0006*	-0.35	0.0028*
2017 GA Lang Farm	41	0.14	0.5090	0.05	0.8316	0.07	0.7415	-0.09	0.684	0.14	0.5167
	32	0.26	0.2270	0.69	0.0002*	-0.07	0.7543	-0.27	0.1961	-0.4	0.0522
	25	0.25	0.2335	0.20	0.3520	0.05	0.8301	-0.38	0.0705	-0.16	0.4471
	Pooled	0.20	0.0854	0.24	0.0408*	0.05	0.6934	-0.22	0.0645	-0.12	0.3069
2017 GA Ponder Farm	43	0.42	0.0637	0.18	0.4476	-0.37	0.1074	-0.37	0.1049	-0.4	0.0787
	31	0.71	0.0005*	0.29	0.2097	-0.15	0.5193	-0.5	0.0256*	-0.45	0.0483*
	24	0.56	0.0100*	0.39	0.0901	-0.08	0.7278	-0.76	0.0001*	-0.79	<0.0001*
	Pooled	0.55	< 0.0001*	0.26	0.0408*	-0.12	0.3635	-0.55	<0.0001*	-0.49	<0.0001*
2016 NC	35	0.11	0.6122	0.63	0.0009*	-0.07	0.7317	NA	NA	-0.05	0.8163
	20	0.23	0.278	0.69	0.0002*	0.27	0.1968	NA	NA	-0.03	0.8748
	Pooled	0.17	0.2553	0.55	<0.0001*	0.05	0.7219	NA	NA	-0.03	0.862
2017 NC	37	-0.32	0.1297	0.45	0.0282*	0.23	0.2831	NA	NA	-0.14	0.5047
	28	-0.56	0.0041*	-0.15	0.4884	0.35	0.0909	NA	NA	-0.55	0.0058*
	Pooled	-0.42	0.0027*	0.12	0.4275	0.27	0.0657	NA	NA	-0.36	0.0121*

[v] Cumulative thrips injury rating (CTIR) was the sum of thrips feeding injury ratings from 3-6 weeks after planting.

[w] Plant biomass measured in mid-season at 14 days after thrips release.

[x] Plant biomass measured at harvest.

[y] Pearson correlation coefficients were obtained for thrips density, plant biomass, and pod weight, while Spearman correlation coefficients were obtained for CTIR.

[z] $P < 0.05$ was notated with a "*", which indicates significant correlation between number of thrips released and the respective variable.

Table 2.5. Significant regression models between number of thrips released and harvest pod weight from individual peanut plants in field cages and percentage of yield reduction for every thrips released in trials conducted in GA and NC

Dataset[v]	Plant age at thrips infestation (DAP)[w]	Linear regression models[y]	R^2	*p*	Yield reduction (%) per thrips release[z]
2016 GA Lang	28	Pod weight = 546.58 - 11.27 Thrips release	0.19	0.0333	2.06
	Pooled[x]	Pod weight = 504.68 - 7.99 Thrips release	0.12	0.0028	1.58
2017 GA Ponder	31	Pod weight = 410.88 - 10.02 Thrips release	0.20	0.0483	2.44
	24	Pod weight = 425.80 - 16.47 Thrips release	0.62	< 0.0001	3.87
	Pooled[x]	Pod weight = 451.23 - 10.93 Thrips release	0.24	< 0.0001	2.42
2017 NC	12	Pod weight = 249.32 - 4.71 Thrips release	0.30	0.0058	1.89
	Pooled[x]	Pod weight = 236.10 - 2.88 Thrips release	0.13	0.0121	1.22

[v] Peanut cultivars Georgia 06G and Bailey were used in GA and NC trials, respectively.

[w] Plant age at thrips infestation effect was created by adjusting planting dates and releasing thrips at the same time; 1st planting plants were older than 2nd planting plants at thrips infestation, and 3rd planting plants were the youngest. The plant age when thrips were released is indicated in paratheses as days after planting (DAP).

[x] Data were pooled across plant ages in the trial for regression analyses.

[y] Number of thrips released were used as a function of pod weight (g) of individual plant in regression analyses.

[z] Percentage of yield reduction for every thrips released was calculated from the associated regression model.

Table 2.6. Means ± SE thrips density, plant biomass, plant size, cumulative thrips injury ratings (CTIR) and yield with summary statistics in small plot field trials conducted in GA in 2016 and 2017

Year	Planting date[w]	Beat cup adult thrips[x] (per cup)		Terminal thrips[x] (per 10 terminals)		Plant biomass[x] (g)		Plant size[xy] (cm^2)		CTIR[z] (0-10 scale; 3~6 WAP)		Yield (kg/ha)	
2016		N = 160		N = 160		N = 160		N = 80		N = 32		N = 32	
	1st	1.54 ± 0.17	a	24.10 ± 2.97	a	4.33 ± 0.48	b	237.08 ± 22.71	b	10.94 ± 1.33		8082.69 ± 141.90	
	2nd	0.93 ± 0.15	b	12.46 ± 2.05	b	6.65 ± 0.73	a	386.92 ± 24.26	a	10.00 ± 1.47		7635.32 ± 185.32	
	Type III test	F (df1, df2)	P > F	F (df1, df2)	P > F	F (df1, df2)	P > F	F (df1, df2)	P > F	F (df1, df2)	P > F	F (df1, df2)	P > F
		6.88 (1, 143)	0.0097	21.55 (1, 143)	< 0.0001	9.96 (1, 143)	0.0019	16.15 (1,143)	< 0.0001	0.26 (1, 15)	0.6143	3.76 (1, 15)	0.0715
2017		N = 240		N = 238		N = 240		N = 120		N = 48		N = 24	
	1st	2.86 ± 0.23	a	43.97 ± 3.08	a	2.49 ± 0.19	b	264.59 ± 24.36		17.29 ± 0.80		7608.49 ± 241.43	
	2nd	1.75 ± 0.18	b	24.96 ± 2.52	b	3.44 ± 0.27	a	275.64 ± 23.29		15.04 ± 0.88		7679.62 ± 111.52	
	Type III test	F (df1, df2)	P > F	F (df1, df2)	P > F	F (df1, df2)	P > F	F (df1, df2)	P > F	F (df1, df2)	P > F	F (df1, df2)	P > F
		15.58 (1, 215)	0.0001	37.95 (1, 213)	< 0.0001	9.59 (1, 215)	0.0022	0.12 (1,215)	0.7324	3.75 (1, 23)	0.0651	0.15 (1,23)	0.7059

[w] The effect of planting date on the variables were analyzed using PROC GLIMMIX procedure in SAS; means followed by different letters indicated significant difference between planting dates; F and *p* values were obtained from type III tests of fixed effects

[x] Means and standard errors of thrips density, plant biomass, and plant size were calculated across sampling dates.

[y] Plant size was calculated as the product of plant height and width.

[z] Cumulative thrips injury rating (CTIR) was the sum of thrips feeding injury ratings from 3~6 weeks after planting based on a 0-10 scale.

Table 2.7. Pearson's correlation coefficients (r) of correlations between cumulative thrips-days (CTDs), cumulative thrips injury ratings (CTIR) and plant biomass, plant size in trials conducted in GA in 2016 and 2017

Variables	Sampling time (week after planting)	Cumulative thrips-days (CTDs)[w]			Cumulative thrips injury ratings (CTIR)[x]		
		n	r[y]	P[z]	n	r[y]	P[z]
Plant biomass	2	78	-0.19	0.0988	-	-	-
	3	78	-0.31	0.0063*	80	-0.27	0.0153*
	4	78	-0.33	0.0035*	80	-0.43	<0.0001*
	5	78	-0.56	<0.0001*	80	-0.51	<0.0001*
	6	78	-0.38	0.0006*	80	-0.32	0.0035*
Plant size	2	78	-0.24	0.0316*	-	-	-
	3	78	-0.33	0.0029*	80	-0.22	0.0520
	4	78	-0.48	<0.0001*	80	-0.35	0.0014*
	5	78	-0.51	<0.0001*	80	-0.32	0.0037*
	6	78	-0.13	0.2590	80	0.00	0.9809

[w] Cumulative thrips-days (CTDs) was calculated based on the equation in Ruppel (1983) using weekly thrips number from 10 terminals per plot from 2 weeks after planting to the respective week.

[x] Cumulative thrips injury rating (CTIR) was the sum of thrips feeding injury ratings from 2 weeks after planting to the respective week.

[y] Pearson's correlation coefficients.

[z] P value notated with an '*' indicated a significant correlation at $p < 0.05$.

Table 2.8. Means ± SE thrips density, plant biomass, plant size, cumulative thrips injury rating (CTIR) and yield with summary statistics in small plot field trials conducted in NC in 2016 and 2017

Year	Planting date[w]	Terminal thrips[x] (per 10 terminals)		Plant biomass[x] (g)		Plant size[xy] (cm2)		CTIR[z] (0-10 scale; 3~6 WAP)		Yield (kg/ha)	
		N = 120		N = 120		N = 120		N = 24		N = 24	
	1st	14.68 ± 2.49	a	2.23 ± 0.19	b	184.35 ± 13.06	b	12.33 ± 2.07	a	4976.64 ±145.76	b
2016	2nd	8.12 ± 1.21	b	4.46 ± 0.44	a	274.90 ± 23.46	a	8.67 ± 1.55	b	6001.97 ± 225.55	a
	Type III test	F (df1, df2)	P > F	F (df1, df2)	P > F	F (df1, df2)	P > F	F (df1, df2)	P > F	F (df1, df2)	P > F
		8.14 (1, 107)	0.0052	26.58 (1, 107)	< 0.0001	12.11 (1, 107)	0.0007	7.60 (1,11)	0.0186	15.80 (1, 11)	0.0022
		N = 96		N = 96		N = 96		N = 24		N = 24	
	1st	14.15 ± 2.16	a	2.29 ± 0.26	b	230.81 ± 21.76		17.00 ± 2.16	a	5775.32 ± 449.35	
2017	2nd	6.84 ± 0.89	b	3.85 ± 0.40	a	248.33 ± 23.37		11.00 ± 1.89	b	5955.32 ± 404.43	
	Type III test	F (df1, df2)	P > F	F (df1, df2)	P > F	F (df1, df2)	P > F	F (df1, df2)	P > F	F (df1, df2)	P > F
		16.12 (1, 83)	0.0001	11.88 (1, 83)	0.0009	0.30 (1, 83)	0.5848	15.19 (1,11)	0.0025	1.84 (1, 11)	0.2021

[w] The effect of planting date on the variables were analyzed using PROC GLIMMIX procedure in SAS; means followed by different letters indicated significant difference between planting dates; F and *p* values were obtained from type III tests of fixed effects

[x] Means and standard errors of thrips density, plant biomass, and plant size were calculated across sampling dates.

[y] Plant size was calculated as the product of plant height and width.

[z] Cumulative thrips injury rating (CTIR) was the sum of thrips feeding injury ratings from 3~6 weeks after planting based on a 0-10 scale.

Table 2.9. Pearson's correlation coefficients (r) of correlations between cumulative thrips-days (CTDs), cumulative thrips injury ratings (CTIR) and plant biomass, plant size in trials conducted in NC in 2016 and 2017

Variables	Sampling time (week after planting)	Cumulative thrips-days (CTDs)[w]			Cumulative thrips injury ratings (CTIR)[x]		
		n	r[y]	P[z]	n	r[y]	P[z]
Plant biomass	3	48	-0.26	0.0791	48	-0.18	0.2101
	4	48	-0.34	0.0168*	48	-0.10	0.4966
	5	48	-0.53	<0.0001*	48	-0.33	0.0222*
	6	48	-0.46	0.0011*	48	-0.36	0.0116*
	7	24	-0.45	0.0291*	24	-0.47	0.0200*
Plant size	3	48	0.28	0.0578	48	0.44	0.0020
	4	48	-0.26	0.0762	48	-0.30	0.0366*
	5	48	-0.31	0.0309*	48	-0.01	0.9515
	6	48	-0.37	0.0104*	48	-0.07	0.6574
	7	24	-0.45	0.0291*	24	-0.55	0.0052*

[w] Cumulative thrips-days (CTDs) was calculated based on the equation in Ruppel (1983) using weekly thrips number from 10 terminals per plot from 2 weeks after planting to the respective week.

[x] Cumulative thrips injury rating (CTIR) was the sum of thrips feeding injury ratings from 2 weeks after planting to the respective week.

[y] Pearson's correlation coefficients.

[z] P value notated with an '*' indicated a significant correlation at $p < 0.05$.

Table 2.10. Multilinear regression models used for EIL calculation in trials conducted in GA in 2016 and 2017

Year	Planting date	n	Regression models[x]	F	df	R^2	P[y]	EIL models[z]
2016	1st planting	16	Yield = 8279.22 - 25.37 CTIR	0.86	1, 14	0.06	0.3691	NA
		16	CTIR = 1.9735 + 0.0117 CTDs	57.54	1, 14	0.80	< 0.0001*	
		16	thrips # = -0.6587 + 0.0028 CTDs	17.33	1, 14	0.55	0.001*	
	2nd planting	16	Yield = 7483.41 - 7.56 CTIR	0.05	1, 14	0.00	0.8239	NA
		16	CTIR = 5.1533 + 0.0136 CTDs	16.94	1, 14	0.55	0.001*	
		16	thrips # = 0.2051 + 0.0010 CTDs	2.57	1, 14	0.16	0.1311	
2017	1st planting	23	Yield = 9199.79 - 133.21 CTIR	9.24	1, 21	0.31	0.0062 *	Model I
		22	CTIR = 6.8726 + 0.0053 CTDs	73.97	1, 20	0.79	< 0.0001*	
		22	thrips # = 3.1248 + 0.0012 CTDs	0.92	1, 20	0.04	0.3481	
	2nd planting	24	Yield = 8374.49 - 60.91 CTIR	4.56	1, 22	0.17	0.0441*	Model II
		24	CTIR = 7.9417+ 0.0060 CTDs	41.07	1, 22	0.65	< 0.0001*	
		24	thrips # = 1.6267 + 0.0019 CTDs	3.93	1, 22	0.15	0.0602	
GA Overall[w]		79	Yield = 8125.14 - 43.25 CTIR	7.17	1, 77	0.09	0.009 *	Model III
		76	CTIR = 6.5433 + 0.0062 CTDs	158.88	1, 74	0.68	< 0.0001*	
		78	thrips # = 0.5123 + 0.0026 CTDs	32.54	1, 76	0.30	< 0.0001*	

[w] Data were pooled across four trials conducted in GA in 2016 and 2017.

[x] CTIR: cumulative thrips injury rating from 3~6 weeks after planting; CTDs: cumulative thrips-days from 3~6 weeks after planting; thrips #: adult thrips number by beat cup sampling at 2 weeks after planting.

[y] Regression models with $p < 0.05$ were significant and denoted with an "*".

[z] EIL were developed using slopes of significant regression models between yield and CTIR and between CTIR and CTDs; EIL models were assigned to datasets having both relationships significant.

Table 2.11. Economic injury levels (EILs) for *Frankliniella fusca* in runner type peanut cultivar Georgia-06G in (a) cumulative thrips-days (CTDs) and (b) adult thrips number per beat cup at two weeks after planting based on three EIL models obtained from trials conducted in 2016 and 2017 in GA.

(a)

Cost of control[y] (USD/ha)	Model I: 2017 1st planting			Model II: 2017 2nd planting			Model III: GA Overall		
	Peanut price (USD/kg)			Peanut price (USD/kg)			Peanut price (USD/kg)		
	$0.30	$0.40	$0.50	$0.30	$0.40	$0.50	$0.30	$0.40	$0.50
$40	189	142	113	365	274	219	497	373	298
$50	236	177	142	456	342	274	622	466	373
$60	283	212	170	547	410	328	746	559	448

(b)

Cost of control[z] (USD/ha)	Model III: GA Overall		
	Peanut price (USD/kg)		
	$0.30	$0.40	$0.50
$40	1.81	1.48	1.29
$50	2.13	1.72	1.48
$60	2.45	1.97	1.68

[y] The unit of EILs was cumulative thrips-days (CTDs) per 10 terminals from 3-6 weeks after planting.

[z] The unit of EILs was adult thrips number by beat cup sampling at 2 weeks after planting.

Table 2.12. Multilinear regression models used for EIL calculation in trials conducted in NC in 2016 and 2017

Year	Planting date	n	Regression models[x]	F	df	R^2	P[y]	EIL models[z]
2016	1st planting	12	Yield = 5072.74 - 11.83 CTIR	0.30	1, 10	0.03	0.5977	NA
		12	CTIR = 2.7478 + 0.0229 CTDs	75.48	1, 10	0.883	< 0.0001*	
	2nd planting	12	Yield = 7071.17 - 130.29 CTIR	44.59	1, 10	0.82	< 0.0001*	Model IV
		12	CTIR = 1.7603 + 0.0304 CTDs	22.81	1, 10	0.70	0.0008*	
2017	1st planting	12	Yield = 7083.05 - 80.32 CTIR	1.79	1, 10	0.15	0.2108	NA
		12	CTIR = 8.1204 + 0.0267 CTDs	6.23	1, 10	0.38	0.0317*	
	2nd planting	12	Yield = 7471.16 - 143.21 CTIR	8.50	1, 10	0.46	0.0154*	Model V
		12	CTIR = 1.7702+ 0.0614 CTDs	13.69	1, 10	0.58	0.0041*	
NC Overall[w]		48	Yield = 6524.85 - 73.83 CTIR	11.76	1, 46	0.20	0.0013*	Model VI
		48	CTIR = 5.5500 + 0.0237 CTDs	44.04	1, 46	0.49	< 0.0001*	

[w] Data were pooled across four trials conducted in GA in 2016 and 2017.

[x] CTIR: cumulative thrips injury rating from 3~6 weeks after planting; CTDs: cumulative thrips-days from 3~6 WAP

[y] Regression models with $p < 0.05$ were significant and denoted with an "*".

[z] EIL were developed using slopes of significant regression models between yield and CTIR and between CTIR and CTDs; EIL models were assigned to datasets having both relationships significant.

Table 2.13. Economic injury levels (EILs) for *Frankliniella fusca* in Virginia type peanut cultivar Bailey in cumulative thrips-days (CTDs) based on three EIL models obtained from trials conducted in 2016 and 2017 in NC.

Cost of control[z] (USD/ha)	Model IV: 2016 2nd planting			Model V: 2017 2nd planting			Model VI: NC Overall		
	Peanut price (USD/kg)			Peanut price (USD/kg)			Peanut price (USD/kg)		
	$0.30	$0.40	$0.50	$0.30	$0.40	$0.50	$0.30	$0.40	$0.50
$40	34	25	20	15	11	9	76	57	46
$50	42	32	25	19	14	11	95	71	57
$60	50	38	30	23	17	14	114	86	69

[z] The unit of EILs was cumulative thrips-days (CTDs) per 10 terminals from 3-6 weeks after planting.

CHAPTER 3

SPATIAL AND TEMPORAL SPREAD OF TOMATO SPOTTED WILT ORTHOTOSPOVIRUS (TSWV) IN TWO RUNNER TYPE TSWV-RESISTANT PEANUT CULTIVARS[1]

[1]Lai, P.-C., Buntin, G. D., Bag, S., Abney, M. R., and Srinivasan, R. To be submitted to *Plant Disease*

Abstract

Tomato spotted wilt orthotospovirus (TSWV) is a thrips-transmitted virus that causes spotted wilt disease in peanut, which is a major yield-limiting factor in peanut production in the southeastern United States. TSWV spatial and temporal spread (epidemics) especially in TSWV-resistant peanut cultivars is not completely understood. Replicated field experiments were conducted to characterize TSWV spread in two runner-type peanut cultivars with field resistance to TSWV (Georgia Green and Georgia-06G), monitor in-field thrips activity, and determine the relationship between the onset of symptom expression, symptom severity, and yield. TSWV incidence progressed continuously throughout the growing season, while thrips activity above the peanut canopy peaked only once at/before 49 days after planting (DAP). Temporal TSWV spread was best fitted by the monomolecular model and the Gompertz model when the overall incidence was low to moderate (<50%) and high (>50%), respectively. Based on the binary power law and SADIE analysis, aggregation was found and often occurred in mid- to late season, and spatiotemporal association was found as TSWV progressed throughout the season. Results of TSWV spread suggested substantial secondary spread even when using TSWV-resistant cultivars. TSWV reduced yield by 0-99.65% depending on time of symptom expression. Early-season TSWV infection had more severe symptoms and less yield when compared with late-season infection. Significant yield losses caused by TSWV were observed in plants expressing symptoms before 91 DAP. Yield reduction following TSWV infection was higher in Georgia Green than Georgia-06G, although the incidence and symptom severity were mostly not different between the two cultivars. Overall, secondary spread contributing to mid-to late-season infection has been demonstrated to cause significant yield reduction in this study, which prompts exploration of options for season-long management.

Introduction

Tomato spotted wilt orthotospovirus is the type species of the genus *Orthotospovirus* in the family *Tospoviridae* and order *Bunyavirales*. TSWV has a broad host range of over 1000 plants, including economically important crops, weeds, and native plants (Parrella et al. 2003; Pappu et al. 2009; Persley et al. 2006; Peters 2013). TSWV inflicts tens of millions of dollars in losses annually in peanut production in the southeastern United States where over 85% of U.S. peanuts are produced (USDA-NASS 2020). TSWV infection in peanut causes spotted wilt disease. Typical TSWV symptoms include concentric ringspots and patterns of chlorosis on leaflets; overall stunting, small and misshaped pegs, pods, and kernels; and reddish discoloration of testa (Halliwell and Philley 1974; Culbreath and Srinivasan 2011). TSWV is exclusively transmitted by nine thrips species in a persistent and propagative manner (Riley et al. 2011; Rotenberg et al. 2015; Whitfield et al. 2005). Thrips must acquire TSWV at their first and/or early second instar larval stages to successfully transmit TSWV (Nagata et al. 2002, 1999; Van De Wetering et al. 1996). Tobacco thrips, *Frankliniella fusca* (Hinds), and western flower thrips, *Frankliniella occidentalis* (Pergande), are two vector species commonly found on peanut in the southeastern U.S. (Culbreath et al. 2003; Srinivasan et al. 2017). *Frankliniella fusca* colonizes peanut plants at seedling stages when peanuts are more susceptible to TSWV and can be found on peanut in foliage and flowers throughout the season, while *Frankliniella occidentalis* is predominantly a flower feeder mostly found later in the season (Buiel and Parlevliet 1996; Lowry et al. 1992; Riley et al. 2011; Shrestha et al. 2015; Todd et al. 1995). Hence, *F. fusca* is considered the major TSWV vector species in peanut in this region (Culbreath et al. 2003; Culbreath and Srinivasan 2011; Lowry et al. 1995; Todd et al. 1995, 1996).

TSWV is not seed transmitted (Culbreath et al. 2003; Ghanekar et al. 1979; Pappu et al. 1999). Viruliferous thrips, presumably from weed hosts, immigrating to peanut fields typically initiate TSWV infection (Groves et al. 2001; Morsello et al. 2008). TSWV epidemics progress in peanut fields throughout the growing season (Culbreath et al. 1996; Culbreath, Todd, Demski, et al. 1992; Camann et al. 1995; Culbreath et al. 1997). Early-season TSWV infection often results in more severe symptoms than late-season infection, and yield losses are often directly proportional to the symptom severity (Culbreath, Todd, and Demski 1992; Narendrappa and Siddaramaiah 1986; Saharan et al. 1983, Culbreath and Srinivasan 2011). However, it is not clear if mid- to late-season TSWV infection also leads to quantifiable yield losses. Thrips are most abundant on peanut in the early season (Todd et al. 1995). The relevance of primary (early season) and secondary (mid- to late-season) TSWV spread to peanut yield loss is not completely understood, in part, due to very few studies conducted in the distant past. Camann et al. (1995) observed random infection clusters and TSWV progression supported by the monomolecular model indicated that primary spread of TSWV was the main driver of the epidemic. In contrast, Lowry et al. (1995) detected viruliferous thrips in terminals and blooms of TSWV-infected plants from mid-season to harvest, long past early season thrips activity; dispersal of these viruliferous thrips could lead to secondary spread.

TSWV spread is typically managed by using a suite of cultural and chemical tactics mainly aimed at reducing thrips landing on peanut plants and minimizing thrips-mediated inoculation of TSWV (Brown et al. 2005; Culbreath et al. 2003). These measures are intended to suppress the primary spread of TSWV by immigrating viruliferous thrips but not secondary spread by in-field thrips population and inoculum. However, weekly insecticide applications resulted in 50% reduced TSWV incidence when compared with early season thrips management

(Todd et al. 1994, 1996). This suggested the occurrence of secondary spread of TSWV in peanut. Black et al. (1993) found higher TSWV incidence when more in-field inoculum was available, providing additional evidence for the importance of secondary spread of the virus.

Since the mid 1990s, cultivars with some degree of field resistance have been routinely planted to mitigate TSWV-induced losses (Culbreath et al. 2003; Srinivasan et al. 2017). Breeding for TSWV resistance has resulted in the release of cultivars with increasing levels of resistance over the years. For simplicity, field-resistant cultivars released in the 1990s are referred to as first-generation cultivars, those released in the 2000s are referred to as second-generation cultivars, and those released after 2010 are referred to as third-generation TSWV-resistant cultivars (Srinivasan et al. 2017). Host resistance in peanut cultivars against thrips is marginal at best and does not significantly contribute to thrips management (Shrestha et al. 2013; Srinivasan et al. 2017; Sundaraj et al. 2014).

TSWV resistance in peanut is not characterized by single gene-governed hypersensitive response as in other crops (Srinivasan et al. 2017). In contrast, resistance in peanut is typified by reduced symptom severity and yield loss in comparison with susceptible cultivars (Culbreath et al. 2003, 2016; Srinivasan et al. 2017). In some instances, field resistant cultivars accumulated reduced amounts of TSWV than susceptible cultivars (Shrestha et al. 2013). In addition, thrips acquired less virus from field-resistant cultivars than from TSWV susceptible cultivars (Shrestha et al. 2013). These suggested that TSWV-infected resistant cultivars might not function as effective inoculum sources and not effectively facilitate the secondary spread of TSWV in comparison with susceptible cultivars. Currently, more than 90% of the production acreage is planted to second-generation resistant cultivar Georgia-06G in Georgia. Despite planting this field-resistant cultivar, TSWV incidence has been on an upward trend, and yield losses continue

to occur (Little 2017, 2019a, 2019b, 2020). The role of second- and third-generation TSWV resistant peanut cultivars in suppressing primary and/or secondary spread of the virus and the contribution of primary (early-season) and secondary (mid- to late-season) spread to quantifiable yield loss remain unknown.

In this study, field experiments were conducted in multiple locations in 2019 and 2020 to assess the extent of spatial and temporal spread of TSWV in the first- and second-generation TSWV-resistant peanut cultivar, Georgia Green (Branch 1996) and Georgia-06G (Branch 2007), respectively. Georgia Green was the predominant TSWV-resistant cultivar planted intensively from the late 1990s to early 2000s (Culbreath and Srinivasan 2011; Srinivasan et al. 2017). Georgia-06G is currently the predominant peanut cultivar planted since late 2000s (Srinivasan et al. 2017). Alongside, thrips activity was monitored throughout the peanut growing season, and the relationship between the onset of TSWV symptoms, symptom severity, and yield loss was investigated.

Materials and Methods

Experimental design and field trials. Field experiments were conducted at the University of Georgia Tifton campus in Tifton, GA and Attapulgus Research and Education Center in Attapulgus, GA in 2019 and 2020. All the field locations have a history of severe TSWV infection in peanut. Naturally occurring TSWV infection was monitored. Runner-type peanut cultivars Georgia Green (Branch 1996) and Georgia-06G (Branch 2007) were planted. Experiments were arranged in a randomized complete block design with four replications. Peanut seeds were planted six seed per 0.3 m in late April to facilitate seedling emergence during predicted peak flights of *F. fusca* (Chappell et al. 2020). Peanut was planted in eight-row plots

that were approximately 9 m long on 23rd and 24th April 2019 in Attapulgus and at the Lang Farm in Tifton, respectively, and on 28th and 29th April 2020 at the Lang Farm and Ponder Farm, respectively, in Tifton.

Thrips monitoring. Thrips activity was monitored using yellow sticky card traps (7.6 x 12.7 cm^2; two sides exposed). Traps were fixed to bamboo sticks with metal clips and adjusted to a height of 10 cm above the ground before peanut emergence and above the peanut canopy afterward. A sticky card trap was placed in the center of each plot. In 2019, sticky card traps were placed in the field starting at ~21 days after planting (DAP) and replaced at 2-week intervals until 133 DAP. In 2020, sticky card traps were placed in the field the day after planting and replaced every week until 49 DAP; afterward, sticky card traps were replaced at 2-week intervals until 133 DAP. Thrips trapped on both sides of the sticky cards were counted under a dissecting microscope (100X) (MEIJI TECHNO, Santa Clara, CA). The number of female *F. fusca* was determined, and data were normalized to *F. fusca* counts per seven days.

Thrips count data were subjected to generalized linear mixed model analysis with a two-way factorial design using PROC GLIMMIX procedure with a negative binomial distribution and log link function in SAS (SAS Institute Inc., Cary, NC). Cultivar and sampling date were the fixed effects, while replication was the random effect. Least square means (LS-means) were used for comparing thrips counts between cultivars and among sampling dates at $\alpha = 0.05$ significance level with Tukey-Kramer adjustment.

Temporal TSWV spread. TSWV incidence in the center four rows of each plot was monitored over the course of the growing seasons. Beginning at ~35 DAP, experimental plots were examined for plants showing TSWV symptoms at 2-week intervals (seven times overall). A vinyl survey flag was placed adjacent to each symptomatic plant, and flagging tape was attached

to the plant's main stem. All symptomatic plants found on a given date were flagged with the same color, and a different color was used for each sampling date. Sampling date and a plant specific identification number were written on the flagging tape of each symptomatic plant, so that individual plants could be identified at the end of the season.

Leaf tissue samples were collected from five randomly selected symptomatic plants from each plot on every sampling date. The samples were tested for TSWV infection via double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) in the laboratory to confirm the visual diagnosis of TSWV infection (Clark and Adams 1977). DAS-ELISA was performed using the protocol described by Shrestha et al. (2013). Samples were considered positive when the final absorbance value was greater than the threshold value calculated as the average absorbance value of negative controls plus four standard deviations. To be more stringent, an adjusted threshold value of 0.1 was adopted for positive samples when the calculated threshold was less than 0.1.

The number of plants per 3 m in two rows of each plot was counted between 20 and 40 DAP and used to extrapolate the total number of plants per plot. TSWV incidence was calculated as the proportion of the plant population exhibiting TSWV symptoms. Cumulative TSWV incidence was compared between cultivars at each sampling date and also compared among seven sampling dates for each cultivar within each trial. TSWV incidence data were subjected to generalized linear mixed model analysis using the PROC GLIMMIX procedure with a beta distribution and logit link function in SAS. The experiment used a two-way factorial design with repeated measures considering the cultivar and sampling time as the fixed effects. LS-means were used to identify differences in cumulative TSWV incidence between cultivars and among sampling dates at $\alpha = 0.05$ significance level with Tukey-Kramer adjustment.

Cumulative TSWV incidence over time was fitted to commonly used temporal pathogen spread models (exponential, logistic, monomolecular, and Gompertz models) using PROC REG procedure in SAS. The back-transformed/recalculated coefficient of determination (R^2) and mean squared error (MSE) were compared among model fits. The best model fit for TSWV incidence in each plot was determined as that with the highest recalculated R^2 and the lowest MSE. The best fit temporal spread model for most replications across cultivars in each trial was selected. Using the best model fit of each trial, the progression rates were compared between cultivars using PROC GLIMMIX procedure with a normal distribution and identity link function in SAS. LS-means were used to identify differences in progression rates between cultivars at α = 0.05 significance level with Tukey-Kramer adjustment.

Spatial TSWV spread. Throughout the growing season, the position of each symptomatic plant was recorded as its distance from the beginning of the row. A spatial map was created for each sampling date. The sampling area (center four rows) of each plot was divided into contiguous quadrats of 1.5 m (row width) x 0.3 m.

Heterogeneity analysis-Binary power law. The binary form of Taylor's power law model (Taylor 1961; Hughes and Madden 1992) was fitted to TSWV incidence data to examine the heterogeneity or aggregation of TSWV incidence and determine whether the aggregation varied systematically with mean TSWV incidence (Turechek and Madden 1999, 2000). The binary power law can be expressed as a relationship between the observed sample variance of TSWV incidence (V_{obs}) and the theoretical variance of a random distribution. For binary data characterized by a binomial distribution, the variance could be expressed as $p(1-p)/n$, where n was the sample size, and p was the mean incidence ($0 \leq p \leq 1$). After logarithmic transformation, the relationship was written as $\ln(V_{obs}) = \ln(A_p) + b \ln[p(1-p)/n]$ in which $\ln(A_p)$ and b were the

intercept and slope of the straight line, respectively; A_p and b were the parameters estimates. When fitting data to the equation by regression analyses using PROC REG in SAS, V_{obs} and the estimated p were calculated from the data set of each plot; a range of TSWV incidence and the associated variances from seven sampling dates were used for the analysis. When A_p and b are not significantly different from 1, randomness is indicated as described by the binomial distribution. When $b = 1$ and $A_p > 1$, there is aggregation, but the degree of aggregation does not depend on TSWV incidence (p). When A_p and b are greater than 1, the degree of aggregation changes with TSWV incidence (p). When b is less than 1, a uniform or regular distribution is indicated (Hughes and Madden 1992; Taylor 1961; Turechek and Madden 1999).

Correlation-type analysis- SADIE. The spatial distribution of TSWV symptomatic plants was further analyzed using the Spatial Analysis by Distance Indices (SADIE) (Li et al. 2012; Perry 1995, 1998; Perry et al. 1999; Xu and Madden 2005; Winder et al. 2019). SADIE program used the location of the sampling units and the counts of TSWV symptomatic plants to evaluate the spatial pattern of symptomatic plants. The distance to regularity (D_r), which was defined as the number of "moves" required for counts in sampling units (i.e., numbers of TSWV symptomatic plants per quadrat) to produce a regular distribution across a designated area, was calculated for observed data sets. Significance of the observed D_r was determined by performing a randomization test. The observed count data in sampling units were randomly reassigned to new sets of sampling units, and D_r was recalculated. A frequency distribution of D_r was then derived from multiple randomizations, and the proportion of randomizations (P_a) with distance to regularity larger than observed D_r was obtained. Significance of the spatial randomness for a two-sided test was determined at $\alpha = 0.05$ calculated as $2 \times P_a$ (Turechek and Madden 1999). The mean distance to regularity (E_a) for a random pattern was obtained and used to calculate the

index of aggregation (I_a). The index of aggregation was written as $I_a = D_r / E_a$. A random spatial pattern is suggested when I_a equals to 1, an aggregated pattern when I_a was greater than 1, and a regular pattern when I_a was smaller than 1.

For each sampling date, SADIE analysis was performed using the original SADIE program (SADIEShell Version 2.0) built by Perry and Conrad (Perry 1995). The maximum number of randomizations of the program (permutations = 5967) was used for each data set. Patch and gap clustering indices are extensions of the SADIE analysis where the program assigned each sampling unit with a value based on the observed counts and the distance to regularity from the randomization process to denote the consistently high (patch) and low (gap) counts. Clustering indices were used to create contour maps representing the degree of clustering and the locations of patches and gaps over seven sampling dates.

The temporal effect on spatial pattern was further analyzed using a SADIE spatial association tool (AssocBatchSetup01) to calculate association indices (X). The spatiotemporal associations between successive sampling dates (i.e., 35 vs. 49 DAP, 49 vs. 63 DAP, etc.) were determined. Local association indices were first calculated by comparing clustering indices between two data sets. Association indices (X) were calculated as the mean of local association indices, which was equivalent to the correlation coefficient of the local clustering indices for each sampling unit between pairs of data sets (Winder et al. 2019). The significance of X was tested by randomizations with adjustment for small-scale spatial autocorrelation in the population at both sampling dates of the pairs using the Dutilleul adjustment (Dutilleul et al. 1993).

TSWV symptom severity. For all trials, five symptomatic plants from each plot on every sampling date were rated for TSWV symptom severity. Typical foliar symptoms of

TSWV, such as concentric ring spots and chlorosis, and stunting of the plants were observed and assessed on an arbitrary 0-10 scale where 0 = no visible symptoms, 1 = 10% or less of foliage showing symptoms, 2 = 20% of foliage showing symptoms, 3 = 30% of foliage showing symptoms, 4 = 40% of foliage showing symptoms, 5 = 50% of foliage showing symptoms and/or plant stunting, 6 = 60% of foliage showing symptoms and/or plant stunting, 7 = 70% of foliage showing symptoms and/or plant stunting, 8 = 80% of foliage showing symptoms and plant stunting, 9 = 90% of foliage showing symptoms and plant stunting, 10 = more than 90% of foliage showing symptoms and plant stunting or a dead plant. Selected plants were rated twice; the first TSWV severity rating was made when symptomatic plants were first detected, and the final TSWV severity rating on the same plant was made one week before harvest. The effects of cultivar and time of first symptom observation on TSWV severity ratings were evaluated. Data were subjected to Wilcoxon score analysis and Kruskal-Wallis test (median one-way ANOVA statistics for Wilcoxon score analysis) using PROC NPAR1WAY procedure in SAS. The effect of cultivar was analyzed within each sampling date (timing of symptom observation), and the effect of sampling date was analyzed within each cultivar.

Harvest and peanut yield. The five TSWV infected plants per plot from each of the seven sampling dates that had previously been tested by DAS-ELISA were manually harvested at 139 to 145 DAP (n = 35 TSWV-infected plants per plot). Ten asymptomatic plants were also harvested from each plot for yield comparison. Leaf samples from asymptomatic plants were tested by DAS-ELISA, and only plants that tested negative for TSWV were used for yield comparison. Manual harvest involved collection of whole plants, including above- and below-ground parts. Each plant was placed in a kraft paper bag (20L) and dried in a heated, forced air-dryer. After drying, whole plant biomass was measured. Peanut pods were removed, counted,

and weighed. Pods were sized and shelled, and the kernels and hulls were separated. Kernels were collected and graded using three screen sizes: 21/64 x ¾", 18/64 x ¾", and 16/64 x ¾". Kernels were graded into five categories including the three screen sizes, sound splits, and other (smaller than the smallest screen). For each sample, weight and number of kernels were recorded for each grading category. Kernels larger than the 16/64 x ¾" size slot were considered marketable.

The effect of timing of symptom observation on whole plant dried biomass, pod weight, and marketable kernel weight was evaluated within each cultivar. Non-infected plants were included in the analyses. Data were analyzed using PROC GLIMMIX procedure with negative binomial distribution and the log link function in SAS. Timing of symptom observation was treated as a fixed effect, while replication was a random effect. Multiple comparisons were conducted using LS-means at $\alpha = 0.05$ significance level with Tukey-Kramer adjustment.

TSWV severity and peanut yield. The final TSWV severity rating and marketable kernel weight data were subjected to Pearson correlation analyses using PROC CORR procedure in SAS to investigate the relationship between the two variables.

Results

Thrips monitoring. The number of *F. fusca* captured by yellow sticky traps above the peanut canopy did not vary by cultivar in any of the trials but varied by sampling date (Table 3.1, Fig. 3.1). In all four trials, a single peak of *F. fusca* count was observed in the early season, and *F. fusca* count remained low during mid- to late season (Fig. 3.1). In the 2019 Tifton trial, the peak *F. fusca* count was between 21 and 49 DAP (Fig. 3.1a). In the 2019 Attapulgus trial and the 2020 Lang Farm trial, the peak *F. fusca* count was observed between 21 and 35 DAP (Fig.

3.1bc). In the 2020 Ponder Farm trial, the peak *F. fusca* count was observed between 21 and 28 DAP (Fig. 3.1d).

Temporal TSWV spread. TSWV infection was confirmed by DAS-ELISA in 99.11% and 99.64% of the tested symptomatic plants in 2019 and 2020, respectively. Overall, the final TSWV incidence was higher in 2019 than 2020 (Fig. 3.2). In the 2019 Tifton trial, TSWV incidence significantly increased over time ($F_{6, 39} = 168.74$, $p < 0.0001$) (Fig. 3.2a). The effect of cultivar on TSWV incidence was significant ($F_{6, 39} = 7.83$, $p = 0.0079$), while the interaction effect between cultivar and sampling date was not significant ($F_{6, 39} = 1.36$, $p = 0.2537$). TSWV incidence in Georgia Green was significantly higher than in Georgia-06G (Fig. 3.2a). In the 2019 Attapulgus trial, TSWV incidence significantly increased over time ($F_{6, 39} = 101.06$, $p < 0.0001$) (Fig. 3.2b). The effect of cultivar on TSWV incidence was not significant ($F_{1, 39} = 2.02$, $p = 0.1630$), and the interaction effect between cultivar and sampling date on TSWV incidence was not significant either ($F_{6, 39} = 0.45$, $p = 0.8419$). For both trials in 2020, TSWV incidence significantly increased over time (Lang Farm: $F_{6, 39} = 79.28$, $p < 0.0001$; Ponder Farm: $F_{6, 39} = 52.38$, $p < 0.0001$), but cultivar did not significantly affect TSWV incidence (Lang Farm: $F_{1, 39} = 0.02$, $p = 0.8960$; Ponder Farm: $F_{1, 39} = 0.40$, $p = 0.5309$). There was no interaction between cultivar and sampling date in either trial (Lang Farm: $F_{6, 39} = 0.67$, $p = 0.6709$; Ponder Farm: $F_{6, 39} = 0.38$, $p = 0.8853$) (Fig. 3.2cd).

In 2019, the Gompertz model was the best fit for temporal TSWV spread data and accounted for 87.5% and 100% of TSWV spread in the Tifton and Attapulgus trial, respectively. In the Tifton trial, the progression rate ranged from 0.1532 to 0.2778 and varied by cultivar ($F_{1, 3} = 19.99$, $p = 0.0208$). The progression rate in Georgia Green (mean ± SE: 0.2323 ± 0.0166) was significantly higher than in Georgia-06G (mean ± SE: 0.1860 ± 0.0166). In the Attapulgus trial,

the progression rate ranged from 0.1469 to 0.2723, and the mean (± SE) progression rate in Georgia Green and Georgia-06G was 0.1712 (± 0.0094) and 0.2034 (± 0.0235), respectively. However, the progression rate in Georgia-06G and Georgia Green was not statistically different ($F_{1, 3} = 2.16$, $p = 0.2381$).

In 2020, the monomolecular model was the best model fit for the temporal TSWV spread data and accounted for 87.5% and 100% of TSWV spread at the Lang Farm and Ponder Farm, respectively. The progression rate of TSWV spread fitted to the monomolecular model was not different between cultivars in either trial (Lang Farm: $F_{1, 3} = 3.42$, $p = 0.1613$; Ponder Farm: $F_{1, 3} = 1.91$, $p = 0.2609$). In the Lang Farm trial, the progression rate ranged from 0.0031 to 0.0113, and the mean (±SE) progression rate in Georgia Green and Georgia-06G was 0.0047 (± 0.0006) and 0.0066 (± 0.0017), respectively. In the Ponder Farm trial, the progression rate ranged from 0.0035 to 0.0128, and the mean (±SE) progression rate in Georgia Green and Georgia-06G was 0.0065 (± 0.0016) and 0.0091 (± 0.0013), respectively.

Spatial TSWV spread. *Binary power law.* The binary power law provided a good description of the relationship between observed variance of TSWV incidence and variance of the random distribution on a log scale for all data sets (Table 3.2). In 2019, estimated slope (b) and intercept ($\ln(A_p)$) were significantly greater than 1 ($p < 0.0001$) and 0 ($p < 0.05$) in 75% and 50% of TSWV spread , respectively, in the Tifton trial; in 87.5% and 100% of TSWV spread, respectively, in the Attapulgus trial. In 2020, b and $\ln(A_p)$ were also significantly greater than 1 ($p < 0.0001$) and 0 ($p < 0.05$) in 87.5% and 75% of TSWV spread, respectively, in the Lang Farm trial; in 62.5% and 100% of TSWV spread, respectively, in the Ponder Farm trial (Table 3.2). The results indicated significant aggregation of TSWV incidence across the majority of data sets of TSWV spread.

SADIE. Spatial distribution of TSWV symptomatic plants at seven sampling dates for TSWV spread in each plot was analyzed by SADIE. In 2019, I_a ranged from 0.739 to 2.468 in the Tifton trial and from 0.671 to 2.602 in the Attapulgus trial across cultivars and sampling dates. Overall, random distribution of TSWV symptomatic plants was found most of the time as I_a was not significantly different from 1; however, aggregation was found occasionally (Table 3.3ab). In the Tifton trial, I_a was significantly greater than 1 ($p < 0.0025$), indicating an aggregated distribution of symptomatic plants, in two of the four Georgia-06G replications at 105 and 119 DAP and one of the four Georgia Green replications at 49 DAP (Table 3.3a). In the Attapulgus trial, an aggregated distribution of symptomatic plants was found in one replication of each cultivar; symptomatic plants were aggregated at 105 and 119 DAP in a Georgia-06G replication; while aggregation of symptomatic plants was found at 49 DAP and after 91 DAP in a Georgia Green replication (Table 3.3b). TSWV distribution from 35 to 119 DAP was visualized in contour maps based on clustering indices as one example for each cultivar in the 2019 Tifton trial was presented (Fig. 3.3 & 3.4). TSWV seemed to be ubiquitous across sampling units as the clustering indices varied slightly within a small range (i.e., -5.5/5.5 in Fig. 3.3a; -5/5 in Fig. 3.4a).

Aggregation of TSWV was more often found in 2020 than 2019. In 2020, the range of I_a values was 0.598-3.603 at the Lang farm and 0.603-3.216 at the Ponder farm across cultivars and sampling dates. In the Lang farm trial, significant aggregation of symptomatic plants, indicated by $I_a > 1$ ($p < 0.0025$), was found in three of the four Georgia-06G replications and all four Georgia Green replications (Table 3.3c). An aggregated distribution of symptomatic plants was first found between 49 and 77 DAP in one Georgia-06G replication and three Georgia Green replications, and aggregated patterns of symptomatic plants existed throughout the season.

Symptomatic plants were aggregated at 63 and 77 DAP and at 49 and 77-105 DAP in two other Georgia-06G replications, while an aggregated pattern was only found at 91 DAP in the other Georgia Green replication (Table 3.3c). In the Ponder farm trial, significant aggregation of symptomatic plants with $I_a > 1$ ($p < 0.0025$) was found in one Georgia Green and three Georgia-06G replications (Table 3.3d). TSWV symptomatic plants were first found to be aggregated at 63 and 77 DAP and they remained aggregated for the rest of the growing season (Table 3.3d). TSWV distribution from 35 to 119 DAP was presented in contour maps, and an example for each cultivar in the 2020 Lang Farm trial was shown (Fig. 3.5 & 3.6). Distinctive patches (clusters with high TSWV incidence) and gaps (clusters with low TSWV incidence) were observed as the clustering indices across sampling units varied in a big range (i.e., -15/15 in Fig. 3.5a; -8.5/8.5 in Fig. 3.6a).

Temporal association in spatial patterns of TSWV symptomatic plants between successive sampling dates was evaluated. In 2019, based on the association indices (X), significant ($p < 0.05$) spatial associations between successive sampling dates were found 91.67% and 95.83% of the time among all the comparisons in both cultivars and all replications in Tifton (Table 3.4a) and Attapulgus (Table 3.4b), respectively. In 2020, significant spatiotemporal associations were found 100% and 93.75% of the time at the Lang Farm (Table 3.4c) and the Ponder Farm trials (Table 3.4d), respectively. Spatial patterns of symptomatic plants that were not temporally associated between successive sampling dates were observed between 35 and 49 DAP in three and two replications of Georgia-06G and Georgia Green, respectively, in 2019; and in two and one replications of Georgia-06G and Georgia Green, respectively, in 2020 (Table 3.4). The spatiotemporal association of symptomatic plants between 49 and 63 DAP was not significant in only one replication of Georgia Green in the Ponder Farm trial in 2019. These

results indicated the spatial distribution of TSWV symptomatic plants was temporally associated between successive sampling dates especially in mid- to late season.

TSWV symptom severity. TSWV symptom severity in Georgia-06G and Georgia Green rated when symptoms first observed (first rating) and one week before harvest (final rating) was significantly affected by timing of symptom observation (Table 3.5). In general, TSWV symptom severity rating was higher in plants that showed symptoms earlier in the season followed by a gradual decrease in severity as the timing of onset of symptoms increased (Fig. 3.7). TSWV symptom severity was compared between Georgia-06G and Georgia Green at each timing of symptom observation within each trial. Most of the time (>85% of severity rating x time of first symptom observation x trial combinations), TSWV severity rating did not differ by cultivar. TSWV severity varied with cultivar in a few instances; Georgia Green had more severe symptoms than Georgia-06G in plants with symptom observed at 77 and 119 DAP in the 2019 trials, but Georgia-06G had more severe symptoms than Georgia Green in plants with symptom observed at 35, 77, and 119 DAP in the 2020 trials (Fig. 3.7).

TSWV infection and peanut yield. Peanut whole plant biomass, pod weight, and marketable kernel weight were significantly affected by timing of onset of symptoms for both cultivars in all trials (Table 3.6). In general, earlier symptom onset resulted in lower whole plant biomass, pod weight, and marketable kernel weight (Fig. 3.8).

Georgia-06G. In the 2019 Tifton trial, TSWV infected Georgia-06G had significantly lower whole plant biomass and pod weight than non-infected plants when symptoms were observed before 63 DAP. Marketable kernel weight of infected plants was significantly reduced compared with non-infected plants when symptoms were observed before 77 DAP (Fig. 3.8a). In the 2019 Attapulgus trial, whole plant biomass of Georgia-06G was significantly lower than non-

infected plants when symptoms were first observed before 77 DAP, while pod and marketable kernel weights were significantly reduced when symptoms were first observed before 63 DAP (Fig. 3.8b). Whole plant biomass, pod weight, and marketable kernel weight of TSWV infected Georgia-06G were significantly reduced compared with non-infected plants when symptoms were observed before 77 and 91 DAP in the 2020 Ponder Farm and Lang Farm trials, respectively (Fig. 3.8cd).

Georgia Green. Whole plant biomass, pod weight, and marketable kernel weight of TSWV infected plants were significantly lower than non-infected plants when symptoms were observed before 63 DAP in the 2019 Attapulgus trial (Fig. 3.8b), and before 77 DAP in the 2019 Tifton trial (Fig. 3.8a) and the 2020 Ponder Farm trial (Fig. 3.8d). In the 2020 Lang Farm trial, TSWV infected Georgia Green had significantly lower whole plant biomass than non-infected plants when symptoms were observed before 77 DAP, while pod and marketable kernel weights were significantly reduced when symptoms were observed before 91 DAP (Fig. 3.8c).

Overall, TSWV infected plants with symptom observed before 91 DAP (range 63-91) consistently resulted in significant yield reduction across cultivars and trials. Significant pod and marketable kernel weight reduction was also observed occasionally in TSWV infected plants when symptoms were observed later in the season. Georgia-06G pod weight was significantly reduced when symptoms were observed at 105 DAP in the 2019 Attapulgus trial when compared with non-infected plants (Fig. 3.8b). Georgia Green with symptoms observed at 119 DAP had significantly reduced pod and marketable kernel weight in the 2019 Attapulgus trial (Fig. 3.8b) and significantly reduced pod weight in the 2020 Lang Farm trial (Fig. 3.8c) when compared with non-infected plants.

The average reduction of peanut whole plant biomass, total pod weight, and marketable kernel weight across timing of TSWV symptom observation ranged from 35.74-59.43% and varied by cultivar and trial in 2019 and 2020 (Table 3.7). When the effect of timing of symptom observation was considered, the reduction ranged from 0-99.65%. In general, higher reduction in the three yield variables was associated with Georgia-06G than Georgia Green (Table 3.7).

TSWV severity and peanut yield. Significant negative correlation was found between final TSWV severity rating and marketable kernel weight of Georgia-06G and Georgia Green in all trials (Table 3.8); the correlation coefficients ranged from -0.5617 to -0.7352. When final TSWV severity rating of a TSWV infected plant was high, the marketable kernel weight was low, and the negative correlation was consistent across cultivars.

Discussion

Peanut cultivars with field resistance to TSWV express less severe symptoms and accumulate less virus than susceptible cultivars upon TSWV infection, which suggested that TSWV resistant cultivars might not serve as efficient inoculum sources contributing to TSWV spread (Shrestha et al. 2013). In this scenario, most of the TSWV infection would be expected to occur early in the season mainly driven by primary spread. However, in both susceptible and resistant cultivars, TSWV incidence has been observed to occur throughout the growing season, which suggested that secondary spread might also play an important role in TSWV epidemics (Camann et al. 1995; Culbreath, Todd, Demski, et al. 1992; Culbreath et al. 1996, 1997, 2010). Temporal and spatial spread of TSWV in peanut cultivars with field resistance to the virus has rarely been studied in the past thirty years, and the relative importance of primary and secondary spread to TSWV epidemics in TSWV-resistant cultivars is unknown. In addition, quantifiable

yield losses in relation to TSWV spread has not been investigated in TSWV-resistant cultivars. TSWV spatial and temporal spread in a first-generation and a second-generation TSWV resistant peanut cultivar were characterized in the current study. TSWV inoculation by incoming viruliferous thrips is the only natural means to initiate epidemics in peanut fields (Culbreath et al. 2003). Monitoring of thrips indicated that there was only one thrips peak in the early season between 21 and 49 DAP. Those thrips were considered the source of initial inoculum and responsible for primary spread of TSWV. Peak occurrence of thrips in early season was also found responsible for primary spread of groundnut bud necrosis orthotospovirus in peanut in India two to three weeks after the peak occurrence of *Frankliniella schultzei* (Reddy et al. 1983). In the southeastern United States, the first TSWV symptoms are generally observed at 30-45 DAP, which is approximately three to five weeks after emergence (Culbreath, Todd, Demski, et al. 1992). Primary TSWV spread during peak immigration of viruliferous thrips in the early season likely contributed to TSWV symptoms observed in early- to mid-season (before 63~70 DAP).

Besides primary/early-season spread of TSWV, TSWV incidence continuously increased throughout the growing season, and new symptomatic plants were found even in late season at about three weeks before harvest in our study. In addition, 68-84% of the total TSWV incidence occurred in the mid- to late season (i.e., after 63 DAP). Because thrips' activity above peanut canopy only peaked once with constantly low number of thrips captured in mid- to late season as observed in our study, the amount of mid- to late-season infection is unlikely to be explained by primary spread from a continual influx of immigrating thrips; secondary spread from in-field inoculum and thrips populations is a more logical explanation. Immigrating thrips can enter peanut fields as soon as seedlings emerge and build up population on peanut. Although the in-

field thrips population usually peaks at around 30 DAP, immature and adult *F. fusca* can be found in leaf terminals and/or blooms throughout the growing season enabling secondary spread of TSWV (Marasigan et al. 2016, 2018; Todd et al. 1995).

When TSWV incidence was high, as in 2019, the Gompertz model was the best fit for TSWV temporal spread; when TSWV incidence was relatively low (<50%), as in 2020, the monomolecular model was the best fit. Camann et al. (1995) also used the monomolecular model to describe temporal TSWV spread in a susceptible and a resistant cultivar at 0.8-30% TSWV incidence. The monomolecular model is often used to describe monocyclic epidemics with the fastest progression rate occurring at the beginning of the epidemic, and the progression rate decreases as the density of healthy plants declines (Madden 1980; Jagger and Richards 2007). In addition, the monomolecular model implies that infected plants do not serve as a source of in-field inoculum for further infections in the season. Although the monomolecular model was the best fit for TSWV epidemics occurred in 2020 in our study, most of the typical characteristics of the model mentioned above were not observed. Firstly, TSWV incidence progressed most rapidly during the mid-season (63-91 DAP) as indicated by the steepest slope in those epidemics fitted by the monomolecular model, which was not at the beginning of the epidemic as expected under the monomolecular model. Secondly, while TSWV spread slowed after mid-season, the decrease in progression rate was unlikely due to a decline in density of non-infected plants because there were more than 70% of the plants that were non-infected by TSWV at harvest. The discrepancy between the assumptions associated with monomolecular and our data raised speculations about the implications for virus spread. On the other hand, the Gompertz model is often associated with polycyclic virus epidemics. The Gompertz model possesses features of the exponential and monomolecular model in the earlier and later part of the epidemic, respectively

(Jagger and Richards 2007). TSWV spread was best fitted by the Gompertz model in this study when the highest TSWV progression rate (i.e., the steepest slope of the temporal spread) was observed at 30-50% TSWV incidence during the mid-season, which was expected with epidemics fitted to the Gompertz model. This suggested the occurrence of polycyclic epidemics in 2019, aided relatively high TSWV incidence and secondary spread.

Spatial distribution of TSWV symptomatic plants was found aggregated throughout the season based on heterogeneity analysis (i.e., binary power law) regardless of the overall TSWV incidence. TSWV incidence from 63 to 119 DAP increased by 2.3 to 3 folds when the average final incidence was 62.67% in 2019 and increased by 2.1 to 5.3 folds when the average final incidence was 38.12% in 2020. Those results indicated that TSWV spread in mid- to late season was likely driven by secondary spread contributing to spatial aggregation, and the secondary spread occurred more often in 2020 when the overall incidence was relatively low than in 2019 when incidence was high. On the other hand, SADIE analysis found both random or aggregated spatial distribution of TSWV depending on overall TSWV incidence and different time points during the epidemics. Regardless of overall TSWV incidence, random distribution of TSWV was found in the early season. The landing pattern of immigrating viruliferous thrips early in the season was likely a major reason for the random distribution of TSWV before secondary spread started to take place. When the overall incidence was greater than 50%, as in 2019, aggregation was seldom detected by SADIE and mostly in the late season when found. With the severe primary spread in 2019, the lack of aggregation in the mid- to late season was likely due to ubiquitous TSWV infection and merging of TSWV aggregates as secondary spread expanded the individual aggregates toward the end of the season. When the overall incidence was less than 50%, as in 2020, SADIE analysis identified aggregation of TSWV symptomatic plants in the

mid- to late season, which suggested substantial secondary spread toward later part of the epidemics; those results could likely explain the up to five times increase of TSWV incidence from mid-season to late season in 2019 although the temporal spread model fit, the monomolecular model, did not support secondary spread. Significant spatial aggregation of TSWV infected peanut plants was also detected in 1990s with the epidemics fitted by the monomolecular model when the overall incidence was relatively low (Camann et al. 1995). Immigrating thrips repeatedly inoculate surrounding plants in the area where they landed could be another explanation for observed aggregation if secondary spread was not heavily involved. However, the longevity of adult *F. fusca* ranged from 6.27 to 10.26 days with higher longevity observed when temperature was lower (Lowry et al. 1992). With a single thrips peak in the early season, the significant increase of TSWV incidence in late season could hardly be explained by repeated inoculations by immigrating thrips from the early season.

The spatiotemporal spread of TSWV in peanut could be confounded by host resistance to TSWV. For instance, TSWV resistance suppresses virus accumulation following primary infection, and could in turn likely reduce the secondary spread of TSWV by impairing virus acquisition by thrips (Shrestha et al. 2013). However, TSWV spread between the two cultivars did not differ in our study, which suggested that the level of resistance in Georgia Green and Georgia-06G might not be very different. A peanut cultivar with a higher level of TSWV resistance, could likely reduce secondary spread in seasons with high TSWV incidence and needs to be examined.

Regardless of host resistance, early (primary)- and late (secondary)- season infection influenced the severity of TSWV symptoms. In both Georgia Green and Georgia-06G in our study, early-season infection induced typical TSWV symptoms. On the contrary, mid- to late-

season infection generally induced milder TSWV symptoms than early season symptoms. Consequently, early-season infection caused higher reduction in whole plant biomass and yield than late-season infection. TSWV infection reduced peanut marketable kernel yield ranging from 0-99.65%, which depended on timing of symptom expression and symptom severity. Significant yield reduction was observed in a TSWV infected susceptible peanut cultivar when symptoms appeared as late as 105 DAP in a previous study (Culbreath, Todd, and Demski 1992). In our study, when TSWV symptoms were observed after 91 DAP, yield loss was uncommon, and in some cases the "cut-off" date for yield loss was much earlier (as early as 63 DAP). The most common "cut-off" date for yield loss was 77 DAP across trials in our study. Using 77 DAP to divide TSWV epidemics into early and late season, early-season infection on average caused 80% yield loss while late-season infection caused 25% yield loss when compared with non-infected plants. Nevertheless, yield reduction of TSWV infection in late season (after 77 DAP) was as high as 53%, which indicated a potential of substantial effect of late-season infection on peanut yield. Mature plant resistance could have contributed to the observed differences in the effects of early- and late-season infection on yield. Early infection of TSWV generally resulted in more severe symptom development and yield reduction in tomato and tobacco (Moriones et al. 1998; Mandal et al. 2007; Riley et al. 2012; Chaisuekul et al. 2003). Mature plant resistance to TSWV in peanut was demonstrated in a greenhouse study that TSWV incidence was higher in younger than older plants after thrips or mechanical inoculation (Shrestha et al. 2015). While TSWV spread was not affected by cultivar in our study, Georgia-06G, the cultivar with presumably higher field resistance, had lower average yield reduction than Georgia Green, which indicated that Georgia-06G could better tolerate TSWV infection with less yield reduction than Georgia Green.

The current study identified that secondary spread of TSWV in peanut could be an important driver of virus spread. Despite the use of peanut cultivar with field resistance to TSWV, secondary spread or mid- to late-season infection occurred and contributed to substantial yield reduction regardless of TSWV incidence. Almost all the available management tactics for TSWV and thrips in peanut production are designed to mitigate primary spread and are likely to have minimal impact on secondary spread. The current management practices are useful to curtail primary spread but certainly not sufficient to reduce secondary spread when the primary spread was not successfully suppressed in the early season. TSWV incidence was reduced by 50% when season-long foliar insecticide applications were adopted (Todd et al. 1996, 1994). While season-long spray programs for thrips and TSWV management in peanut might be considered impractical under current standards, the yield losses accompanying secondary spread of TSWV as demonstrated in this study spark a new debate on the relevance prolonged thrips and TSWV management in peanut production.

Literature Cited

Black, M. C., Andrews, T. D., and Smith, O. D. 1993. Interplot interference in field experiments with spotted wilt disease of peanut. In *Proc. Am. Peanut Res. Ed.,* , p. 65.

Branch, W. D. 2007. Registration of 'Georgia-06G' Peanut. J. Plant Regist. 1:120–120.

Branch, W. D. 1996. Registration of 'Georgia Green' Peanut. Crop Sci. 36:806–806.

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

Buiel, A. A. M., and Parlevliet, I. E. 1996. Mature plant and tissue resistance in the groundnut-

peanut bud necrosis virus system. Euphytica. 91:213–217.

Camann, M. A., Culbreath, A. K., Pickering, J., Todd, J. W., and Demski, J. W. 1995. Spatial and temporal patterns of spotted wilt epidemics in peanut. Phytopathology. 85:879–885.

Chaisuekul, C., Riley, D. G., and Pappu, H. R. 2003. Transmission of tomato spotted wilt virus to tomato plants of different ages. J. Entomol. Sci. 38:127–136.

Chappell, T. M., Ward, R. V, DePolt, K. T., Roberts, P. M., Greene, J. K., and Kennedy, G. G. 2020. Cotton thrips infestation predictor: a practical tool for predicting tobacco thrips (Frankliniella fusca) infestation of cotton seedlings in the south-eastern United States. Pest Manag. Sci.

Clark, M. F., and Adams, A. N. 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., Selph, A. C., Williams, B. W., Kemerait, R. C., Srinivasan, R., Abney, M. R., et al. 2016. Effects of new field resistant cultivars and in-furrow applications of phorate insecticide on tomato spotted wilt of peanut. Crop Prot. 81:70–75.

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

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

Culbreath, A. K., Todd, J. W., and Brown, S. L. 2003. Epidemiology and Management of Tomato Spotted Wilt in Peanut. Annu. Rev. Phytopathol. 41:53–75.

Culbreath, A. K., Todd, J. W., and Demski, J. W. 1992. Productivity of Florunner Peanut Infected with Tomato Spotted Wilt Virus. Peanut Sci. 19:11–14.

Culbreath, A. K., Todd, J. W., Demski, J. W., and Chamberlin, J. R. 1992. Disease Progress of Spotted Wilt in Peanut Cultivars Florunner and Southern Runner. Phytopathology. 82:766.

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

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

Dutilleul, P., Clifford, P., Richardson, S., and Hemon, D. 1993. Modifying the t Test for Assessing the Correlation Between Two Spatial Processes. Biometrics. 49:305.

Ghanekar, A. M., Reddy, D. V. R., Iizuka, N., Amin, P. W., and Gibbons, R. W. 1979. Bud necrosis of groundnut (Arachis hypogaea) in India caused by tomato spotted wilt virus. Ann. Appl. Biol. 93:173–179.

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

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

Hughes, G., and Madden, L. V. 1992. Aggregation and incidence of disease. Plant Pathol. 41:657–660.

Jagger, M., and Richards, K. 2007. Temporal Analysis I: Quantifying and Comparing Epidemics. In *The Study of Plant Disease Epidemics*, eds. Laurence V. Madden, Gareth Hughes, and

Frank van den Bosch. The American Phytopathological Society, p. 63–116.

Li, B., Madden, L. V., and Xu, X. 2012. Spatial analysis by distance indices: An alternative local clustering index for studying spatial patterns. Methods Ecol. Evol. 3:368–377.

Little, E. L. 2017. *2015 Georgia Plant Disease Loss Estimates*.

Little, E. L. 2019a. *2016 Georgia Plant Disease Loss Estimates*.

Little, E. L. 2019b. *2017 Georgia Plant Disease Loss Estimates*.

Little, E. L. 2020. *2018 Georgia Plant Disease Loss Estimates*.

Lowry, V. K., Smith, J. W., and Mitchell, F. L. 1992. Life-Fertility Tables for Frankliniella fusca (Hinds) and F. occidentals (Pergande) (Thysanoptera: Thripidae) on Peanut. Ann. Entomol. Soc. Am. 85:744–754.

Lowry, V. K., Smith, J. W., Mitchell, F. L., and Crumley, C. R. 1995. Thrips Vectors Responsible for the Secondary Spread of Tomato Spotted Wilt Virus in South Texas Peanut. In *Thrips Biology and Management*, Springer US, p. 167–170.

Madden, L. V. 1980. Quantification of disease progression. Prot. Ecol. 2:159–176.

Mandal, B., Wells, M. L., Martinez-Ochoa, N., Csinos, A. S., and Pappu, H. R. 2007. Symptom development and distribution of Tomato spotted wilt virus in flue-cured tobacco. Ann. Appl. Biol. 151:67–75.

Marasigan, K., Toews, M., Kemerait, R., Abney, M. R., Culbreath, A., and Srinivasan, R. 2018. Evaluation of alternatives to an organophosphate insecticide with selected cultural practices: Effects on thrips, frankliniella fusca, and incidence of spotted wilt in peanut farmscapes. J. Econ. Entomol. 111:1030–1041.

Marasigan, K., Toews, M., Kemerait, R., Abney, M. R., Culbreath, A., and Srinivasan, R. 2016. Evaluation of alternatives to carbamate and organophosphate insecticides against thrips and

tomato spotted wilt virus in peanut production. J. Econ. Entomol. 109:544–557.

Moriones, E., Aramburu, J., Riudavets, J., Arnó, J., and Laviña, A. 1998. Effect of plant age at time of infection by tomato spotted wilt tospovirus on the yield of field-grown tomato. Eur. J. Plant Pathol. 104:295–300.

Morsello, S. C., Groves, R. L., Nault, B. A., and Kennedy, G. G. 2008. Temperature and precipitation affect seasonal patterns of dispersing tobacco thrips, Frankliniella fusca, and onion thrips, Thrips tabaci (Thysanoptera: Thripidae) caught on sticky traps. Environ. Entomol. 37:79–86.

Nagata, T., Inoue-Nagata, A. K., van Lent, J., Goldbach, R., and Peters, D. 2002. Factors determining vector competence and specificity for transmission of Tomato spotted wilt virus. J. Gen. Virol. 83:663–671.

Nagata, T., Inoue-Nagata, A. K., Smid, H. M., Goldbach, R., and Peters, D. 1999. Tissue tropism related to vector competence of Frankliniella occidentalis for tomato spotted wilt tospovirus. J. Gen. Virol. 80:507–515.

Narendrappa, T., and Siddaramaiah, A. L. 1986. Estimation of loss in yield due to bud necrosis of groundnut. Plant Pathol. Newsl. 4:14–15.

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

Pappu, S. S., Pappu, H. R., Culbreath, A. K., and Todd, J. W. 1999. Localization of Tomato Spotted Wilt Virus (Genus Tospovirus , Family Bunyaviridae) in Peanut Pods. Peanut Sci. 26:98–100.

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

Perry, J. N. 1998. Measures of spatial pattern for counts. Ecology. 79:1008–1017.

Perry, J. N. 1995. Spatial Analysis by Distance Indices. J. Anim. Ecol. 64:303.

Perry, J. N., Winder, L., Holland, J. M., and Alston, R. D. 1999. Red-blue plots for detecting clusters in count data. Ecol. Lett. 2:106–113.

Persley, D. M., Thomas, J. E., and Sharman, M. 2006. Tospoviruses - An Australian perspective. Australas. Plant Pathol. 35:161–180.

Peters, D. 2013. Tospoviruses. In *Virus and virus-like diseases of major crops in developing countries*, Kluwer Academic Publishers: Dordrecht, p. 719–742.

Reddy, D. V. R., Amin, P. W., Mcdonald, D., and Ghanekar, A. M. 1983. Epidemiology and control of groundnut bud necrosis and other diseases of legume crops in India caused by tomato spotted wilt virus. In *Plant Virus Epidemiology*, eds. R. T. Plumb and J. M. Thresh. Blackwell Scientific Publications, Oxford, p. 93–102.

Riley, D. G., Joseph, S. V., and Srinivasan, R. 2012. Temporal Relationship of Thrips Populations to Tomato Spotted Wilt Incidence in Tomato in the Field. J. Entomol. Sci. 47:65–75.

Riley, D. G., Joseph, S. V., Srinivasan, R., and Diffie, S. 2011. Thrips vectors of Tospoviruses. J. Integr. Pest Manag. 1:1–10.

Saharan, G. S., Kaushik, C. D., Kaushik, J. C., and Singh, H. V. 1983. Occurrence effect on yield and yield components and vectors of bud necrosis virus of groundnut. Proc. Natl. Semin. Manag. Dis. oilseed Crop. :7–9.

Shrestha, A., Srinivasan, R., Riley, D. G., and Culbreath, A. K. 2012. Direct and indirect effects of a thrips-transmitted tospovirus on the preference and fitness of its vector, frankliniella fusca. Entomol. Exp. Appl. 145:260–271.

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

Shrestha, A., Sundaraj, S., Culbreath, A. K., Riley, D. G., Abney, M. R., and Srinivasan, R. 2015. Effects of Thrips Density, Mode of Inoculation, and Plant Age on Tomato Spotted Wilt Virus Transmission in Peanut Plants. Environ. Entomol. 44:136–143.

Srinivasan, R., Abney, M. R., Culbreath, A. K., Kemerait, R. C., Tubbs, R. S., Monfort, W. S., et al. 2017. Three decades of managing Tomato spotted wilt virus in peanut in southeastern United States. Virus Res. 241:203–212.

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

Taylor, L. R. 1961. Aggregation, variance and the mean. Nat. 189:732–735.

Todd, J., Culbreath, A., Rogers, D., and Demski, J. 1994. Contraindications of insecticide use relative to vector control of spotted wilt disease in peanut. Proc. Amer. Peanut Res. Educ. 26:42.

Todd, J. W., Culbreath, A. K., and Brown, S. L. 1996. Dynamics of vector populations and progress of spotted wilt disease relative to insecticide use in peanuts. In *Acta Horticulturae*, International Society for Horticultural Science, p. 483–490.

Todd, J. W., Culbreath, A. K., Chamberlin, J. R., Beshear, R. J., and Mullinix, B. G. 1995. Colonization and Population Dynamics of Thrips in Peanuts in the Southern United States. In

Thrips Biology and Management, Springer US, p. 453–460.

Turechek, W. W., and Madden, L. V. 2000. Analysis of the association between the incidence of two spatially aggregated foliar diseases of strawberry. Phytopathology. 90:157–170.

Turechek, W. W., and Madden, L. V. 1999. Spatial pattern analysis of strawberry leaf blight in perennial production systems. Phytopathology. 89:421–433.

USDA-NASS. 2020. *Crop Production 2019 Summary*.

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

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

Winder, L., Alexander, C., Griffiths, G., Holland, J., Woolley, C., and Perry, J. 2019. Twenty years and counting with SADIE: Spatial Analysis by Distance Indices software Twenty years and counting with SADIE: Spatial Analysis by Distance Indices software and review of its adoption and use. Rethink. Ecol. 4:1–16.

Xu, X., and Madden, L. V. 2005. Interrelationships among SADIE indices for characterizing spatial patterns of organisms. Phytopathology. 95:874–883.

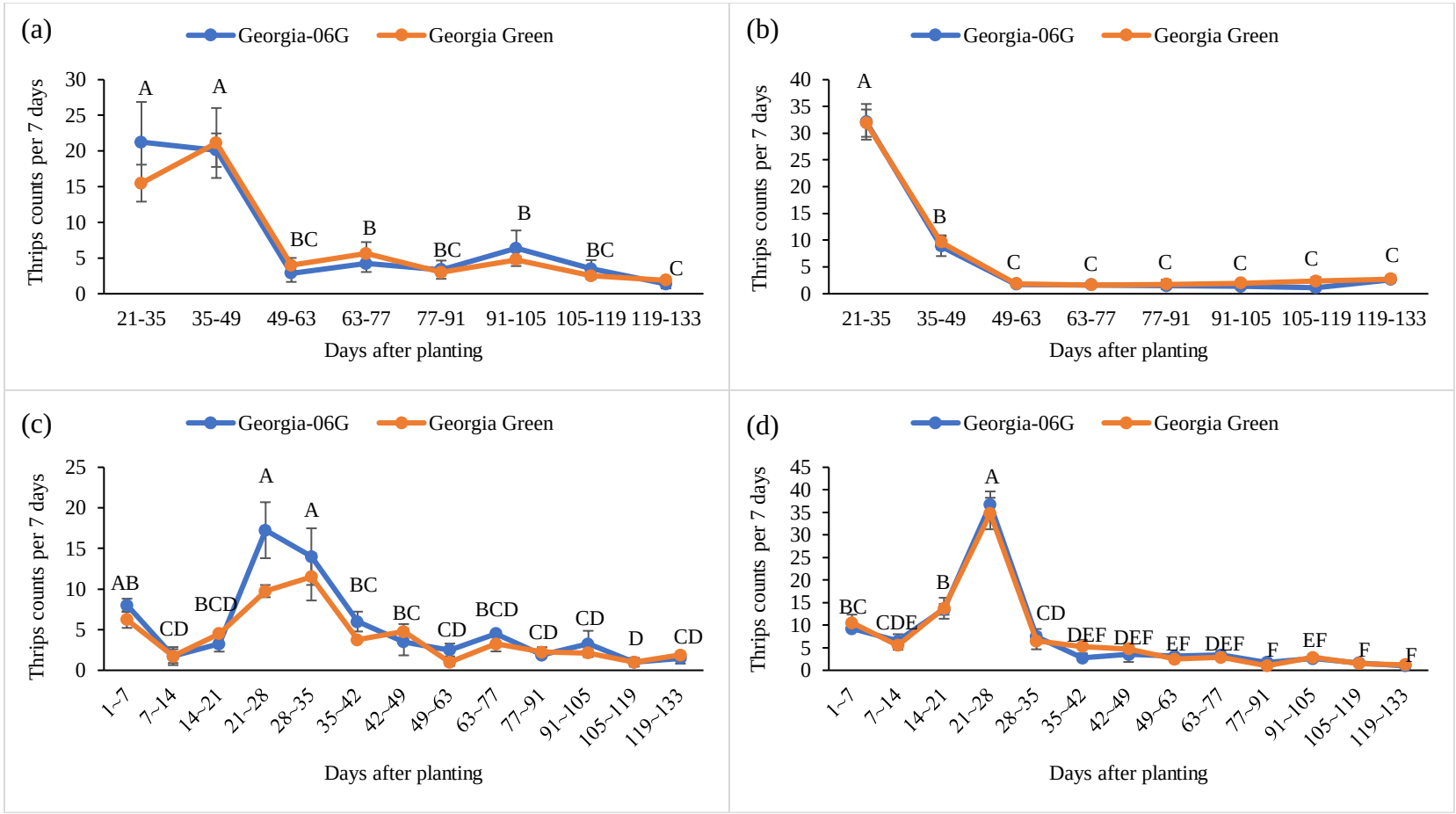


Fig. 3.1. Mean (± SE) number of *F. fusca* per sticky card trap in peanut cultivar Georgia-06G and Georgia Green (n = 4 for each sampling date) from 21 to 133 days after planting (DAP) in (a) Tifton and (b) Attapulgus in 2019, and from 1 to 133 DAP at (c) Lang Farm and (d) Ponder Farm in 2020. Thrips activity was monitored by sticky card traps placed at 10 cm above peanut canopy. Data were normalized to thrips counts per 7 days on both sides of a sticky card. Letters denote groupings of significant differences between sampling dates in number of female *F. fusca* across cultivars. Means within variables notated with different letters are significantly different ($p < 0.05$).

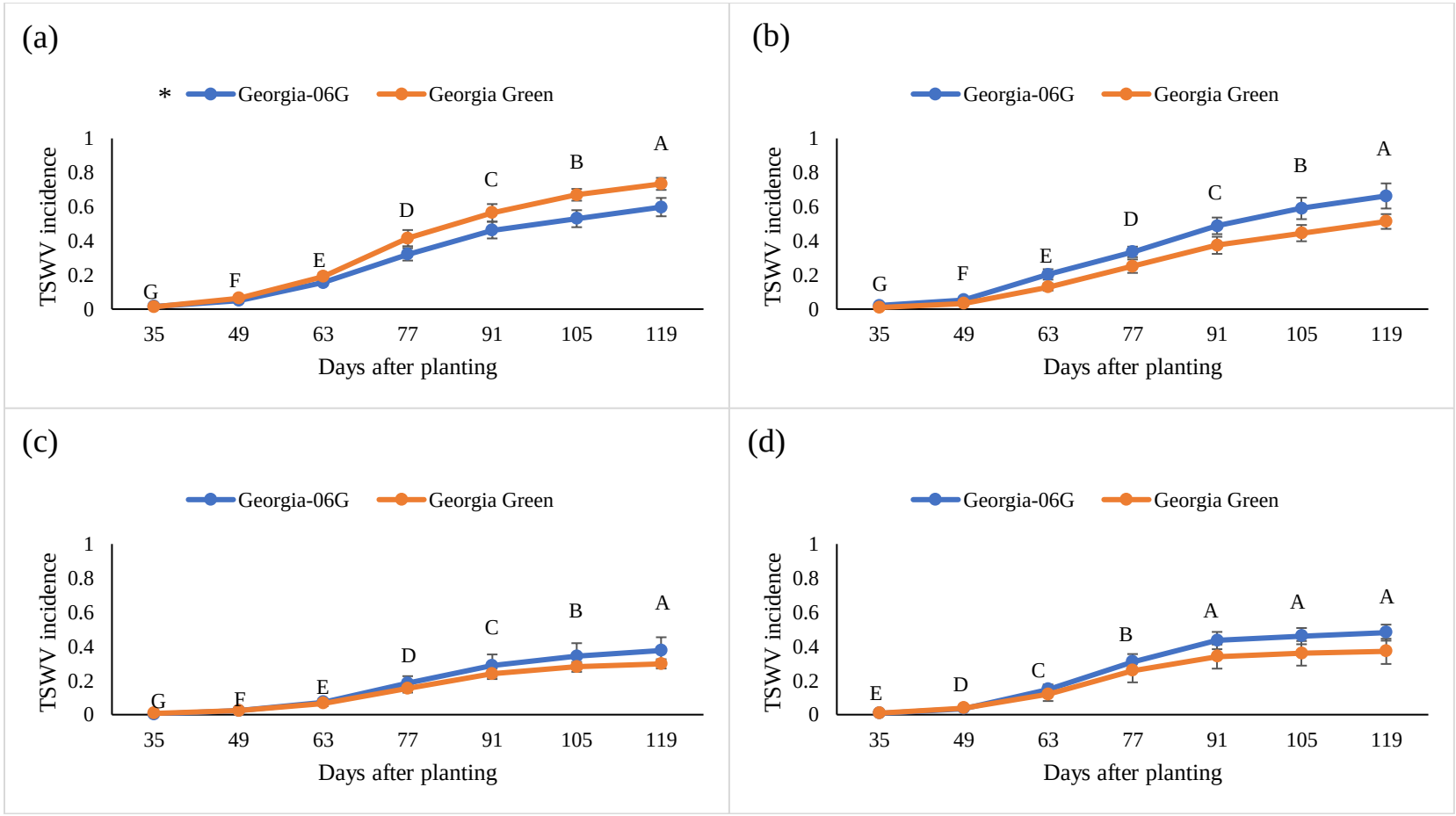
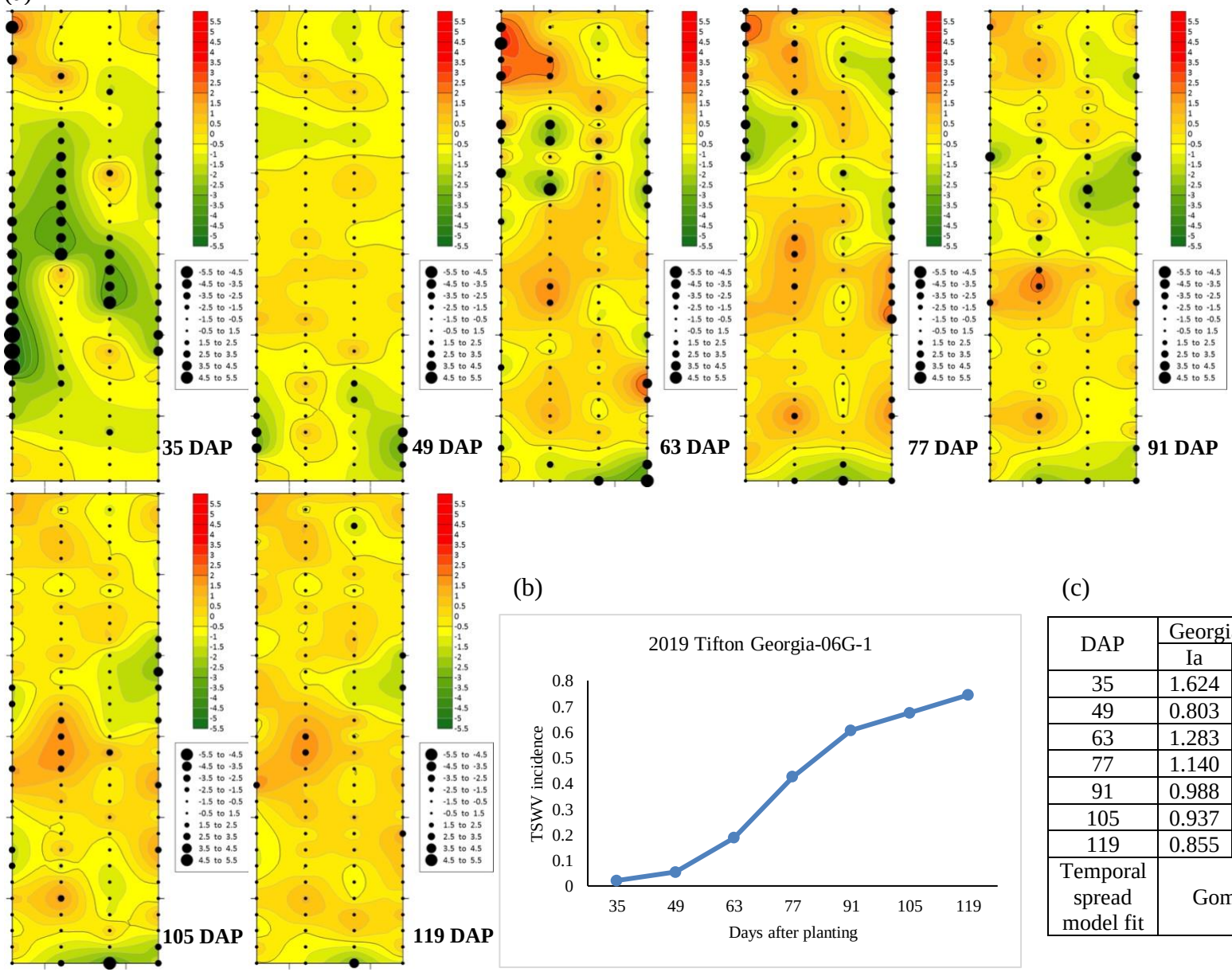


Fig. 3.2. Temporal TSWV spread in Georgia-06G and Georgia Green from 35 to 119 days after planting in trial in (a) Tifton and (b) Attapulgus in 2019, and at (c) Lang Farm and (d) Ponder Farm in Tifton, GA in 2020. Cumulative mean (±SE) TSWV incidence was presented (n = 4 for each cultivar in each trial) over time. Letters denote groupings of significant differences in TSWV incidence between sampling dates across cultivars. Means notated with different letters are significantly different ($p < 0.05$). TSWV incidence was significantly different between cultivars as notated with "*" in (a) ($p < 0.05$).

(a)



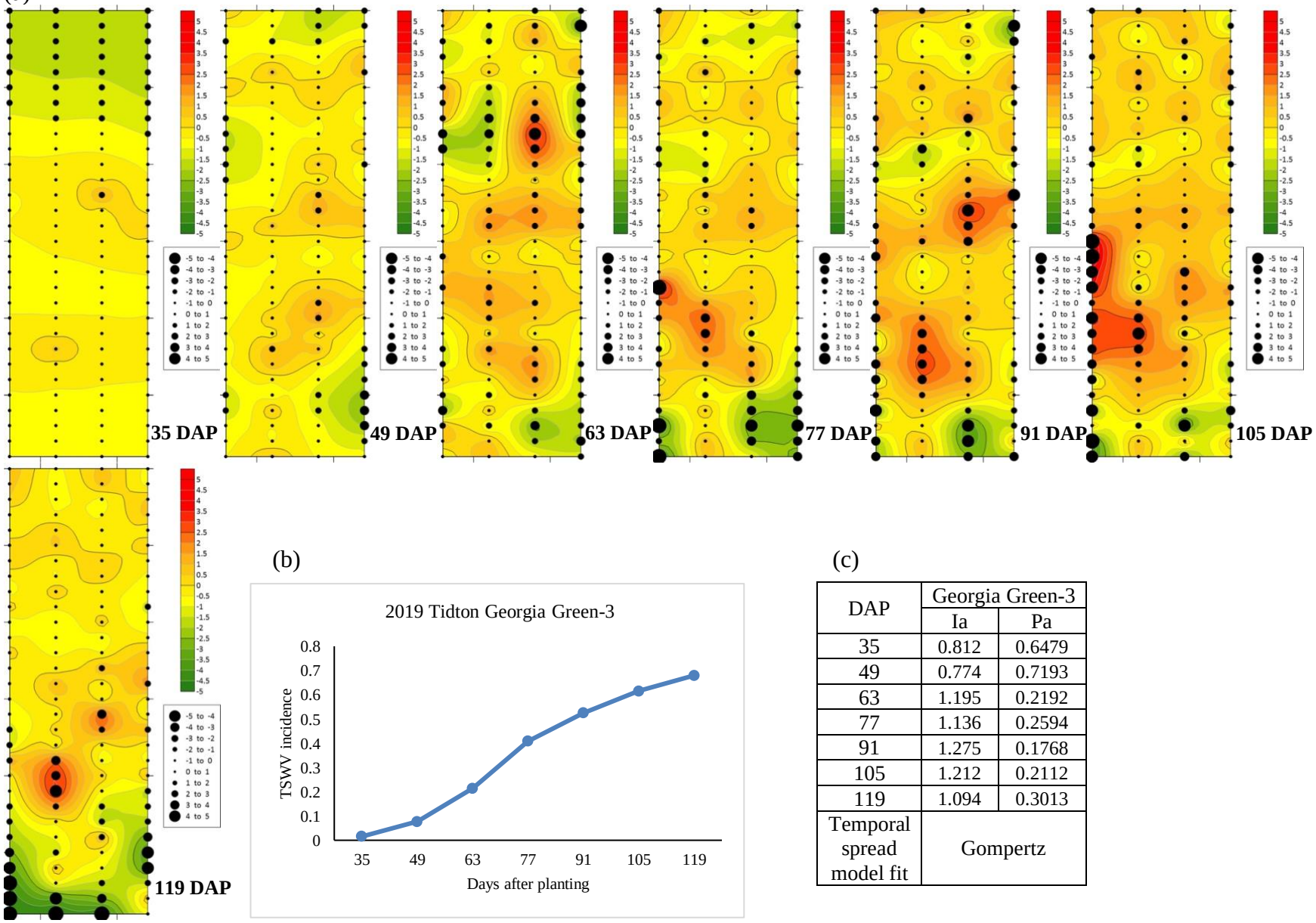
(b)

(c)

DAP	Georgia-06G-1	
	Ia	Pa
35	1.624	0.0598
49	0.803	0.6630
63	1.283	0.1689
77	1.140	0.2616
91	0.988	0.3979
105	0.937	0.4748
119	0.855	0.5866
Temporal spread model fit	Gompertz	

Fig. 3.3. (a) Contour maps of TSWV distribution and spread in the Georgia-06G-1 plot in 2019 Tifton trial from 35 to 119 DAP. SADIE assigns each location an index of clustering using the mean count of TSWV. Positive cluster indices indicate potential patches with high counts of TSWV-infected plants (red), while negative cluster indices indicate potential gaps with low counts of TSWV-infected plants (green); the larger the value, the greater is the evidence for clustering locally. The size of the circle is proportional to the absolute value of cluster index. (b) Cumulative TSWV incidence was plotted over time with a final incidence of 74.4 %. (c) The temporal TSWV spread was best fitted by the Gompertz model, and no significant aggregation was found based on aggregation indices (*Ia*) SADIE.

(a)



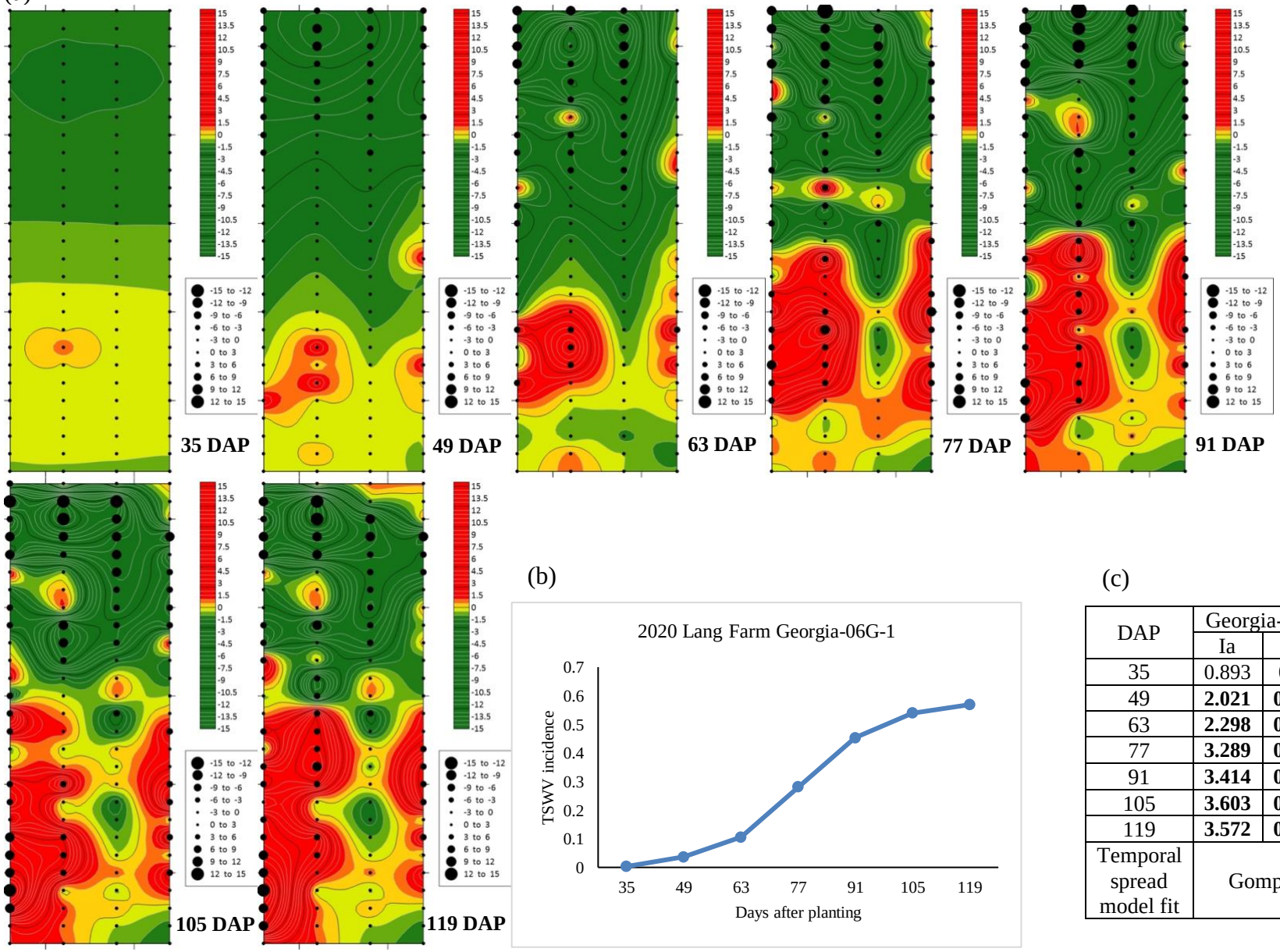
(b)

(c)

DAP	Georgia Green-3	
	Ia	Pa
35	0.812	0.6479
49	0.774	0.7193
63	1.195	0.2192
77	1.136	0.2594
91	1.275	0.1768
105	1.212	0.2112
119	1.094	0.3013
Temporal spread model fit	Gompertz	

Fig. 3.4. (a) Contour maps of TSWV distribution and spread in the Georgia Green-3 plot in 2019 Tifton trial from 35 to 119 DAP. SADIE assigns each location an index of clustering using the mean count of TSWV. Positive cluster indices indicate potential patches with high counts of TSWV-infected plants (red), while negative cluster indices indicate potential gaps with low counts of TSWV-infected plants (green); the larger the value, the greater is the evidence for clustering locally. The size of the circle is proportional to the absolute value of cluster index. (b) Cumulative TSWV incidence was plotted over time with a final incidence of 68 %. (c) The temporal TSWV spread was best fitted by the Gompertz model, and no significant aggregation was found based on aggregation indices (*Ia*) SADIE.

(a)



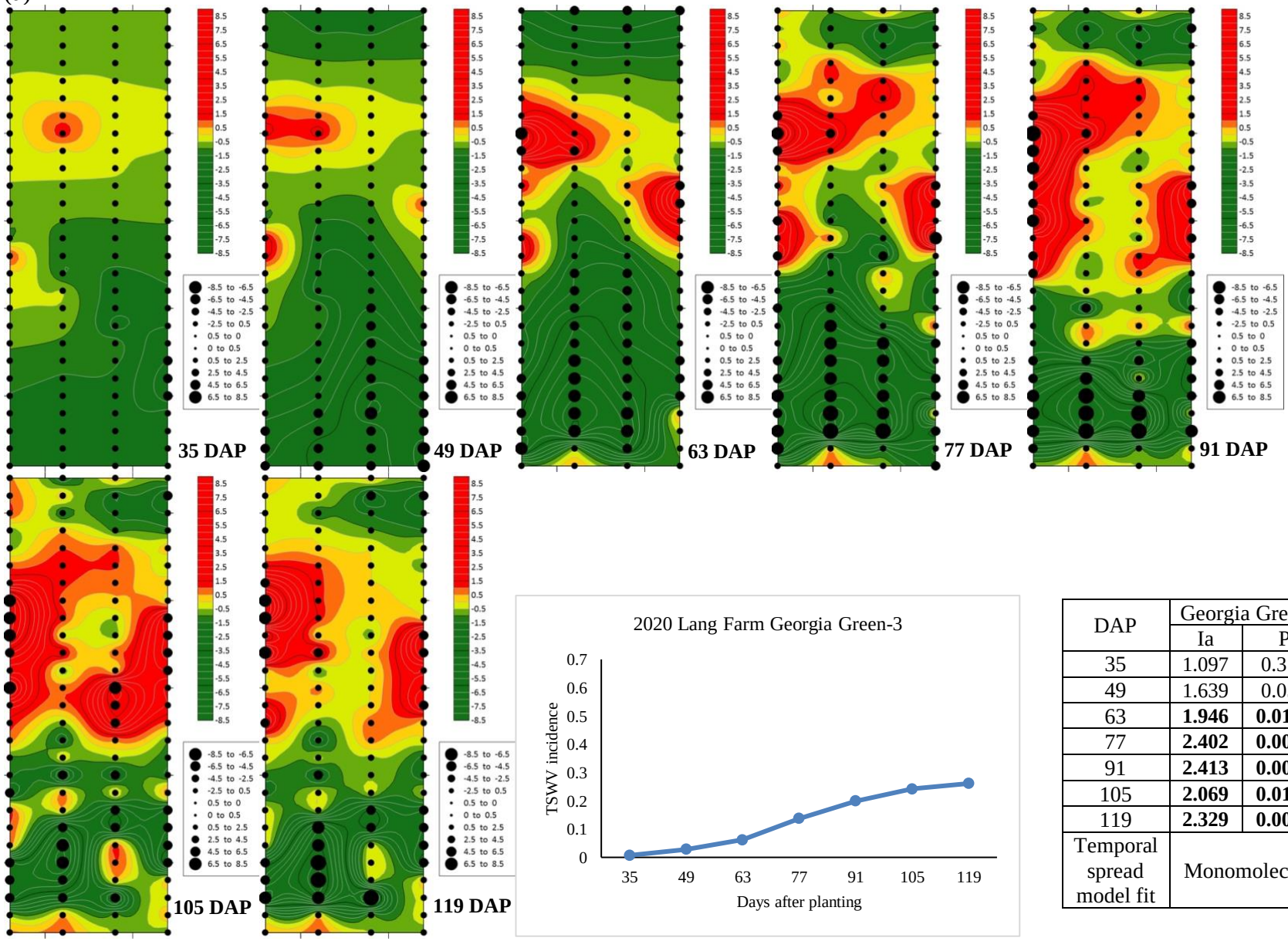
(b)

(c)

DAP	Georgia-06G-1	
	Ia	Pa
35	0.893	0.5939
49	**2.021**	**0.0097***
63	**2.298**	**0.0040***
77	**3.289**	**0.0002***
91	**3.414**	**0.0002***
105	**3.603**	**0.0002***
119	**3.572**	**0.0002***
Temporal spread model fit	Gompertz	

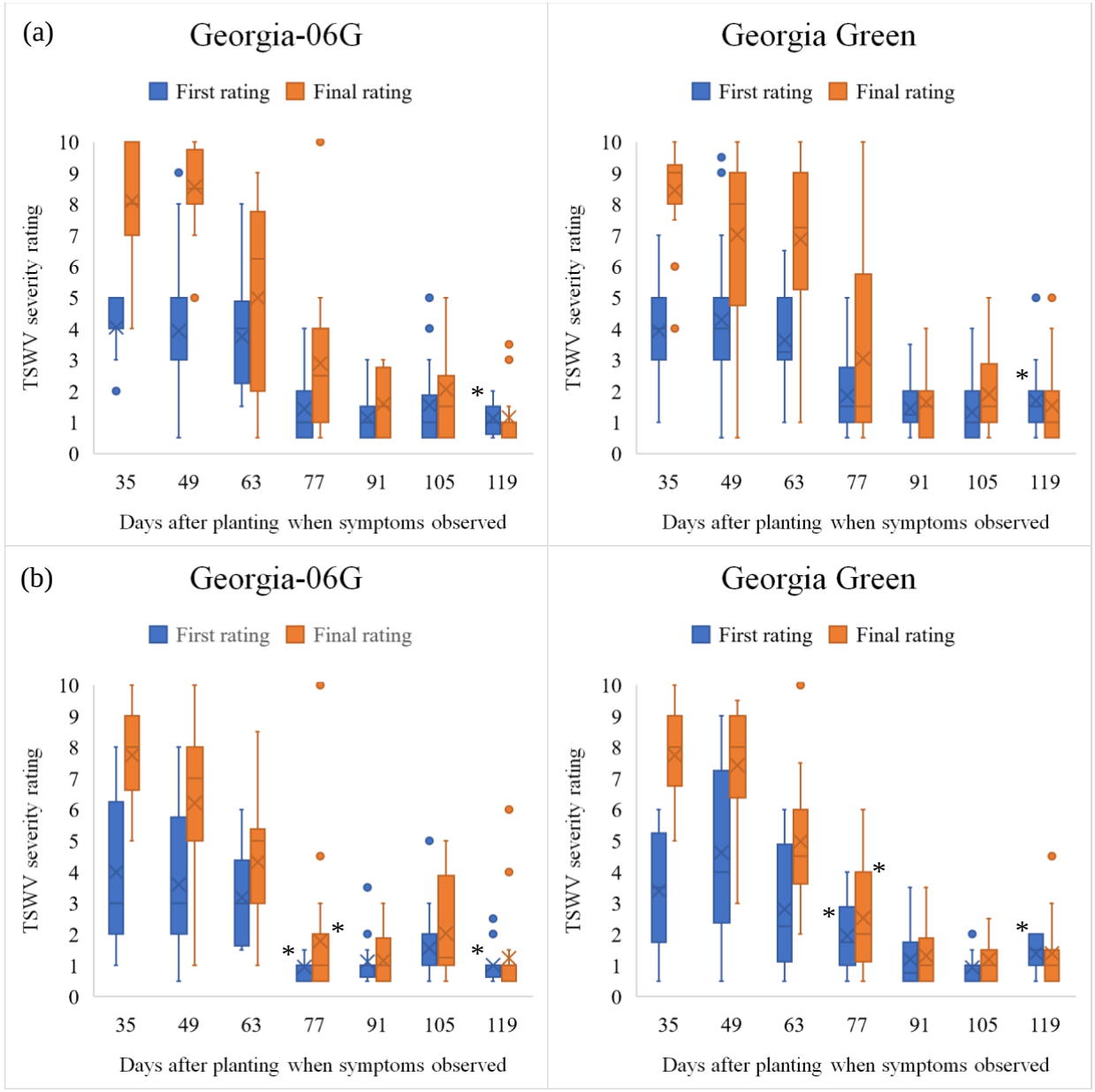
Fig. 3.5. (a) Contour maps of TSWV distribution and spread in the Georgia-06G-1 plot in 2020 Lang Farm trial from 35 to 119 DAP. SADIE assigns each location an index of clustering using the mean count of TSWV. Positive cluster indices indicate potential patches with high counts of TSWV-infected plants (red), while negative cluster indices indicate potential gaps with low counts of TSWV-infected plants (green); the larger the value, the greater is the evidence for clustering locally. The size of the circle is proportional to the absolute value of cluster index. (b) Cumulative TSWV incidence was plotted over time with a final incidence of 56.9 %. (c) The temporal TSWV spread was best fitted by the Gompertz model, and aggregation was found at 49 to 119 DAP based on aggregation indices (*Ia*) SADIE (significant Ia and Pa were bold and notated with ”*”).

(a)



DAP	Georgia Green-3	
	Ia	Pa
35	1.097	0.3107
49	1.639	0.0561
63	**1.946**	**0.0173***
77	**2.402**	**0.0030***
91	**2.413**	**0.0022***
105	**2.069**	**0.0124***
119	**2.329**	**0.0034***
Temporal spread model fit	Monomolecular	

Fig. 3.6. (a) Contour maps of TSWV distribution and spread in the Georgia Green-3 plot in 2020 Lang Farm trial from 35 to 119 DAP. SADIE assigns each location an index of clustering using the mean count of TSWV. Positive cluster indices indicate potential patches with high counts of TSWV-infected plants (red), while negative cluster indices indicate potential gaps with low counts of TSWV-infected plants (green); the larger the value, the greater is the evidence for clustering locally. The size of the circle is proportional to the absolute value of cluster index. (b) Cumulative TSWV incidence was plotted over time with a final incidence of 26.1 %. (c) The temporal TSWV spread was best fitted by the Monomolecular model, and aggregation was found at 49 to 119 DAP based on aggregation indices (*Ia*) SADIE (significant Ia and Pa were bold and notated with ”*”).


(a)
Georgia-06G
First rating
Final rating
TSWV severity rating
Days after planting when symptoms observed
Georgia Green
(b)
Georgia-06G
Georgia Green
35
49
63
77
91
105
119

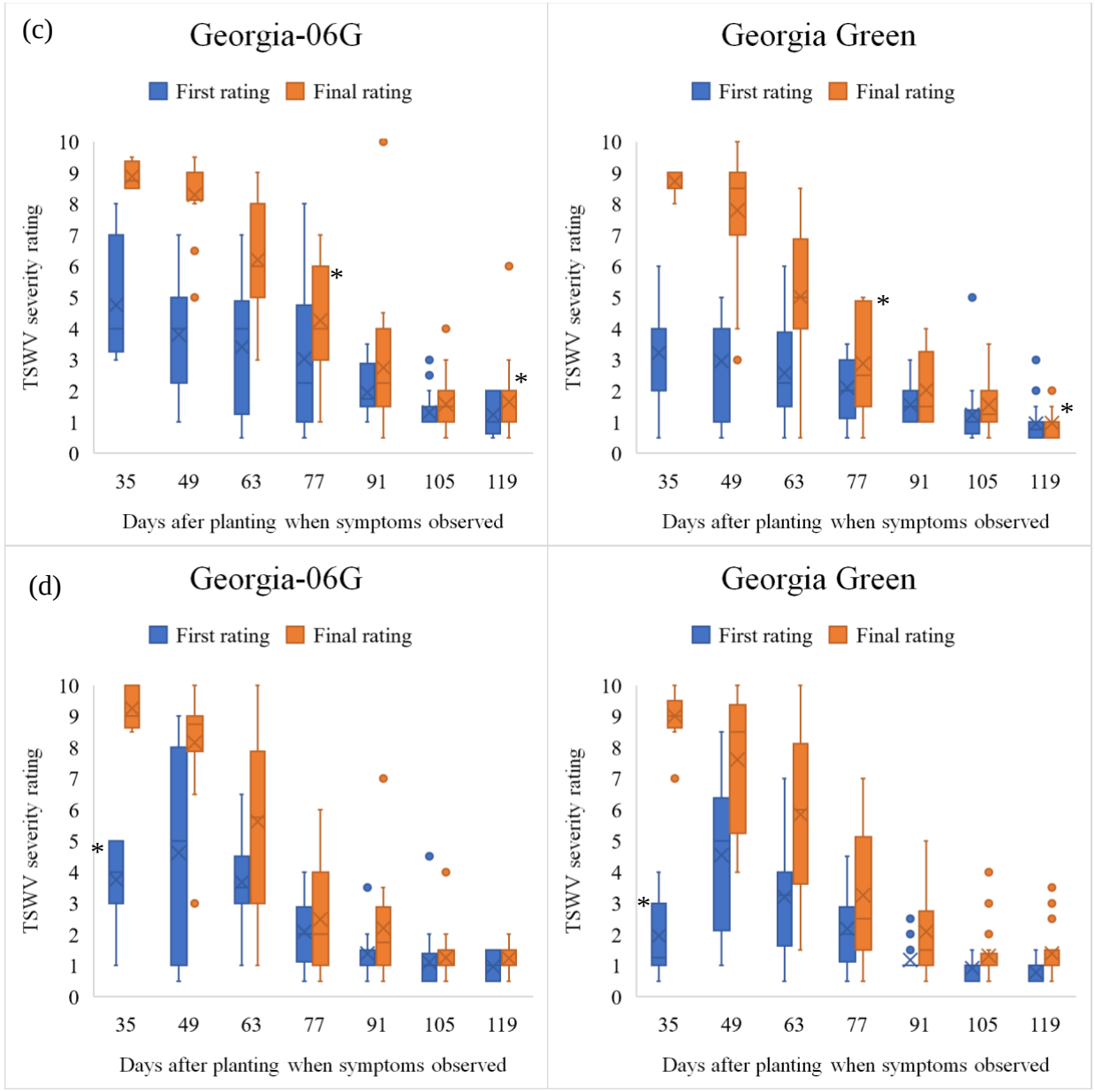
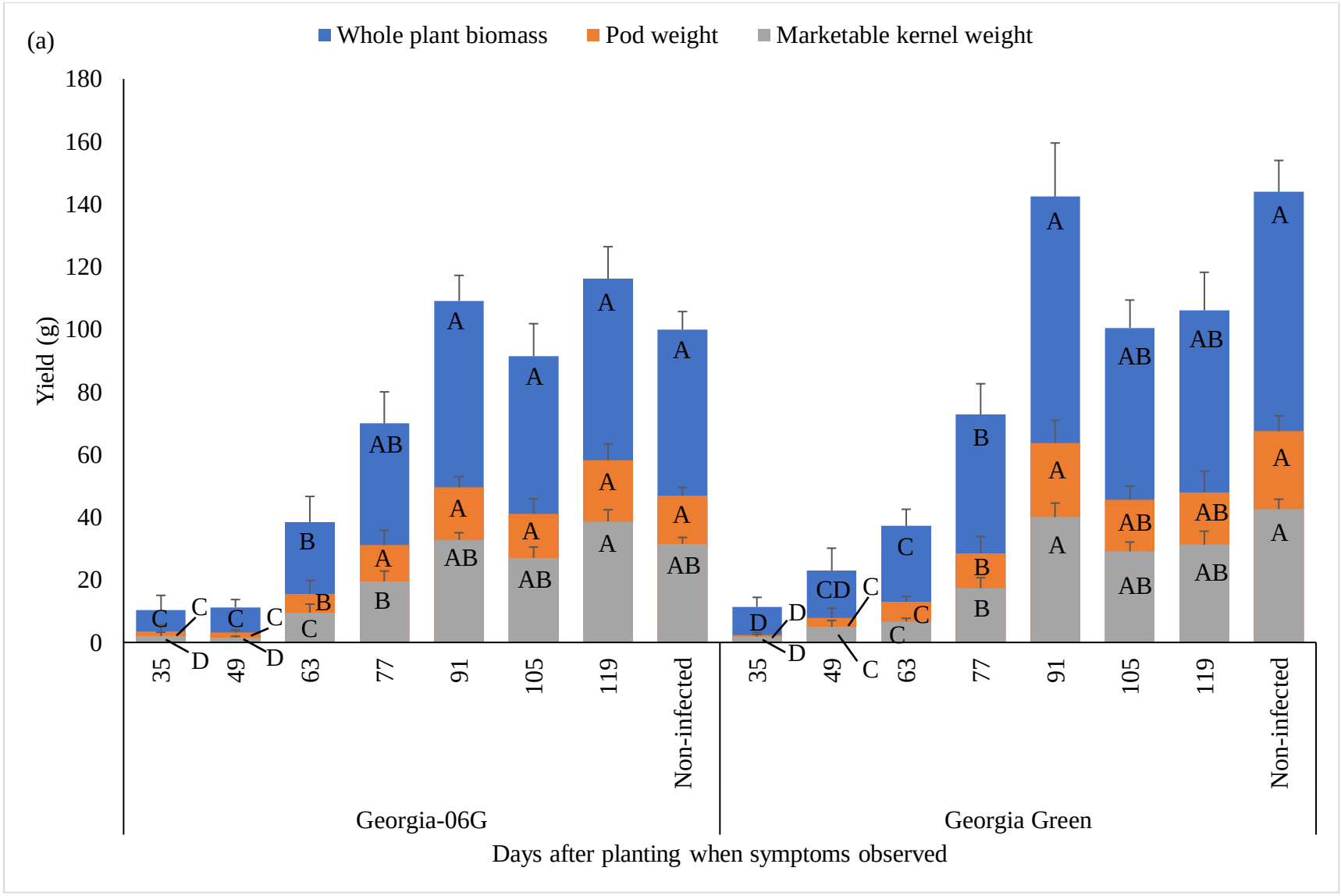
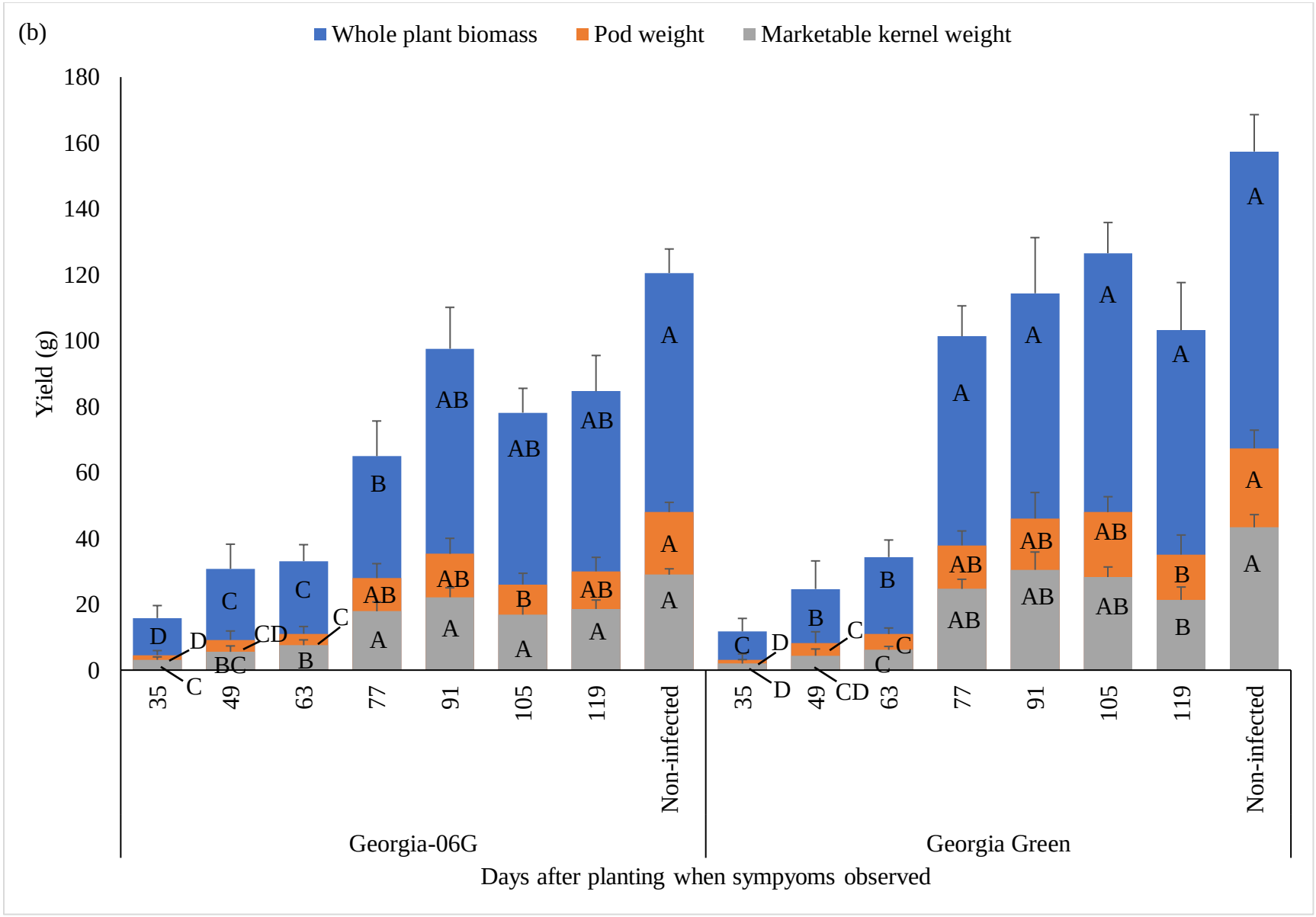
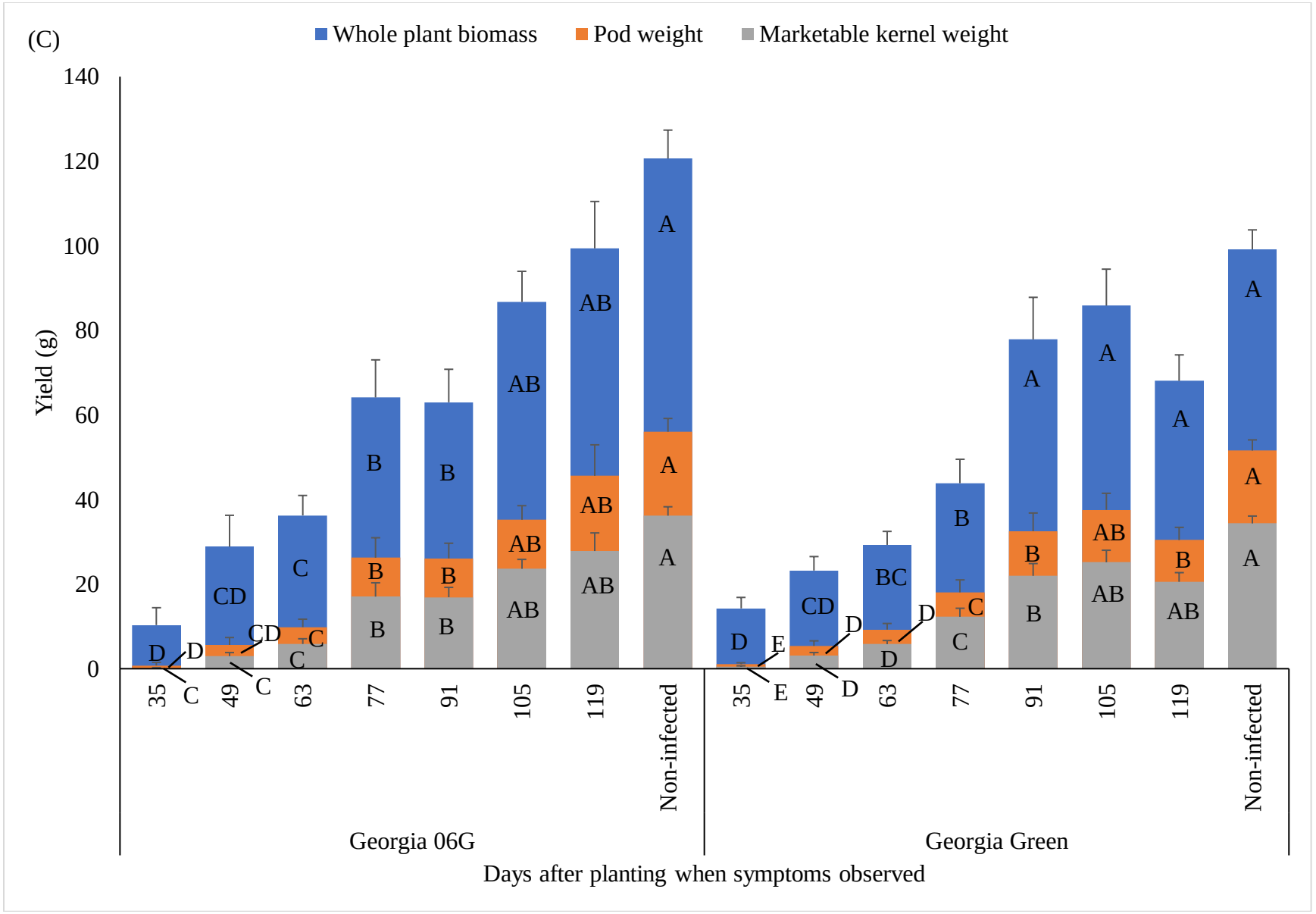

(c)
Georgia-06G
First rating
Final rating
TSWV severity rating
Days afer planting when symptoms observed
Georgia Green
Days after planting when symptoms observed
(d)
Georgia-06G
Georgia Green
35
49
63
77
91
105
119

Fig. 3.7. Median TSWV severity ratings of symptomatic plants with different time of first symptom observation in Georgia-06G and Georgia Green in trial in (a) Tifton and (b) Attapulgus in 2019, and at (c) Lang Farm and (d) Ponder Farm in Tifton, GA in 2020. First severity ratings were rated at the time of first detection, and final severity ratings were rated a week before harvest. TSWV severity was rated on a 0-10 scale where 0 = no visible symptoms and 10 = more than 90% of foliage showing symptoms and stunted plant or a dead plant. Median and mean were labeled as the line and "X" in the box. Data points outside the boxes were outliers. Boxes notated with "*" indicate significant cultivar effect on severity ratings ($p < 0.05$).


(a)
Whole plant biomass
Pod weight
Marketable kernel weight
Yield (g)
180
160
140
120
100
80
60
40
20
0
35
49
63
77
91
105
119
Non-infected
Georgia-06G
Georgia Green
Days after planting when symptoms observed


(b)
Whole plant biomass
Pod weight
Marketable kernel weight
Yield (g)
180
160
140
120
100
80
60
40
20
0
35
49
63
77
91
105
119
Non-infected
Georgia-06G
Georgia Green
Days after planting when sympyoms observed


(C)
Whole plant biomass
Pod weight
Marketable kernel weight
Yield (g)
140
120
100
80
60
40
20
0
35
49
63
77
91
105
119
Non-infected
Georgia 06G
Georgia Green
Days after planting when symptoms observed

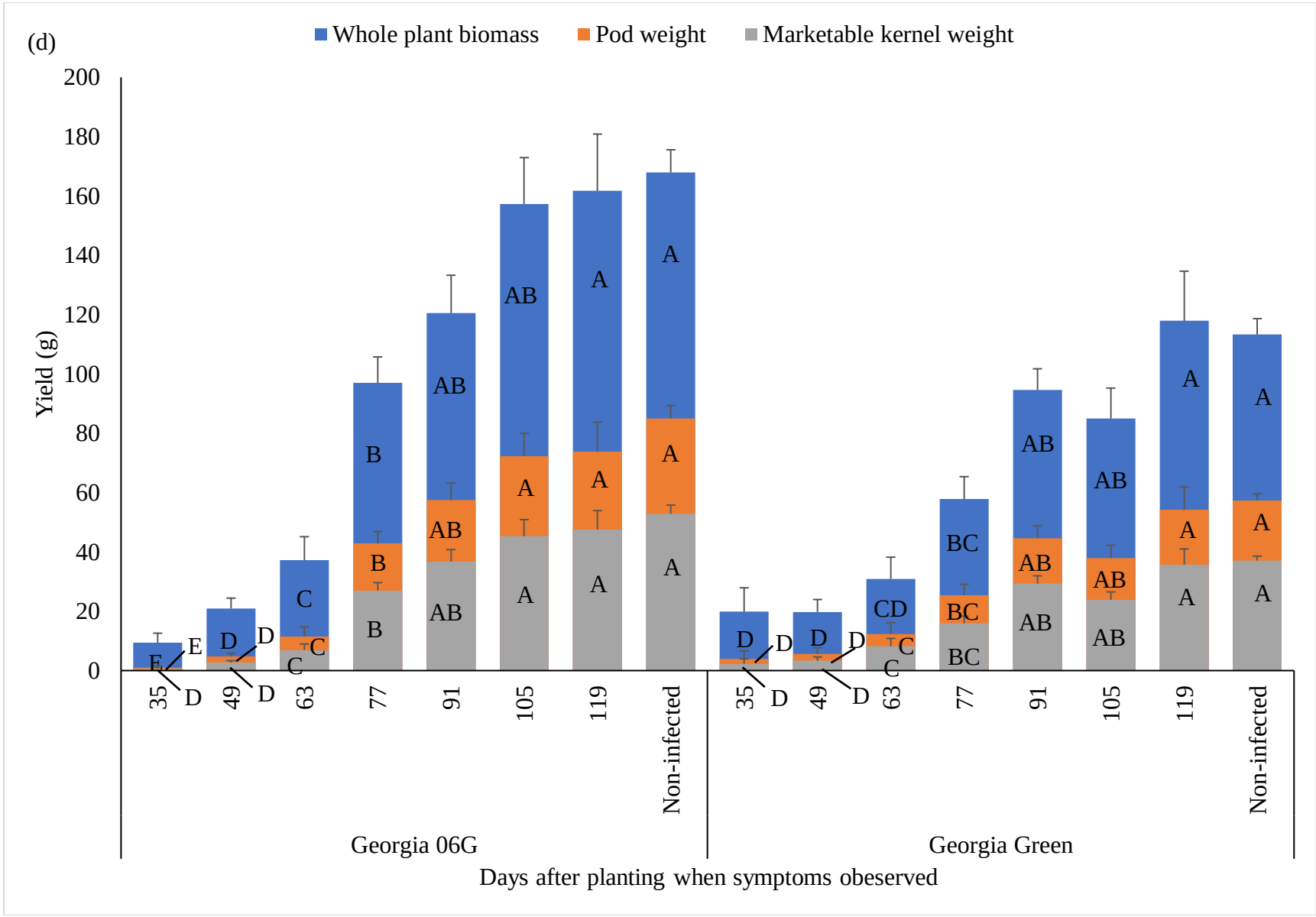

(d)
Whole plant biomass
Pod weight
Marketable kernel weight
Yield (g)
200
180
160
140
120
100
80
60
40
20
0
35
49
63
77
91
105
119
Non-infected
Georgia 06G
Georgia Green
Days after planting when symptoms obeserved

Fig. 3.8. Mean (± SE) whole plant dry biomass, pod weight, and marketable kernel weight of TSWV infected and non-infected Georgia-06G and Georgia Green at harvest. TSWV infected plants with different time of first symptoms observation and non-infected plants were collected from (a) Tifton and (b) Attapulgus in 2019 and at (e) Lang Farm and (f) Ponder Farm in Tifton, GA in 2020. Means within variable notated with different letters are significantly different ($p < 0.05$).

Table 3.1. Effect of cultivar and sampling date on thrips counts captured by sticky cards above peanut canopy in four independent trials in 2019 and 2020.

Trial	Source of variations		All thrips		*F. fusca* (female)	
		df	F	P >F[z]	F	P >F[z]
2019 Tifton	Cultivar	1, 45	0.15	0.6976	0.01	0.9346
	Sampling date	7, 45	17.08	< 0.0001	27.77	< 0.0001
	Cultivar x sampling date	7, 45	1.00	0.4432	0.58	0.7661
2019 Attapulgus	Cultivar	1, 45	1.75	0.1921	1.18	0.2838
	Sampling date	7, 45	46.68	< 0.0001	77.61	< 0.0001
	Cultivar x sampling date	7, 45	0.92	0.5009	0.28	0.9575
2020 Lang Farm	Cultivar	1, 45	2.64	0.1087	1.70	0.1969
	Sampling date	12, 45	11.61	< 0.0001	19.26	< 0.0001
	Cultivar x sampling date	12, 45	0.56	0.8703	0.86	0.5857
2020 Ponder Farm	Cultivar	1, 45	1.18	0.2813	0.00	0.9725
	Sampling date	12, 45	55.18	< 0.0001	62.66	< 0.0001
	Cultivar x sampling date	12, 45	1.13	0.3488	0.52	0.8971

[z] Data were analyzed using PROC GLIMMIX in SAS; the effect was significant when $p < 0.05$.

Table 3.2. TSWV incidence data from four independent trials over two years fitted to the binary power law

Trial	Data set	ln(*Ap*) (SE)[y]	*Ap*	*b* (SE)[y]	R^{2z}
2019 Tifton	Georgia-06G-1	0.226 (0.112)	1.254	1.038 (0.031)**	0.996
	Georgia-06G-2	0.771 (0.114)*	2.162	1.118 (0.029)**	0.997
	Georgia-06G-3	0.733 (0.130)*	2.080	1.117 (0.034)**	0.996
	Georgia-06G-4	0.481 (0.104)*	1.618	1.078 (0.027)**	0.997
	Georgia Green-1	-0.037 (0.178)	0.964	0.976 (0.046)**	0.989
	Georgia Green-2	0.090 (0.207)	1.095	0.998 (0.055)**	0.985
	Georgia Green-3	0.243 (0.101)	1.275	1.041 (0.029)**	0.996
	Georgia Green-4	0.523 (0.082)*	1.688	1.084 (0.020)**	0.998
2019 Attapulgus	Georgia-06G-1	1.156 (0.148)*	3.178	1.206 (0.040)**	0.995
	Georgia-06G-2	1.145 (0.106)*	3.143	1.230 (0.030)**	0.997
	Georgia-06G-3	1.025 (0.171)*	2.786	1.180 (0.045)**	0.993
	Georgia-06G-4	0.886 (0.140)*	2.426	1.182 (0.038)**	0.995
	Georgia Green-1	0.967 (0.214)*	2.631	1.123 (0.053)**	0.989
	Georgia Green-2	0.493 (0.191)*	1.637	1.047 (0.044)**	0.991
	Georgia Green-3	0.515 (0.216)	1.673	1.062 (0.057)**	0.986
	Georgia Green-4	1.257 (0.235)*	3.514	1.206 (0.055)**	0.990
2020 Lang Farm	Georgia-06G-1	0.778 (0.198)*	2.177	1.082 (0.045)**	0.991
	Georgia-06G-2	0.424 (0.145)*	1.528	0.953 (0.034)**	0.994
	Georgia-06G-3	-0.619 (0.300)	0.538	0.795 (0.034)**	0.991
	Georgia-06G-4	0.536 (0.140)*	1.709	1.058 (0.030)**	0.996
	Georgia Green-1	0.782 (0.157)*	2.186	1.073 (0.040)**	0.993
	Georgia Green-2	1.115 (0.331)*	3.050	1.126 (0.076)**	0.978
	Georgia Green-3	1.116 (0.275)*	3.053	1.145 (0.065)**	0.984
	Georgia Green-4	0.435 (0.128)*	1.545	1.069 (0.027)**	0.997
2020 Ponder Farm	Georgia-06G-1	0.521 (0.095)	1.684	1.062 (0.023)**	0.998
	Georgia-06G-2	0.812 (0.372)	2.252	1.060 (0.081)**	0.972
	Georgia-06G-3	0.545 (0.112)*	1.725	1.080 (0.025)**	0.997
	Georgia-06G-4	0.416 (0.213)	1.516	1.039 (0.049)**	0.989
	Georgia Green-1	1.025 (0.097)*	2.787	1.145 (0.023)**	0.998
	Georgia Green-2	0.521 (0.234)*	1.684	1.057 (0.067)**	0.980
	Georgia Green-3	1.077 (0.155)*	2.936	1.184 (0.037)**	0.995
	Georgia Green-4	1.132 (0.232)*	3.102	1.149 (0.052)**	0.990

[y] $\ln(A_p)$ and b are the estimated intercepts and slopes, respectively, of the best fitting line based on least squares regressions with standard errors in parentheses; estimates notated with "*" and "**"are significant at $p < 0.05$ and $p < 0.0001$, respectively.

[z] Coefficient of determination (%).

Table 3.3. Characterization of spatial distribution of TSWV symptomatic plants with SADIE index of aggregation (I_a) in Georgia-06G and Georgia Green peanut field in trial in (a) Tifton and (b) Attapulgus, GA in 2019, and (c) Lang farm and (d) Ponder farm, Tifton GA in 2020

(a)

DAP[y]	Georgia-06G-1		Georgia-06G-2		Georgia-06G-3		Georgia-06G-4		Georgia Green-1		Georgia Green-2		Georgia Green-3		Georgia Green-4	
	Ia	*Pa*	*Ia*	*Pa*	*Ia*	*Pa*	*Ia*	*Pa*	*Ia*	*Pa*	*Ia*	*Pa*	*Ia*	*Pa*	*Ia*	*Pa*
35	1.624	0.0598	1.759	0.0402	0.748	0.7340	0.801	0.6596	0.79	0.6817	1.117	0.2867	0.812	0.6479	0.688	0.8274
49	0.803	0.6630	0.938	0.4667	0.875	0.5589	1.230	0.2066	1.584	0.0702	1.286	0.1656	0.774	0.7193	**2.468**	**0.0018***
63	1.283	0.1689	0.739	0.7905	1.199	0.2229	1.308	0.1565	0.752	0.761	1.017	0.3715	1.195	0.2192	1.704	0.0444
77	1.140	0.2616	1.611	0.0571	1.221	0.2061	1.371	0.1282	1.712	0.0407	1.231	0.1959	1.136	0.2594	1.396	0.1237
91	0.988	0.3979	1.478	0.0922	1.790	0.0342	1.766	0.0401	0.765	0.7434	1.298	0.1627	1.275	0.1768	1.117	0.2722
105	0.937	0.4748	1.479	0.0922	**2.025[z]**	**0.0139***	**1.964**	**0.0208***	0.829	0.6246	1.389	0.1222	1.212	0.2112	1.184	0.2304
119	0.855	0.5866	1.258	0.1823	**2.176**	**0.0097***	**1.919**	**0.0238***	0.761	0.7419	1.540	0.0778	1.094	0.3013	1.212	0.2142
Temporal spread model fit	Gompertz		Monomolecular		Gompertz		Gompertz		Gompertz		Gompertz		Gompertz		Gompertz	

(b)

DAP[y]	Georgia-06G-1		Georgia-06G-2		Georgia-06G-3		Georgia-06G-4		Georgia Green-1		Georgia Green-2		Georgia Green-3		Georgia Green-4	
	Ia	*Pa*	*Ia*	*Pa*	*Ia*	*Pa*	*Ia*	*Pa*	*Ia*	*Pa*	*Ia*	*Pa*	*Ia*	*Pa*	*Ia*	*Pa*
35	0.741	0.7622	1.259	0.1884	0.877	0.5452	0.752	0.7484	0.671	0.8421	0.876	0.5544	0.977	0.4205	1.050	0.3477
49	1.225	0.2112	0.849	0.5899	0.901	0.5192	1.061	0.3216	1.349	0.1480	0.769	0.7124	**2.208**	**0.0072***	1.004	0.3881
63	1.527	0.0835	1.126	0.2703	1.270	0.1849	1.437	0.1061	1.412	0.1140	1.514	0.0848	1.742	0.0396	1.047	0.3472
77	1.341	0.1425	1.595	0.0652	1.336	0.1404	1.788	0.0335	1.351	0.1374	1.646	0.0565	1.649	0.0530	1.123	0.2715
91	1.708	0.0412	1.602	0.0617	1.406	0.1133	1.607	0.0618	1.675	0.0481	1.485	0.0937	**2.303**	**0.0045***	1.621	0.0627
105	**2.179**	**0.0080***	1.322	0.1530	1.431	0.1073	1.826	0.0295	1.489	0.0861	1.664	0.0514	**2.602**	**0.0008***	1.381	0.1347
119	**2.123**	**0.0085***	1.263	0.1792	1.229	0.2066	1.707	0.0471	1.544	0.0754	1.291	0.1654	**2.403**	**0.0028***	1.627	0.0592
Temporal spread model fit	Gompertz		Gompertz		Gompertz		Gompertz		Gompertz		Gompertz		Gompertz		Gompertz	

(c)

DAP[y]	Georgia-06G-1		Georgia-06G-2		Georgia-06G-3		Georgia-06G-4		Georgia Green-1		Georgia Green-2		Georgia Green-3		Georgia Green-4	
	Ia	*Pa*	*Ia*	*Pa*	*Ia*	*Pa*	*Ia*	*Pa*	*Ia*	*Pa*	*Ia*	*Pa*	*Ia*	*Pa*	*Ia*	*Pa*
35	0.893	0.5939	1.246	0.1926	-	-	1.021	0.4176	0.598	0.9593	1.068	0.3456	1.097	0.3107	1.230	0.2180
49	**2.021[b]**	**0.0097***	1.411	0.1217	1.070	0.3702	**1.957**	**0.0158***	1.095	0.3087	1.221	0.2123	1.639	0.0561	1.414	0.1193
63	**2.298**	**0.0040***	1.297	0.1657	**1.883**	**0.0209***	1.808	0.0310	1.007	0.3813	1.638	0.0543	**1.946**	**0.0173***	1.459	0.1004
77	**3.289**	**0.0002***	1.462	0.0959	**2.400**	**0.0018***	**1.968**	**0.0147***	1.336	0.148	**2.473**	**0.0012***	**2.402**	**0.0030***	**2.048**	**0.0109***
91	**3.414**	**0.0002***	1.700	0.0411	1.775	0.0345	**1.917**	**0.0199***	**1.915**	**0.0181***	**2.268**	**0.0052***	**2.413**	**0.0022***	**2.177**	**0.0089***
105	**3.603**	**0.0002***	1.388	0.1210	1.516	0.0808	**1.921**	**0.0189***	1.783	0.032	**2.634**	**0.0007***	**2.069**	**0.0124***	**2.367**	**0.0034***
119	**3.572**	**0.0002***	1.451	0.1016	1.351	0.1332	1.746	0.0384	1.806	0.0308	**2.800**	**0.0005***	**2.329**	**0.0034***	**2.513**	**0.0013***
Temporal spread model fit	Gompertz		Monomolecular		Monomolecular		Monomolecular		Monomolecular		Monomolecular		Monomolecular		Monomolecular	

(d)

DAP[y]	Georgia-06G-1		Georgia-06G-2		Georgia-06G-3		Georgia-06G-4		Georgia Green-1		Georgia Green-2		Georgia Green-3		Georgia Green-4	
	Ia	*Pa*	*Ia*	*Pa*	*Ia*	*Pa*	*Ia*	*Pa*	*Ia*	*Pa*	*Ia*	*Pa*	*Ia*	*Pa*	*Ia*	*Pa*
35	0.885	0.6090	1.407	0.1314	1.205	0.2318	0.842	0.5889	0.767	0.9311	0.916	0.5019	0.807	0.6511	1.028	0.3818
49	1.710	0.0481	0.603	0.9645	1.264	0.1937	1.793	0.0327	1.264	0.1921	1.048	0.3447	0.75	0.7580	1.460	0.1059
63	**2.897**	**0.0002***	1.766	0.0416	1.576	0.0677	**2.328**	**0.0032***	1.906	0.0256	0.973	0.4245	0.861	0.5758	1.634	0.0623
77	**3.216**	**0.0002***	**2.093**	**0.0106***	1.042	0.3392	**2.607**	**0.0015***	1.837	0.0307	1.212	0.2105	0.867	0.5708	**2.707**	**0.0005***
91	**3.036**	**0.0002***	**2.234**	**0.0065***	1.027	0.3660	**2.598**	**0.0005***	1.216	0.2086	1.438	0.1076	0.975	0.4151	**2.403**	**0.0037***
105	**2.788**	**0.0002***	**1.906**	**0.0240***	1.065	0.3199	**2.793**	**0.0005***	1.079	0.307	1.452	0.0969	0.990	0.3972	**2.235**	**0.0039***
119	**2.898**	**0.0002***	**2.232**	**0.0059***	1.163	0.2435	**2.696**	**0.0007***	1.072	0.3228	1.471	0.0937	1.101	0.2908	**2.377**	**0.0025***
Temporal spread model fit	Monomolecular		Monomolecular		Monomolecular		Monomolecular		Monomolecular		Monomolecular		Monomolecular		Monomolecular	

[y] Sampling dates presented in days after planting (DAP).

[z] Index of aggregation (I_a) in bold indicates significant aggregated pattern with $I_a > 1$ according to a two-tail test at $p < 0.025$*.

Table 3.4. Spatiotemporal associations of TSWV distribution determined by association indices (*X*) in Georgia-06G and Georgia Green peanut field in (a) Tifton and (b) Attapulgus, GA in 2019 and (c) Lang farm and (d) Ponder farm, Tifton GA in 2020

(a)

Sampling date (DAP)[y]		Georgia-06G-1		Georgia-06G-2		Georgia-06G-3		Georgia-06G-4		Georgia Green-1		Georgia Green-2		Georgia Green-3		Georgia Green-4	
		X	*p*	*X*	*p*	*X*	*p*	*X*	*p*	*X*	*p*	*X*	*p*	*X*	*p*	*X*	*p*
35	49	0.1293	0.0857[z]	0.2652	0.0045	0.3416	0.064	0.2633	0.0218	0.302	0.0006	0.5051	<.0001	0.175	0.0539	-0.008	0.5233
49	63	0.3622	<.0001	0.5891	<.0001	0.529	<.0001	0.4638	<.0001	0.3744	<.0001	0.4067	0.0001	0.5582	<.0001	0.5292	<.0001
63	77	0.49	<.0001	0.481	<.0001	0.6519	<.0001	0.6038	<.0001	0.4555	<.0001	0.5342	<.0001	0.5072	<.0001	0.6594	<.0001
77	91	0.6102	<.0001	0.7754	<.0001	0.6601	<.0001	0.5947	<.0001	0.5782	<.0001	0.5609	<.0001	0.6113	<.0001	0.7208	<.0001
91	105	0.7565	<.0001	0.8596	<.0001	0.7775	<.0001	0.8061	<.0001	0.4306	<.0001	0.8144	<.0001	0.7264	<.0001	0.7277	<.0001
105	119	0.7717	<.0001	0.8107	<.0001	0.7006	<.0001	0.6448	<.0001	0.8248	<.0001	0.5045	<.0001	0.4239	<.0001	0.5293	<.0001

(b)

Sampling date (DAP)[y]		Georgia-06G-1		Georgia-06G-2		Georgia-06G-3		Georgia-06G-4		Georgia Green-1		Georgia Green-2		Georgia Green-3		Georgia Green-4	
		X	*p*	*X*	*p*	*X*	*p*	*X*	*p*	*X*	*p*	*X*	*p*	*X*	*p*	*X*	*p*
35	49	0.4778	<.0001	0.4184	<.0001	0.1308	0.1367	0.4577	0.0001	0.3619	0.0012	0.6344	<.0001	0.4537	0.001	0.6739	0.0004
49	63	0.4652	<.0001	0.4414	<.0001	0.6283	<.0001	0.576	<.0001	0.4575	<.0001	0.0491	0.3353	0.4342	<.0001	0.5057	<.0001
63	77	0.7111	<.0001	0.6862	<.0001	0.7011	<.0001	0.6493	<.0001	0.6496	<.0001	0.553	<.0001	0.6377	<.0001	0.6867	<.0001
77	91	0.7297	<.0001	0.6963	<.0001	0.7689	<.0001	0.6518	<.0001	0.7186	<.0001	0.6682	<.0001	0.7448	<.0001	0.6841	<.0001
91	105	0.7219	<.0001	0.6357	<.0001	0.8387	<.0001	0.6973	<.0001	0.8534	<.0001	0.7769	<.0001	0.7812	<.0001	0.6765	<.0001
105	119	0.6008	<.0001	0.746	<.0001	0.608	<.0001	0.6813	<.0001	0.924	<.0001	0.8144	<.0001	0.827	<.0001	0.8744	<.0001

(c)

Sampling date (DAP)[y]		Georgia-06G-1		Georgia-06G-2		Georgia-06G-3		Georgia-06G-4		Georgia Green-1		Georgia Green-2		Georgia Green-3		Georgia Green-4	
		X	*p*	*X*	*p*	*X*	*p*	*X*	*p*	*X*	*p*	*X*	*p*	*X*	*p*	*X*	*p*
35	49	0.8436	<.0001	0.448	<.0001	-	-	0.8231	<.0001	0.6852	<.0001	0.5778	<.0001	0.8033	<.0001	0.5094	0.0007
49	63	0.7418	<.0001	0.6497	<.0001	0.6719	<.0001	0.5666	<.0001	0.528	<.0001	0.8403	<.0001	0.6411	<.0001	0.5529	<.0001
63	77	0.7608	<.0001	0.5765	<.0001	0.3962	<.0001	0.6473	<.0001	0.6315	<.0001	0.7379	<.0001	0.8522	<.0001	0.6886	<.0001
77	91	0.7872	<.0001	0.7929	<.0001	0.4494	<.0001	0.8022	<.0001	0.6574	<.0001	0.7433	<.0001	0.8517	<.0001	0.6789	<.0001
91	105	0.8076	<.0001	0.75	<.0001	0.7774	<.0001	0.8542	<.0001	0.8472	<.0001	0.6159	<.0001	0.7946	<.0001	0.8192	<.0001
105	119	0.8685	<.0001	0.9105	<.0001	0.794	<.0001	0.7321	<.0001	0.9558	<.0001	0.9201	<.0001	0.853	<.0001	0.7931	<.0001

(d)

Sampling date (DAP)[y]		Georgia-06G-1		Georgia-06G-2		Georgia-06G-3		Georgia-06G-4		Georgia Green-1		Georgia Green-2		Georgia Green-3		Georgia Green-4	
		X	*p*	*X*	*p*	*X*	*p*	*X*	*p*	*X*	*p*	*X*	*p*	*X*	*p*	*X*	*p*
35	49	-0.4139	0.9997[z]	0.5425	<.0001	0.2642	0.0780	0.3747	0.0036	0.2584	0.1960	0.5122	0.0002	0.4741	0.0007	0.6201	<.0001
49	63	0.5627	<.0001	0.5284	<.0001	0.3034	0.0127	0.7711	<.0001	0.5986	0.0001	0.5886	<.0001	0.4899	<.0001	0.601	<.0001
63	77	0.7831	<.0001	0.6766	<.0001	0.53	<.0001	0.6682	<.0001	0.8304	<.0001	0.6021	<.0001	0.5504	<.0001	0.719	<.0001
77	91	0.8362	<.0001	0.7229	<.0001	0.6098	<.0001	0.7453	<.0001	0.7672	<.0001	0.7369	<.0001	0.7153	<.0001	0.763	<.0001
91	105	0.9342	<.0001	0.8391	<.0001	0.9227	<.0001	0.8547	<.0001	0.9577	<.0001	0.787	<.0001	0.8492	<.0001	0.8995	<.0001
105	119	0.913	<.0001	0.8374	<.0001	0.9282	<.0001	0.9603	<.0001	0.9642	<.0001	0.9347	<.0001	0.865	<.0001	0.9713	<.0001

[y] Spatiotemporal Analysis was conducted between successive sampling dates presented in days after planting (DAP).
[z] Underlined association indices (*X*) indicate no spatiotemporal association between the two sampling dates.

Table 3.5. Effect of time of first symptom observation on TSWV severity ratings of Georgia-06G and Georgia Green in four independent trials in 2019 and 2020

Trial	Cultivar[w]	First rating[x]			Final rating[y]		
		χ^{2z}	df	$P > \chi^2$	χ^2	df	$P > \chi^2$
2019 Tifton	Georgia-06G	73.48	6	<0.0001	85.62	6	<0.0001
	Georgia Green	53.54	6	<0.0001	78.12	6	<0.0001
2019 Attapulgus	Georgia-06G	75.70	6	<0.0001	86.46	6	<0.0001
	Georgia Green	45.37	6	<0.0001	92.00	6	<0.0001
2020 Lang Farm	Georgia-06G	41.53	6	<0.0001	80.38	6	<0.0001
	Georgia Green	42.05	6	<0.0001	88.81	6	<0.0001
2020 Ponder Farm	Georgia-06G	58.50	6	<0.0001	82.71	6	<0.0001
	Georgia Green	67.20	6	<0.0001	91.08	6	<0.0001

[w] Georgia-06G is a second-generation TSWV resistant cultivar and Georgia Green is a first-generation TSWV resistant cultivar.
[x] First TSWV severity ratings were obtained when symptom first observed.
[y] Final TSWV severity ratings were obtained a week before harvest.
[z] Severity ratings were subjected to Wilcoxon score analysis and Kruskal-Wallis test (median one-way ANOVA); the effect of time of first symptom observation was significant when $p < 0.05$.

Table 3.6. Effect of time of first symptom observation on whole plant biomass, pod weight, marketable kernel weight of Georgia-06G and Georgia Green in four independent trials in 2019 and 2020[u]

			Whole plant biomass[w]		Pod weight[x]		Marketable kernel weight[y]	
Trial	Cultivar[v]	df	F	P > F[z]	F	P > F[z]	F	P > F[z]
2019 Tifton	Georgia-06G	7, 160	32.85	<0.0001	44.22	<0.0001	43.52	<0.0001
	Georgia Green	7, 158	34.85	<0.0001	36.91	<0.0001	34.69	<0.0001
2019 Attapulgus	Georgia-06G	7, 169	26.03	<0.0001	26.14	<0.0001	22.55	<0.0001
	Georgia Green	7, 161	40.89	<0.0001	33.98	<0.0001	33.60	<0.0001
2020 Lang Farm	Georgia-06G	7, 149	25.06	<0.0001	32.91	<0.0001	31.76	<0.0001
	Georgia Green	7, 159	41.41	<0.0001	52.44	<0.0001	46.62	<0.0001
2020 Ponder Farm	Georgia-06G	7, 153	52.43	<0.0001	70.47	<0.0001	61.02	<0.0001
	Georgia Green	7, 159	25.90	<0.0001	31.48	<0.0001	34.82	<0.0001

[u] Whole plant biomass, pod weight, marketable kernel weight were compared between TSWV infected plants with different time of first symptom observation (seven sampling dates) and TSWV non-infected plants.

[v] Georgia-06G is a second-generation TSWV resistant cultivar and Georgia Green is a first-generation TSWV resistant cultivar.

[w] TSWV infected and non-infected plants were harvested manually and dried to obtain whole plant dried biomass.

[x] All peanut pods were removed and weighed after whole plant biomass was measured.

[y] Kernels were graded using three screen sizes: 21/64 x ¾", 18/64 x ¾", and 16/64 x ¾"; kernels larger than the 16/64 x ¾" size slot were considered marketable.

[z] Data were analyzed using PROC GLIMMIX in SAS; the effect of time of first symptom observation was significant when $p < 0.05$.

Table 3.7. Percentages of averaged, minimum, and maximum reduction of whole plant biomass, pod weight, marketable kernel weight in TSWV infected Georgia-06G and Georgia Green across time of first symptom observation in four independent trials in 2019 and 2020

Trial	Cultivar[w]	Averaged reduction (min, max) (%)[v]		
		Whole plant biomass[x]	Pod weight[y]	Marketable kernel weight[z]
2019 Tifton	Georgia-06G	35.74 (0.00, 89.68)	38.22 (0.00, 93.31)	40.12 (0.00, 95.06)
	Georgia Green	50.14 (1.03, 92.11)	55.03 (5.56, 96.57)	55.13 (5.96, 95.99)
2019 Attapulgus	Georgia-06G	52.51 (19.07, 86.89)	57.10 (26.42, 90.52)	54.79 (23.57, 89.19)
	Georgia Green	50.90 (19.62, 92.54)	57.81 (28.76, 95.36)	59.42 (29.75, 95.42)
2020 Lang Farm	Georgia-06G	48.24 (17.61, 91.45)	56.09 (18.62, 98.66)	56.87 (23.12, 99.65)
	Georgia Green	47.57 (13.41, 85.61)	59.70 (27.16, 97.99)	59.43 (26.78, 98.65)
2020 Ponder Farm	Georgia-06G	44.29 (3.74, 94.43)	51.55 (13.15, 98.85)	50.76 (10.00, 98.79)
	Georgia Green	44.83 (0.00, 82.55)	52.54 (5.66, 93.06)	52.50 (3.90, 93.85)

[v] Whole plant biomass, pod weight, marketable kernel weight of TSWV non-infected plants were used to obtained reduction percentages in TSWV infected plants with different time of first symptom observation (seven sampling dates).

[w] Georgia-06G is a second-generation TSWV resistant cultivar and Georgia Green is a first-generation TSWV resistant cultivar.

[x] TSWV infected and non-infected plants were harvested manually and dried to obtain whole plant dried biomass.

[y] All peanut pods were removed and weighed after whole plant biomass was measured.

[z] Kernels were graded using three screen sizes: 21/64 x ¾", 18/64 x ¾", and 16/64 x ¾"; kernels larger than the 16/64 x ¾" size slot were considered marketable.

Table 3.8. Summary of Pearson correlation analyses between final TSWV symptom severity rating and marketable kernel weight of TSWV infected Georgia-06G and Georgia Green collected from four independent trials in 2019 and 2020[w]

Trial	Cultivar[x]	Sample size	Correlation coefficient (r)[y]	*p*[z]
2019 Tifton	Georgia-06G	170	-0.7352	< 0.0001
	Georgia Green	169	-0.7053	< 0.0001
2019 Attapulgus	Georgia-06G	180	-0.5957	< 0.0001
	Georgia Green	172	-0.5617	< 0.0001
2020 Lang Farm	Georgia-06G	160	-0.7047	< 0.0001
	Georgia Green	168	-0.6892	< 0.0001
2020 Ponder Farm	Georgia-06G	164	-0.6874	< 0.0001
	Georgia Green	169	-0.6810	< 0.0001

[w] Final TSWV severity rating was obtained a week before harvest; kernels were graded using three screen sizes: 21/64 x ¾", 18/64 x ¾", and 16/64 x ¾"; kernels larger than the 16/64 x ¾" size slot were considered marketable.

[x] Georgia-06G is a second-generation TSWV resistant cultivar and Georgia Green is a first-generation TSWV resistant cultivar.

[y] Negative values of correlation coefficients indicated a negative correlation between final TSWV severity rating and marketable kernel weight.

[z] Correlations between final TSWV severity rating and marketable kernel weight were significant when $p < 0.05$.

CHAPTER 4

THE RELIABILITY OF DAS-ELISA FOR DETECTING TOMATO SPOTTED WILT ORTHOTOSPOVIRUS IN LEAF AND ROOT TISSUE FROM SYMPTOMATIC AND ASYMPTOMATIC PEANUT PLANTS[1]

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Abstract

Thrips-transmitted tomato spotted wilt orthotospovirus (TSWV) causes spotted wilt disease in peanut. Double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) is commonly used to detect TSWV. Previous studies have detected higher percentages of TSWV infection by DAS-ELISA in peanut root tissue than leaf tissue samples and have suggested that root tissue might be a better sink for TSWV than leaf tissue. However, whether the higher percentage of TSWV detection in root tissue than leaf tissue samples is due to root tissue being a better sink for TSVW than leaf tissue or is an overestimation of TSWV infection by DAS-ELISA in root than leaf tissue samples is unclear. Asymptomatic infection of TSWV has been documented in peanut, and it is not clear if TSWV detection in asymptomatic plants is also affected by the tissue type. To address these questions, TSWV detection in leaf and root tissue samples from symptomatic and asymptomatic plants by DAS-ELISA was cross-validated with reverse transcription polymerase chain reaction (RT-PCR) and quantitative RT-PCR (qRT-PCR). TSWV was also quantitated by qRT-PCR in leaf and root tissue samples. TSWV was detected by DAS-ELISA, RT-PCR, and qRT-PCR with similar efficiency in leaf and root tissue samples of symptomatic plants and in leaf tissue of asymptomatic plants. However, TSWV was detected at a significantly higher percentage in root tissue samples of asymptomatic plants via DAS-ELISA than by RT-PCR and qRT-PCR indicating that DAS-ELISA usage for TSWV detection in root tissue samples could result in overestimation of TSWV infection. Leaf tissue samples had more TSWV loads than root tissue samples from symptomatic plants, while TSWV loads in leaf and root tissue samples from asymptomatic plants were equally low when compared with symptomatic plants. These findings suggest that leaf tissue is a better choice than root tissue for TSWV detection in peanut using DAS-ELISA.

Introduction

Spotted wilt disease of peanut (*Arachis hypogaea* L.) is caused by the tomato spotted wilt orthotospovirus (TSWV), and TSWV is exclusively transmitted by nine thrips species in the order Thysanoptera and family Thripidae (Culbreath et al. 2003; Riley et al. 2011; Rotenberg et al. 2015). *Tomato spotted wilt orthotospovirus* is the type species in the genus *Orthotospovirus*, family *Tospoviridae*, and order *Bunyavirales*. TSWV infection in peanut was first reported in the United States in Texas in 1971 (Halliwell and Philley 1974). The virus has since become ubiquitous across major peanut-producing states in the Southeast (Black et al. 1986; Culbreath, Todd, Demski, et al. 1992; Garcia et al. 2000; Hagan 1990; Reed and Sukamto 1995). In Georgia, the top U.S. peanut-producing state, the annual peanut yield loss to TSWV was estimated at over US $20 million from 2015 to 2018 (Little 2017, 2019a, 2019b, 2020; USDA-NASS 2020).

The most prominent symptoms of spotted wilt disease are concentric ring spots and chlorosis on peanut foliage. In severe cases of TSWV infection, peanut plants are stunted. Other symptoms such as small or misshaped pegs, pods, kernels, and reddish discoloration of the seed coats can also be found on below-ground plant parts (Culbreath et al. 1992a, 1992b; Culbreath et al. 2003; Halliwell and Philley 1974). Visual assessment of symptoms is commonly used to evaluate spotted wilt incidence and severity (Culbreath et al. 2010; Cantonwine et al. 2006; Wells et al. 2002). However, biotic or abiotic factors including temperature/water stress, nutrient deficiency, pest infestation, and other pathogen infection often confound spotted wilt disease symptoms induced by TSWV infection. Oftentimes, other TSWV detection techniques are used to confirm the presence of the virus. Among the available techniques, serology-based double

antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) is most commonly used to detect TSWV in peanut tissues; another less-frequently used technique is nucleic acid-based reverse transcription polymerase chain reaction (RT-PCR) (Culbreath et al. 1991; Dang et al. 2009; Jain et al. 1998; Kresta et al. 1995; Murakami et al. 2006; Pappu, Pappu, et al. 1999; Rowland et al. 2005; Wang et al. 2007). Quantitative reverse transcription polymerase chain reaction (qRT-PCR) is another technique that has become available relatively recently to detect TSWV and/or to quantitate TSWV loads in peanut tissue samples (Shrestha et al. 2013, 2015; Sundaraj et al. 2014).

TSWV incidence is often evaluated to assess TSWV susceptibility of cultivars/genotypes in breeding trials and the efficacy of TSWV management practices (Culbreath et al. 2000, 2008, 2013; Murakami et al. 2006; Wang et al. 2007). DAS-ELISA has become the standard plant virus detection method due to its relatively low cost and scalability for larger sample sizes (Boonham et al. 2014; Torrance and Jones 1981; Ward et al. 2004). In DAS-ELISA, the TSWV capsid protein in peanut leaf/root tissue samples is selectively trapped by TSWV capsid protein-specific monoclonal antibody coated on a polystyrene microtiter plate. The captured TSWV then interacts with another TSWV-specific antibody conjugated with an enzyme (i.e., alkaline phosphatase). The enzyme-conjugated antibody-TSWV capsid protein complex induces a colorimetric response in the presence of the enzyme substrate para-nitrophenyl phosphate (PNP) (Clark and Adams 1977; Sherwood 1989). RT-PCR is more specific and sensitive than DAS-ELISA but is less frequently used, as it requires total RNA extraction from peanut tissue samples, specialized equipment, advanced operational skills, and is more expensive than DAS-ELISA (Boonham et al. 2014; Putnam 1995). In RT-PCR, the total RNA extracted from the test sample is subjected to reverse transcription to synthesize complementary DNA (cDNA). The

cDNA is then used as the template in PCR with TSWV capsid protein gene-specific primers to amplify the TSWV capsid protein gene, which can be visualized through agarose gel electrophoresis (Jain et al. 1998; Schochetman et al. 1988). QRT-PCR differs from RT-PCR in that the PCR product is detected in real time during amplification. This is commonly achieved using non-specific fluorescent dyes that intercalate with double-stranded DNA or complementary fluorescent probes that bind to the amplicon by base-pairing (Mackay et al. 2002). The amount of the template (i.e., TSWV capsid protein gene) can be determined by absolute/relative quantitation through qRT-PCR (Boonham et al. 2002; Rotenberg et al. 2009). Absolute quantitation compares the cycle threshold (Ct) (when the number of templates from amplification is enough to trigger exponential phase of the reaction) of test samples to serially diluted copies of the target gene representing a standard curve. Relative quantitation uses Ct values to determine the relative changes in template quantity between the gene of interest and a housekeeping gene (Mackay et al. 2002; Pfaffl 2004).

TSWV infection in peanut is systemic, and TSWV has been detected in multiple peanut plant tissues, including leaves, pegs, pods, and roots (Pappu et al. 1999; Rowland et al. 2005). Peanut leaf tissue is typically used for TSWV detection via DAS-ELISA; however, one study indicated that distribution of TSWV in peanut leaves was not uniform (Kresta et al. 1995). In other studies, TSWV was detected at a higher percentage in root tissue samples than in leaf tissue samples (Culbreath et al. 1991; Culbreath et al. 1992a; Murakami et al. 2006; Rowland et al. 2005) . This led to the presumption that root tissue could serve as a better sink for TSWV than leaf tissue. Nevertheless, *in planta* movement and accumulation of TSWV in peanut is not completely understood. It is not clear if the difference in TSWV detection between root and leaf tissue samples via DAS-ELISA is due to higher virus loads in root versus leaf tissue samples, or

if the difference is the result of the DAS-ELISA producing false positives when root tissue samples are tested. A more recent study compared TSWV detection by DAS-ELISA and RT-PCR using root tissue samples from randomly collected field-grown peanut plants and found good congruence between the two methods (Dang et al. 2009). However, thus far no study has tested TSWV symptomatic leaf and root tissue samples with DAS-ELISA in comparison with RT-PCR. Besides symptomatic TSWV infection, asymptomatic infection of TSWV is often detected. Asymptomatic TSWV infection has been identified by biological assays in diploid peanut species following TSWV inoculation using asymptomatic leaf tissues (Lyerly et al. 2002). In some instances, the incidence of TSWV asymptomatic infection based on DAS-ELISA of root tissue samples was as high as the incidence of symptomatic infection (Culbreath et al. 1991; Culbreath and Srinivasan 2011; Murakami et al. 2006). DAS-ELISA based virus detection in leaf and root tissue samples could be confounded by numerous extraneous factors and warrants cross validation to assess whether DAS-ELISA based TSWV detection in asymptomatic plant tissue samples is accurate or an over estimation due to detection of false positives (Lacroix et al. 2016; Mahmoud 2011; Van Schadewijk et al. 2011; Sikora et al. 1999). Thus far no such cross validation has been performed with asymptomatic plant tissue samples.

To clarify the effectiveness of TSWV detection techniques in relevant tissue types from symptomatic and asymptomatic plants, this study compared TSWV detection efficiency via DAS-ELISA, RT-PCR, and qRT-PCR across leaf and root tissue samples from symptomatic and asymptomatic plants. In addition, this study also used qRT-PCR to quantitate TSWV loads in both tissue type samples to assess if root tissue is a better TSWV sink than leaf tissue.

Materials and Methods

Sample collection. Samples of leaf and root tissue from TSWV symptomatic and asymptomatic peanut plants were used for TSWV detection. Samples were collected from four peanut fields established at the University of Georgia research farms in Tifton and Attapulgus, GA in 2018 and 2019. From symptomatic plants, peanut leaves with distinct spotted wilt symptoms at growing points (top 10-15 cm) were collected. Leaf tissue samples from asymptomatic plants were randomly collected at growing points. Sections (~10 cm) of the primary root (i.e., taproot) cut immediately below the crown of the plants from which leaf samples were collected were used for TSWV detection. Samples of leaf and root tissue were placed in resealable bags and transported to the laboratory on ice. Field collected samples were stored at 4°C for up to five days before being processed and tested by DAS-ELISA. Afterwards, field samples were stored at -80°C and later tested by RT-PCR and qRT-PCR.

In 2018, samples of leaf and root tissue from 20 TSWV symptomatic plants (cultivar Georgia-06G) were collected from a peanut field planted on 30th April at the Ponder Farm in Tifton, GA. Symptomatic plants were sampled at 35 (n=2), 49 (n=2), 63 (n=5), 77 (n=6), and 91 (n=6) days after planting (DAP). In addition, samples of leaf and root tissue were collected from asymptomatic plants in the same field at 140 DAP (n=1) and in a field planted on the same date at the research station in Attapulgus at 141 DAP (n=8). Asymptomatic samples were collected from four peanut cultivars: Georgia-06G, Georgia Green, Georgia-16HO, and TUFRunner 511 (Branch 1996, 2007, 2017; Tillman and Gorbet 2017).

In 2019, samples of leaf and root tissue from 48 symptomatic plants were collected from a peanut field planted on 24th April at the Lang Farm in Tifton, GA. Six plants of each cultivar namely Georgia-06G and Georgia Green were randomly sampled on each sampling day (35, 49,

63, and 77 DAP). Samples of leaf and root tissue were also collected from 21 asymptomatic plants at 141 DAP from the same field in Tifton, and another 21 asymptomatic plants were sampled at 139 DAP from a peanut field planted on 23rd April at the research station in Attapulgus, GA.

Detection of TSWV in leaf and root tissue samples by DAS-ELISA, RT-PCR, and qRT-PCR. Samples of leaf and root tissue from symptomatic and asymptomatic plants were tested for the presence of TSWV using DAS-ELISA, RT-PCR, and qRT-PCR. TSWV loads in leaf and root tissue samples were quantitated by qRT-PCR.

DAS-ELISA was performed in 96-well microtiter plates. A positive control (frozen, symptomatic leaf tissue of field-grown tobacco plants) and negative control (leaf tissue of greenhouse-grown, non-infected peanut plants) with two replications each were included in each plate along with test samples. Approximately 0.03 g of either leaf or root tissue samples were ground in 300 □l of sample extraction buffer. Primary (anti-TSWV IgG) and secondary (anti-TSWV IgG conjugated with alkaline phosphatase) antibodies were used at a 1:200 dilution ratio (Agdia, Elkhart, IN). The assay steps, including enzyme coating, incubation, and washing were followed as per the manufacturer's instructions. An hour after adding the substrate, final absorbance values were measured by a photometer at 405 nm (model Elx 800, Bio-Tek, Kocherwaldstr, Germany). Samples were considered positive when the final absorbance value was greater than the threshold value of average absorbance value of negative control samples plus four standard deviations. To be more stringent, a value of 0.1 was adopted to define positive samples when the calculated threshold value was less than 0.1.

For RT-PCR and qRT-PCR, total RNA from leaf and root tissue samples was extracted using RNeasy plant mini kit (Qiagen, Valencia, CA) according to the protocol provided by the

manufacturer. Approximately 0.1 g of leaf or root tissue samples was used for RNA extraction. The concentration and the quality of the total RNA extracts were evaluated by NanoDrop™ 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA). The concentration of total RNA in samples was normalized to 50 (±5) ng/µl. Complimentary DNA (cDNA) was synthesized using normalized total RNA samples with Go-Script reverse transcription system (Promega Corporation, Madison, WI) following the manufacturer's instructions. Oligo dT primers were used for cDNA synthesis, and cDNA from each sample was used as a template for RT-PCR and qRT-PCR. Total RNA obtained from a TSWV positive leaf tissue sample collected from an infected peanut plant in Tifton, GA was used as a positive control. Total RNA obtained from a leaf tissue sample collected from a non-infected, greenhouse grown peanut plant was used as a negative control.

PCR was performed in a DNA engine thermocycler (Bio-Rad Laboratories, Hercules, CA). The reaction mix included 5 µl of GoTaq Green Master Mix (Promega Corporation, Madison, WI), 0.5 µl (0.5 µM) of each forward and reverse primer specific to the TSWV-N gene (forward: 5'-ATGTCTAAGGTTAAGCTC-3'; reverse: 5'-TTAAGCAAGTTCTGTGAG-3') (Jain et al. 1998), 1 µl of synthesized cDNA (1µg/µl cDNA from 2.5ng/µl total RNA), and the total reaction volume was adjusted to 10 µl by adding nuclease-free water. The PCR program started with an initial activation step at 95°C for 15 min, followed by 35 cycles of 94°C for 1 min, 52°C for 45 s, and 72°C for 1 min with a final extension step at 72°C for 10 min. The presence of the targeted amplicons (~ 800 bp) was visualized by agarose gel (1 %) electrophoresis.

The cDNA from leaf and root tissue samples were used for qPCR. QPCR was performed in a Realplex Mastercycler (Eppendorf, Hamburg, Germany). The reaction mix consisted of 2 µl

of synthesized cDNA (5ng total RNA), 12.5 µl of GoTaq qPCR 2X MasterMix (Promega Corporation, Madison, WI), 0.5 µl of each forward and reverse primers (0.2 µM), and the final volume was brought to 25 µl by adding nuclease-free water. A pair of TSWV-N gene specific primers were used (forward: 5'-GCTTCCCACCCTTTGATTC-3'; reverse: 5'-ATAGCCAAGACAACACTGATC-3') (Rotenberg et al. 2009). Each sample, including test samples and positive and negative controls, was duplicated per qPCR run. The reaction program started with an initial step of 95°C for 2 mins, followed by 40 cycles of 95°C for 15 s, 55°C for 60 s, and 72°C for 20 s. The reaction program was extended with a melting curve analysis, which involved incubating the reaction mix at 95°C for 15 s, 60°C for 15 s and increasing the temperature by 0.5°C per min for 20 min, with a final step of 95°C for 15 s. Melting curve analysis was used to rule out non-specific binding of primers. TSWV loads in the samples were quantitated using the standard curve protocol with plasmid carrying TSWV-N gene inserts described by Shrestha et al. (2013).

Statistical analyses. Whether TSWV detection varied by tissue type and/or detection method was determined for samples from both symptomatic and asymptomatic plants. Data from symptomatic and asymptomatic plants were analyzed separately. The experiment was a two-way factorial design. Data were subjected to analysis of variance using PROC GLIMMIX procedure with binomial distribution in SAS (SAS Enterprise 9.4, SAS Institute, Cary, NC). Tissue type and detection method were the fixed effects, while year and replication were random effects in the analysis. Least square means were used to identify differences in TSWV detection at α = 0.05 significance level with Tukey-Kramer adjustment.

TSWV loads obtained from qRT-PCR were compared between leaf and root tissue samples from symptomatic and asymptomatic plants. Data from symptomatic and asymptomatic

plants were analyzed separately and subjected to analysis of variance using PROC GLIMMIX procedure with negative binomial distribution in SAS. The tissue type served as a fixed effect while year and replication served as random effects. Least square means of TSWV copies were used to identify differences between tissue types at $\alpha = 0.05$ significance level with Tukey-Kramer adjustment.

Results

Detection of TSWV in leaf and root tissues of symptomatic plants. TSWV detection in symptomatic leaf tissue samples was 100.00%, 89.71%, 97.06% via DAS-ELISA, RT-PCR, and qRT-PCR, respectively (Fig. 4.1a). TSWV detection in root tissue samples from symptomatic plants by DAS-ELISA, RT-PCR, and qRT-PCR was 82.35%, 77.94%, and 91.18%, respectively (Fig. 4.1a). TSWV detection was significantly affected by detection method ($F_{2,401} = 3.17$, $p = 0.0432$) but did not vary by tissue type ($F_{1,401} = 0$, $p = 0.9888$), and the interaction (tissue type x detection technique) effect was not significant ($F_{2,401} = 0.03$, $p = 0.9701$). Detection of TSWV in symptomatic plants was high for all three methods across tissue types. While TSWV detection by qRT-PCR was significantly higher than RT-PCR, TSWV detection by DAS-ELISA was not different from RT-PCR or qRT-PCR (Fig. 4.1b). In addition, when comparing detection methods within each tissue type, TSWV detection was not different among the three methods in either leaf tissue samples or root tissue samples (Fig. 4.1a).

Detection of TSWV in leaf and root tissues of asymptomatic plants. TSWV detection in leaf tissue samples from asymptomatic plants was 23.53%, 11.76%, 17.65% for DAS-ELISA, RT-PCR, and qRT-PCR, respectively (Fig. 4.2). Detection of TSWV in root tissue samples from asymptomatic plants was 90.20%, 1.96%, 9.80% for DAS-ELISA, RT-PCR, and qRT-PCR,

respectively (Fig. 4.2). A significant interaction effect between detection method and tissue type on TSWV detection was found ($F_{2,299} = 16.63$, $p < 0.0001$). TSWV detection percentages for the three detection methods were compared within each tissue type. TSWV detection in leaf tissue from asymptomatic plants was not different among the three detection methods ($F_{2,299} = 1.25$, $p = 0.2877$) (Fig. 4.2). However, percent detection of TSWV significantly varied by detection method in root tissue from asymptomatic plants ($F_{2,299} = 24.64$, $p < 0.0001$). TSWV detection by DAS-ELISA was significantly higher than RT-PCR and qRT-PCR (Fig. 4.2). Mean percentage of TSWV detection via DAS-ELISA was 46 times higher than RT-PCR and 9 times higher than qRT-PCR.

TSWV accumulation in leaf and root tissue of symptomatic and asymptomatic plants. TSWV loads in leaf and root tissue samples from symptomatic and asymptomatic plants were compared. Overall, TSWV loads were higher in symptomatic plant samples than asymptomatic plant samples ($F_{1,137} = 11.05$, $p = 0.0011$) irrespective of tissue type (Fig. 4.3a). TSWV loads were significantly higher in leaf tissue samples than root tissue samples from symptomatic plants ($F_{1,125} = 15.56$, $p = 0.0001$) (Fig. 4.3b). However, TSWV loads in leaf and root tissue samples from asymptomatic plants were not significantly different ($F_{1,11} = 0.13$, $p = 0.7215$) (Fig. 4.3c).

Discussion

Evaluation of spotted wilt disease caused by TSWV in peanut is commonly accomplished by visual assessments of typical TSWV-induced symptoms, such as yellowing, concentric ring spots, and stunting (Culbreath et al. 2003; Halliwell and Philley 1974). Foliar symptom-based screening can be confounded by biotic factors such as infection of other pathogens, arthropod

infestation, TSWV resistance status, and timing of infection. For example, infection of impatiens necrotic spot virus (INSV) and peanut mottle virus (PMV) can produce foliar symptoms akin to TSWV infection in peanut (Sreenivasulu 1988; Hoffmann et al. 1998; Pappu, Black, et al. 1999). Abiotic factors such as environmental conditions and chemical injury can also lead to foliar symptoms that resemble TSWV infection (Brecke et al. 1996; Mandal et al. 2002; Morichetti and Ferrell 2010). In such instances, it is useful to confirm TSWV infection with either a serology-based detection technique such as DAS-ELISA or a nucleic acid-based detection technique such as RT-PCR, with the former being used more often than the later. A few previous studies found that TSWV was more often detected by DAS-ELISA in peanut root tissue than leaf tissue samples (Rowland et al. 2005; Murakami et al. 2006; Culbreath et al. 1991; Culbreath, Todd, and Demski 1992a). However, DAS-ELISA is prone to producing false detection results when unstandardized tissue types are used, and it is unclear whether the high frequency of TSWV detection in root tissue samples was a reflection of true TSWV incidence or an overestimation due to false positives. This study attempts to cross validate DAS-ELISA detection results from both tissue types with more sensitive nucleic acid-based detection assays.

In this study, TSWV infection in leaf and root tissue samples from symptomatic and asymptomatic peanut plants was assessed by DAS-ELISA, RT-PCR, and qRT-PCR. TSWV accumulation in leaf and root tissue samples was also quantitated using qRT-PCR. Results from this study show that percent TSWV detection did not vary between leaf and root tissue samples or among the three detection methods in each tissue type from symptomatic plants. In addition, symptomatic leaf tissue samples had higher TSWV loads than root tissue samples from symptomatic plants. Dang et al. (2009) also found congruency in TSWV detection between DAS-ELISA and RT-PCR while using peanut root tissue samples. However, it was not clear if

the root tissue samples used for detection were collected from symptomatic and/or asymptomatic plants. Also, that study did not compare TSWV detection between root and leaf tissue samples using the same two techniques.

In general, TSWV detection in asymptomatic leaf and/or root tissue samples was significantly lower than in symptomatic tissue samples. For samples from asymptomatic plants, this study found similar percentages of detection between DAS-ELISA, RT-PCR, and qRT-PCR in leaf tissue samples, but percentages of detection varied with methods in root tissue samples. TSWV detection via DAS-ELISA was significantly higher than detection by RT-PCR and qRT-PCR in root tissue samples of asymptomatic plants. The inconsistency in TSWV detection between detection methods using root tissue samples from asymptomatic plants could, in part, be explained by two possible scenarios: either DAS-ELISA overestimated TSWV infection (false positive), or RT-PCR and qRT-PCR underestimated TSWV infection (false negative). Low detection sensitivity of a detection assay could cause underestimation of TSWV infection. Sensitivity can be defined as the capability of the method/assay to reliably detect the lowest number of pathogen copies per test sample (López et al. 2003). Generally, the sensitivity of nucleic acid-based detection assays with the use of gene-specific primers is higher than serology-based assays (Ward et al. 2004; López et al. 2003; Schaad et al. 2003). While the sensitivity of DAS-ELISA and RT-PCR has not been evaluated and compared specifically for TSWV detection in peanut, such comparisons have been documented in other crops and pathosystems. Results from multiple studies indicate that the sensitivity of PCR can range from 2 to 625 fold higher than ELISA (Hu et al. 1995; Adams et al. 1999; Sanchez-Navarro et al. 1998; Mekuria et al. 2003; Mumford et al. 1994; Webster et al. 2017). Thus, it is unlikely that RT-PCR and qRT-PCR underestimated TSWV infection when compared with DAS-ELISA, and detection

sensitivity is unlikely to explain the differences in TSWV detection among detection methods observed in this study.

The reliability of ELISA can be affected by factors leading to inaccurate detection results, even though ELISA is generally sensitive and specific with the use of monoclonal antibodies (Boonham et al. 2014). Well-recognized causes of inaccurate detection results include non-homogenous virus distribution in plants, interference of plant extracts, failure to detect certain virus serotypes, and cross reactivity with other closely related viruses (Van Schadewijk et al. 2011). In addition, physiological and biochemical characteristics of the host plant and tissue type chosen for virus detection are important factors that are known to interfere with serological reactions in ELISA (Lacroix et al. 2016; Gunn and Pares 1988; Mahmoud 2011; Sikora et al. 1999). Non-specific antigen-antibody interaction due to the presence of plant proteins could lead to false positives in ELISA (Naidu and Hughes 2001; Mumford et al. 1994). Detection of potato leafroll virus (PLRV) in potato tubers via DAS-ELISA resulted in 70% overestimation of PLRV infection due to false positives (Gunn and Pares 1988). Gunn and Pares (1988) speculated that the false positives could have been caused by non-specific antibodies originating from co-purification of non-virus antigens (plant proteins) along with PLRV in the PLRV-specific antibody production process. While blocking reagents, such as ovalbumin, bovine serum albumin (BSA), and polyvinylpyrrolidone (PVP) are typically included in the commercial DAS-ELISA kits to reduce non-specific reactions, false positives could still arise from non-virus antigen-antibody interactions. Plant proteins such as pathogenesis-related proteins, lectins, and sesquiterpenoids, are often present in virus-infected plants, and these plant-originated proteins could induce non-specific binding in ELISA (Redolfi 1983; Etzler 1985; Gunn and Pares 1988). For example, non-specific reactions in DAS-ELISA for cucumber mosaic virus detection in

ornamental plants and wild weed species resulted in false positives (Mahmoud 2011; Sikora et al. 1999). The authors demonstrated that components in plant extracts bound to microtiter plates and interacted with antibodies even under conditions that were unsuitable for antigen binding (i.e., the neutral pH and the presence of Tween) (Mahmoud 2011; Sikora et al. 1999). Lectins are commonly present in peanut root tissue where they serve as defense proteins and play an important role for rhizobia agglutination in legume roots (Kijne et al. 1997; Peumans and Van Damme 1994). Plant roots are known to secrete chemical compounds, such as phenolics, terpenoids, and associated secondary metabolites, for defense against pathogenic microorganisms in soil (Baetz and Martinoia 2014). While such proteins could play a role in overestimating TSWV infection in peanut, no empirical studies have been conducted to implicate their interference in DAS-ELISA-based detection.

Over or under estimation of TSWV infection by DAS-ELISA could also be affected by the test threshold used. Threshold absorbance values to determine virus infection via DAS-ELISA have varied across studies ranging from two or three times the average absorbance value of negative controls (i.e., non-infected plant tissue) to the average absorbance value of negative controls plus three or four times the standard deviation (Sutula et al. 1986). It has been demonstrated that setting a more stringent detection threshold reduces false positives and increases false negatives. In one study where a range of threshold absorbance values were calculated using different methods, the lowest threshold value (0.040) resulted in 0.00% false negatives and 9.09% false positives, whereas the highest threshold values (0.131) resulted in 11.11% and 2.04% false negatives and false positives, respectively (Sutula et al. 1986). In this study, a high threshold value was selected using either a value of 0.1 or the mean of negative controls plus four standard deviations that was higher than 0.1. The stringency of the threshold

value should help avoid false positives. In addition, both inarguably high (>3.0) and low (<0.01) absorbance values were obtained from root samples with none of the values being ambiguous or close to the baseline threshold (0.1) used in this study. If indeed DAS-ELISA was overestimating the TSWV infection in root tissue samples, it was likely due to non-specific antigen-antibody interactions rather than false positives related to threshold values.

Over or under estimation of virus infection could also occur with nucleic acid-based detection assays. False negative results commonly occur due to inhibitors interfering with the reaction by binding to DNA templates, interacting or competing with cofactor ions, and denaturing or degrading DNA polymerase (Rådström et al. 2008). Inhibitors are substances that are unintentionally extracted along with the nucleic acids of test samples; examples include phenolic compounds and heavy metals from the environment, cell debris, and residual reagents from extraction procedure (e.g. phenol, EDTA, ethanol, and isopropanol) (Wilson 1997; Lacroix et al. 2016). PCR inhibitors were unlikely a concern in the current study as a column-based RNA extraction was utilized. In addition, RT-PCR and qRT-PCR efficiently detected TSWV in root tissue samples from symptomatic plants using the same extraction method. Using column-based extraction can improve the purity of nucleic acid extracts; however, it can also increase the risk of losing nucleic acid extracts, which may lead to false negatives (Rådström et al. 2004; Lacroix et al. 2016). Loss of nucleic acids of the pathogen of interest can be especially problematic when the original quantity is relatively low, such as in asymptomatic plants. Asymptomatic plants generally accumulated less virus when compared with symptomatic plants (Zhu et al. 2010; Schaad and Frederick 2002). This phenomenon was also observed in the current study, as TSWV loads were 3-fold higher in symptomatic than asymptomatic tissue samples across tissue types.

According to the qRT-PCR results, TSWV loads were higher in leaf tissue samples than in root tissue samples of symptomatic plants, while TSWV loads were not significantly different between leaf and root tissue samples of asymptomatic plants. The results indicate that it would be easier to detect TSWV in leaf than root tissue samples of symptomatic plants due to higher TSWV accumulation in leaf tissue. When detecting TSWV in asymptomatic plants, leaf tissue samples are a better choice than root tissue samples especially when DAS-ELISA is used due to the possibility of false positives with root tissue samples. DAS-ELISA has a tendency to overestimate TSWV in root tissue samples, and results from DAS-ELISA should be cross validated with other detection methods to avoid overestimation.

This study demonstrated that DAS-ELISA might not be suitable for TSWV detection in peanut root tissue samples and could potentially lead to overestimation of TSWV infection. Nevertheless, DAS-ELISA could still be a reliable TSWV detection assay for peanut leaf tissue samples and good for large-scale screening in symptomatic/asymptomatic plants if appropriate tissue type is used. Root tissue does not seem to be a better sink than leaf tissue for TSWV either in symptomatic or asymptomatic plants. In fact, it is possible to overestimate TSWV incidence in root tissue samples on some occasions if DAS-ELISA is used, possibly due to non-specific plant antigen-TSWV antibody interactions.

Literature Cites

Adams, A. N., Guise, C. M., and Crossley, S. J. 1999. Plum pox virus detection in dormant plum trees by PCR and ELISA. Plant Pathol. 48:240–244.

Baetz, U., and Martinoia, E. 2014. Root exudates: The hidden part of plant defense. Trends Plant Sci. 19:90–98.

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

Boonham, N., Kreuze, J., Winter, S., van der Vlugt, R., Bergervoet, J., Tomlinson, J., et al. 2014. Methods in virus diagnostics: From ELISA to next generation sequencing. Virus Res. 186:20–31.

Boonham, N., Smith, P., Walsh, K., Tame, J., Morris, J., Spence, N., et al. 2002. The detection of Tomato spotted wilt virus (TSWV) in individual thrips using real time fluorescent RT-PCR (TaqMan). J. Virol. Methods. 101:37–48.

Branch, W. D. 2007. Registration of 'Georgia-06G'' Peanut.' J. Plant Regist. 1:120.

Branch, W. D. 2017. Registration of 'Georgia-16HO' Peanut. J. Plant Regist. 11:231–234.

Branch, W. D. 1996. Registration of 'Georgia Green' Peanut. Crop Sci. 36:806–806.

Brecke, B. J., Funderburk, J. E., Teare, I. D., and Gorbet, D. W. 1996. Interaction of early-season herbicide injury, tobacco thrips injury, and cultivar on peanut. Agron. J. 88:14–18.

Cantonwine, E. G., Graduate Student, F., Culbreath, A. K., and Stevenson, K. L. 2006. Integrated Disease Management of Leaf Spot and Spotted Wilt of Peanut.

Clark, M. F., and Adams, A. N. 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., Csinos, A. S., Brenneman, T. B., Demski, J. W., and Todd, J. W. 1991. Association of Tomato Spotted Wilt Virus with Foliar Chlorosis of Peanut in Georgia. Plant Dis. 75:863C.

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

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

Culbreath, A. K., Todd, J. W., and Brown, S. L. 2003. Epidemiology and Management of Tomato Spotted Wilt in Peanut. Annu. Rev. Phytopathol. 41:53–75.

Culbreath, A. K., Todd, J. W., and Demski, J. W. 1992a. Comparison of hidden and apparent spotted wilt epidemics in peanut. Proc. Am. Peanut Res. Educ. Soc. 24:39.

Culbreath, A. K., Todd, J. W., and Demski, J. W. 1992b. Productivity of Florunner Peanut Infected with Tomato Spotted Wilt Virus. Peanut Sci. 19:11–14.

Culbreath, A. K., Todd, J. W., Demski, J. W., and Chamberlin, J. R. 1992. Disease Progress of Spotted Wilt in Peanut Cultivars Florunner and Southern Runner. Phytopathology. 82:766.

Culbreath, A. K., Todd, J. W., Gorbet, D. W., Brown, S. L., Baldwin, J., Pappu, H. R., et al. 2000. Reaction of Peanut Cultivars to Spotted Wilt. Peanut Sci. 27:35–39.

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

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

Etzler, M. E. 1985. *PLANT LECTINS: MOLECULAR AND BIOLOGICAL ASPECTS*.

Garcia, L. E., Brandenburg, R. L., and Bailey, J. E. 2000. Incidence of Tomato spotted wilt virus (Bunyaviridae) and tobacco thrips in Virginia-type peanuts in North Carolina. Plant Dis.

84:459–464.

Gunn, L. V., and Pares, R. D. 1988. Effect of potato physiology on the interpretation of ELISA results for potato leafroll virus. Plant Pathol. 37:516–521.

Hagan, A. K., Weeks, J. R., French, J. C., Gudauskas, R. T., Mullen, J. M., Gazaway, W. S., et al. 1990. Tomato Spotted Wilt Virus in Peanut in Alabama. Plant Dis. 74:615.

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

Hoffmann, K., Geske, S. M., and Moyer, J. W. 1998. Pathogenesis of tomato spotted wilt virus in peanut plants dually infected with peanut mottle virus. Plant Dis. 82:610–614.

Hu, J. S., Li, H. P., Barry, K., Wang, M., and Jordan, R. 1995. Comparison of dot blot, ELISA, and RT-PCR assays for detection of two cucumber mosaic virus isolates infecting banana in Hawaii. Plant Dis. 79:902–906.

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

Kijne, J. W., Bauchrowitz, M. A., and Diaz, C. L. 1997. Root lectins and rhizobia. Plant Physiol. 115:869–873.

Kresta, K. K., Mitchell, F. L., and Smith, J. W. 1995. Survey by ELISA of Thrips (Thysanoptera: Thripidae) Vectored Tomato Spotted Wilt Virus Distribution in Foliage and Flowers of Field-Infected Peanut. Peanut Sci. 22:141–149.

Lacroix, C., Renner, K., Cole, E., Seabloom, E. W., Borer, E. T., and Malmstrom, C. M. 2016. Methodological guidelines for accurate detection of viruses in wild plant species. Appl. Environ. Microbiol. 82:1966–1975.

Little, E. L. 2017. *2015 Georgia Plant Disease Loss Estimates*.

Little, E. L. 2019a. *2016 Georgia Plant Disease Loss Estimates*.

Little, E. L. 2019b. *2017 Georgia Plant Disease Loss Estimates*.

Little, E. L. 2020. *2018 Georgia Plant Disease Loss Estimates*.

López, M. M., Bertolini, E., Olmos, A., Caruso, P., Gorris, M. T., Llop, P., et al. 2003. Innovative tools for detection of plant pathogenic viruses and bacteria. Int. Microbiol. 6:233–243.

Mackay, I. M., Arden, K. E., and Nitsche, A. 2002. Real-time PCR in virology. Nucleic Acids Res. 30:1292–1305.

Mahmoud, S. Y. M. 2011. Detection of cucumber mosaic virus in some ornamental plants and elimination of nonspecific ELISA reactions. Arch. Phytopathol. Plant Prot. 44:1410–1424.

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

Mekuria, G., Ramesh, S. A., Alberts, E., Bertozzi, T., Wirthensohn, M., Collins, G., et al. 2003. Comparison of ELISA and RT-PCR for the detection of Prunus necrotic ring spot virus and prune dwarf virus in almond (Prunus dulcis). J. Virol. Methods. 114:65–69.

Morichetti, S., and Ferrell, J. 2010. Diagnosing Herbicide Injury in Peanut. EDIS. 2010.

Mumford, R. A., Barker, I., and Wood, K. R. 1994. The detection of tomato spotted wilt virus using the polymerase chain reaction. J. Virol. Methods. 46:303–311.

Murakami, M., Gallo-Meagher, M., Gorbet, D. W., and Meagher, R. L. 2006. Utilizing immunoassays to determine systemic tomato spotted wilt virus infection for elucidating field resistance in peanut. Crop Prot. 25:235–243.

Naidu, R. A., and Hughes, J. d'A. 2001. Methods for the detection of plant virus diseases. Plant Virol. sub-Saharan Africa Proc. a Conf. Organ. by IITA. :233–260.

Pappu, S. S., Black, M. C., Pappu, H. R., Brenneman, T. B., Culbreath, A. K., and Todd, J. W. 1999. First Report of Natural Infection of Peanut (Groundnut) by Impatiens Necrotic Spot Tospovirus (Family Bunyaviridae). Plant Dis. 83:966–966.

Pappu, S. S., Pappu, H. R., Culbreath, A. K., and Todd, J. W. 1999. Localization of Tomato Spotted Wilt Virus (Genus Tospovirus , Family Bunyaviridae) in Peanut Pods. Peanut Sci. 26:98–100.

Peumans, W. J., and Van Damme, E. J. M. 1994. Lectins as PIant Defense Proteins '. Arch. Microbiol. 1:347–352.

Pfaffl, M. W. 2004. Quantification strategies in real-time PCR. In *A-Z of quantitative PCR*, , p. 87–112.

Putnam, M. L. 1995. Evaluation of selected methods of plant disease diagnosis. Crop Prot. 14:517–525.

Rådström, P., Knutsson, R., Wolffs, P., Lövenklev, M., and Löfström, C. 2004. Pre-PCR processing: Strategies to generate PCR-compatible samples. Appl. Biochem. Biotechnol. - Part B Mol. Biotechnol. 26:133–146.

Rådström, P., Löfström, C., Lövenklev, M., Knutsson, R., and Wolffs, P. 2008. Strategies for overcoming PCR inhibition. Cold Spring Harb. Protoc. 3:pdb.top20.

Redolfi, P. 1983. Occurrence of pathogenesis-related (b) and similar proteins in different plant species. Netherlands J. Plant Pathol. 89:245–254.

Reed, J. T., and Sukamto, S. 1995. Thrips and Tomato Spotted Wilt Virus in a Mississippi Peanut Field. In *Thrips Biology and Management*, Springer US, p. 171–173.

Riley, D. G., Joseph, S. V., Srinivasan, R., and Diffie, S. 2011. Thrips vectors of Tospoviruses. J. Integr. Pest Manag. 1:1–10.

Rotenberg, D., Jacobson, A. L., Schneweis, D. J., and Whitfield, A. E. 2015. Thrips transmission of tospoviruses. Curr. Opin. Virol. 15:80–89.

Rotenberg, D., Kumar, N. K. K., Ullman, D. E., Montero-Astúa, M., Willis, D. K., German, T. L., et al. 2009. Variation in Tomato spotted wilt virus titer in Frankliniella occidentalis and its association with frequency of transmission. Phytopathology. 99:404–410.

Rowland, D., Dorner, J., Sorensen, R., Beasley, J. P., and Todd, J. 2005. Tomato spotted wilt virus in peanut tissue types and physiological effects related to disease incidence and severity. Plant Pathol. 54:431–440.

Sanchez-Navarro, J. A., Aparicio, F., Rowhani, A., and Pallás, V. 1998. Comparative analysis of ELISA, nonradioactive molecular hybridization and PCR for the detection of prunus necrotic ringspot virus in herbaceous and Prunus hosts. Plant Pathol. 47:780–786.

Schaad, N. W., and Frederick, R. D. 2002. Real-time PCR and its application for rapid plant disease diagnostics. Can. J. Plant Pathol. 24:250–258.

Schaad, N. W., Frederick, R. D., Shaw, J., Schneider, W. L., Hickson, R., Petrillo, M. D., et al. 2003. Advances in molecular-based diagnostics in meeting crop biosecurity and phytosanitary issues. Annu. Rev. Phytopathol. 41:305–324.

Van Schadewijk, A. R., Meekes, E. T. M., Verbeek, M., and Verhoeven, J. T. J. 2011. Validation of plant virus detection. In *Acta Horticulturae*, International Society for Horticultural Science, p. 81–86.

Schochetman, G., Ou, C.-Y., and Jones, W. K. 1988. *Polymerase Chain Reaction*.

Sherwood, J. L. 1989. Use of Monoclonal Antibodies in Detection of Tomato Spotted Wilt

Virus. Phytopathology. 79:61.

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

Shrestha, A., Sundaraj, S., Culbreath, A. K., Riley, D. G., Abney, M. R., and Srinivasan, R. 2015. Effects of Thrips Density, Mode of Inoculation, and Plant Age on Tomato Spotted Wilt Virus Transmission in Peanut Plants. Environ. Entomol. 44:136–143.

Sikora, E. J., Andrianifahanana, M., and Murphy, J. F. 1999. Detection of cucumber mosaic cucumovirus in weed species: A cautionary report on nonspecific reactions in ELISA. Can. J. Plant Pathol. 21:338–344.

Sreenivasulu, P. 1988. Tomato Spotted Wilt Virus (TSWV) and Strains of Peanut Mottle Virus that Mimic TSWV Symptoms in Peanut in Georgia. Plant Dis. 72:546.

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

Sutula, C. L., Gillett, J. M., Morrissey, S. M., and Ramsdell, D. C. 1986. Interpreting ELISA Data and Establishing the Positive-Negative Threshold. Plant Dis. 70:722.

Tillman, B. L., and Gorbet, D. W. 2017. Registration of ‘TUFRunner “511” ’ Peanut. J. Plant Regist. 11:235–239.

Torrance, L., and Jones, R. A. C. 1981. Recent developments in serological methods suited for use in routine testing for plant viruses. Plant Pathol. 30:1–24.

USDA-NASS. 2020. *Crop Production 2019 Summary*.

Wang, M. L., Pinnow, D. L., and Pittman, R. N. 2007. Preliminary screening of peanut germplasm in the US collection for TSWV resistance and high flavonoid content. Plant Pathol. J. 6:219–226.

Ward, E., Foster, S. J., Fraaije, B. A., and McCartney, H. A. 2004. Plant pathogen diagnostics: Immunological and nucleic acid-based approaches. Ann. Appl. Biol. 145:1–16.

Webster, C. G., Turechek, W. W., Li, W., Kousik, C. S., and Adkins, S. 2017. Development and evaluation of ELISA and qRT-PCR for identification of squash vein yellowing virus in cucurbits. Plant Dis. 101:178–185.

Wells, M. L., Culbreath, A. K., Todd, J. W., Brown, S. L., and Gorbet, D. W. 2002. A regression approach for comparing field resistance of peanut cultivars to tomato spotted wilt tospovirus. Crop Prot. 21:467–474.

Wilson, I. G. 1997. Inhibition and facilitation of nucleic acid amplification. Appl. Environ. Microbiol. 63:3741–3751.

Zhu, Y. J., Lim, S. T. S., Schenck, S., Arcinas, A., and Komor, E. 2010. RT-PCR and quantitative real-time RT-PCR detection of Sugarcane yellow leaf virus (SCYLV) in symptomatic and asymptomatic plants of Hawaiian sugarcane cultivars and the correlation of SCYLV titre to yield. Eur. J. Plant Pathol. 127:263–273.

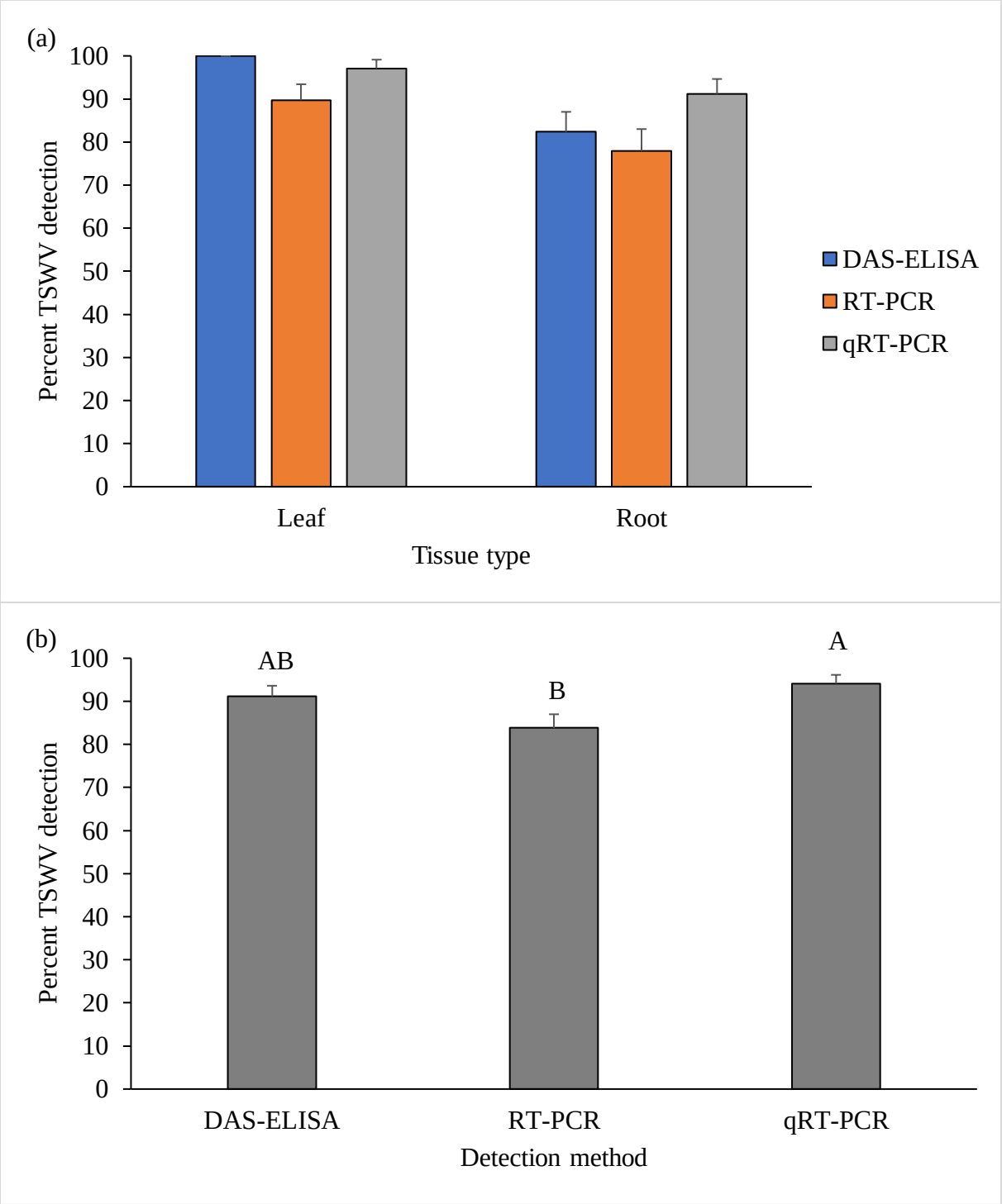

Fig. 4.1. Mean percentage (±SE) of TSWV infection ascertained via DAS-ELISA, RT-PCR, and qRT-PCR in (a) leaf and root tissue samples of symptomatic peanut plants (n = 68) and (b) across tissue types (n = 136). Data were pooled across 2018 and 2019. TSWV symptomatic leaf at the growing points and the primary root of the same plants were sampled for TSWV detection.

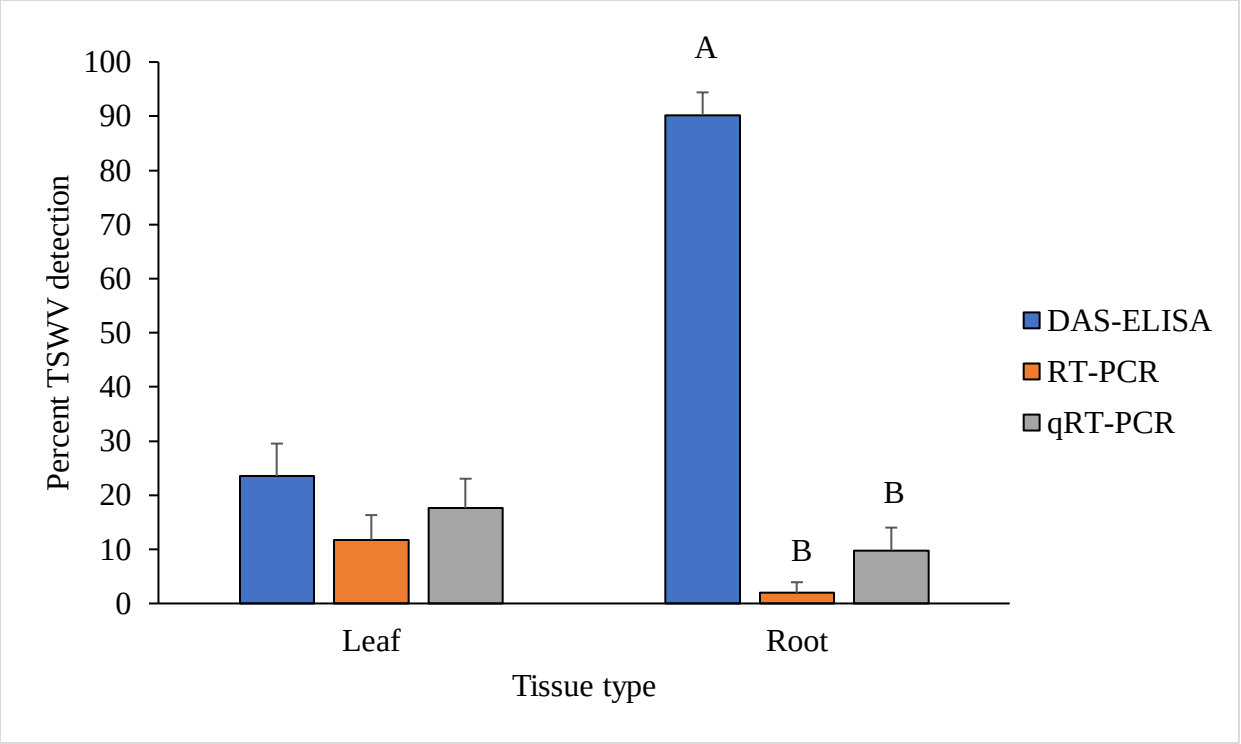

Fig. 4.2. The percentage (±SE) of TSWV infection ascertained via DAS-ELISA, RT-PCR, and qRT-PCR in leaf and root tissue of asymptomatic peanut plants (n = 51). Data were pooled across 2018 and 2019. Leaf at the growing points and the primary root of the same plants were sampled for TSWV detection.

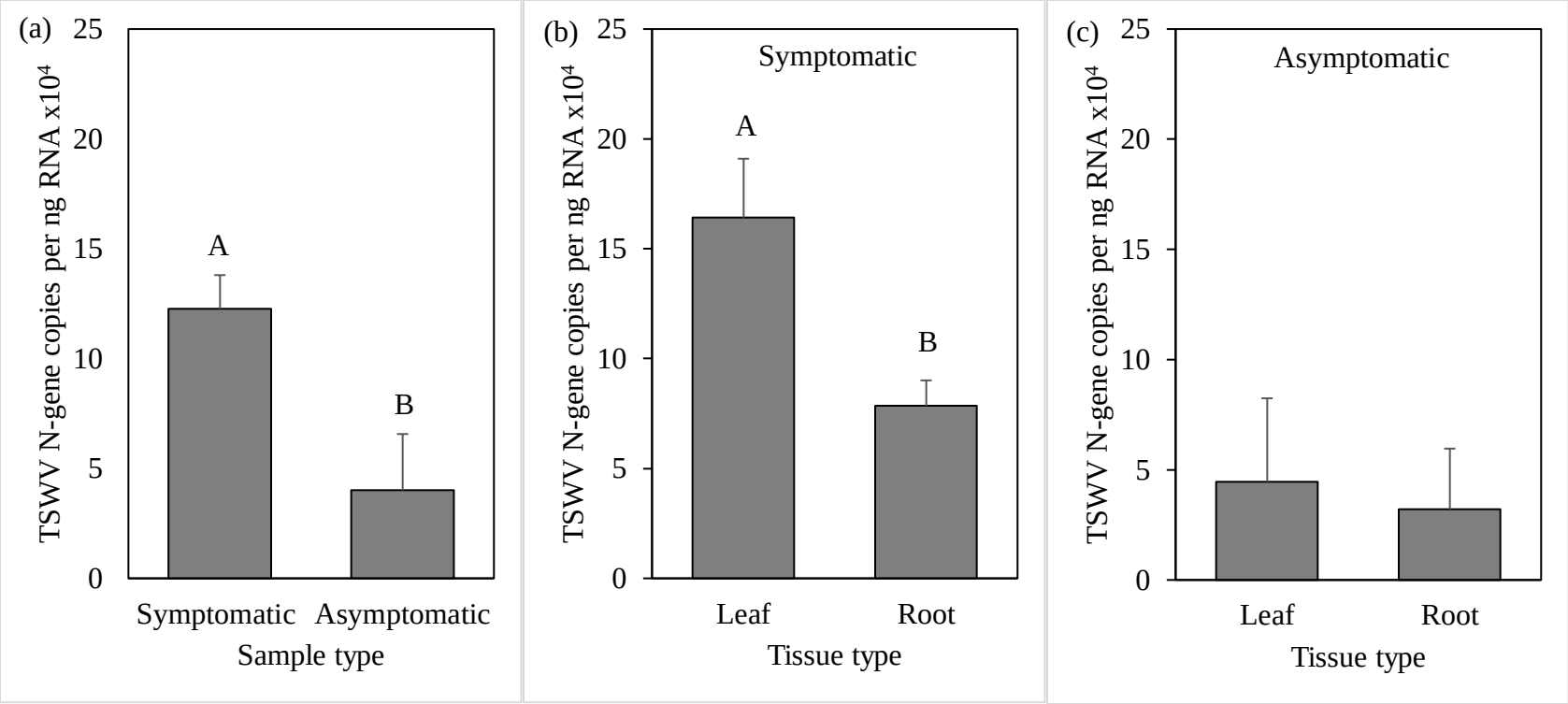

Fig. 4.3. TSWV accumulation in (a) symptomatic (n = 128) and asymptomatic plants (n = 14) across tissue types and in leaf and root tissue samples from (b) symptomatic plants (n = 66, 62 for leaf and root samples, respectively) and (c) asymptomatic plants (n = 9, 5 for leaf and root samples, respectively). Data were pooled across 2018 and 2019. TSWV-N gene copies were estimated by qRT-PCR. A standard curve was generated using linearized plasmids with a TSWV N-gene insert. Threshold cycle (Ct) for each sample was calculated, and TSWV N-gene copies in test samples were estimated from the standard curve.

CHAPTER 5

IMPACT OF HOST RESISTANCE TO TOMATO SPOTTED WILT ORTHOTOSPOVIRUS IN PEANUT CULTIVARS ON VIRUS POPULATION GENETICS AND THRIPS FITNESS[1]

[1]Lai, P.-C., Buntin, G. D., Bag, S., Abney, M. R., and Srinivasan, R. To be submitted to *Pathogens*

Abstract

Thrips-transmitted tomato spotted wilt orthotospovirus (TSVW) is a major constraint to peanut production in the southeastern United States. As the most important management tactic, cultivars with field resistance to TSWV have been widely used for over twenty years. The intensive use of TSWV resistant cultivars has raised concerns about possible selection pressure to TSWV and a likelihood of resistance breakdown. Population genetics of TSWV isolates collected from peanut cultivars with varying levels of TSWV resistance was investigated using all five TSWV genes. Haplotype networks did not indicate host resistance-based clustering of TSWV isolates. Genetic variation in TSWV isolates and neutrality tests suggested the occurrence of recent population expansion. Mutation and purifying selection seem to be the major forces driving TSWV evolution. While positive selection was found in N and RdRp genes, evidence suggested that positive selection was not influenced by TSWV resistance in peanut. Population differentiation occurred between isolates collected from 1998, 2010, and 2016-2019 but not between susceptible and resistant peanut cultivars. Evaluated TSWV resistant peanut cultivars differed, albeit not substantially, in their susceptibility to thrips. Thrips reproduction was reduced, and development was delayed in some cultivars without effects on oviposition. Overall, no evidence was found to support exertion of selection pressure on TSWV by host resistance in peanut, and some TSWV resistant cultivars differentially affected thrips fitness than others.

Introduction

Resistant cultivars often form the first line of defense against arthropod-borne plant viruses such as the thrips-transmitted tomato spotted wilt orthotospovirus (TSWV). TSWV causes substantial economic losses in various crops such as tomato, pepper, tobacco, and peanut

(Culbreath et al. 1991, 2003; Gitaitis et al. 1998; Pappu et al. 2009; Riley and Pappu 2004; Srinivasan et al. 2017). TSWV infection leads to spotted wilt disease in peanut, which has been a severe limiting factor in peanut production in the southeastern United States. TSWV infection in peanut was first reported in Texas in 1971 and has since spread to other southern states (Culbreath et al. 2003; Halliwell and Philley 1974).

In the 1990s, TSWV became a yield-limiting problem in peanut in the southeastern United States (Culbreath et al. 2003; Culbreath and Srinivasan 2011; Garcia et al. 2000; Hagan et al. 1990). Observations then indicated variation in susceptibility to TSWV among peanut cultivars. For example, the commonly grown cultivar 'Florunner' was highly susceptible to TSWV, while another cultivar 'Southern Runner' was less susceptible to TSWV (Black and Smith 1987; Culbreath et al. 1992). Intensive screening and breeding efforts over the next three decades led to consistent release of peanut cultivars with incremental increases in levels of TSWV resistance (Srinivasan et al. 2017). Resistance to TSWV in peanut is commonly referred to as field resistance or tolerance and is typified by milder symptoms following TSWV infection and increased yield compared with TSWV susceptible cultivars, especially under high virus pressure (Culbreath et al. 1996, 2003). The mode of TSWV resistance in peanut is unknown and is different from crops such as tomato and pepper. In pepper and tomato, dominant genes such as *Tsw* and *Sw-5* confer resistance via hypersensitive response (HR) characterized by rapid death of cells around virus entry sites causing local necrotic lesions without systemic symptoms (Moury et al. 1997; Thomas-Carroll and Jones 2003). However, HR was not observed in TSWV-resistant peanut cultivars, instead TSWV infection in field-resistant peanut cultivars resulted in systemic symptom expression, albeit to a lesser degree than in susceptible cultivars (Culbreath et al. 2003; Shrestha et al. 2013; Srinivasan et al. 2017). These responses suggested that field resistance is

more likely to be governed by multiple quantitative traits in peanut as opposed to single gene-governed resistance in Solanaceae hosts. Several major quantitative trait loci (QTLs) have thus far been linked to TSWV resistance in peanut cultivars (Agarwal et al. 2018; Khera et al. 2016; Pandey et al. 2017; Tseng et al. 2016). Yet, the mechanism of TSWV resistance in peanut remains to be characterized.

TSWV strains have overcome resistance conferred by single genes such as *Tsw* in pepper and *Sw-5* in tomato in several places worldwide (Latham and Jones 1998; Moury et al. 1997; Roggero et al. 1999, 2002; Sharman and Persley 2006). Resistance-breaking (RB) TSWV strains originated from mutation and/or reassortment events followed by positive selection (Almási et al. 2017; López et al. 2011; Margaria et al. 2015; Tentchev et al. 2011). While TSWV RB strains have not been reported in peanut, the potential threat of emergence of RB strains remains a concern. TSWV management in peanut relies heavily on TSWV-resistant cultivars with an estimated >95% of the peanut acreage planted with them (Srinivasan et al. 2017). As stated earlier, these field-resistant peanut cultivars display less severe symptoms and accumulate less TSWV following TSWV infection than susceptible cultivars (Shrestha et al. 2013). Whether the prolonged resistant cultivar-TSWV interactions over time in the peanut production landscape could lead to development of new strains that can overcome resistance remains to be assessed.

Sundaraj et al. (2014) did not find evidence of positive selection pressure on the N gene of TSWV isolates collected from TSWV-resistant peanut cultivars. TSWV has a tripartite genome consisting of the large (8.9 kb), medium (2.8 kb), and small (2.9 kb) segment with coding regions for five genes (Whitfield et al. 2005). The large segment encodes for the RNA-dependent RNA polymerase (RdRp), which plays a crucial role in genome replication, in the negative sense (Adkins et al. 1995; De Haan et al. 1991). The medium segment encodes for a

nonstructural protein (NSm) in the positive sense and the Gn/Gc glycoprotein precursor in the negative sense (Kormelink et al. 1992). NSm is involved in cell-to-cell movements in plant hosts (Kormelink et al. 1994; Soellick et al. 2000; Storms et al. 1995). The Gn/Gc glycoproteins play a role in maturation and assembly of virions as well as thrips transmission (Bandla et al. 1998; Whitfield et al. 2005). The small segment encodes for another nonstructural protein (NSs) in the positive sense and the nucleocapsid protein (N) in the negative sense (De Haan et al. 1990). NSs was identified as RNA silencing suppressors during plant infection (Takeda et al. 2002), and the N protein encapsidates the RNA segments to form ribonucleoprotein, which serves as the template for replication and transcription (Richmond et al. 1998). Heterogeneity in nucleotide sequences of NSs and NSm between wild type and RB strains of TSWV leading to positive selection was associated with resistance breakdown in pepper and tomato (Almási et al. 2017; López et al. 2011; Tentchev et al. 2011). The effect of possible selection pressure from resistance in peanut cultivars on other genes in the TSWV genome remains unclear.

TSWV is exclusively transmitted by thrips in a persistent and propagative mode under natural conditions, and resistance or tolerance against the vector could also influence the susceptibility of these cultivars to TSWV (Garzo et al. 2020; Jacobson and Kennedy 2013; Maris et al. 2003a; Maris et al.2003b). Resistance to thrips could impact thrips preference, feeding, reproduction, development, and ultimately affect virus acquisition and inoculation (Shrestha et al. 2013; Sundaraj et al. 2014; Srinivasan et al. 2018). Therefore, it is possible that the observed field resistance to TSWV could be due to effects against the virus and/or the vector.

In this study, TSWV isolates collected from peanut cultivars with varying levels of TSWV field resistance were studied by fully or partially sequencing the five TSWV genes and assessing numerous population genetics parameters. In addition, whether peanut cultivars

possessed any resistance against thrips that could interfere with TSWV transmission was also investigated.

Materials and Methods

TSWV isolates. TSWV symptomatic leaves were collected from 22 peanut cultivars with varying levels of field resistance to TSWV during the growing season from 2016 to 2019 in Georgia. Foliage samples (n=59) were collected from peanut fields on research farms at the University of Georgia Tifton campus and Attapulgus Research and Education Center in Georgia. Peanut type, cultivar, collection year and location, and TSWV susceptibility status of each cultivar are listed in Table 5.1.

RNA extraction, cDNA synthesis, PCR, and sequencing. Total RNA from symptomatic leaf samples from susceptible and resistant peanut cultivars was extracted using RNeasy plant mini kit (Qiagen, Valencia, CA) following the manufacturer's protocol. Approximately 0.1 g of leaf tissue per sample was used for RNA extraction. The total RNA extract was used as the template for complimentary DNA (cDNA) synthesis using Go-Script reverse transcription system (Promega Corporation, Madison, WI) with oligo dT primers following manufacturer's instructions. The synthesized cDNA served as the template for PCR. PCR was conducted in a DNA engine thermo cycler (Bio-Rad Laboratories, Hercules, CA) using 50 µl volume reactions. Primers were designed to amplify full length of N gene and partial regions of N, NSs, NSm, Gn/Gc, and RdRp genes according to the reference sequences of the TSWV genomes in GenBank (accession numbers: NC_002050 to NC_002052 & KT160280-KT160282). Primer pairs, annealing temperatures, and amplicon sizes are listed in Table 5.2. The reaction mix consisted of 25 µl of GoTaq Green Master Mix (Promega Corporation, Madison,

WI), 2.5 µl (0.5µM) of each forward and reverse primer, 5 µl of synthesized cDNA, and 15 µl nuclease-free water. The PCR program started with an initial activation step at 95°C for 2 min, followed by 35 cycles of amplification and a final extension step at 72°C for 5 min. The amplification cycle included denaturation at 94°C for 1 min, annealing at primer-specific temperature for 45 s, and extension at 72°C for 50s (N, NSs, NSm, Gn/Gc genes) or 90 s (RdRp gene). The presence of targeted amplicons in the PCR product was visualized by agarose gel (1 %) electrophoresis. The PCR product was purified using the GeneJET PCR purification kit (Thermo Scientific™, Thermo Fisher Scientific, Waltham, MA) according to the manufacturer's protocol. Purified PCR products were sequenced in both directions using SimpleSeq™ DNA sequencing service from Eurofins Genomics (Eurofins MWG Operon Inc., Louisville, KY). Consensus sequences were assembled from sequences of both directions and edited in Geneious Prime® (version 2019.2.3) (Kearse et al. 2012). Sequences of full-length N gene and partial NSs, NSm, Gn/Gn, and RdRp genes obtained in this study were deposited in the GenBank with accession numbers MW519186-MW519472 (Appendix A).

TSWV isolates and sequence alignments. Nucleotide sequences were aligned using Clustal W with default settings in Geneious Prime®. Sequence alignments were manually corrected when necessary. For N gene (777 bp), a nucleotide alignment of 150 TSWV isolates collected in Georgia, including 59 isolates collected in this study, 82 isolates collected in 2010 (GenBank accession numbers: HQ40603-HQ406984), and 9 isolates collected in 1998 (GenBank accession numbers: AF048714-AF018716 and AF064469-AF064474), were used for the analysis. The nine isolates were collected from peanut and solanaceous crops in Tift County in 1998 (Pappu et al. 1998); while the 80 isolates were collected from peanut in ten counties in Georgia, and two isolates were from solanaceous crops collected in Tift County in 2010

(Sundaraj et al. 2014). Sequence alignments of partial NSs (869 bp) and GnGc (756 bp) genes were obtained from 59 TSWV isolates and used for the analysis. Sequence alignments for partial NSm gene (672 bp) and RdRp (1416bp) genes were obtained from 58 and 52 TSWV isolates, respectively.

Haplotype network construction. Haplotype networks were used to visualize genealogical relationships among TSWV isolates using DNA sequence alignments of the five TSWV genes. Median-joining networks were constructed using POPART haplotype network software (Leigh and Bryant 2015). Median-joining network combines features of minimum spanning trees by favoring short connections among isolates and maximum-parsimony heuristic algorithm by adding "median vectors" (ancestral nodes) in the network to infer evolutionary history (Bandelt et al. 1999; Leigh and Bryant 2015). A haplotype network was constructed for each TSWV genes.

Genetic diversity and test of neutrality. Genetic variation in each gene among TSWV isolates was evaluated. The haplotype (gene) diversity, nucleotide diversity (π, the pairwise average number of nucleotide differences per site) (Nei 1987), and the Watterson's estimator (θ_w, a measure of the population mutation rate based on segregating sites) (Watterson 1975; Nei 1987) were estimated using DnaSP (v6.12.03) (Rozas et al. 2017). Population neutrality, the hypothesis of all mutation being selectively neutral, was tested by Tajima's D (Tajima 1989) and Fu and Li's D* and F* statistics (Fu and Li 1993). Tajima's D test statistic is based on the relationship between the number of segregating sites (total number of mutations) and the pairwise average number of nucleotide differences; the significance of Tajima's D statistic is determined by a two-tailed test under the beta distribution (Tajima 1989). The Fu and Li's D* and F* test statistics are based on the relationship between numbers of external and internal

mutations in the genealogy of sequences from a population. D* statistic measures the differences between the number of singletons (sites of nucleotide variants that only appear in one sequence of the population) and total number of mutations. F* statistic assesses the differences between the singleton number and the pairwise average number of nucleotide differences (Fu and Li 1993). Statistical significance of Fu and Li's D* and F* statistics were determined using critical values obtained from simulated distribution of each statistic over the interval of mutation rate (θ) from 2 to 20 (Fu and Li 1993). When Tajima's D and Fu and Li's D* and F* statistics are indistinguishable from zero, neutral variation is implied indicating that there is no evidence for changes in population size or directional selections. Negative statistics indicate the occurrence of population expansion or purifying selection and an excess of rare mutations, while positive statistics imply a population bottleneck with deficiency of rare mutations. Fu's Fs statistic is a more sensitive and powerful test to detect population expansion and genetic hitchhiking; the test statistic examines neutrality of mutations under conditions when excess of rare alleles and young mutations exist (Fu 1997).

Recombination detection and identification of selection. All sequences of TSWV genes were analyzed using the Recombination Detection Program v.4.100 (RDP4) (Martin et al. 2015) to detect recombination. The program used seven recombination detection methods to search for recombinants; significant recombination event was determined when more than three methods were significant.

Selection pressure on codons of TSWV genes were assessed using the Fast, Unconstrained Bayesian AppRoximation (FUBAR) test in Hypothesis Testing using Phylogenies (HyPhy) software package. FUBAR uses a Bayesian approach to infer non-synonymous (dN) and synonymous (dS) substitution rate on a per codon basis (Murrell et al. 2013). Positive

selection is inferred when the posterior non-synonymous substitution rate (β) is higher than synonymous substitution rate (α) at a given codon site; in contrast, negative (purifying) selection is indicated when substitution rate is higher for synonymous substitution than non-synonymous substitution. Significance of selection was determined by the posterior probability of the difference between the rates of synonymous and non-synonymous substitution with a significant level of Prob = 0.900. Bayes factors indicated the strength of evidence in favor of positive selection; a higher Bayes factors provided stronger support for positive selection.

Test of gene flow and population differentiation. Haplotype-based test statistics, including Hs and Hst, and nucleotide-based statistics, including Ks, Kst, and Snn (Hudsonet al. 1992a), were computed using Gene Flow and Genetic Differentiation analysis module in DnaSP v.6. Significance tests for haplotype- and sequence-based statistics were fulfilled by randomization (permutation) tests with 1000 replications. The extent of genetic differentiation between TSWV populations was examined by evaluating the fixation index (Fst) (Hudson, et al. 1992b).

Thrips feeding injury and survival. The effect of TSWV resistant peanut cultivars on thrips feeding injury and survival was evaluated through replicated no-choice tests in a greenhouse. *Frankliniella fusca* from a laboratory colony established in 2009 at the University of Georgia were used for the experiment; *F. fusca* was reared on greenhouse-grown Georgia Green leaflets maintained in small Petri dishes (35 mm diameter) with wet cotton rounds. The colony was maintained in a growth chamber (Thermo Fisher Scientific, Waltham, MA) at 25~30 ℃ with L14:D10 photoperiod (Marasigan et al. 2016; Shrestha et al. 2012, 2013, 2015). Six peanut cultivars with varying degrees of TSWV susceptibility were used, which included Florunner (Norden et al. 1969), Georgia Green (Branch 1996), Georgia-06G (Branch 2007), Georgia-12Y

(Branch 2013), and Georgia-16HO (Branch 2017), and Tifguard (Holbrook et al. 2008). For each cultivar, six peanut seedlings (at two-node stage) were placed in a thrips-proof cage (Megaview Science Co., Taichung, Taiwan). Ten thrips (adult female up to three days old) were released on each peanut plant. A trace of pine pollen was dusted on the surface of leaves to supplement thrips development (Angelella and Riley 2010). Plants were evaluated for thrips injury, and surviving thrips were counted at three-day intervals for up to 30 days after thrips release. Thrips larvae and adults on leaves and stems of the plants were counted. Thrips feeding injury was rated on a scale of 0-3 where 0 represented no injury, and 1 to 3 represented <25%, 25~50%, >50% leaf area of individual leaflets having feeding scars, respectively. Feeding damage index (FDI) was calculated based on a formula originally proposed by Maris et al. (2003) and modified by Sundaraj et al. (2014): FDI = (number of leaflets with feeding injury/total number of leaflets in a plant) x injury rating. The experiment was conducted two times (n=12 per cultivar).

Thrips count and feeding damage index data were pooled from the experiments for statistical analysis. Thrips count data were subjected to generalized linear mixed model analysis with repeated measure using PROC GLIMMIX procedure with the negative binomial distribution and the log link function in SAS (SAS Enterprise 9.4, SAS Institute, Cary, NC). Peanut cultivar served as a fixed effect, and experiment and replication were random effects. Least square means (LS-means) were used for multiple comparisons at significant level of α = 0.05 with Tukey-kramer adjustment to determine significant differences between peanut cultivars. Feeding damage index data were subjected to the Wilcoxon Score tests using PROC NPAR1WAY procedure in SAS, and the significance of cultivar effect was determined by Kruskal-Wallis test (one-way ANOVA test for the Wilcoxon Score tests) at α = 0.05. Data were analyzed by evaluating date. When the cultivar effect was significant on feeding damage

index, multiple pairwise Wilcoxon two-sample tests and Kruskal-Wallis tests were conducted among cultivars.

Thrips development, reproduction, and oviposition. The effect of TSWV resistant peanut cultivars on thrips developmental time, number of adult thrips produced, and number of eggs laid were evaluated through microcosm experiments. Thrips and peanut cultivars used in these experiments were the same as those used for evaluating thrips injury and survival. Six Munger cages (Munger 1942) were set up for each cultivar, and the experiment was conducted two times (n = 12 per cultivar). Ten thrips (adult female up to three days old) were transferred onto two peanut leaflets with a trace of pollen in a Munger cage using a fine paintbrush. Adult female thrips were removed from the Munger cages after 72 h. The cages were monitored daily under a dissecting microscope (40X) (MEIJI TECHNO, Santa Clara, CA). Adult thrips emerging from each cage was counted at 24 h interval and removed from the cage. Developmental time (adult to adult) for each thrips emerged was recorded. The adult thrips removed from the Munger cages were further used for oviposition on peanut leaflets of the same peanut cultivar. Five female adult thrips (up to three days old) were transferred onto two leaflets with a trace of pollen in a small Petri dish with a wet cotton round. Cages were secured by rubber bands to avoid thrips escape, and adult thrips were allowed to oviposit for 72 h and removed. During the experiments, all cages were maintained in a growth chamber (Thermo Fisher Scientific, Waltham, MA) at 25~30 ℃ with L14:D10 photoperiod. Subsequently, peanut leaflets were stained for egg counting using a staining method described by Ben-Mahmoud et al. (2018). Peanut leaflets were immersed in the McBride's solution (0.2% acid fuchsin in 1:1 ethanol: glacial acetic acid) and shaken in a benchtop orbital shaker (MAXQ4450, Thermo Scientific, Waltham, MA) at a low speed (145 rpm) for 24 h. Leaflets were then transferred to clean vials and soaked in a de-

staining solution (1:1:1 lactic acid: glycerol: water). After shaken for 3 h, the vials were moved to an incubator (Isotemp Oven Model 630G, Fisher Scientific, Waltham, MA) at 80 ℃ for 24 h. Leaflets were allowed to cool at room temperature, and thrips eggs (partially or fully embedded in leaf tissues) were stained red and counted under a dissecting microscope (100X) (MEIJI TECHNO, Santa Clara, CA).

Adult thrips counts, thrips developmental time from adult to adult, and egg counts were pooled across experiments for statistical analysis. Adult thrips counts and egg counts were subjected to generalized linear mixed model analysis using the PROC GLIMMIX procedure with the Poisson distribution and the log link function in SAS. Peanut cultivars served as a fixed effect, while experiment and replication were random effects. LS-means were used for multiple comparisons with Tukey-kramer adjustment to determine significant differences between peanut cultivars. Median developmental time of thrips was subjected to the Wilcoxon Score tests using PROC NPAR1WAY procedure in SAS, and the significance of cultivar effect was determined by Kruskal-Wallis test at $\alpha = 0.05$.

Results

Haplotype network construction. Median-joining (MJ) networks were constructed based on nucleotide polymorphisms observed in full length of N gene, and partial NSs, NSm, Gn/Gc, and RdRp gene of TSWV isolates. The MJ network of TSWV N gene includes 150 sequences of TSWV isolates obtained from 1998, 2010 and 2016-2019 (Fig. 5.1). TSWV isolates collected from 1998 appeared to parse from a major cluster with isolates from 2010 at the center, and the other major cluster had ancestral sequences of new isolates collected from 2016 to 2019 at the center. However, many isolates from 2010 and from 2016 to 2019 were dispersed within

the two major clusters (Fig. 5.1). Isolates collected from susceptible cultivars after 2010 were blended within isolates from resistant cultivars without any obvious clustering (Fig. 5.1). The MJ network of TSWV NSs (Fig. 5.2), NSm (Fig. 5.3), Gn/Gc (Fig. 5.4), and RdRp gene (Fig. 5.5) showed radiations of sequences from ancestral sequences that composed of isolates from resistant cultivars, susceptible and resistant cultivars, or unsampled sequences. However, TSWV isolates collected from susceptible or resistant cultivars did not form any distinct clusters in the networks. Overall, nucleotide polymorphisms in TSWV isolates reflected in the MJ networks did not seem to be associated with host susceptibility to TSWV (Fig. 5.1-5).

Genetic diversity and population neutrality. The parameters describing genetic diversity including number of segregating sites (S), number of haplotypes (h), haplotype diversity (Hd), average number of nucleotide differences (K), nucleotide diversity (π), and population mutation rate (θ_w) are presented in Table 5.3. Across all the TSWV genes and population subgroups, TSWV isolates exhibited high haplotype diversity ranging from 0.978 to 1.000, while low nucleotide diversity was observed ranging from 0.0072 to 0.0132 (Table 5.3). Mutations were observed in all five genes as indicated by detection of segregating sites. The number of segregating sites and haplotypes was proportional to the sample size (Table 5.3). Genetic diversity was compared among TSWV isolates collected from 1998, 2010, and 2016-2019 using N gene sequences. TSWV isolates collected in 1998 had slightly higher nucleotide diversity but lower haplotype diversity than isolates collected in 2010 and in 2016-2019. The Gn/Gc gene had the highest population mutation rate (θ_w), followed by the RdRp gene (Table 5.3). On the other hand, the RdRp gene had the highest average number of nucleotide differences (K) followed by the Gn/Gc and NSm gene. Average number of nucleotide differences (K) is Tajima's estimate of population mutation rate. Across genes, the resistant subgroup had higher

θ_w than susceptible subgroup, while the comparison of K in susceptible and resistant subgroups was inconsistent. Higher K in susceptible than resistant subgroup was observed in N, NSs, and RdRp genes, while higher K in resistant than susceptible subgroup was observed in NSm and Gn/Gc genes. However, some differences of K between susceptible and resistant subgroups were very small.

Statistics for testing the neutrality of genetic variation in TSWV isolates in all TSWV genes were listed in Table 5.4. For all the population subgroups across the five genes, negative values of Tajima's D and Fu and Li's D* and F* statistics were found, and most of the statistics were significant except for a few subgroups with a smaller sample size (Table 5.4). Negative values of the statistics Tajima's D and Fu and Li's D* and F* tests suggest recent population expansion events or populations under purifying selection. Negative values of Fu's Fs statistics also support the occurrence of recent population expansion.

Recombination and selection in TSWV genes. Recombination events and recombinants were not detected in all sequences of the five TSWV genes from isolates collected in this study.

Selection tests found eight codon positions of the N gene sequence with overabundance of non-synonymous substitutions indicated by positive values of posterior $\beta-\alpha$ (β: posterior non-synonymous substitution rate; α: posterior synonymous substitution rate), while only one codon position 7 (T $\rightarrow$ I, F) was determined to be significantly driven by positive selection (Table 5.5). Among 258 codons of the N gene sequence, negative selection (i.e., purifying selection) was found at 63 sites (24%). Selection tests showed that nine, two, and six codon positions had excess of non-synonymous substitutions in NSs, NSm, and Gn/Gc sequences, respectively; however, none were significant, and no positive selection was determined in those

three genes (Table 5.5). Among 286, 224, 251 codons of the NSs, NSm, GnGc sequences, significant negative selection was observed at 17 (6%), 29 (13%), 47 (18%) codon sites, respectively. Selection tests found 12 codon positions of RdRp sequence with overabundance of non-synonymous substitutions, and positive selection was determined at codon position 149 (K → R), 207 (Q → R), 368 (G → R, E, A), and 397 (Q → L, H). Among 471 codons of the RdRp sequence, 110 (23%) codon positions were found with evidence of negative (purifying) selection.

Genetic differentiation between subgroups of TSWV isolates. According to the haplotype-based (Hs and Hst) and nucleotide-based population differentiation statistics (Ks and Kst, and Snn) on N gene sequences, significant genetic differentiation was observed between the subgroups of isolates collected in 1998, 2010 and in this study from 2016 to 2019 (Table 5.6). The extent of differentiation was higher between 1998 isolates and the later subgroups of isolates (2010, 2016-2019, and after 2010) than between 2010 isolates and new isolates collected in 2016 to 2019 indicated by Fst values, which was 10-fold higher in the former than the later subgroup comparisons (Table 5.6). Except for the significant Snn statistic found between susceptible and resistant subgroup from 2010 isolates with the N gene, population differentiation was not found in the rest of the comparisons between susceptible and resistant subgroups as none of the differentiation test statistics were significant across the five genes (Table 5.6). Small Fst values of the comparisons between susceptible and resistant subgroups indicated that nucleotide differences were similar between pairwise sequences from within and between subpopulations with no evidence of population differentiation.

Thrips feeding injury and survival. Thrips feeding injury on peanut leaves, presented as feeding damage index, was monitored from 3-30 days after thrips released (DAT). Thrips feeding injury varied with peanut cultivars at 3 ($\chi^2 = 11.73$, $p = 0.0385$), 12 ($\chi^2 = 25.86$, $p <$

0.0001), 15 (χ^2 = 19.29, p = 0.0017), 18 (χ^2 = 24.09, p = 0.0002), and 21 (χ^2 = 13.66, p = 0.0179) DAT (Fig. 5.6). At 3 DAT, Georgia-12Y had lower thrips feeding injury than Georgia-06G and Georgia Green. At 12 DAT, Georgia-12Y and Georgia-06G had lower thrips feeding injury than Georgia-16HO and Georgia Green; Georgia-12Y also had lower thrips feeding injury than Tifguard, and Florunner had lower thrips feeding injury than Georgia-16HO. At 15 DAT, Georgia-12Y and Georgia-06G had lower thrips feeding injury than Georgia-16HO, Georgia Green, and Tifguard, while Florunner had lower thrips feeding injury than Georgia Green. At 18 DAT, Georgia-12Y, Georgia-06G, and Florunner had lower thrips feeding injury than Georgia-16HO, Georgia Green, and Tifguard. At 21 DAT, Tifguard had higher thrips feeding injury than Georgia-12Y, Georgia-06G, Georgia Green, and Florunner, while Georgia-16HO had higher thrips feeding injury than Georgia-06G (Fig. 5.6).

Thrips survival was evaluated by counting all adult and immature thrips on each plant. Number of thrips surviving varied with peanut cultivar ($F_{5, 583}$ = 2.52, p = 0.0286), and the variation among cultivars differed by recording time ($F_{45, 583}$ = 3.09, p < 0.0001) (Table 5.7). Significant differences in thrips survival among peanut cultivars were only observed at 9 and 12 DAT. At 9 DAT, Georgia-12Y had the lowest thrips number, while at 12 DAT, Georgia-12Y and Florunner had lower thrips numbers than other cultivars (Table 5.7).

Overall, our results suggested that the susceptibility of peanut cultivars to *F. fusca* was similar with some minor variations in thrips feeding injury and survival during the experimental period, and Georiga-12Y and Georgia-06G reduced fitness of *F. fusca* more than other tested cultivars.

Thrips development, reproduction, and oviposition. The median developmental time for *F. fusca* to complete one generation varied with peanut cultivars (p < 0.05) with very little

differences. The median developmental time of adult thrips on Georgia-12Y and Tifguard was one day longer than the rest of the cultivars (Table 5.8). Thrips reproduction was affected by peanut cultivars (Table 5.9). The number of adult thrips emerged per one thrips released was significantly lower on Georgia-12Y than on Florunner, Georgia Green, and Georgia-16HO. Oviposition rate of thrips was significantly affected by peanut cultivars ($F_{5,55} = 2.45$; $p = 0.0451$); however, mean number of eggs among cultivars was not separated by conserved post-hoc multiple comparison (Tukey-Kramer adjustment) likely due to high variations of egg counts within cultivars (Table 5.9).

Discussion

Development of highly-virulent isolates due to host resistance-induced selection pressure has led to breakdown of host resistance to arthropod-borne viruses such as TSWV and has seriously impacted management (Fabre et al. 2012; Kobayashi et al. 2014). The increase in TSWV incidence in recent years despite the intense use of resistant cultivars over twenty years in a majority of the acreage created concerns about the stability of field resistance in peanut and possible changes in TSWV virulence (Culbreath et al. 2003; Culbreath and Srinivasan 2011; Little 2017, 2019a, 2019b, 2020; Srinivasan et al. 2017). An earlier study found no direct evidence to support the hypothesis that field resistance in peanut exerts selection pressure on TSWV, but that study based the evaluations on the TSWV N gene alone (Sundaraj et al. 2014). It is possible that alterations in other genes could also influence the virulence of isolates/strains (Almási et al. 2017; López et al. 2011; Tentchev et al. 2011). The main objective of this study was to investigate if TSWV resistance in peanut exerts any selection pressure on all the five genes of TSWV, namely N, NSs, Gn/Gc, NSm, and RdRp. In addition, this study examined the

various mechanisms shaping the genetic structure of local TSWV populations and whether the mechanisms vary with virus genes.

According to haplotype networks, nucleotide polymorphisms were observed in all five TSWV genes, but no distinct cluster of TSWV isolates based on host resistance in peanut cultivars was found in any of the genes evaluated. On the other hand, N gene sequences of TSWV isolates collected in1998 diverged from a cluster of isolates with ancestral sequences collected in 2010, and another radiation of sequences was form with ancestral sequences from isolates collected in 2016-2019 at the center. Our results demonstrated a significant temporal effect from 1998 to 2019 on TSWV population in Georgia.

Overabundance of non-synonymous substitutions were found at codon positions in all five TSWV genes, while only one codon in N gene and four codons in RdRp were under positive selection. On the contrary, significant negative selection (i.e., purifying selection) was found in up to 24 % of the codons in the five TSWV genes. These results are similar to the findings in previous studies where limited positive selection at codon sites was found across the five TSWV genes with the background of predominant purifying selection (Tsompana et al. 2005; Kaye et al. 2011; Sundaraj et al. 2014). Non-synonymous substitutions were found in both TSWV susceptible and resistant cultivars, which indicated that those positive selection may not be related to TSWV susceptibility of the peanut hosts. Among the five genes of TSWV, NSm had the fewest codon sites with overabundance of non-synonymous substitutions. Tsompana et al. (2005) also found that NSm gene was the only gene in which no evidence of positive selection was found. NSm interacts with nucleocapsid protein and is proven to be involved in cell-to-cell movement through plasmodesmata in host plants (Kormelink et al. 1994; Storms et al. 1995, 1998). NSm gene of TSWV was identified as an avirulence determinant of *Sw-5* gene-based

resistance in tomato (Hoffmann et al. 2001; Peiró et al. 2014). In addition, non-synonymous substitution of a codon in the NSm gene was found to be positively selected in TSWV resistance-breaking isolates against the *Sw-5* gene in tomato (López et al. 2011). On the other hand, TSWV NSs gene was identified as an avirulence determinant for *Tsw*-based resistance in pepper (de Ronde et al. 2013, 2014; Margaria et al. 2007). *Tsw*-based resistance in pepper was overcome by a single mutation in the NSs gene (Almási et al. 2017). NSs gene encodes a protein with functions of RNA silencing suppression in infected plant hosts (Takeda et al. 2002). Nevertheless, positive selection was not found in TSWV NSs gene in this study. In another study, positive selection was found in one codon site each of N, NSm, and RdRp gene of TSWV isolates collected from peanut in the mid-Atlantic states; those isolates with positive selection were collected in a certain year but not from other years, which suggesting a possible strong environmental influence (Kaye et al. 2011). However, positive selection on N and RdRp genes was found in isolates from different collection years and locations in our study suggesting that the source of selection pressure might appear commonly in local geographic regions over time. Relatively more purifying selection was found in N and RdRp genes. N protein encapsidates the viral RNA to form ribonucleoproteins with putative structural and regulatory functions in replication cycles (Richmond et al. 1998; Whitfield et al. 2005). RdRp contains nucleic acid motifs for polymerase activities (De Haan et al. 1991; Chapman et al. 2003). The higher frequency of purifying selection in N and RdRp genes is likely due to preservation of their crucial functions in replications and transcription.

Population genetic structure of all five TSWV genes possessed high haplotype diversity and low nucleotide diversity, which is a signature of recent population expansion. These results are consistent with sequences of N gene from isolates collected in Georgia (Sundaraj et al. 2014),

sequences of N, NSm, and RdRp from isolates collected in North Carolina and Virginia (Kaye et al. 2011), as well as sequences of N, NSs, NSm, and Gn/Gc from isolates in different geographic regions worldwide (Tsompana et al. 2005). Furthermore, the negative and significant neutrality test statistics, including Tajima' D, Fu and Li's D* and F*, suggested that TSWV populations deviated from neutrality and likely have experienced population expansion and/or purifying selection. Negative Fu's Fs statistics also supported the occurrence of recent population expansion and/or genetic hitchhiking events.

Temporal population differentiation between TSWV subgroups of isolates sampled during a 20-year period was evident by both haplotype and nucleotide-based statistics. However, no such effect was observed in any of the TSWV genes between subgroups of TSWV isolated from susceptible and resistant peanut cultivars. These results echo previous findings and reaffirm the significance of temporal effect on population genetic structure leading to population differentiation of TSWV isolates collected from different years (Sundaraj et al. 2014). Altogether, this study reiterates previous findings that population expansion, purifying selection, and population differentiation are the major mechanisms shaping population genetics of TSWV (Kaye et al. 2011; Tsompana et al. 2005).

Mutation, recombination, and reassortment are major mechanisms contributing to genetic variation in plant viruses (Roossinck 1997; García-Arenal et al. 2001). All the mutations in TSWV genes identified in this study were from nucleotide substitutions and not from insertions or deletions (data not shown). Substitutions, indicated by number of segregating sites, were found in all TSWV genes, and the substitution rate varied among TSWV genes. Population mutation rate of each TSWV gene estimated in this study was similar or slightly lower than those reported from other geographic regions (Tsompana et al. 2005; Kaye et al. 2011). These results

supported the previous finding that mutation is another significant evolutionary factor for shaping population genetic structure of TSWV. Population mutation rate (θ_w) also varied by subgroups of TSWV susceptibility of peanut cultivars. The mutation rate was higher in TSWV isolates collected from resistant cultivar than susceptible cultivars. However, these results need to be interpreted carefully as θ_w might be affected by the sample size. Alternatively, Kaye et al. (2011) suggested that the average number of pairwise nucleotide differences (K), which is Tajima's estimate of the population mutation rate, is more appropriate than θ_w for estimating mutation rates of TSWV isolates collected from peanut hosts because the authors found the nonequilibrium state of the population due to the occurrence of bottlenecks. Tajima's estimate indicated that the mutation rates varied between subgroups of susceptibility of peanut cultivars but without a constant trend as to which subgroup having higher mutation rates. Recombination was not detected in any of the five TSWV genes in our study as all the sequences are highly similar among TSWV isolates. Although recombination in negative-sense RNA viruses is much rarer, recombination in TSWV has been detected in TSWV genes and genome in previous studies (Chare and Holmes 2006; Kaye et al. 2011; Lian et al. 2013). On the other hand, reassortment has been found to play an important role in creating genetic variation in the TSWV genome (Lian et al. 2013). TSWV has been shown to utilize reassortment to overcome TSWV N gene-derived resistance (Qiu et al. 1998; Qiu and Moyer 1999). Since analyses were conducted on individual gene sequences but not with whole genome sequences in our study, reassortment cannot be tested.

Alongside TSWV resistance, previous studies also have shown significant impact of TSWV-resistant peanut cultivars on thrips biology and fitness (Shrestha et al. 2013; Sundaraj et al. 2014). In greenhouse no-choice tests conducted in this study, thrips feeding injury and

survival was not different among peanut cultivars at the end of the experiment when all the cultivars had fairly high level of thrips injury and thrips densities, which suggested that none of the tested cultivars possessed a high level of resistance to *F. fusca*. Georgia-12Y had reduced *F. fusca* feeding injury and survival than other tested cultivars. In addition, a slightly longer median developmental time of *F. fusca* to complete one generation and reduced reproduction on leaflets of Georgia-12Y both support the negative effect of Georgia-12Y on *F. fusca* development and fitness. However, oviposition rate of *F. fusca* on Georgia-12Y was not different from other cultivars. In contrast, negative fitness effects of TSWV-resistant peanut cultivars on thrips resulted in reduced developmental time (Shrestha et al. 2013). Peppers possess resistance to thrips also reduced thrips developmental time (Maharijaya et al. 2012). Divergent effects of host plant resistance on thrips developmental time could be related to the differences in resistance mechanisms. Common mechanisms of thrips resistance in other crops came from morphological traits of the plants such as leaf thickness, waxiness, and amount of pubescence; biochemical traits such as alkaloids and other secondary metabolites are also known to contribute to resistance to thrips (Srinivasan et al. 2018). Factors causing differences in susceptibility of peanut cultivars to thrips are largely unknown and required further in-depth research.

The current study demonstrated that nucleotide substitutions were the important sources of genetic variation in TSWV. Population expansion and purifying selection were substantial factors driving TSWV evolution, while positive selection was occasionally found in N and RdRp genes. Overall, we did not find evidence of TSWV resistance in peanut cultivars exerting substantial selection pressure on any of the five TSWV genes. Quantitative resistance is generally more durable than qualitative resistance (i.e., resistance conferred by a single resistance gene) likely because of the partial resistance effect exerting a low selection pressure

on the pathogen. If the quantitative resistance is conferred by a combination of resistance mechanisms with multiple genes involved, the resistance would be more difficult to overcome (Mundt 2014; Pilet-Nayel et al. 2017). As the underlying mechanisms of TSWV resistance in peanut has just started to be revealed, much more information is needed to properly assess the durability of the quantitative resistance in peanut. Monitoring for resistance-breaking strains in peanut is still necessary as quantitative resistance could also be adapted by the pathogen (Montarry et al. 2012). TSWV-resistant peanut cultivars tested in this study mostly possess similar susceptibility to *F. fusca* except that development was delayed and reproduction was reduced in Georgia-12Y. The negative impact of some TSWV-resistant peanut cultivars on thrips fitness could likely contribute to the overall success of TSWV resistance cultivars. Interaction between thrips vectors and peanut is a crucial part of TSWV pathosystem, and thrips resistance in peanut cultivars in addition to TSWV resistance could be an extra layer to reduce selection pressure on TSWV from peanut hosts.

Literature Cited

Adkins, S., Quadt, R., Choi, T. J., Ahlquist, P., and German, T. 1995. An RNA-dependent RNA polymerase activity associated with virions of tomato spotted wilt virus, a plant- and insect-infecting bunyavirus. Virology. 207:308–311.

Agarwal, G., Clevenger, J., Pandey, M. K., Wang, H., Shasidhar, Y., Chu, Y., et al. 2018. High-density genetic map using whole-genome resequencing for fine mapping and candidate gene discovery for disease resistance in peanut. Plant Biotechnol. J. 16:1954–1967.

Almási, A., Nemes, K., Csömör, Z., Tóbiás, I., Palkovics, L., and Salánki, K. 2017. A single point mutation in Tomato spotted wilt virus NSs protein is sufficient to overcome Tsw-gene-

mediated resistance in pepper. J. Gen. Virol. 98:1521–1525.

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

Bandelt, H. J., Forster, P., and Röhl, A. 1999. Median-joining networks for inferring intraspecific phylogenies. Mol. Biol. Evol. 16:37–48.

Bandla, M. D., Campbell, L. R., Ullman, D. E., and Sherwood, J. L. 1998. Interaction of tomato spotted wilt tospovirus (TSWV) glycoproteins with a thrips midgut protein, a potential cellular receptor for TSWV. Phytopathology. 88:98–104.

Ben-Mahmoud, S., Smeda, J. R., Chappell, T. M., Stafford-Banks, C., Kaplinsky, C. H., Anderson, T., et al. 2018. Acylsugar amount and fatty acid profile differentially suppress oviposition by western flower thrips, frankliniella occidentalis, on tomato and interspecific hybrid flowers. PLoS One. 13.

Black, M. C., and Smith, D. H. 1987. Spotted wilt and rust reactions in south Texas among selected peanut genotypes. In *Proc. Am. Peanut Res. Ed. Soc*, , p. 31.

Branch, W. D. 2007. Registration of ‘Georgia-06G’ Peanut. J. Plant Regist. 1:120–120.

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

Branch, W. D. 2017. Registration of ‘Georgia-16HO’ Peanut. J. Plant Regist. 11:231–234.

Branch, W. D. 1996. Registration of ‘Georgia Green’ Peanut. Crop Sci. 36:806–806.

Chapman, E. J., Hilson, P., and German, T. L. 2003. Association of L Protein and in vitro Tomato Spotted Wilt Virus RNA-Dependent RNA Polymerase Activity. Intervirology. 46:177–181.

Chare, E. R., and Holmes, E. C. 2006. A phylogenetic survey of recombination frequency in plant RNA viruses. Arch. Virol. 151:933–946.

Culbreath, A. K., Csinos, A. S., Bertrand, P. F., and Demski, J. W. 1991. Tomato Spotted Wilt Virus Epidemic in Flue-Cured Tobacco in Georgia. Plant Dis. 75:483.

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

Culbreath, A. K., Todd, J. W., and Brown, S. L. 2003. Epidemiology and Management of Tomato Spotted Wilt in Peanut. Annu. Rev. Phytopathol. 41:53–75.

Culbreath, A. K., Todd, J. W., Demski, J. W., and Chamberlin, J. R. 1992. Disease Progress of Spotted Wilt in Peanut Cultivars Florunner and Southern Runner. Phytopathology. 82:766.

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

Fabre, F., Rousseau, E., Mailleret, L., and Moury, B. 2012. Durable strategies to deploy plant resistance in agricultural landscapes. New Phytol. 193:1064–1075.

Fu, Y. X. 1997. Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. Genetics. 147:915–925.

Fu, Y. X., and Li, W. H. 1993. Statistical tests of neutrality of mutations. Genetics. 133:693–709.

García-Arenal, F., Fraile, A., and Malpica, J. M. 2001. Variability and genetic structure of plant virus populations. Annu. Rev. Phytopathol. 39:157–186.

Garcia, L. E., Brandenburg, R. L., and Bailey, J. E. 2000. Incidence of Tomato spotted wilt virus (Bunyaviridae) and tobacco thrips in Virginia-type peanuts in North Carolina. Plant Dis. 84:459–464.

Garzo, E., Moreno, A., Plaza, M., and Fereres, A. 2020. Feeding Behavior and Virus-transmission Ability of Insect Vectors Exposed to Systemic Insecticides. Plants. 9:895.

Gitaitis, R. D., Dowler, C. C., and Chalfant, R. B. 1998. Epidemiology of tomato spotted wilt in pepper and tomato in southern Georgia. Plant Dis. 82:752–756.

De Haan, P., Kormelink, R., De Oliveira Resende, R., Van Poelwijk, F., and Peters Goldbach, D. R. 1991. Tomato spotted wilt virus L RNA encodes a putative RNA polymerase. J. Gen. Virol. 72:2207–2216.

De Haan, P., Wagemakers, L., Peters, D., and Goldbach, R. 1990. The S RNA segment of tomato spotted wilt virus has an ambisense character. J. Gen. Virol. 71:1001–1007.

Hagan, A. K., Weeks, J. R., French, J. C., Gudauskas, R. T., Mullen, J. M., Gazaway, W. S., et al. 1990. Tomato Spotted Wilt Virus in Peanut in Alabama. Plant Dis. 74:615.

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

Hoffmann, K., Qiu, W. P., and Moyer, J. W. 2001. Overcoming host- and pathogen-mediated resistance in tomato and tobacco maps to the M RNA of Tomato spotted wilt virus. Mol. Plant-Microbe Interact. 14:242–249.

Holbrook, C. C., Timper, P., Culbreath, A. K., and Kvien, C. K. 2008. Registration of 'Tifguard' Peanut. J. Plant Regist. 2:92–94.

Hudson, R. R., Boos, D. D., and Kaplan, N. L. 1992. A statistical test for detecting geographic subdivision. Mol. Biol. Evol. 9:138–151.

Hudson, R. R., Slatkin, M., and Maddison, W. P. 1992. Estimation of levels of gene flow from DNA sequence data. Genetics. 132:583–589.

Jacobson, A. L., and Kennedy, G. G. 2013. Effect of cyantraniliprole on feeding behavior and virus transmission of Frankliniella fusca and Frankliniella occidentalis (Thysanoptera: Thripidae) on Capsicum annuum. Crop Prot. 54:251–258.

Kaye, A. C., Moyer, J. W., Parks, E. J., Carbone, I., and Cubeta, M. A. 2011. Population genetic analysis of Tomato spotted wilt virus on peanut in North Carolina and Virginia. Phytopathology. 101:147–153.

Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., et al. 2012. Geneious Basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics. 28:1647–1649.

Khera, P., Pandey, M. K., Wang, H., Feng, S., Qiao, L., Culbreath, A. K., et al. 2016. Mapping quantitative trait loci of resistance to tomato spotted wilt virus and leaf spots in a recombinant inbred line population of peanut (Arachis hypogaea L.) from SunOleic 97R and NC94022 ed. Jinfa Zhang. PLoS One. 11:e0158452.

Kobayashi, K., Sekine, K. T., and Nishiguchi, M. 2014. Breakdown of plant virus resistance: Can we predict and extend the durability of virus resistance? J. Gen. Plant Pathol. 80:327–336.

Kormelink, R., De Haan, P., Meurs, C., Peters, D., and Goldbach, R. 1992. The nucleotide sequence of the M RNA segment of tomato spotted wilt virus, a bunyavirus with two ambisense RNA segments. J. Gen. Virol. 73:2795–2804.

Kormelink, R., Storms, M., Van Lent, J., Peters, D., and Goldbach, R. 1994. Expression and Subcellular Location of the NSM Protein of Tomato Spotted Wilt Virus (TSWV), a Putative Viral Movement Protein. Virology. 200:56–65.

Latham, L. J., and Jones, R. A. C. 1998. Selection of resistance breaking strains of tomato spotted wilt tospovirus. Ann. Appl. Biol. 133:385–402.

Leigh, J. W., and Bryant, D. 2015. POPART: Full-feature software for haplotype network construction ed. Shinichi Nakagawa. Methods Ecol. Evol. 6:1110–1116.

Lian, S., Lee, J. S., Cho, W. K., Yu, J., Kim, M. K., Choi, H. S., et al. 2013. Phylogenetic and Recombination Analysis of Tomato Spotted Wilt Virus ed. Darren P. Martin. PLoS One. 8:e63380.

Little, E. L. 2017. *2015 Georgia Plant Disease Loss Estimates*.

Little, E. L. 2019a. *2016 Georgia Plant Disease Loss Estimates*.

Little, E. L. 2019b. *2017 Georgia Plant Disease Loss Estimates*.

Little, E. L. 2020. *2018 Georgia Plant Disease Loss Estimates*.

López, C., Aramburu, J., Galipienso, L., Soler, S., Nuez, F., and Rubio, L. 2011. Evolutionary analysis of tomato Sw-5 resistance-breaking isolates of tomato spotted wilt virus. J. Gen. Virol. 92:210–215.

Maharijaya, A., Vosman, B., Verstappen, F., Steenhuis-Broers, G., Mumm, R., Purwito, A., et al. 2012. Resistance factors in pepper inhibit larval development of thrips (Frankliniella occidentalis). Entomol. Exp. Appl. 145:62–71.

Marasigan, K., Toews, M., Kemerait, R., Abney, M. R., Culbreath, A., and Srinivasan, R. 2016. Evaluation of alternatives to carbamate and organophosphate insecticides against thrips and tomato spotted wilt virus in peanut production. J. Econ. Entomol. 109:544–557.

Margaria, P., Ciuffo, M., Pacifico, D., and Turina, M. 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.

Margaria, P., Ciuffo, M., Rosa, C., and Turina, M. 2015. Evidence of a tomato spotted wilt virus resistance-breaking strain originated through natural reassortment between two evolutionary-distinct isolates. Virus Res. 196:157–161.

Maris, P. C., Joosten, N. N., Goldbach, R. W., and Peters, D. 2003. Restricted Spread of Tomato

spotted wilt virus in Thrips-Resistant Pepper. Phytopathology. 93:1223–1227.

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

Martin, D. P., Murrell, B., Golden, M., Khoosal, A., and Muhire, B. 2015. RDP4: Detection and analysis of recombination patterns in virus genomes. Virus Evol. 1.

Montarry, J., Cartier, E., Jacquemond, M., Palloix, A., and Moury, B. 2012. Virus adaptation to quantitative plant resistance: Erosion or breakdown? J. Evol. Biol. 25:2242–2252.

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

Mundt, C. C. 2014. Durable resistance: A key to sustainable management of pathogens and pests. Infect. Genet. Evol. 27:446–455.

Munger, F. 1942. A Method for Rearing Citrus Thrips in the Laboratory. J. Econ. Entomol. 35:373–375.

Murrell, B., Moola, S., Mabona, A., Weighill, T., Sheward, D., Kosakovsky Pond, S. L., et al. 2013. FUBAR: A fast, unconstrained bayesian AppRoximation for inferring selection. Mol. Biol. Evol. 30:1196–1205.

Nei, M. 1987. *Molecular evolutionary genetics*. Columbia Univ. Press, New York.

Norden, A. J., Lipscomb, R. W., and Carver, W. A. 1969. Registration of Florunner Peanuts 1 (Reg. No. 2). Crop Sci. 9:850–850.

Pandey, M. K., Wang, H., Khera, P., Vishwakarma, M. K., Kale, S. M., Culbreath, A. K., et al. 2017. Genetic dissection of novel QTLs for resistance to leaf spots and tomato spotted wilt

virus in peanut (Arachis hypogaea L.). Front. Plant Sci. 8:25.

Pappu, H., Pappu, S., Jain, R., Bertrand, P., Culbreath, A., McPherson, R., et al. 1998. Sequence characteristics of natural populations of tomato spotted wilt tospovirus infecting flue-cured tobacco in Georgia. Virus Genes. 17:169–177.

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

Peiró, A., Cañizares, M. C., Rubio, L., López, C., Moriones, E., Aramburu, J., et al. 2014. The movement protein (NSm) of Tomato spotted wilt virus is the avirulence determinant in the tomato Sw-5 gene-based resistance. Mol. Plant Pathol. 15:802–813.

Pilet-Nayel, M. L., Moury, B., Caffier, V., Montarry, J., Kerlan, M. C., Fournet, S., et al. 2017. Quantitative resistance to plant pathogens in pyramiding strategies for durable crop protection. Front. Plant Sci. 8:27.

Qiu, W., and Moyer, J. W. 1999. Tomato spotted wilt tospovirus adapts to the TSWV N gene-derived resistance by genome reassortment. Phytopathology. 89:575–582.

Qiu, W. P., Geske, S. M., Hickey, C. M., and Moyer, J. W. 1998. Tomato spotted wilt Tospovirus genome reassortment and genome segment- specific adaptation. Virology. 244:186–194.

Richmond, K. E., Chenault, K., Sherwood, J. L., and German, T. L. 1998. Characterization of the nucleic acid binding properties of tomato spotted wilt virus nucleocapsid protein. Virology. 248:6–11.

Riley, D. G., and Pappu, H. R. 2004. *Tactics for Management of Thrips (Thysanoptera: Thripidae) and Tomato Spotted Wilt Virus in Tomato*.

Roggero, P., Masenga, V., and Tavella, L. 2002. Field isolates of Tomato spotted wilt virus

overcoming resistance in pepper and their spread to other hosts in Italy. Plant Dis. 86:950–954.

Roggero, P., Melani, V., Ciuffo, M., Tavella, L., Tedeschi, R., and Stravato, V. M. 1999. Two Field Isolates of Tomato Spotted Wilt Tospovirus Overcome the Hypersensitive Response of a Pepper (Capsicum annuum) Hybrid with Resistance Introgressed from C. chinense PI152225. Plant Dis. 83:965–965.

de Ronde, D., Butterbach, P., Lohuis, D., Hedil, M., van Lent, J. W. M., and Kormelink, R. 2013. Tsw gene-based resistance is triggered by a functional RNA silencing suppressor protein of the Tomato spotted wilt virus. Mol. Plant Pathol. 14:405–415.

de Ronde, D., Pasquier, A., Ying, S., Butterbach, P., Lohuis, D., and Kormelink, R. 2014. Analysis of Tomato spotted wilt virus NSs protein indicates the importance of the N-terminal domain for avirulence and RNA silencing suppression. Mol. Plant Pathol. 15:185–195.

Roossinck, M. J. 1997. Mechanisms of plant virus evolution. Annu. Rev. Phytopathol. 35:191–209.

Rozas, J., Ferrer-Mata, A., Sanchez-DelBarrio, J. C., Guirao-Rico, S., Librado, P., Ramos-Onsins, S. E., et al. 2017. DnaSP 6: DNA sequence polymorphism analysis of large data sets. Mol. Biol. Evol. 34:3299–3302.

Sharman, M., and Persley, D. M. 2006. Field isolates of Tomato spotted wilt virus overcoming resistance in capsicum in Australia. Australas. Plant Pathol. 35:123–128.

Shrestha, A., Srinivasan, R., Riley, D. G., and Culbreath, A. K. 2012. Direct and indirect effects of a thrips-transmitted tospovirus on the preference and fitness of its vector, frankliniella fusca. Entomol. Exp. Appl. 145:260–271.

Shrestha, A., Srinivasan, R., Sundaraj, S., Culbreath, A. K., and Riley, D. G. 2013. Second

generation peanut genotypes resistant to thrips-transmitted tomato spotted wilt virus exhibit tolerance rather than true resistance and differentially affect thrips fitness. J. Econ. Entomol. 106:587–596.

Shrestha, A., Sundaraj, S., Culbreath, A. K., Riley, D. G., Abney, M. R., and Srinivasan, R. 2015. Effects of Thrips Density, Mode of Inoculation, and Plant Age on Tomato Spotted Wilt Virus Transmission in Peanut Plants. Environ. Entomol. 44:136–143.

Soellick, T. R., Uhrig, J. F., Bucher, G. L., Kellmann, J. W., and Schreier, P. H. 2000. The movement protein NSm of tomato spotted wilt tospovirus (TSWV): RNA binding, interaction with the TSWV N protein, and identification of interacting plant proteins. Proc. Natl. Acad. Sci. U. S. A. 97:2373–2378.

Srinivasan, R., Abney, M. R., Culbreath, A. K., Kemerait, R. C., Tubbs, R. S., Monfort, W. S., et al. 2017. Three decades of managing Tomato spotted wilt virus in peanut in southeastern United States. Virus Res. 241:203–212.

Srinivasan, R., Abney, M. R., Lai, P. C., Culbreath, A. K., Tallury, S., and Leal-Bertioli, S. C. M. 2018. Resistance to thrips in peanut and implications for management of thrips and thrips-transmitted orthotospoviruses in peanut. Front. Plant Sci. 871:1604.

Storms, M. M. H., Kormelink, R., Peters, D., Van Lent, J. W. M., and Goldbach, R. W. 1995. The nonstructural NSm protein of tomato spotted wilt virus induces tubular structures in plant and insect cells. Virology. 214:485–493.

Storms, M. M. H., Van der Schoot, C., Prins, M., Kormelink, R., Van Lent, J. W. M., and Goldbach, R. W. 1998. A comparison of two methods of microinjection for assessing altered plasmodesmal gating in tissues expressing viral movement proteins. Plant J. 13:131–140.

Sundaraj, S., Srinivasan, R., Culbreath, A. K., Riley, D. G., and Pappu, H. R. 2014. Host plant

resistance against tomato spotted wilt virus in peanut (Arachis hypogaea) and its impact on susceptibility to the virus, virus population genetics, and vector feeding behavior and survival. Phytopathology. 104:202–210.

Tajima, F. 1989. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. Genetics. 123:585–595.

Takeda, A., Sugiyama, K., Nagano, H., Mori, M., Kaido, M., Mise, K., et al. 2002. Identification of a novel RNA silencing suppressor, NSs protein of Tomato spotted wilt virus. FEBS Lett. 532:75–79.

Tentchev, D., Verdin, E., Marchal, C., Jacquet, M., Aguilar, J. M., and Moury, B. 2011. Evolution and structure of Tomato spotted wilt virus populations: Evidence of extensive reassortment and insights into emergence processes. J. Gen. Virol. 92:961–973.

Thomas-Carroll, M. L., and Jones, R. A. C. 2003. Selection, biological properties and fitness of resistance-breaking strains of Tomato spotted wilt virus in pepper. Ann. Appl. Biol. 142:235–243.

Tseng, Y. C., Tillman, B. L., Peng, Z., and Wang, J. 2016. Identification of major QTLs underlying tomato spotted wilt virus resistance in peanut cultivar Florida-EPTM “113.” BMC Genet. 17:128.

Tsompana, M., Abad, J., Purugganan, M., and Moyer, J. W. 2005. The molecular population genetics of the Tomato spotted wilt virus (TSWV) genome. Mol. Ecol. 14:53–66.

Ullman, D. E., Cho, J. J., Mau, R. F. L., Hunter, W. B., Westcot, D. M., and Custer, D. M. 1992. Thrips-Tomato Spotted Wilt Virus Interactions: Morphological, Behavioral and Cellular Components Influencing Thrips Transmission. In Springer, New York, NY, p. 195–240.

Watterson, G. A. 1975. On the number of segregating sites in genetical models without

recombination. Theor. Popul. Biol. 7:256–276.

Van De Wetering, F., Hulshof, J., Posthuma, K., Harrewijn, P., Goldbach, R., and Peters, D. 1998. Distinct feeding behavior between sexes of Frankliniella occidentalis results in higher scar production and lower tospovirus transmission by females. Entomol. Exp. Appl. 88:9–15.

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

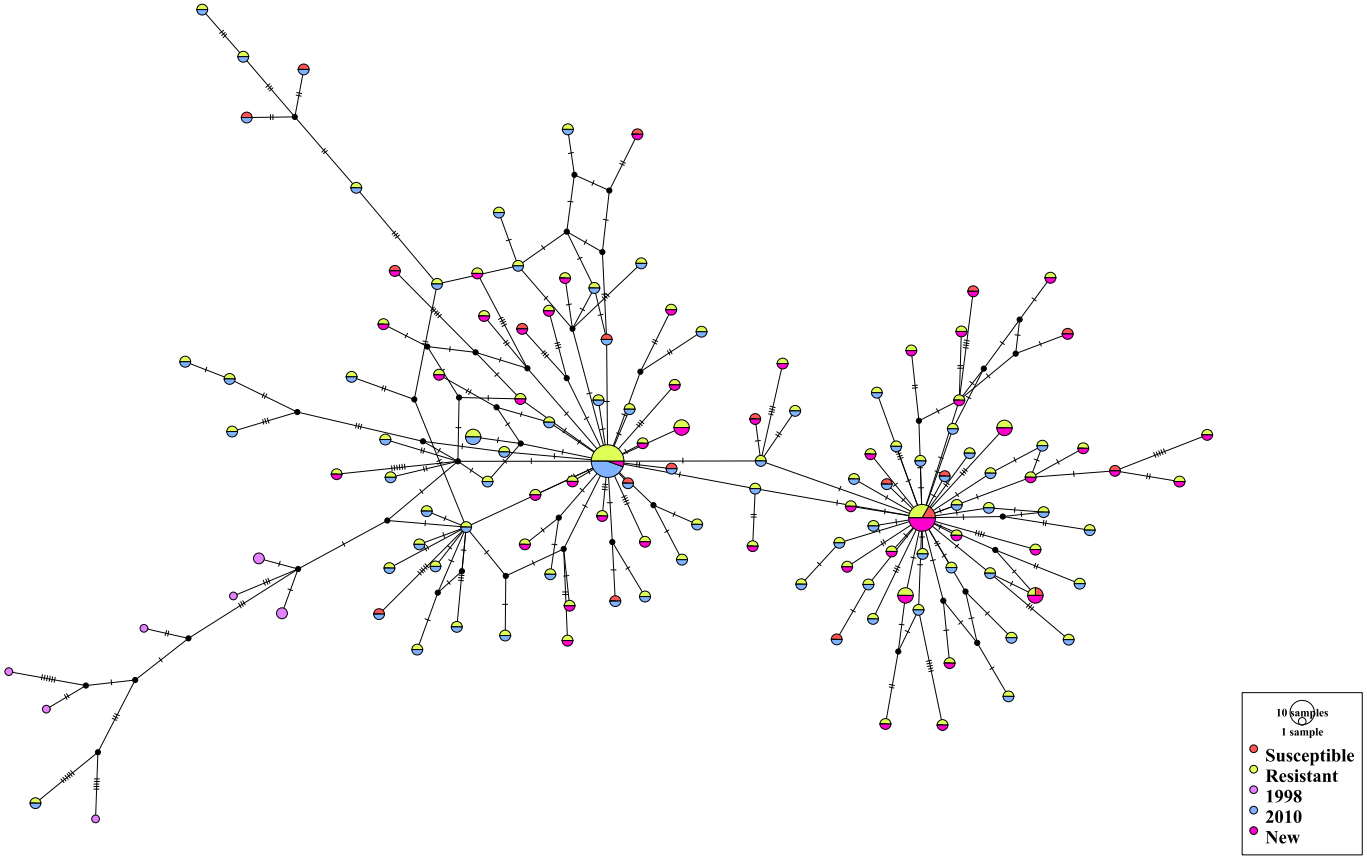

Fig. 5.1. A median-joining network of TSWV isolates from TSWV susceptible and resistant peanut cultivars based on TSWV N gene nucleotide sequences. TSWV isolates collected from 1998 (Pappu et al. 1998), 2010 (Sundaraj et al. 2014), and 2016-2019 (New) in GA were included. Colors indicate haplotype traits as labeled in the legend. Black nodes represent inferred (unsampled) sequences. Hatch marks on the branches represent mutation events. Colors within each individual circle are proportional to number of sequences with the corresponding trait at the node.

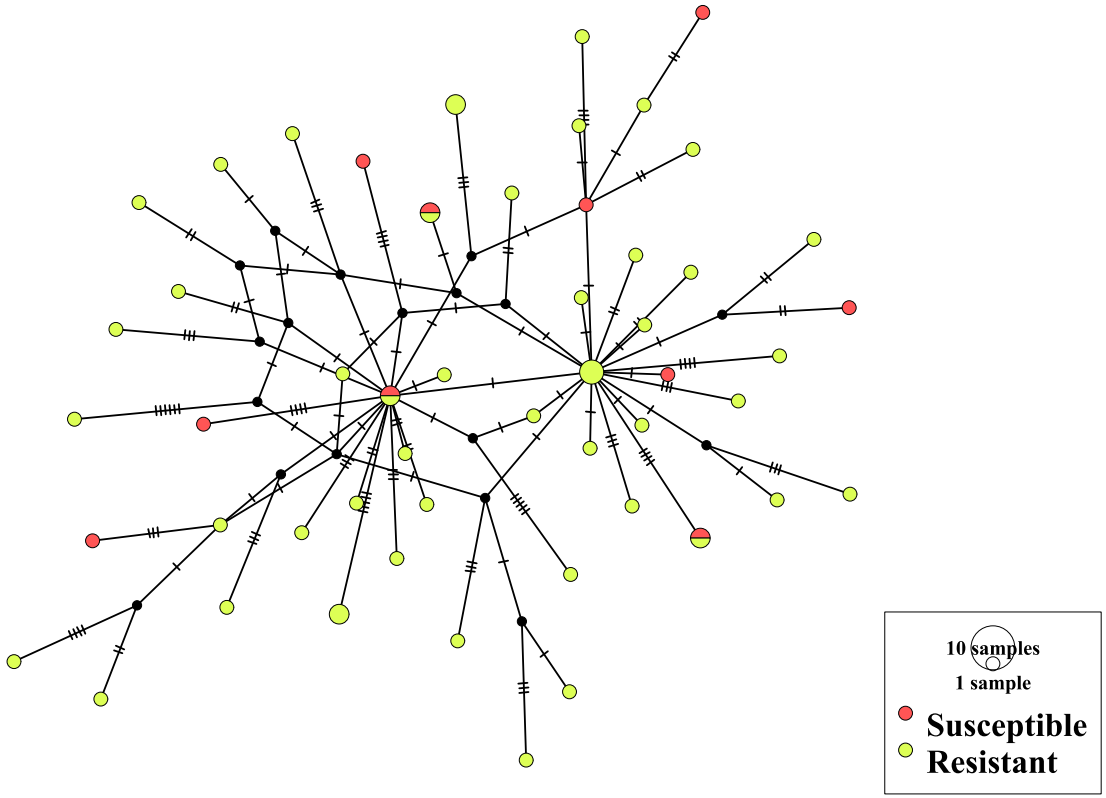

Fig. 5.2. A median-joining network of TSWV isolates from TSWV susceptible and resistant peanut cultivars based on TSWV NSs gene nucleotide sequences. Colors indicate haplotype traits as labeled in the legend. Black nodes represent inferred (unsampled) sequences. Hatch marks on the branches represent mutation events. Colors within each individual circle are proportional to number of sequences with the corresponding trait at the node.

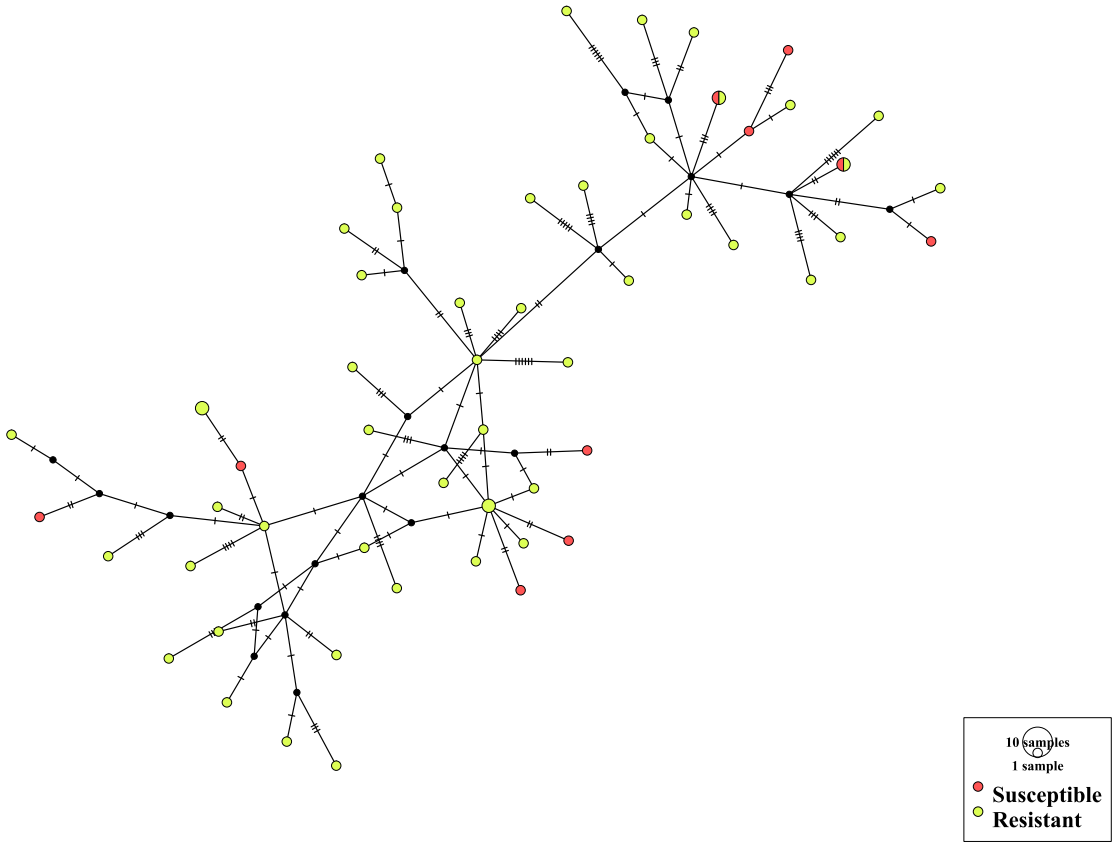

Fig. 5.3. A median-joining network of TSWV isolates from TSWV susceptible and resistant peanut cultivars based on TSWV NSm gene nucleotide sequences. Colors indicate haplotype traits as labeled in the legend. Black nodes represent inferred (unsampled) sequences. Hatch marks on the branches represent mutation events. Colors within each individual circle are proportional to number of sequences with the corresponding trait at the node.

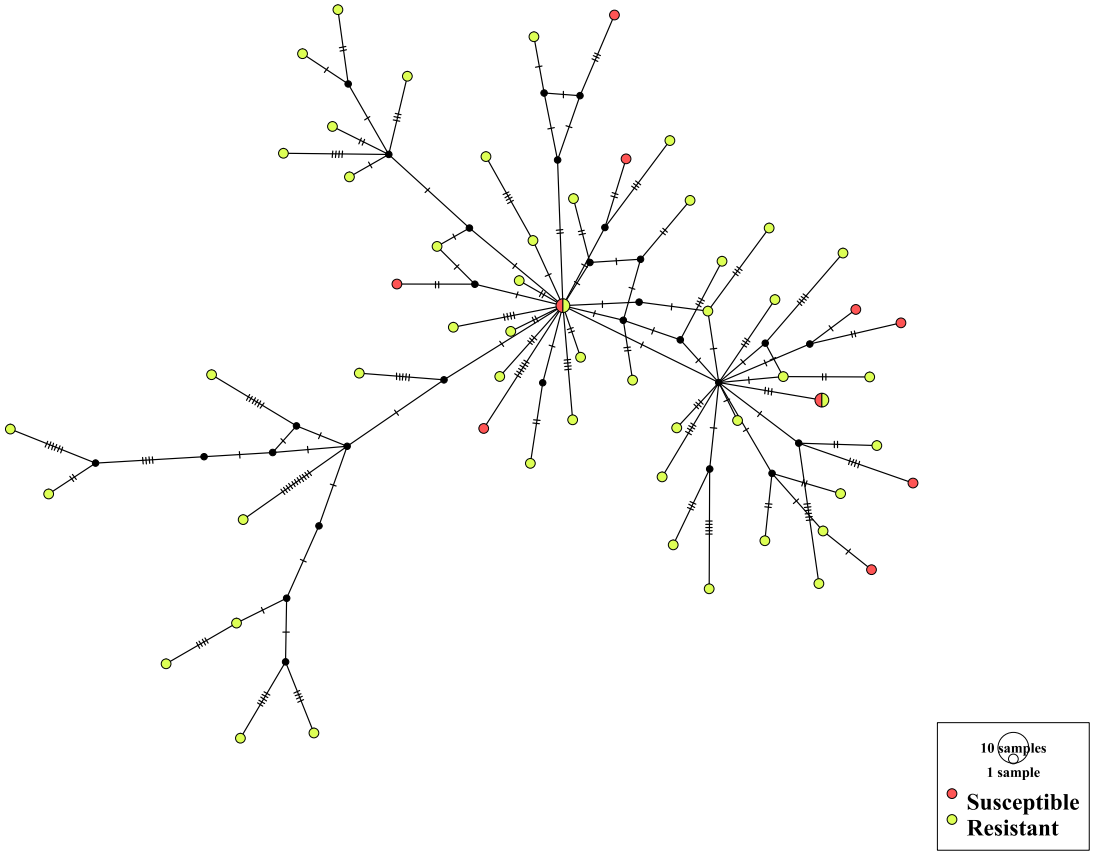

Fig. 5.4. A median-joining network of TSWV isolates from TSWV susceptible and resistant peanut cultivars based on TSWV Gn/Gc gene nucleotide sequences. Colors indicate haplotype traits as labeled in the legend. Black nodes represent inferred (unsampled) sequences. Hatch marks on the branches represent mutation events. Colors within each individual circle are proportional to number of sequences with the corresponding trait at the node.

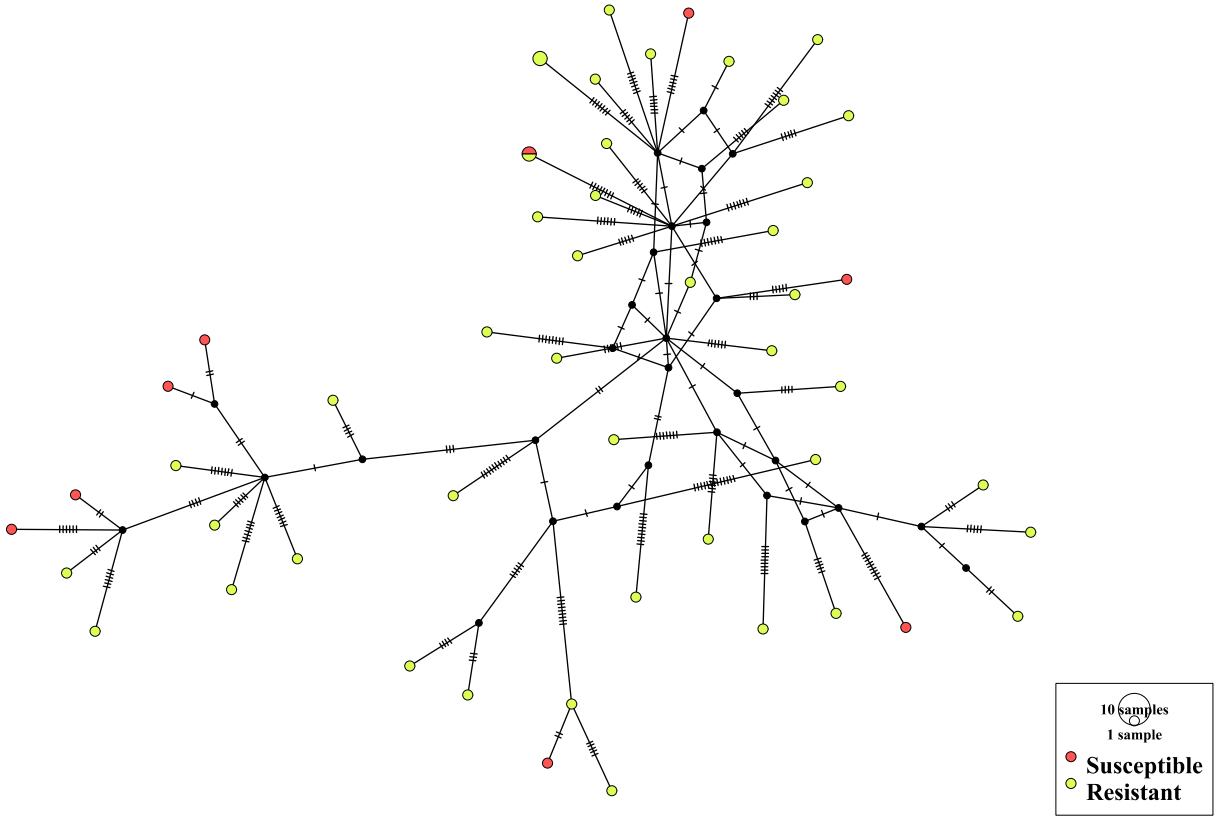

Fig. 5.5. A median-joining network of TSWV isolates from TSWV susceptible and resistant peanut cultivars based on TSWV RdRp gene nucleotide sequences. Colors indicate haplotype traits as labeled in the legend. Black nodes represent inferred (unsampled) sequences. Hatch marks on the branches represent mutation events. Colors within each individual circle are proportional to number of sequences with the corresponding trait at the node.

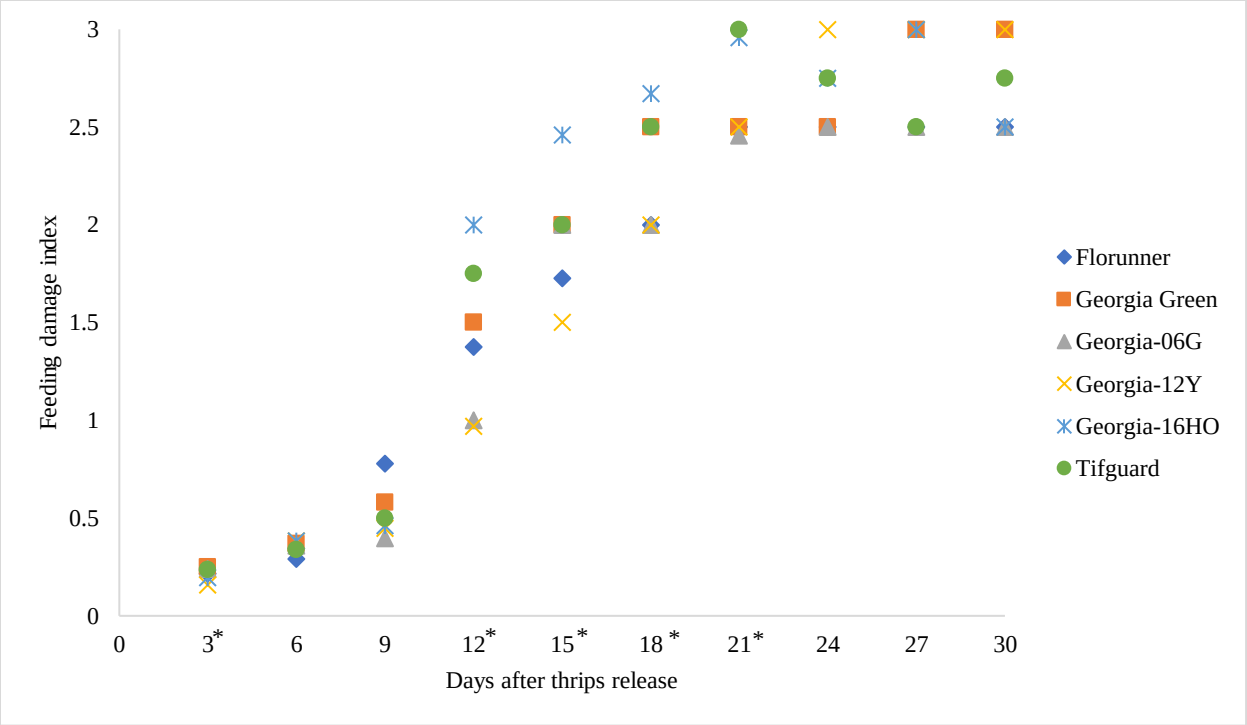

Fig. 5.6. Thrips feeding injury evaluated at 3-day intervals for up to 30 days post thrips released on TSWV susceptible and resistant peanut cultivars. Median feeding damage indices are presented for each evaluating date. Evaluating date denoted with "*" indicate significant cultivar effects on thrips feeding injury at $p < 0.05$ based on the Wilcoxon Score and Kruskal-Wallis tests. Six plants of each cultivar were placed in a thrips-proof cage with ten thrips were released at the base of each plant at the two-node stage. The experiment was conducted two times (n = 12 for each cultivar).

Table 5.1. Information regarding peanut samples from which TSWV isolates were collected and sequenced

Type	Cultivar	Year	Location	Reference	Susceptibility subgroups[z]
Runner	AUNPL-17	2017, 2018, 2019	Tifton	?	Resistant
Runner	FloRun 107	2016, 2017	Tifton	Tillman and Gorbet 2015	Resistant
Runner	FloRun 331	2017, 2018, 2019	Tifton	?	Resistant
Runner	Florunner	2018, 2019	Tifton	Norden et al. 1969	Susceptible
Runner	Georgia Green	2016, 2017, 2018, 2019	Tifton, Attapulgus	Branch 1996	Susceptible
Runner	Georgia-06G	2016, 2017, 2018, 2019	Tifton, Attapulgus	Branch 2007	Resistant
Runner	Georgia-12Y	2018, 2019	Tifton	Branch 2013	Resistant
Runner	Georgia-13M	2016, 2017, 2018	Tifton	Branch 2014	Resistant
Runner	Georgia-14N	2016, 2017, 2018, 2019	Tifton	Branch and Brenneman 2015	Resistant
Runner	Georgia-16HO	2017, 2018, 2019	Tifton, Attapulgus	Branch 2017	Resistant
Runner	Georgia-18RU	2019	Tifton	Branch 2019	Resistant
Runner	Tifguard	2016, 2017, 2018, 2019	Tifton	Holbrook et al. 2008	Resistant
Runner	TifNV-High O/L	2017, 2018, 2019	Tifton	Holbrook et al. 2017	Resistant
Runner	TUFRunner 157	2017	Tifton	?	Resistant
Runner	TUFRunner 297	2017, 2018, 2019	Tifton	Tillman 2018	Resistant
Runner	TUFRunner 511	2017, 2018	Tifton, Attapulgus	Tillman and Gorbet 2017	Resistant
Runner	TUFRunner 727	2016, 2017	Tifton	?	Resistant
Spanish	Georgia-04S	2016	Tifton	Branch 2005	Resistant
Valencia	New Mexico Valencia C	2019	Tifton	Hsi 1980	Susceptible
Virginia	Bailey	2019	Tifton	Isleib et al. 2011	Resistant
Virginia	Georgia-11J	2017, 2019	Tifton, Attapulgus	Branch 2012	Resistant
Virginia	Gregory	2019	Tifton	Isleib et al. 1999	Susceptible

[z] Grouping for TSWV susceptible and resistant subgroups for analyses of population genetics based on relative TSWV susceptibility.

Table 5.2. Primer pairs used for PCR

RNA Fragments	Gene	Primer pairs	Direction	Sequence (5' -> 3')	Annealing Temperature (°C)	Amplicon size (bp)[y]
L	RdRp	L6885	F	CTGTCCTCATTGTCGTGCCT	58	1519
		L8403	R	CAACTAACGCCACCCCTGAT		
M	NSm	M290	F	ACATCTTCCTTTGGAACCTA	53	673
		M962	R	CCTCTTCTTCTCCAACTGAT		
	GnGc	M2565	F	ACCAAGCTTCTTCACATCC	58	756
		M3320	R	TTTATGTTCCAGGCTGTCC		
S	NSs	S574	F	GTCTTGTGTCAAAGAGCATACCTATAA	58	860
		S1433	R	TGATCCCGCTTAAATCAAGCT		
	N[z]	S2057	F	TTAAGCAAGTTCTGTGAG	52	777
		S2833	R	ATGTCTAAGGTTAAGCTC		

[y] Expected amplicon size for each primer pair from PCR based on reference sequence (NC_002050 to NC_002052 & KT160280 to KT160282).

[z] Primers designed by Pappu et al. (1998).

Table 5.3 Summary of parameters for genetic variation in five TSWV genes

Gene	Subgroups	N[p]	S[q]	h[r]	Hd (SD)[s]	K[t]	π (SD)[u]	θ_w[v]
N	**Overall**	150	162	130	0.995 (0.003)	6.410	0.0083 (0.0005)	0.0373
	GA-1998[w]	9	27	7	0.944 (0.070)	7.944	0.0102 (0.0021)	0.0128
	GA-2010[x]	82	100	74	0.991 (0.006)	5.793	0.0075 (0.0006)	0.0259
	2010-S[x]	10	28	10	1.000 (0.045)	7.600	0.0098 (0.0017)	0.0127
	2010-R[x]	72	89	64	0.989 (0.008)	5.555	0.0072 (0.0006)	0.0236
	GA-New[y]	59	93	50	0.989 (0.008)	5.909	0.0076 (0.0006)	0.0258
	New-S[y]	10	29	9	0.978 (0.054)	6.711	0.0086 (0.0016)	0.0132
	New-R[y]	49	79	43	0.992 (0.007)	5.781	0.0075 (0.0006)	0.0228
	After 2010[z]	141	152	123	0.994 (0.003)	5.931	0.0076 (0.0004)	0.0354
	After 2010-S[z]	20	48	19	0.995 (0.018)	7.284	0.0094 (0.0013)	0.0174
	After 2010-R[z]	121	137	106	0.994 (0.004)	5.716	0.0074 (0.0004)	0.0328
NSs	**Overall**	59	108	52	0.995 (0.004)	6.212	0.0072 (0.0005)	0.0268
	Susceptible	10	29	10	1.000 (0.045)	6.289	0.0072 (0.0011)	0.0118
	Resistant	49	97	45	0.996 (0.005)	6.236	0.0072 (0.0005)	0.0250
NSm	**Overall**	58	91	54	0.998 (0.004)	8.469	0.0126 (0.0006)	0.0292
	Susceptible	10	30	10	1.000 (0.045)	8.467	0.0126 (0.0011)	0.0158
	Resistant	48	82	46	0.998 (0.005)	8.492	0.0126 (0.0007)	0.0275
Gn/Gc	**Overall**	59	147	57	0.999 (0.003)	8.731	0.0116 (0.0008)	0.0419
	Susceptible	10	34	10	1.000 (0.045)	7.311	0.0097 (0.0013)	0.0159
	Resistant	49	132	49	1.000 (0.004)	9.009	0.0119 (0.0009)	0.0392
RdRp	**Overall**	52	226	50	0.998 (0.004)	17.417	0.0123 (0.0005)	0.0353
	Susceptible	9	65	9	1.000 (0.052)	18.694	0.0132 (0.0014)	0.0169
	Resistant	43	202	42	0.999 (0.005)	17.099	0.0121 (0.0006)	0.0330

[p] Number of sequences in the subgroup.

[q] Number of segregating sites.

[r] Number of haplotypes.

[s] Haplotype diversity with standard deviation in parentheses; values may vary from 0 to 1.000.

[t] Average number of nucleotide differences between sequences; Tajima's estimate of population mutation rate.

[u] Nucleotide diversity with standard deviation in parentheses; estimates can range from 0 to 0.100.

[v] Watterson's estimator per site based on number of segregating sites.

[w] N gene sequences of TSWV isolates from Pappu et al. (1998).

[x] N gene sequences of TSWV isolates from Sundaraj et al. (2014).

[y] N gene sequences of TSWV isolates collected in this study from 2016 to 2019 and subgroups of isolates from susceptible (S) and resistant (R) cultivars.

[z] N gene sequences of TSWV isolates collected after 2010 from Sundaraj et al. (2014) and this study with subgroups of isolates from susceptible (S) and resistant (R) cultivars.

Table 5.4. Summary of neutrality test statistics for five TSWV genes

Gene	Subgroups	Tajima's D[r]	Fu & Li's D*[s]	Fu & Li's F*[t]	Fu's Fs[u]
N	**Overall**	-2.550***[v]	-5.927**	-5.232**	-242.902
	GA-1998[w]	-1.000	-1.116	-1.220	-0.201
	GA-2010[x]	-2.411**	-5.315**	-4.950**	-115.544
	2010-S[x]	-1.111	-1.012	-1.170	-4.341
	2010-R[x]	-2.390**	-4.843**	-4.634**	-93.219
	GA-new[y]	-2.484**	-4.350**	-4.348**	-57.998
	New-S[y]	-1.656.	-1.903.	-2.078.	-2.673
	New-R[y]	-2.428**	-4.113**	-4.168**	-48.172
	After 2010[z]	-2.574***	-5.648**	-5.089**	-33.181
	After 2010-S[z]	-1.8545*	-2.263	-2.495	-12.064
	After 2010-R[z]	-2.5791***	-5.334**	-4.933**	-33.964
NSs	**Overall**	-2.586***	-4.505**	-4.507**	-62.831
	Susceptible	-1.853*	-2.183*	-2.370*	-5.012
	Resistant	-2.573***	-4.517**	-4.534**	-52.774
NSm	**Overall**	-2.047*	-3.335*	-3.401**	-33.303
	Susceptible	-0.969	-1.113	-1.215	-3.987
	Resistant	-1.979*	-3.013*	-3.142*	-49.038
GnGc	**Overall**	-2.620***	-5.042**	-4.910**	-33.741
	Susceptible	-1.890*	-2.241**	-2.431**	-4.472
	Resistant	-2.571***	-4.910**	-4.824**	-34.172
RdRp	**Overall**	-2.395**	-4.199**	-4.202**	-35.341
	Susceptible	-1.181	-1.304	-1.432	-1.481
	Resistant	-2.378**	-3.715**	-3.851**	-27.922

[r] Tajima's D compares the nucleotide diversity with the proportion of segregating sites; a negative value provides evidence for population expansion and/or purifying selection at the locus.

[s] Fu and Li's D* is based on the differences between the number of singletons and the total number of mutations; a negative value provides evidence for population expansion and/or purifying selection at the locus.

[t] Fu and Li's F* is based on the differences between the number of singletons and the average number of nucleotide differences between sequences; a negative value provides evidence for population expansion and/or purifying selection at the locus.

[u] Fu's Fs is based on the haplotype frequency distribution and the mutation rate; a negative value supports population expansion.

[v] Significance of the value is denoted as ".", $p < 0.1$; "*", $p < 0.05$, "**", $p < 0.01$; "***", $p < 0.001$.

[w] N gene sequences of TSWV isolates from Pappu et al. (1998).

[x] N gene sequences of TSWV isolates from Sundaraj et al. (2014).

[y] N gene sequences of TSWV isolates collected in this study from 2016 to 2019 and subgroups of isolates from susceptible (S) and resistant (R) cultivars.

[z] N gene sequences of TSWV isolates after 2010 from Sundaraj et al. (2014) and this study with subgroups of isolates from susceptible (S) and resistant (R) cultivars.

Table 5.5. Summary of codon sites with amino acid substitutions in five TSWV genes

Gene	Codon site[t]	α[u]	β[v]	$\beta-\alpha$[w]	Prob [$\alpha<\beta$][x]	Bayes Factor [$\alpha<\beta$][y]	Amino acid changes[z]
N	**7**	1.508	6.379	4.871	**0.902***	19.951	T → I, F
	8	0.799	0.833	0.034	0.545	2.591	K → M
	10	0.531	1.180	0.648	0.738	6.115	S → N
	18	0.542	0.681	0.139	0.583	3.030	G → S
	19	0.771	0.806	0.036	0.545	2.595	K → I
	40	2.687	8.793	6.105	0.828	10.395	G → D, E
	174	0.544	0.877	0.333	0.625	3.613	Y → C
	222	0.994	2.559	1.565	0.726	5.743	S → C
NSs	16	1.128	3.666	2.538	0.667	3.547	Q → K
	53	1.049	1.253	0.204	0.582	2.474	S → T
	98	1.066	1.244	0.178	0.580	2.448	I → V
	114	1.035	1.158	0.122	0.576	2.407	T → A
	152	1.144	1.174	0.030	0.567	2.322	E → K
	193	0.849	1.514	0.666	0.623	2.938	N →D
	243	5.451	11.60	6.149	0.709	4.321	T → I, A
	257	1.051	1.099	0.048	0.569	2.342	P → T
	281	0.732	2.528	1.796	0.684	3.839	L → M
NSm	124	1.127	1.462	0.335	0.592	2.619	K → R
	210	1.056	5.211	4.155	0.824	8.475	K → I, T
GnGc	51	0.656	0.971	0.315	0.609	3.142	N → S
	82	1.018	1.138	0.120	0.561	2.575	Y → S
	135	1.401	4.190	2.789	0.753	6.152	S → N
	139	0.656	0.971	0.315	0.609	3.142	N → S
	165	0.883	1.234	0.352	0.588	2.872	L → V
	223	0.619	2.307	1.688	0.790	7.582	D → N
RdRp	51	0.792	3.945	3.153	0.863	15.037	V → I
	149	1.006	6.957	5.951	**0.905***	22.825	K → R
	207	0.902	11.777	10.875	**0.959***	55.579	Q → R
	208	0.967	1.051	0.085	0.545	2.853	N → D
	211	0.689	0.905	0.217	0.580	3.298	I → V
	244	0.796	0.900	0.104	0.555	2.974	E → K
	280	0.699	1.067	0.368	0.596	3.511	N → D
	331	2.785	5.005	2.220	0.639	4.226	I → A
	341	0.699	1.061	0.362	0.595	3.503	N → S
	368	0.865	16.328	15.463	**0.992***	288.727	G → R, E, A
	397	0.665	9.774	9.109	**0.912***	24.825	Q → L, H
	414	0.708	0.791	0.082	0.557	3.000	S → T

[t] Codon positions with overabundance of non-synonymous substitutions.

[u] Mean posterior synonymous substitution rate at a codon site.

[v] Mean posterior non-synonymous substitution rate at a codon site.

[w] Mean posterior $\beta-\alpha$; a positive value indicates an overabundance of non-synonymous substitutions

[x] Posterior probability of positive selection at a codon site; significance is determined at Prob [$\alpha<\beta$] > 0.900.

[y] Empirical Bayes Factor for positive selection at a codon site; a higher value provides stronger support.

[z] Codon changes as a result of substitution.

Table 5.6. Summary of parameter estimates and test statistics for population differentiation

Gene	Comparison[p]	Hs[q]	Hst[q]	*p*(Hs/Hst)[r]	Ks[s]	Kst[s]	*p*(Ks/Kst)[r]	Snn[t]	*p*(Snn)[r]	Fst[u]
N	GA-1998[v] vs. GA-2010[w]	0.9875	0.0050	0.001	6.0059	0.0782	< **0.001**	0.9923	< **0.001**	0.2915
	GA-1998[v] vs. GA-new[x]	0.9840	0.0068	**0.003**	6.1783	0.1230	< **0.001**	1.0000	< **0.001**	0.3493
	GA-1998[v] vs. After 2010[y]	0.9919	0.0029	0.001	6.0517	0.0559	< **0.001**	0.9953	< **0.001**	0.3126
	GA-2010[w] vs. GA-new[x]	0.9903	0.0041	< **0.001**	5.8415	0.0151	< **0.001**	0.6490	< **0.001**	0.0302
	GA-2010 S vs. R[z]	0.9898	0.0015	0.325	5.8045	-0.0020	0.637	0.8438	**0.041**	-0.0081
	GA-new S vs. R[z]	0.9902	-0.0014	0.638	5.9383	-0.0050	0.923	0.6825	0.776	-0.0168
	After 2010 S vs. R[z]	0.9938	0.0005	0.215	5.9385	-0.0013	0.722	0.7590	0.483	-0.0048
NSs	S vs. R[z]	0.9964	-0.0011	0.857	6.2446	-0.0052	0.982	0.6059	0.886	-0.0184
NSm	S vs. R[z]	0.9985	-0.0009	0.936	8.4877	-0.0022	0.559	0.6997	0.614	-0.0077
GnGc	S vs. R[z]	1.0000	-0.0012	1.000	8.7208	0.0012	0.302	0.6439	0.779	0.0044
RdRp	S vs. R[z]	0.9991	-0.0006	0.912	17.3748	0.0024	0.244	0.7212	0.606	0.0080

[p] Comparison between population subgroups of TSWV isolates.
[q] Hs and Hst are haplotype-based test statistics of population differentiation.
[r] Significance of the test statistics is determined when $p < 0.05$.
[s] Ks and Kst are nucleotide-based test statistics of population differentiation.
[t] Test statistic Snn is independent of sample size and diversity; a value close to 1 indicates differentiation.
[u] Fst, a nucleotide-based test statistic, determines the extent of genetic differentiation.
[v] N gene sequences of TSWV isolates from Pappu et al. (1998).
[w] N gene sequences of TSWV isolates from Sundaraj et al. (2014).
[x] N gene sequences of TSWV isolates collected in this study from 2016 to 2019.
[y] N gene sequences of TSWV isolates collected after 2010 from Sundaraj et al. (2014) and this study.
[z] Subgroups of isolates from susceptible (S) and resistant (R) cultivars

Table 5.7. Effect of TSWV susceptible and resistant peanut cultivars on *Frankliniella fusca* survival on whole plants in no-choice tests

Cultivar[x]	Thrips number (mean ± SE)[y]									
	3d	6d	9d	12d	15d	18d	21d	24d	27d	30d
Florunner	2.42 ± 0.47	2.58 ± 0.54	20.08 ±7.38 AB	45.50 ± 6.44 B	47.5 ± 5.21	33.92 ± 4.27	34.08 ± 3.87	29.17 ± 2.53	38.92 ± 3.49	66.58 ± 7.49
Georgia Green	2.17 ± 0.24	3.17 ± 0.39	16.75 ± 3.66 AB	83.83 ± 11.02 A	72.75 ± 7.22	46.17 ± 5.35	39.67 ± 4.48	40.5 ± 7.24	49.00 ± 5.19	60.08 ± 7.07
Georgia-06G	3.08 ± 0.26	2.67 ± 0.36	13.42 ± 3.36 B	59.75 ± 4.49 AB	74.45 ± 8.84	53.36 ± 8.43	47.36 ± 7.01	38.27 ± 5.27	42.45 ± 6.4	68.36 ± 9.99
Georgia-12Y	3.08 ± 0.29	4.00 ± 0.41	3.17 ± 0.34 C	41.25 ± 5.50 B	68.42 ± 5.73	55.67 ± 5.22	46.50 ± 2.99	41.92 ± 4.63	49.33 ± 6.25	56.33 ± 7.86
Georgia-16HO	2.17 ± 0.49	2.25 ± 0.51	24.58 ± 4.05 AB	64.50 ± 6.31 AB	55.25 ± 6.27	42.42 ± 4.65	40.00 ± 6.02	29.83 ± 5.38	41.5 ± 5.57	74.50 ± 9.38
Tifguard	2.50 ± 0.44	3.08 ± 0.56	29.75 ± 4.19 A	62.42 ± 7.36 AB	62.92 ± 9.5	48.17 ± 7.78	37.17 ± 5.59	28.00 ± 3.34	43.42 ± 7.3	64.58 ± 13.33
Type III tests[z]										
F value (df)	0.53 (5, 583)	0.92 (5, 583)	18.43 (5, 583)	3.51 (5, 583)	1.65 (5, 583)	1.69 (5, 583)	0.84 (5, 583)	1.75 (5, 583)	0.48 (5, 583)	0.54 (5, 583)
P > *F*	0.7558	0.47	<0.0001*	0.0039*	0.145	0.1346	0.5237	0.1224	0.7916	0.7475

[x] Florunner and Georgia Green are TSWV susceptible cultivars and Georgia-06G, Georgia-12Y, Georgia-16HO, and Tifguard are TSWV resistant cultivars

[y] Total thrips number on peanut plants counted at 3~30 days post thrips release. Means with their standard errors in a column followed by the same letter are not significantly different from each other.

[z] F values with degrees of freedom in the parentheses; $p < 0.05$ notated with "*" indicated significant cultivar effect.

Table 5.8. Median developmental time of *Frankliniella fusca* to complete one generation (adult to adult) on leaflets of TSWV susceptible and resistant peanut cultivars

Cultivar[x]	N	Median developmental time (day) [y]
Florunner	1244	16 (12-25)
Georgia Green	1185	16 (12-23)
Georgia-06G	911	16 (12-25)
Georgia-12Y	637	17 (12-25)
Georgia-16HO	1156	16 (13-24)
Tifguard	923	17 (13-24)
Kruskal-Wallis test[z]		
χ^2		189.5975
$p > \chi^2$		< 0.0001*

[x] Florunner and Georgia Green are TSWV susceptible cultivars and Georgia-06G, Georgia-12Y, Georgia-16HO, and Tifguard are TSWV resistant cultivars.

[y] Ten female adult thrips were released on leaflets in each Munger cage and allowed to lay eggs for 72h then removed; cages were monitored at 24h intervals to record newly emerged adult thrips.

[z] Data were analyzed by Wilcoxon tests of NPAR1WAY procedure in SAS; significance of the test was determined by Kruskal-Wallis test, and $p < 0.05$ notated with "*" indicated significant cultivar effect.

Table 5.9. Mean and standard error of number of adult *Frankliniella fusca* produced and number of eggs oviposited per female released on leaflets of TSWV susceptible and resistant peanut cultivars

Cultivar[w]	N	Adult thrips produced (per thrips)[x]	Egg number (per thrips)[y]
Florunner	12	10.37 ± 0.89 A	14.67 ± 0.74
Georgia Green	12	9.88 ± 1.41 A	19.08 ± 0.99
Georgia-06G	12	7.59 ± 1.00 AB	17.27 ± 1.20
Georgia-12Y	12	5.31 ± 0.60 B	18.27 ± 1.27
Georgia-16HO	12	9.63 ± 1.27 A	15.10 ± 1.26
Tifguard	12	7.69 ± 0.95 AB	18.63 ± 1.17
Type III test[z]			
F (df)		5.10 (5, 55)	2.45 (5, 55)
P > *F*		0.0007*	0.0451*

[w] Florunner and Georgia Green are TSWV susceptible cultivars and Georgia-06G, Georgia-12Y, Georgia-16HO, and Tifguard are TSWV resistant cultivars

[x] Ten female adult thrips were released on leaflets in each Munger cage and allowed to lay eggs for 72h then removed; cages were monitored at 24h intervals to record newly emerged adult thrips. Mean thrips number followed by the same letter are not significantly different from each other.

[y] Five female adult thrips were released on leaflets in each petri dish cage and allowed to lay eggs for 72h than removed; eggs were stained and counted under a dissecting microscope.

[z] F values with degrees of freedom in the parentheses; $p < 0.05$ notated with "*" indicated significant cultivar effect.

Appendix A. Gene sequences of TSWV isolates collected from susceptible and resistant peanut cultivars with GenBank accession numbers

N		NSs		NSm		Gn/Gc		RdRp	
Isolate ID	GenBank accession	Isolate ID	GenBank accession	Isolate ID	GenBank accession	Isolate ID	GenBank accession	Isolate ID	GenBank accession
FloRun331 2018	MW519186	Tifguard 2017	MW519245	GeorgiaGreen 2018 A	MW519304	Georgia14N 2016	MW519362	Georgia04S 2016	MW519421
GeorgiaGreen 2019 A	MW519187	Florunner 2018 J	MW519246	FloRun331 2018 L	MW519305	Georgia12Y 2019	MW519363	Tifguard 2018 J	MW519422
Georgia12Y 2018 L	MW519188	Georgia04S 2016	MW519247	GeorgiaGreen 2016	MW519306	NMValenciaC 2019	MW519364	Florunner 2018 J	MW519423
AUNPL17 2017	MW519189	Georgia14N 2017	MW519248	Georgia13M 2018 J	MW519307	FloRun107 2016	MW519365	Florunner 2019	MW519424
Georgia13M 2016	MW519190	Georgia18RU 2019	MW519249	GeorgiaGreen 2018 J	MW519308	TUFRunner157 2017	MW519366	Georgia06G 2016	MW519425
Georgia16HO 2019	MW519191	GeorgiaGreen 2019 A	MW519250	Georgia06G 2018 A	MW519309	Bailey 2019	MW519367	GeorgiaGreen 2016	MW519426
Florunner 2018 J	MW519192	Georgia12Y 2019	MW519251	TUFRunner511 2018 L	MW519310	Gregory 2019	MW519368	TUFRunner297 2018 L	MW519427
Georgia16HO 2017	MW519193	Georgia16HO 2017	MW519252	NMValenciaC 2019	MW519311	Georgia13M 2017	MW519369	TUFRunner727 2017	MW519428
Tifguard 2017	MW519194	Georgia16HO 2019	MW519253	Georgia06G 2018 J	MW519312	Georgia06G 2019 A	MW519370	GeorgiaGreen 2018 A	MW519429
FloRun107 2017	MW519195	Georgia06G 2019	MW519254	Bailey 2019	MW519313	GeorgiaGreen 2016	MW519371	Georgia06G 2019 A	MW519430
Georgia06G 2019	MW519196	NMValenciaC 2019	MW519255	Gregory 2019	MW519314	GeorgiaGreen 2018 A	MW519372	Georgia11J 2017	MW519431
Georgia14N 2018 J	MW519197	Georgia14N 2018 J	MW519256	Georgia06G 2019 A	MW519315	TifNVHOL 2017	MW519373	Georgia16HO 2018 P	MW519432
TUFRunner297 2017	MW519198	TUFRunner297 2017	MW519257	Georgia14N 2016	MW519316	Georgia16HO 2018 A	MW519374	Tifguard 2016	MW519433
FloRun107 2016	MW519199	Georgia12Y 2018 L	MW519258	AUNPL17 2017	MW519317	Georgia06G 2018 J	MW519375	TUFRunner511 2018 P	MW519434
Georgia14N 2017	MW519200	TUFRunner511 2018 L	MW519259	Georgia04S 2016	MW519318	TUFRunner511 2018 L	MW519376	FloRun107 2017	MW519435
Georgia18RU 2019	MW519201	Georgia06G 2019 A	MW519260	AUNPL17 2018 J	MW519319	Georgia11J 2019 A	MW519377	GeorgiaGreen 2019	MW519436
Georgia04S 2016	MW519202	Georgia13M 2016	MW519261	TifNVHOL 2019	MW519320	Georgia13M 2018 J	MW519378	TUFRunner297 2019	MW519437
Georgia06G 2017	MW519203	TUFRunner511 2017	MW519262	TUFRunner511 2018 A	MW519321	GeorgiaGreen 2018 J	MW519379	Georgia14N 2016	MW519438
Georgia06G 2016	MW519204	FloRun107 2016	MW519263	GeorgiaGreen 2019	MW519322	Georgia06G 2017	MW519380	Georgia16HO 2018 A	MW519439
AUNPL17 2018 J	MW519205	TUFRunner511 2018 A	MW519264	GeorgiaGreen 2017	MW519323	Georgia14N 2019	MW519381	Georgia14N 2017	MW519440
TUFRunner331 2019	MW519206	AUNPL17 2017	MW519265	Georgia13M 2016	MW519324	TUFRunner727 2017	MW519382	TifNVHOL 2018 J	MW519441
Gregory 2019	MW519207	GeorgiaGreen 2016	MW519266	TUFRunner297 2019	MW519325	FloRun331 2018 L	MW519383	FloRun331 2018 L	MW519442
Georgia11J 2019 A	MW519208	Georgia16HO 2018 P	MW519267	Georgia16HO 2018 P	MW519326	Georgia06G 2018 A	MW519384	Georgia12Y 2018 L	MW519443
Bailey 2019	MW519209	FloRun331 2018 L	MW519268	TifNVHOL 2018 J	MW519327	Georgia04S 2016	MW519385	TUFRunner511 2018 L	MW519444
Georgia11J 2017	MW519210	AUNPL17 2019	MW519269	FloRun107 2017	MW519328	Georgia18RU 2019	MW519386	Georgia06G 2019	MW519445
TifNVHOL 2019	MW519211	Tifguard 2019	MW519270	TUFRunner297 2017	MW519329	Georgia06G 2019	MW519387	AUNPL17 2017	MW519446
Tifguard 2016	MW519212	Georgia06G 2018 A	MW519271	Georgia13M 2017	MW519330	TUFRunner511 2018 A	MW519388	TUFRunner297 2017	MW519447
Georgia13M 2018 J	MW519213	Georgia06G 2018 J	MW519272	TUFRunner511 2017	MW519331	Georgia06G 2016	MW519389	FloRun331 2017	MW519448
GeorgiaGreen 2018 J	MW519214	Georgia14N 2019	MW519273	FloRun331 2019	MW519332	TifNVHOL 2019	MW519390	FloRun107 2016	MW519449
Tifguard 2018 J	MW519215	Georgia11J 2019 A	MW519274	AUNPL17 2019	MW519333	AUNPL17 2018 J	MW519391	Georgia12Y 2019	MW519450
TUFRunner727 2016	MW519216	Georgia11J 2017	MW519275	Tifguard 2019	MW519334	Florunner 2019	MW519392	Georgia18RU 2019	MW519451
Georgia06G 2018 A	MW519217	Tifguard 2016	MW519276	Florunner 2018 J	MW519335	Georgia14N 2018 J	MW519393	TifNVHOL 2017	MW519452
TUFRunner297 2018 L	MW519218	Florunner 2019	MW519277	TUFRunner511 2018 P	MW519336	Georgia16HO 2018 P	MW519394	TUFRunner727 2016	MW519453
AUNPL17 2019	MW519219	GeorgiaGreen 2017	MW519278	Georgia14N 2018 J	MW519337	TUFRunner297 2017	MW519395	Georgia16HO 2017	MW519454
Tifguard 2019	MW519220	TUFRunner297 2019	MW519279	TUFRunner297 2018 L	MW519338	FloRun107 2017	MW519396	TUFRunner157 2017	MW519455
TUFRunner511 2018 P	MW519221	TifNVHOL 2018 J	MW519280	GeorgiaGreen 2019 A	MW519339	TUFRunner511 2018 P	MW519397	Georgia06G 2018 J	MW519456
Florunner 2019	MW519222	GeorgiaGreen 2019	MW519281	Georgia16HO 2019	MW519340	AUNPL17 2019	MW519398	Georgia16HO 2019	MW519457
Georgia14N 2016	MW519223	Georgia13M 2017	MW519282	Tifguard 2016	MW519341	Tifguard 2019	MW519399	NMValenciaC 2019	MW519458
Georgia14N 2019	MW519224	TUFRunner157 2017	MW519283	Florunner 2019	MW519342	Georgia13M 2016	MW519400	FloRun331 2019	MW519459
GeorgiaGreen 2017	MW519225	Bailey 2019	MW519284	Georgia18RU 2019	MW519343	TifNVHOL 2018 J	MW519401	TUFRunner511 2017	MW519460
Georgia16HO 2018 A	MW519226	Gregory 2019	MW519285	Georgia06G 2016	MW519344	TUFRunner297 2019	MW519402	Georgia13M 2018 J	MW519461
GeorgiaGreen 2018 A	MW519227	TifNVHOL 2019	MW519286	TUFRunner727 2016	MW519345	GeorgiaGreen 2019	MW519403	GeorgiaGreen 2018 J	MW519462
TifNVHOL 2017	MW519228	Georgia16HO 2018 A	MW519287	Tifguard 2017	MW519346	GeorgiaGreen 2017	MW519404	AUNPL17 2019	MW519463
Georgia06G 2018 J	MW519229	Tifguard 2018 J	MW519288	Georgia16HO 2017	MW519347	AUNPL17 2017	MW519405	Tifguard 2019	MW519464
Georgia13M 2017	MW519230	TUFRunner297 2018 L	MW519289	FloRun331 2017	MW519348	TUFRunner727 2016	MW519406	Georgia14N 2018 J	MW519465
TUFRunner157 2017	MW519231	TifNVHOL 2017	MW519290	Georgia12Y 2018 L	MW519349	Tifguard 2017	MW519407	Georgia14N 2019	MW519466
TifNVHOL 2018 J	MW519232	Georgia06G 2016	MW519291	Tifguard 2018 J	MW519350	Georgia16HO 2017	MW519408	GeorgiaGreen 2019 A	MW519467
GeorgiaGreen 2019	MW519233	FloRun331 2017	MW519292	Georgia06G 2019	MW519351	FloRun331 2017	MW519409	TifNVHOL 2019	MW519468
TUFRunner297 2019	MW519234	TUFRunner727 2016	MW519293	Georgia11J 2017	MW519352	Georgia11J 2017	MW519410	GeorgiaGreen 2017	MW519469
GeorgiaGreen 2016	MW519235	Georgia14N 2016	MW519294	Georgia14N 2017	MW519353	Georgia14N 2017	MW519411	Tifguard 2017	MW519470
TUFRunner331 2017	MW519236	Georgia06G 2017	MW519295	FloRun107 2016	MW519354	Georgia12Y 2018 L	MW519412	Georgia06G 2017	MW519471
TUFRunner727 2017	MW519237	FloRun331 2019	MW519296	Georgia06G 2017	MW519355	Tifguard 2018 J	MW519413	Georgia13M 2017	MW519472
Georgia06G 2019 A	MW519238	TUFRunner511 2018 P	MW519297	TifNVHOL 2017	MW519356	Georgia16HO 2019	MW519414		
Georgia16HO 2018 P	MW519239	AUNPL17 2018 J	MW519298	Georgia11J 2019 A	MW519357	Tifguard 2016	MW519415		
NMValenciaC 2019	MW519240	Georgia13M 2018 J	MW519299	TUFRunner727 2017	MW519358	TUFRunner297 2018 L	MW519416		
TUFRunner511 2018 L	MW519241	GeorgiaGreen 2018 J	MW519300	Georgia16HO 2018 A	MW519359	GeorgiaGreen 2019 A	MW519417		
Georgia12Y 2019	MW519242	GeorgiaGreen 2018 A	MW519301	Georgia14N 2019	MW519360	FloRun331 2019	MW519418		
TUFRunner511 2018 A	MW519243	TUFRunner727 2017	MW519302	TUFRunner157 2017	MW519361	Florunner 2018 J	MW519419		
TUFRunner511 2017	MW519244	FloRun107 2017	MW519303			TUFRunner511 2017	MW519420		