Evaluation of Management Methods for Thrips and Tomato Spotted Wilt Virus in Alabama Peanuts

by

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Abstract

Tomato spotted wilt virus (TSWV) is a plant disease with a broad host range, infecting several economically important crops such as tomatoes, peppers, and peanuts, and maintains its presence in the environment through several weedy hosts. This virus is transmitted via select species of thrips, which acquire TSWV by feeding on infected plants as larvae and then spread it to other plants as adults. While thrips feeding alone can be highly damaging to seedling-stage plants, the addition of TSWV causes necrosis, leaf cupping, and stunting throughout the growing season, even after thrips have left the plant. In peanuts, TSWV can cause yield losses of 40-60% in both moderately field resistant and susceptible varieties. Thus, thrips management is essential to control TSWV. Some potential control variables include selecting varieties with field resistance to TSWV, planting date selection, insecticide applications, tillage practices, and herbicide applications. This project aims to assess these variables to determine their impact on thrips and TSWV management and yield. Additionally, thrips were collected from two hostplants (peanuts and white clover) and assessed using gel electrophoresis to determine the presence or absence of TSWV, depending on the hostplant and year they were collected from. This information will aid in formulating integrated management systems for TSWV and in developing a deeper understanding of the ecology of TSWV in southern Alabama.

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List of Abbreviations

AU-NPL 17	Auburn University-National Peanut Lab peanut variety (2017)
DAP	Days After Planting
FGCC	Fractional Green Canopy Closure
GA-06G	Georgia peanut variety (2006)
GA-12Y	Georgia peanut variety (2012)
ORF	Open Reading Frame
PCR	Polymerase Chain Reaction
REC	Research & Education Center
TSWV	Tomato Spotted Wilt Virus

Chapter 1: Introduction and Review of Literature

Introduction and Problem Statement

Various factors influence the management of thrips and their transmission of TSWV, a highly damaging plant virus that can cause devastating yield loss in peanuts. These factors include the use of at-plant insecticides, timing of planting, selection of suitable varieties, planting in cover crop residue, and utilization of herbicide applications. At-plant insecticides play a crucial role in controlling thrips populations and potentially enhancing plant defenses against thrips, optimal planting dates can mitigate thrips populations during plant emergence by avoiding peak migratory flight periods, utilization of TSWV field resistant peanut varieties, and cover crops offer a means to deter thrips and promote soil health. Other management options, such as herbicide applications, could have adverse effects on the overall health of peanut plants. While research has examined these management factors, thrips populations, weather conditions, varieties, and pesticide resistance continue to evolve. Therefore, ongoing research is vital to identify the most effective methods for TSWV control and understand the interactions of different management practices. Additionally, given the broad host range of thrips and TSWV, testing for TSWV presence or absence in thrips populations both within research plots and the surrounding environment, can provide insight into virus ecology.

Arachis hypogaea, also known as the peanut (or “goober”, derived from the Congo name “nguba”), was brought to North America by African slaves during the 1700s (Catalano 2022). At this time, peanuts were regarded as a lower-class food due to how they were eaten. Having to crack and discard pods to consume the nut was considered barbaric to upper-class individuals (Dixon 2009). During the Civil War, peanuts grew in popularity as they became valued by troops due to their nourishment and high caloric value. Peanuts were consumed raw, roasted, boiled, and even used in pies and peanut coffee (Dixon 2009). In 1895, John Harvey Kellogg invented peanut butter to serve as a protein source for elderly people, who had trouble chewing, before it made its public debut in 1904 (Dixon 2009; Catalano 2022). By this time, the public abandoned the association of peanuts with the lower class and made them a new staple for American diets (Dixon 2009).

Peanuts thrive in the warm, humid climate of the southeastern United States – especially Georgia, Florida, and Alabama (Dixon 2009). Unfortunately, Alabama farmers initially struggled to find value in growing peanuts, as bank loans were only provided for farmers growing cotton during the 1800s (Dixon 2009). This changed, however, after the introduction of the boll weevil (*Anthonomus grandis*) and its the century-long destruction of cotton fields from the 1890s until its eradication in 1994. The cost of cotton production and yield losses from the boll weevil pressured many farmers into seeking alternative options for crops to replace cotton (Wrenshall 1949). The timing of the peanut's integration into American diets was nearly perfect, and banks agreed to help finance the switch. In 2023, Alabama grew approximately 175,000 acres of peanuts, producing 480.5 million pounds in yield and generating over 119 million dollars per year (U.S. National Agricultural Statistics Service NASS 2023). Alabama also hosts the National Peanut Festival in Dothan (considered the "Peanut Capital of the World") that spans ten days and draws an average of 200,000 visitors (Dixon 2009). Runner peanuts make up 80% of peanut production and they are primarily used for roasted peanuts and peanut butter (National Peanut Board 2023). Virginia peanuts create larger nuts and are used for snacking peanuts and gourmet peanut butters (National Peanut Board 2023). These account for 15% of production and are primarily grown in Virginia, North Carolina, and South Carolina (National Peanut Board 2023). The remaining common peanut types, Spanish and Valencia, account for the remaining 5% of production (National Peanut Board

2023). Nationally, peanut production was valued at $1.5 billion in the year 2022, comparable to crops such as tobacco ($1 billion), oranges ($1.5 billion), and tomatoes ($1.8 billion) (USDA Economic Research Service 2024). Although peanuts are immune to the boll weevil, there are several other threats to their production.

Pests of peanuts include animals, nematodes, fungi, weeds, and insects. These pests can cause yield loss by foliar damage, root damage, peanut consumption, sunlight or soil nutrient deprivation, peanut quality decrease, and virus transmission. Grazing animals, like deer, have become an emerging issue as they reduce plant stand and yield by eating seedlings and new growth (Ober and Kane 2018). Later in the season, field mice and birds may feed on peanuts that have been dug up and left to dry before harvest or in grain storage (Besser 1986). Nematodes, including root-knot, sting, and ring nematodes, can infest the roots of peanut plants and decrease yield quality and quantity (Luc et al. 2005). Fungal diseases can decrease yield by causing defoliation, especially during wet years or in fields where peanuts are grown consecutively, while other fungi such as *Penicillium* spp. and *Aspergillus* spp. can produce mycotoxins, decreasing the quality of peanut harvest and potentially making it completely unmarketable (Zorzete et al. 2011). Weeds also pose a threat by competing with peanuts for soil nutrients. Additionally, because peanuts are so low growing, they are also vulnerable to tall or broad-leafed weeds shading them out of sunlight (Jat et al. 2011). Several arthropod species, such as spider mites, rootworms, burrower bugs, and thrips can feed on the foliage and/or pods. Thrips, however, not only cause feeding damage, but can also transmit TSWV (Strayer-Scherer and Graham 2021; Hollis 2023).

Thrips

Tobacco thrips (*Frankliniela fusca*) are the predominant species of thrips that infest row crops in Alabama (Strayer-Scherer and Graham 2021). Tobacco thrips are a generalist insect that feed on a variety of host plants, including cash crops such as tobacco, peanuts, tomato, and onion, as well as weedy plants such as chickweed, clover, and rye (Salguero Navas et al. 1991; Todd et al. 1995; Sparks et al. 2010; Cook et al. 2011). Thrips are well known as an early-season pest of seedling cotton, where they cause severe damage, stunting and stand loss (Cook et al. 2011). Although thrips are small, one to two millimeters in length, they congregate in large numbers on host plants, where females produce up to ten eggs per day during their ~30 day adult lifespan. Total development time depends on temperature and other abiotic factors, but a range of 20-35°C is required to progress to each life stage (Lowry et al. 1992; Shrestha et al. 2012; Huseth et al. 2017; Riley and Sparks 2022). The progression from egg to adult typically takes 11.5 to 23.9 days, with development time decreasing as temperature rises from 20-35°C (Lowry et al. 1992). Eggs are usually embedded in the plant tissues of emerging leaves, where they hatch after 3 to 5 days (Skarlinsky and Funderburk 2016; LaTora et al. 2021). Newly hatched larvae are white bodied with enlarged red eyes, and a head that is larger compared to the thorax than in later life stages (LaTora et al. 2021). These first instar larvae feed for two days before molting into the second instar larvae, which are more yellow in color and have seven pairs of dorsal setae on the pronotum, as well as a dark band extending from the marginal teeth to the campaniform sensilla on the ninth tergite (Skarlinksy and Funderburk 2016). After three to five days, larvae enter a three-day inactive prepupal stage – in which no feeding occurs – before entering the final pupal stage (LaTora et al. 2021). Tobacco thrips feed for three days during this last pupal stage, followed by their final molt into adults (Strayer-Scherer and Graham 2021). Female tobacco thrips can be differentiated from males by their dark colored and elongated bodies (1.2-1.8mm long) (Nakao et al. 2011; Strayer-Scherer and Graham 2021; Riley and Sparks 2022). Males are similar in appearance but are shorter in length (1.0-1.3 mm long) and yellow to light brown (Nakao et al. 2011; Strayer-Scherer and

Graham 2021). Immature thrips lack wings but have wing pads that are seen in the prepupal and pupal stages (Kucharczyk and Kucharczyk 2013; Riley and Sparks 2022).

Most thrips feeding occurs during the larval stages, and is concentrated on soft tissues such as buds, blooms, and new growth at the terminal which is tender and easier for them to break through with their rasping-sucking mouthparts (Todd et al. 1995). Thrips feeding can be recognized by silvery stippling/flecking injury on the leaf surface, wrinkled leaves, and black flecks of frass (Jordan et al. 2006; Srinivasan et al. 2018; Riley and Sparks 2022). Though rare in the southeastern USA, excessive thrips feeding early in the season can result in stunting, delayed maturity, yield loss, and death in peanuts (Todd et al. 1995; Drake et al. 2009). Unlike in other crops, like cotton, yield loss in peanuts is not usually directly attributed to injury from feeding injury or stand reduction, since peanuts are hardy plants that can outgrow injury from most thrips infestations (Kichler 2022). Instead, yield loss is largely caused by Tomato spotted wilt virus (TSWV) (Strayer-Scherer and Graham 2021).

Tomato Spotted Wilt Virus

Tomato spotted wilt virus, the causal agent of spotted wilt, is a species of *Orthotospovirus* in the family *Bunyaviridae* (Riley and Sparks 2022). The virus was first reported in peanuts in 1974, with field-wide epidemics soon following in 1985 before reaching Alabama in 1988 (Halliwel and Philley 1974; Black et al. 1986; Gudauskas et al. 1988). It is transmitted exclusively by thrips in a persistent and propagative manner, and is most commonly transmitted by species in the genus *Frankliniella*, such as *F. fusca*, *F. occidentalis*, and *F. bispinosa*, but TSWV may also be transmitted by *Thrips setosus* and *T. tabacci* (Sakimura 1963; Wijkamp et al. 1993; de Borbón et al. 1999; Tsuda et al. 1996; Nagata et al. 2004; Avila et al. 2006; Ohnishi et al. 2006; Rotenberg et al. 2015). In the southeastern USA, *F. fusca* and *F.*

occidentalis are the most common and efficient vectors, with *F. fusca* being the most economically impactful because it infests and infects early in the season, while *F. occidentalis* primarily infests flowers later in the season (Todd et al. 1995; McPherson et al. 1999; Groves et al. 2001; Chaisuekul et al. 2003; Joost and Riley 2004; Riley et al. 2012). Though the virus is transmitted by thrips in the adult stage, it can only be acquired by during the first or second instar (Wijkamp and Peters 1993).

Like thrips, TSWV has a broad host range and can be found in peppers, squash, tomatoes, and other economically important plants. Additionally, many common weed species host this virus, such as pigweed, morning glory, and lambsquarters (Groves et al. 2001; Srinivasan et al. 2014). Because weeds create a year-round reservoir for TSWV, the virus is difficult to manage by crop rotations, an otherwise viable method to control for many other pests and diseases. Additionally, there is potential for coinfection of TSWV with other viruses, especially peanut mottle (PMV), but also peanut stripe (PStV) and peanut stunt (PSV) (Gudauskas et al. 1993). In peanut fields, the first occurrences of TSWV show at random, but eventually develop into larger clusters of infected plants that continue to spread down the row (Strayer-Scherer and Graham 2021). Symptoms of TSWV in peanuts can begin showing as soon as 21 days after emergence and include stunted growth, pod malformation, chlorotic rings on the leaves, reddening of seed coats and reduced yield (Culbreath et al. 2003; Sundaraj et al. 2014; Strayer-Scherer and Graham 2021). New growth may be smaller, wilted or crinkled in appearance, and will have upright rather than lateral growth direction (Strayer-Scherer and Graham 2021). Vine collapse and general yellowing of the foliage can develop later in the season, leading to a reduction in vigor and even plant death (Culbreath et al. 2003; Sundaraj et al. 2014). By the time symptoms are observed, however, it is too late for treatment options (Cabrera 2020). Management of TSWV is entirely preventative, with the most effective methods based on either selecting peanut varieties that are high-yielding or field resistant to TSWV, or by avoiding thrips feeding by use of insecticides and choosing planting dates outside of peak thrips migration times (Cabrera 2020). Up-to-date information on field resistant varieties and management methods can be found on the Peanut Rx Guide. The Peanut Rx Guide was released in 2005 as a combination of the Spotted Wilt Index and the Peanut Fungal Disease Risk Index, and allows growers and researchers to calculate the risk index of their crop to not only TSWV, but also nematodes and a variety of fungal diseases. Peanut Rx is updated continuously thanks to efforts from the University of Georgia, the University of Florida, Clemson University, Mississippi State University, and Auburn University. With these research efforts and implementation of these management methods, average peanut yields almost doubled within a 20 year timespan, increasing from <2800 kg/ha in 1995 to ~5000 kg/ha in 2015 (Srinivasan et al. 2017).

Insecticides

There are several insecticides commercially available for thrips management, such as aldicarb, acephate, spinetoram, imidacloprid, and phorate. Among these, imidacloprid is most used by farmers due to its economic value, but it could have negative effects in preventing tomato spotted wilt (Kichler 2022). Studies have shown that imidacloprid can increase thrips feeding and probing incidences, and it is even labeled as being a risk for increasing transmission of TSWV (Joost and Riley 2007). Phorate, however, is thought to induce plant defense responses, reducing the severity of TSWV infection (Cabrera 2020). Motta et al. (1998) found that peanuts treated with phorate applied at 5 lb/acre reduced both the incidence and severity of TSWV compared to non-treated peanuts.

Planting Date

Thrips are a migratory species with relatively consistent annual migratory patterns; thus, planting date can aid in "dodging" most thrips damage. Tobacco thrips typically have a peak migration time at around late April (Brown et al. 2005) while flower thrips peak at around the first week of June in Alabama (Brown et al. 2005; Frank et al. 2020). Todd et al. (1995) reported that 52 experiments performed in Georgia over a six-year period showed that planting in May or June rather than in April resulted in lower tobacco thrips populations in peanuts. Today, the use of planting dates is used for TSWV risk mitigation as part of the Peanut Rx guide (Peanut RX, UGA). Peanut Rx provides up-to-date information to aid farmers in choosing the best options to reduce the risk of TSWV and other diseases in their fields. For planting date, Peanut Rx currently recommends planting between May 11th and May 31st for the best TSWV risk mitigation (PeanutRx 2021).

Temperature, humidity, and other weather conditions can cause variation in thrips migrations year-by-year, with warm winters and springs speeding up their lifecycle, which could result in an earlier infestation (North Carolina State University 2021). Thus, monitoring weather patterns is important to predict thrips flights and evaluate planting dates to ensure farmers are receiving up-to-date advice on the risk of TSWV incidence in given planting windows.

Varieties

Due to the economic impacts of TSWV, considerable research has gone into breeding varieties with field resistance to the virus. Currently, Georgia-06G, released in 2006, is the most widely used runner-type cultivar in the USA (Monfort 2020), which is regarded for its field resistance to TSWV, large seed size, and high yield potential. Georgia-12Y, released in 2012, is another popular variety that is also high yielding. Although highly field resistant to TSWV, GA-12Y has a less valuable, medium-sized seed, and is a late-maturity variety, making it less ideal for later planting dates (after May 15th).

Other commercially available varieties include Georgia-09B, Georgia-13M, Georgia-14N, TUFRunner™ 511, and TUFRunner™ 297, all of which are considered high-oleic varieties (Monfort 2020). High-oleic varieties have a longer shelf life due to their greater production of oleic acid. Oleic acid increases shelf life by decreasing oxidization (which causes rancidity) and allows peanuts to maintain their crunch and flavor longer than other varieties (Peanut Company of Australia 2016). These high-oleic peanuts come with the risk of being more susceptible to leaf spot diseases and TSWV. Additionally, these varieties have smaller seed sizes and limited availability depending on the variety. However, some farmers still seek them out due to the increased sale value of high-oleic peanuts (Monfort 2020).

AU-NPL-17 is a newly released variety developed by Auburn University's peanut breeding program, which functions in joint with the College of Agriculture's Department of Crop, Soil, and Environmental Sciences and the USDA's National Peanut Research Lab in Dawson, Georgia (Hollis 2023). This is a high-yielding variety with a medium maturity bred for growing conditions in the southeastern US, making it ideal for planting outside of thrips migratory windows. Future releases of this variety are planned, with traits that would increase yield and disease resistance (Hollis 2023).

Breeding peanuts with TSWV field resistance benefits farmers because, as field resistance increases, farmers can plant earlier in the planting window. Eventually, farmers may be able to begin planting in better conditions around mid-April, which would maximize their yield without with less concern of TSWV outbreaks (Nuti et al. 2014).

Cover Crops

Cover crops are grown temporarily between cash crops and are terminated or "burned down" prior to the cash crop emergence using one of several different methods, such as herbicide applications, mowing, or rolling. Cover crops offer several benefits to cash crops, such as creating ground residue to control weeds, improving soil structure and moisture, and increasing soil nutrients (Balkcom et al. 2007). Weed control may be provided by chemical suppression (allelopathy) or by preventing emergence due to the residue covering the ground. The roots of cover crops improve the soil's ability to soak in water, while the residue above the soil makes it cooler than soils without cover crop residue, thus reducing evaporation (Munawar et al. 1990; Bauer and Busscher 1993; Marois and Wright 2003; Williams and Weil 2004; Coppens et al. 2006; Rowland et al. 2006; Simoes et al. 2009; Wright et al. 2009). Cover crop roots and residue also help reduce soil erosion and chemical runoff, maintaining soil quality and preventing chemicals from leaching into groundwater or unwanted areas (Doran and Smith 1991; Sainju et al. 1997; Dabney et al. 2001; Truman and Williams 2001; Tubbs 2003; Coppens et al. 2006).

The type or mixture of cover crops that are planted also plays a vital role in pest control, as grasses and brassicas reduce nitrogen leaching in legume, soaking it up in their biomatter so it doesn't run off into groundwater (Balkcom et al. 2007; Sánchez and White 2022). Following burn down, the nitrogen from cover crop biomatter can then be utilized by the cash crop (Sánchez and White 2022). Additionally, while cover crops do harbor many insects and diseases, these interactions can be investigated so a cover crop species that does not share the crop's pest can be selected (Balkcom et al. 2007). Cover crop residues have also been found to reduce disease incidence of TSWV, as well as other diseases such as leaf spot and white mold (Balkcom et al. 2007; Wright et al. 2009). Higher residues result in less damage from thrips, correlating with lower incidence of TSWV (Balkcom et al. 2007). The use of cover crops can have varying results. In a study performed by Campbell et al. (2001) at the Wiregrass research station in Headland, AL, peanuts were planted in sandy loam soil following cotton. A variety of forage systems were planted as winter cover crops, including wheat, rye, oats, ryegrass, wheat/ryegrass, rye/ryegrass, oats/ryegrass, and fallow (no cover crop). The peanuts were planted following herbicidal burn down and ratings were taken for insects, TSWV severity, and yield (Campbell et al. 2001). The results showed that insect and TSWV ratings showed no significant differences, though TSWV ratings did have some broad ranges. (Campbell et al. 2001). Ryegrass had the lowest TSWV severity at an average rating of 2.5, wheat with the highest severity at 6.5, and fallow falling in third highest at 4.3 (Campbell et al. 2001). Cover crops significantly impact yield, with rye having the highest yield at 5687 lbs per acre and fallow the lowest at 4943 lbs per acre (Campbell et al. 2001).

Herbicides

Several herbicides are used on peanut crops to manage weeds, including pre-emergence herbicides such as pendimethalin, s-metolachlor, flumioxazin, and dimethenamid, as well as post-emergence herbicides, such as imazapic, acifluorfen, clethodim, and bentazon (Kimura et al. 2004). The herbicide, or herbicide mixture, used is largely dependent on local regulations and on what weed species are present. Some herbicides, such as paraquat are broad-spectrum and work on a variety of weed species, while others may be more specialized for grasses or broadleaf weeds (Kimura et al. 2004).

The effects of herbicides on TSWV incidence in peanuts is not fully understood and there is limited research on the topic. In a 2002 study done across five locations in Georgia and Florida, some fields had a significant increase in TSWV incidence, as well as lower yield, when the herbicide chlorimuron was applied at 46, 63, and 80 days after emergence (Prostko et al. 2002). Other locations showed an increase in TSWV, but no decrease in yield with chlorimuron applications. Additional herbicides that were assessed, which included imazapic, bentazon, sulfentrazone, diclosulam, flumioxazin, axifluoren, and paraquat, did not show any effect in increasing or decreasing TSWV incidence.

Other Management Options

Other methods of TSWV management include plant density, row patterns, avoid planting in low temperatures, soil moisture, and tillage (Strayer-Scherer and Graham 2021). Higher plant density can help dilute thrips populations, as opposed to lower densities that cause more thrips to congregate on the fewer available plants – increasing the risk of TSWV transmission (Tillman et al. 2006; Strayer-Scherer and Graham 2021). Planting peanuts in twin rows rather than single rows is beneficial for the same plant density reason (Tillman et al. 2006). Growing conditions that are optimal for peanuts have been found to be at lower risk to TSWV, so low temperatures and overly wet soils should be avoided (Strayer-Scherer and Graham 2021).

TSWV Presence in Thrips Populations

Many species of thrips are capable of transmitting TSWV. Three of the four thrips species found in Alabama peanuts are known vectors of this disease: tobacco thrips, western flower thrips (*F. occidentalis*), and (possibly) *F. bispinosa* (Salguero Navas et al. 1991; Webb et al. 1997; Riley and Pappu 2000). Among these, tobacco thrips are the most abundant, though western flower thrips can persist in higher numbers later in the season, mostly feeding on blooms (Todd et al. 1995; McPherson et al. 1999; Groves et al. 2001; Chaisuekul et al. 2003; Joost and Riley 2004; Riley et al. 2012). Tobacco thrips numbers are usually highest two to four weeks after peanuts emerge, but populations quickly decline approximately six weeks post-emergence (Riley and Sparks 2022). The first pupal stage of larvae is the most important for acquiring the virus, while the more mobile adult stage is the most efficient for transmitting it (Strayer-Scherer and Graham 2021).

Both thrips and TSWV demonstrate an exceptionally wide host range, with their impact spanning across not only various cash crops but also extending to weeds (Groves et al., 2001; Srinivasan et al., 2014). Weeds play a crucial role as reservoirs for both thrips and TSWV, providing environments that sustain their populations in the vicinity of agricultural fields. Consequently, managing these pests becomes challenging as they persist despite conventional methods such as tillage or crop rotations. Notably, Batuman et al. (2020) highlighted that while the prevalence of TSWV in most weed species remains relatively low, certain species, such as rough-seeded buttercup (*Ranunculus muricatus*), exhibit strikingly high infection rates, exceeding 85%. Additionally, bridge crops like radicchio have been identified as unexpected reservoirs for TSWV, further complicating efforts to control its spread (Batuman et al., 2020).

Considering the persistence of thrips and TSWV in the environment, monitoring local thrips populations is a valuable strategy to gauge the prevalence of the virus within a specific area or host plant. Timing can also be a variable of TSWV incidence in thrips populations. Batuman et al. (2020) conducted biweekly sampling of thrips between March and July spanning the years 2008-2011 and found differences in TSWV presence depending on the time sampled. Their study revealed that thrips populations sampled during the early stages of the season (March through May), predominantly tested negative for TSWV, with only sporadic occurrences (1 positive per 20 samples each year). In contrast, thrips populations sampled in the later months of June and July exhibited higher incidences of TSWV, with a frequency of one positive per eight samples. This trend suggests a gradual accumulation of TSWV within thrips populations over the course of the growing season, suggesting TSWV may build within populations as the growing season progresses. Because of these temporal differences, timing the sampling to ensure it matches the emergence of the research crop is ideal in ensuring the TSWV incidence results align with the growing season.

Summary

There are a variety of methods that can be used to help manage TSWV in peanuts, such as at-plant insecticides, planting date, varietal selection, cover crops, and herbicide usage. Insecticides control thrips populations and could increase plant immune responses against thrips, choosing the right planting date can reduce thrips populations at plant emergence by avoiding migratory flight windows, planting TSWV field resistant varieties, utilizing cover crops can deter thrips and increase soil health for the plants, and careful herbicide application is important to ensure peanut plants aren't being damaged.

While these effects have been studied, thrips populations, weather patterns, varieties, and pesticide resistances are constantly evolving. Thus, it is important to continue researching and documenting what methods are the most effective in TSWV control, as well as how these different management methods interact with each other. In addition, monitoring TSWV incidence in thrips populations within and nearby the research field can provide information on virus ecology.

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Chapter 2: Evaluating the efficacy of peanut variety, planting date, and insecticide use as methods to manage TSWV in peanuts

ABSTRACT

Peanuts grown in Alabama are at risk of thrips injury and tomato spotted wilt virus (TSWV) infection, which could result in reduced yield and seed quality at harvest. Feeding from thrips can cause severe seedling injury and potential stand loss, but the primary concern is transmission of tomato spotted wilt virus. To control this disease, management practices such as planting date, varietal selection, and insecticide use are utilized. The objective of this experiment was to evaluate how three planting dates of varying thrips infestation risk, three varieties of varying susceptibility to TSWV, and the use of Thimet insecticide impacts thrips injury, TSWV incidence, and yield. A field study was done at the Wiregrass REC in Headland, AL in 2022 and 2023. Treatments included three varieties (GA-06G, GA-12Y and AU-NPL 17) planted at high (April), low (May) and mid (June) TSWV risk planting dates with and without Thimet in-furrow insecticide. Overall, planting date was the driving factor for thrips injury, TSWV incidence and yield. Peanuts planted in April tended to have higher injury and TSWV with lower yields. While it did not significantly impact TSWV incidence, peanuts treated with Thimet had significantly lower injury ratings and higher yields than those not treated, when averaged across planting dates and varieties. Based on these data, peanut growers in Alabama should focus on avoiding April planting dates to reduce their risk of TSWV infection and maintain yield potential.

INTRODUCTION

Peanuts thrive in the warm, humid climate of the southeastern United States, particularly in states like Georgia, Florida, and Alabama and are a valuable cash crop with a variety of uses, including human consumption, animal feed, and oils (Wrenshall 1949; Dixon 2009; National

Peanut Board 2023). Different peanut types, such as runner, Virginia, Spanish, and Valencia, contribute to the diverse peanut market. In Alabama, runner peanuts make up the majority of production acreage and are primarily roasted or made into peanut butter (National Peanut Board 2023). Approximately 175,000 acres of peanuts are grown in Alabama, producing 480.5 million pounds in yield with a value of 119 million dollars per year (U.S. National Agricultural Statistics Service NASS 2023).

Peanuts are susceptible to thrips feeding and subsequent injury during the seedling stage (Strayer-Scherer and Graham 2021; Hollis 2023). In addition to foliar injury and potential stand loss, thrips can also transmit Tomato spotted wilt virus (TSWV) (Hollis 2023). This virus causes a wide range of symptoms, including stunted growth, pod malformation, chlorotic rings on the leaves, reddening of seed coats, and reduced yield (Culbreath et al. 2003; Sundaraj et al. 2014; Strayer-Scherer and Graham 2021). TSWV is exclusively transmitted to peanuts by thrips, which acquire the virus in the larval stage by feeding on an infected plant. Infected thrips can then transmit TSWV in the adult stage to a non-infected plant upon dispersal (Wijkamp and Peters 1993). TSWV has an extremely broad host range, extending to over 900 species of weeds, cash crops, and ornamentals, and is difficult to control with conventional methods, like crop rotation or tillage (Business Queensland 2023). Once plants are infected with TSWV, it is not possible to cure (Groves et al. 2001; Srinivasan et al. 2014; Cabrera 2020). Thus, management methods for TSWV are entirely based on prevention, either by selecting field resistant varieties that are less susceptible to TSWV, selecting planting dates outside of expected thrips flight windows, or by using insecticides targeted to reduce thrips populations (Cabrera 2020).

To mitigate the economic impact of TSWV, breeding efforts have focused on developing field resistant varieties. Currently, GA-06G and GA-12Y are popular TSWV field resistant varieties across the southeastern US, with GA-06G being widely used for its large seeds and high yield (Monfort 2020). The recently released AU-NPL 17 is another field resistant variety from the peanut breeding program at Auburn University is a high-yielding, medium-maturity cultivar ideal for southeastern US conditions (Hollis 2023). While GA-12Y may not be as high yielding as GA-06G or AU-NPL 17, it does boast a higher field resistance to TSWV according to the Peanut Rx guide (Peanut RX, UGA) (Monfort 2020). A study by Sundaraj et al. (2013) aimed to determine the mechanism of resistance to TSWV in peanut varieties across several field resistant and susceptible species, including Tifguard, Georgia Green and Georgia-06G. This study found that, while field resistant varieties had little to no effect of mechanical inoculation rates or virus attributes such as nucleocapsid gene copies or positive selection, there was a difference in the thrips (Sundaraj et al. 2013). Choice and no-choice tests showed that field resistant varieties had lower feeding incidences and lower thrips survival compared to susceptible varieties (Sundaraj et al. 2013). This results in reduced virus infection rates in field resistant peanuts, as well as reduced viral accumulation in thrips populations (Sundaraj et al. 2013).

Thrips follow consistent annual migratory patterns, making planting dates crucial for minimizing damage. Tobacco thrips peak migration occurs in late April, while flower thrips peak in early June in Alabama (Brown et al. 2005; Frank et al. 2020). A study by Todd et al. (1995) in Georgia found that planting in May or June reduces tobacco thrips populations in peanuts. Planting date recommendations for TSWV risk mitigation are provided by the Peanut Rx guide, currently suggesting May 11th to May 31st for optimal protection. Weather conditions, especially warm winters and springs, can influence thrips migrations, impacting their lifecycle and potentially leading to earlier infestations (North Carolina State University 2021). Producers can monitor weather patterns for thrips flights and adjusting planting dates to mitigate TSWV by utilizing resources such as the North Carolina State Tobacco Thrips Flight and TSWV Intensity Predictor (North Carolina State Climate Office 2021).

Insecticides like aldicarb, acephate, imidacloprid, and phorate are used for thrips management (Greene et al. 2021). Imidacloprid is popular among farmers due to its economic value, but studies indicate potential negative effects in preventing tomato spotted wilt (Kichler 2022). Research suggests imidacloprid may increase thrips feeding and TSWV transmission risk (Kichler 2022). In contrast, phorate is believed to trigger plant defense responses, potentially reducing TSWV severity (Cabrera 2020). Motta et al. (1998) found that peanuts treated with 5lb/acre of phorate showed reduced TSWV incidence and severity compared to untreated peanuts.

The goal of this study was to evaluate the impacts of planting date, variety and Thimet usage on thrips injury, TSWV incidence and yield. Based on the Peanut Rx Guide, three planting dates were selected (late April, mid-May, and mid-June), as well as three peanut varieties (GA-12Y, GA-06G, and AU-NPL 17). In addition to this, peanuts were also treated with Thimet or did not receive an at-plant insecticide.

MATERIALS AND METHODS

Experimental Design

A field trial was conducted at the Wiregrass Research and Extension Center in Headland, AL during the 2022 and 2023 growing seasons. The experiment was planted in a split-split plot design within a randomized complete block with four replications. Main plots consisted of 24 rows and contained three planting dates: late April (high risk to thrips infestation), mid-May (low risk), and mid-June (moderate risk). These risk levels were determined by the Georgia Peanut Rx guide (Kemerait et al 2004). The sub-plot consisted of eight rows and contained three varieties selected based on the Peanut Rx guidelines: GA-12Y (field resistant to TSWV), GA-06G (moderately field resistant, but historically high yielding), and AU-NPL 17 (moderately field resistant). Lastly, the sub-sub plot was four rows, either treated or not treated with an in-furrow application of Thimet according to the product label (20 g phorate, 5 lbs/acre). Plots were 9.1 m long and spaced 0.9 m apart and planted at a seeding rate of 20 seed per row meter. The peanuts were non-irrigated for the 2022 season and irrigated during 2023. The managed for high yield with regards to insect, weed and disease management according to recommendations from the Alabama Cooperative Extension System.

Data Collection

In both years, the percentage stand was evaluated by randomly sampling the number of plants per 9 meters of row in the center two rows of each plot at 14 days after planting (DAP). Whole plot vigor and thrips injury ratings were made at 14, 21, and 28 DAP. Vigor was rated on a 0 to 10 scale, with 0 being no vigor and 10 being maximum vigor, and is based on the overall health, growth, and fullness of the plants in the plot. Thrips injury was rated on a 0 to 5 scale, with 0 being no injury and 5 being complete absence of leaf tissue, measured based on the severity of thrips damage symptoms (silvery speckling, leaf crinkling, etc.) seen in the plot.

Fractional green canopy clover (FGCC) and virus incidence was recorded at 42, 49, and 56 DAP. FGCC was recorded with the Canopeo application on iPhone in the center two rows of each plot to assess for growth stunting and general lack of vitality. The Canopeo app was developed at Oklahoma State University App Centre and works by analyzing R/G and B/G ratios and the excess green index to produce a binary image, wherein white pixels represent green matter and black pixels represent all other colors (Patrignani and Oschner 2015). Canopeo then quantifies the FGCC as a percentage, which is then used to estimate canopy coverage. This method has been found to be a fast, reliable, and nondestructive way to accurately assess plant health in small-plot cotton and peanut trials (Graham et al. 2019; Chauhan et al. 2022). Percent virus incidence was recorded by dividing the number of row meters that contained plants which exhibited symptoms of TSWV (stunted growth, leaf chlorosis) by the total number of row meters (18.3 m) in the center two rows of each plot. Finally, the center two rows of each plot were harvested and yield was taken at approximately 150 DAP. These data will be used to determine the presence of TSWV in the field and how well the plants are managing its symptoms, given different treatment conditions.

Statistical Analysis

Combined percent stand counts, seedling vigor, thrips injury ratings, Canopeo ratings, TSWV incidence and yield data were analyzed with a mixed model of variance (PROC GLIMMIX, SAS 9.4, SAS Institute Inc. Cary, NC). Planting date, variety, insecticide and their interactions were considered fixed effects in the model. Replication, replication by planting date and plant date nested in replication by year were considered random effects. Degrees of freedom were estimated using the Kenward Rogers Method. Means and standard errors were calculated with PROC MEANS. Means were separated using LSMEANS and were considered significant at α=0.05. Unless indicated, two- and three-way interactions were not significant and are not discussed.

RESULTS

Early Season Results

Variety (F = 7.37; df = 2, 18; P = 0.005) and planting date (F = 34.56; df = 2, 6; P < 0.001) significantly impacted percent stand. Varieties GA-12Y and GA-06G had significantly higher stands than AU-NPL17 (Table 1). Peanuts planted at early (April) and mid (May) planting dates had significantly higher stand than peanuts planted at the late (June) plant date (Table 1).

No significant difference was observed for insecticide (*F* = 0.49; df = 1, 99; *P* = 0.485) (Table 1).

Similarly, variety (*F* = 18.15; df = 2, 18; *P* <0.001) and planting date (*F* = 7.65; df = 2, 6; *P* <0.001) significantly impacted seedling vigor. GA-12Y and GA-06G again significantly outperformed AU-NPL17 (Table 1). However, the early (April) and late (June) planted peanuts both averaged ≈0.3 vigor points higher than peanuts planted at the Mid (May) date (Table 1). No significance was found for insecticide treatment (*F* = 0.24; df = 1, 99; *P* = 0.624) for seedling vigor.

A significant two-way interaction of variety by planting date was observed for thrips injury (*F* = 6.27; df = 2, 99; *P* = 0.003). In general, Early (April) planted peanuts had higher thrips ratings than Mid (May) or Late (June) planted peanuts. However, at both the Mid (May) and Late (June) planting dates AU-NPL17 had more thrips injury than either GA-12Y or GA-06G (Table 2). There was also a significant interaction of insecticide by planting date (F = 6.27; df = 2, 99; P = 0.003) for thrips injury. While Thimet significantly reduced injury in the early (April) and mid (May) planting dates, there was no reduction in injury for Thimet in the late (June) planting date (Table 3).

Late Season Results

There was no impact of variety (F =1.41; df = 2, 18; P = 0.270) or insecticide (F = 0.40; df = 1, 96; P = 0.527) on Canopeo ratings. However, there was an effect of planting date (F = 59.71; df = 2, 6; P = <0.001), where late planted peanuts had the highest Canopeo ratings, followed by mid and early planted peanuts (Table 4).

Significant differences for variety (F = 10.85; df = 2, 18; P = 0.001) and planting date (F = 11.95; df = 2, 6; P = 0.008) were found for TSWV incidence. The highest TSWV incidence was found in GA-06G, with GA-12Y and AU-NPL17 showing similar and lower TSWV incidence (Table 4). Planting date (F = 1.83; df = 1, 96; P = 0.180) also had a significant effect on TSWV incidence, where peanuts planted at the early (April) plant date had significantly higher TSWV incidence than those planted at the mid (May) or late (June) planting dates (Table 4). No significant effect of insecticide treatments (F = 1.83; df = 1, 96; P = 0.180) was observed for TSWV incidence.

A significant difference was found for yield in insecticide (F = 7.9; df = 1, 114.2; P = 0.006) and variety (F = 3.03; df = 2, 18; P = 0.073) treatments. Plots that received Thimet had significantly higher yield than plots without Thimet treatment (Table 4). Mid-planted peanuts had significantly higher yield than Early-planted peanuts, with the Late-planted date falling between the other two dates.

DISCUSSION

Early Season

In our study, AU-NPL17 had significantly lower stands than GA-12Y and GA-06G. These results are similar to those found by Faske et al. (2020) who observed AU-NPL17 to have lower stands than GA-12Y and GA-06G. We also found that percent stand counts were reduced at the late (June) planting date. Zurweller et al. (2023) also reported lower stand in later planted peanuts (May 30). In that study, the authors suggested lower stands could have been due to warmer soils and less moisture. In our study, we did not monitor soil temperatures, but we did have adequate moisture for germination.

AU-NPL 17 had significantly lower average vigor than GA-12Y and GA-06G, which could be attributed to lower stands observed with this variety, which could suggest less seedling vigor. Peanuts at the mid (May) planting date had significantly lower vigor than planting dates, however the differences were minor (0.3 points on 0-10 scale) and statistical differences may be a result of the trial design.

Two interactions were observed for thrips injury. In the first, variety by planting date, the mid and late-planted GA12-Y and GA-06G had significantly less thrips injury than AU-NPL 17 at these planting dates. This may again be attributed to the low stand in AU-NPL 17. Lower plant populations are known to correlate with higher incidences of TSWV (Tillman et al. 2006; Strayer-Scherer and Graham 2021). AU-NPL 17 likely had more thrips injury at the mid and late planting dates due to the reduced stands at these dates, which has been seen previously (Zurweller et al. 2023). Peanut stands with fewer plants down the row are thought to result in higher thrips populations on individual plants, resulting in higher injury (Brown et al. 2005). In the second interaction, insecticide by planting date, Thimet usage correlated with significant reductions in thrips injury at the early and mid-planting dates, but not at the late planting date. Despite Peanut Rx guidelines suggesting the late plant date is at moderate risk compared to the mid plant date (low risk), late planted peanuts were observed to have the least amount of thrips injury in both years of this study (University of Georgia 2018). Based on thrips injury trends for planting date over the two years assessed, Thimet correlated with a significant reduction in feeding injury when thrips pressure was high but did not result in reduced damage when pressure was low.

Late Season

The late plant date showed the highest canopy closure across treatments. This differs from previous research, which shows later planting dates correlate with decreased canopy closure (Bateman et al. 2020). While the decreased stand at the late plant date would lead to the assumption that it would also have decreased canopy closure, a study done by Plumblee et al.

(2018) observed no correlation between seed density and canopy closure. Plumblee et al. (2018) also concluded that factors such as thrips population, flight patterns, feeding injury must be driving differences in canopy closure, which aligns with our observations of significantly lower thrips injury followed by significantly higher canopy closure, and vice versa.

The higher field resistant variety, GA-12Y, had significantly lower virus incidence than GA-06G or AU-NPL 17, reflective of Peanut Rx guidelines on TSWV resistant varieties. AU-NPL 17 and GA-06G did not significantly separate for virus incidence, but the highest incidence was found to be in GA-06G (12.9%) followed by AU-NPL 17 (10.7%). Though the Peanut Rx guide states GA-06G and AU-NPL 17 are of equal susceptibility to TSWV, other sources state that AU-NPL 17 may be more field resistant, especially given its high yield potential (Hollis 2017). While peanuts planted at the mid and late planting dates did not separate as they did for thrips injury and canopy closure, the early planting date was observed to have significantly higher TSWV incidence. The lack of separation between mid and late plant dates for TSWV incidence could be attributed to the less populous, but more efficient TSWV vectors, western flower thrips (*F. occidentalis*), have been found to migrate through Alabama at higher densities in late May to early June (Brown et al. 2005; Cook et al. 2007; Frank et al. 2020).

Insecticide treatment was found to have a significant impact on yield in this study. Plots that received an in-furrow application of Thimet yielded ≈350 kg/ha higher than plots without Thimet treatment (Table 4). The overall trend of peanuts treated with Thimet having higher yields than non-treated peanuts is similar to other studies (Brandenburg 2017, Mahoney et al. 2018, Zurweller et al. 2023). This suggests that thrips injury can reduce yields, regardless of TSWV incidence in Alabama and thrips should be managed to maintain yields. Yield results also showed a significant interaction for planting date. Yield was significantly lower for early-planted peanuts than for any other treatment. This aligns with the literature which states TSWV causes significant yield loss (Culbreath et al. 2003; Sundaraj et al. 2014; Strayer-Scherer and Graham 2021). The reduced yield during the early plant date correlates with the significantly higher virus incidence observed at that date. The Mid-planted peanuts had the highest yield out of the three, which aligns with the Peanut Rx recommendations to plant in mid-May.

CONCLUSION

This study shows the importance of a systematic approach to TSWV management in peanuts to maximize yield. We found that planting date was the most important factor in thrips injury and TSWV incidence. Overall, the use of Thimet was important, but more so at the early planting date (April) when thrips injury and TSWV infection risk is the highest. These results demonstrate that farmers should plant high yielding cultivars in Mid-May with Thimet in areas with high TSWV incidence to reduce virus pressure and maintain yield potential.

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TABLES

Table 1. Impact of peanut variety, planting date and at-plant insecticide on percent stand (Mean ± SEM), seedling vigor, and thrips injury for peanuts planted in Headland, AL (2022-23).

Variety	Planting Date	Insecticide	% Stand	Vigor (0-10)	Thrips Injury** (0-5)
GA-12Y	-	-	68.7 (2.3) a	7.8 (0.1) a	1.3 (0.1) b
GA-06G	-	-	67.9 (2.2) a	7.7 (0.1) a	1.2 (0.1) b
AU NPL17	-	-	59.2 (2.5) b	7.3 (0.1) b	1.6 (0.1) a
P>F			*<0.01*	*<0.01*	*<0.01*
-	Early (April)	-	73.9 (1.27) a	7.7 (0.1) a	2.1 (0.04) a
-	Mid (May)	-	79.6 (2.0) a	7.4 (0.1) b	1.3 (0.1) b
-	Late (June)	-	52.4 (2.3) b	7.7 (0.1) a	0.7 (0.1) c
P>F			*<0.01*	*0.02*	*<0.01*
-	-	Non-Treated	66.1 a	7.6 a	1.5 (0.1) a
-	-	Thimet[1]	64.5 a	7.6 a	1.2 (0.1) b
P>F			*0.485*	*0.24*	*<0.01*

Means within a column followed by a common letter are not significantly different (FPLSD *P=0.05).*

[1]1.68 kg ai/ha (phorate).

*Indicates significant interactions.

Table 2. Interaction of variety and planting date on thrips injury (Mean ± SEM) for peanuts planted in Headland, AL (2022-23).

Variety	Planting Date	Thrips Injury (0-5)
GA-12Y	Early (April)	2.1 (0.1) a
GA-12Y	Mid (May)	1.2 (0.1) c
GA-12Y	Late (June)	0.5 (01) d
GA-06G	Early (April)	2.0 (0.1) a
GA-06G	Mid (May)	1.2 (0.1) c
GA-06G	Late (June)	0.5 (0.04) d
AU NPL17	Early (April)	2.2 (0.1) a
AU NPL17	Mid (May)	1.4 (0.1) b
AU NPL17	Late (June)	1.1 (0.1) c
P>F		*0.047*

Means within a column followed by a common letter are not significantly different (FPLSD *P=0.05)*.

Table 3. Interaction of planting date and at-plant insecticide on thrips injury (Mean ± SEM) for peanuts planted in Headland, AL (2022-23).

Planting Date	Insecticide	Thrips Injury (0-5)
Early (April)	Thimet[1]	2.0 (0.1) b
Mid (May)	Thimet[1]	1.0 (0.1) d
Late (June)	Thimet[1]	0.7 (0.1) e
Early (April)	Non-Treated	2.2 (0.1) a
Mid (May)	Non-Treated	1.5 (0.1) c
Late (June)	Non-Treated	0.7 (0.1) e
P>F		*<0.01*

Means within a column followed by a common letter are not significantly different (FPLSD *P=0.05).*

[1]1.68 kg ai/ha (phorate).

Table 4. Impact of peanut variety, planting date and at-plant insecticide on fractional green canopy closure (FGCC) (Mean ± SEM), percent tomato spotted wilt virus (TSWV) incidence, and yield (kg/ha) for peanuts planted in Headland, AL (2022-23).

Variety	Planting Date	Insecticide	% FGCC	% TSWV Incidence	Yield** (kg/ha)
GA-12Y	-	-	66.6 a	7.5 (0.6) b	4803.3 a
GA-06G	-	-	63.7 a	12.9 (0.9) a	4606.3 a
AU NPL17	-	-	64.5 a	10.7 (1.1) a	4983.9 a
P>F			*0.270*	*<0.01*	*0.07*
-	Early (April)	-	47.5 (1.2) c	13.4 (1.3) a	4455.3 (157.3) b
-	Mid (May)	-	67.1 (1.7) b	7.7 (0.6) b	5124.2 (93.8) a
-	Late (June)	-	80.1 (0.9) a	10.0 (0.6) b	4813.9 (94.6) ab
P>F			*<0.01*	*<0.01*	*0.04*
-	-	Non-Treated	64.4 a	11.0 a	4622.6 (106.7) b
-	-	Thimet[1]	65.4 a	9.7 a	4973.0 (92.4) a
P>F			*0.527*	*0.180*	*0.01*

Means within a column followed by a common letter are not significantly different (FPLSD *P=0.05)*.

[1] 1.68 kg ai/ha (phorate).

*Indicates significant interactions.

Chapter 3: Evaluation of how cover crop, variety, insecticide, and herbicide treatment effects thrips and TSWV peanuts

ABSTRACT

Peanut cultivation in Alabama faces the threat of thrips damage and infection by tomato spotted wilt virus (TSWV), potentially leading to decreased yield and seed quality during harvest. Thrips feeding can inflict significant harm to seedlings, potentially causing stand loss, but the main worry is the transmission of TSWV. The use of insecticides and field resistant varieties are well documented methods of controlling thrips and TSWV, but the effects of cover crop and herbicides on managing this pest and disease isn't well known. It's hypothesized that cover crops could deflect thrips and improve plant health, while herbicides could damage peanut plants and make them more vulnerable to TSWV. This study aims to assess how an insecticide (Thimet), popular peanut varieties (GA-12Y and GA-06G), cover crop residue (crimson clover, radish, and rye mixture), and herbicide use (Gramoxone, 2-4DB, and Dual Magnum) can impact plant health, thrips injury, TSWV incidence, and yield in peanuts grown in Headland, AL in 2022. In this study, treatments with cover crop residue and the GA-12Y variety showed significantly higher yield than plots without cover crop residue or GA-06G. GA-12Y especially seemed to benefit from being planted in cover crop residue, showing higher stand, vigor, and canopy closure. The only significant factor found involving herbicide was canopy closure, in which plots that received herbicide treatment had significantly reduced fractional green canopy closure (FGCC). The insecticide treatment likewise only significantly impacted one effect, in which Thimet was found to significantly reduce thrips injury. Based on these results, variety choice and cover crop usage seem to be the most impactful factors in improving plant health and yield.

INTRODUCTION

Cover crops, grown temporarily between and terminated before cash crop emergence, provide multiple benefits to the cash crop (Balkcom et al. 2007). Cover crops can aid in weed control through ground cover, enhanced soil structure and moisture, and increased soil nutrients (Balkcom et al. 2007). Cover crops also help reduce soil erosion and chemical runoff, thereby maintaining soil quality and preventing chemicals from leaching into groundwater or unwanted areas (Doran and Smith 1991; Sainju et al. 1997; Dabney et al. 2001; Truman and Williams 2001; Tubbs 2003; Coppens et al. 2006). The type or mixture of cover crop plays a crucial role in nitrogen cycling, with grasses and brassicas reducing nitrogen leaching by accumulating it into their biomass, making it available for the cash crop after burn down (Balkcom et al. 2007; Sánchez and White 2022). While cover crops could negatively impact the field by harboring insects and diseases, proper selection can mitigate these issues (Balkcom et al. 2007). Cover crop residues have been found to reduce disease incidence, including tomato spotted wilt virus (TSWV), leaf spot, and white mold (Balkcom et al. 2007; Wright et al. 2009). A study done by Campbell et al. (2001) at the Wiregrass research station in Headland, AL, showed different types of forage systems resulted in varied impacts on insect and TSWV ratings. Ryegrass had the lowest severity, with wheat having the highest, and fallow having the third highest average rating (Campbell et al. 2001). Cover crops significantly influenced yield, with rye yielding the highest (6374 kg/ha) and fallow the lowest (5540 kg/ha) (Campbell et al. 2001).

Various herbicides, both pre-emergence (pendimethalin, s-metolachlor, flumioxazin, dimethenamid) and post-emergence (imazapic, acifluorfen, clethodim, bentazon), are employed in peanut cultivation (Kimura et al. 2004). The choice of herbicide or mixture depends on local regulations and the weed species present. Some herbicides, like paraquat, offer broad-spectrum effectiveness across various weed types, while others may specialize in grasses or broadleaf weeds (Kimura et al. 2004). The impact of herbicides on TSWV incidence in peanuts is not fully understood. It is hypothesized that herbicides could have a negative impact on disease control, as a study by Prostko et al. (2002) found the herbicide chlorimuron correlated with increased TSWV severity and reduced yield in peanuts in some field trial locations. The study showed that other herbicides, including imazapic, bentazon, sulfentrazone, diclosulam, flumioxazin, axifluoren, and paraquat, did not exhibit any consistent effect on TSWV incidence (Prostko et al. 2002).

Peanut variety choice can also mitigate TSWV, with breeding efforts focusing on the development of field resistant cultivars. Varieties GA-12Y and GA-06G are both popular TSWV field resistant varieties, with GA-06G often being favored for its large seeds and high yield (Monfort 2020). Although the newer GA-12Y variety does not have as large of a seed as GA-06G, it does have greater field resistance to TSWV (Monfort 2020). With both varieties having their pros and cons, it's important to continue assessing them in order to keep track of their performance in the field and make accurate recommendations to growers.

For insecticidal management of thrips, imidacloprid is often favored by farmers for its economic benefits but has raised concerns due to potential adverse effects on preventing tomato spotted wilt (Kichler 2022). Studies suggest that imidacloprid might heighten thrips feeding and increase the risk of TSWV transmission (Kichler 2022). Conversely, phorate is believed to induce plant defense responses, potentially mitigating the severity of TSWV (Cabrera 2020). Motta et al. (1998) observed that peanuts treated with 8.1 g/ha phorate exhibited reduced TSWV incidence and severity compared to untreated peanuts.

Due to limited research on the impact of cover crop residues and herbicide use on thrips feeding and TSWV incidence, the objective of this experiment was to increase our understanding of these factors. We also incorporated varietal selection (GA-12Y and GA-06G) and insecticide usage (Thimet) to further explore potential interactions among these variables. The experiment was conducted at the same site as the previous study (Headland, AL) as well as an additional location in Fairhope, AL in order to provide two site years of research data in 2022. While the field data collected was identical to that of the previous experiment, the decision was made to conduct two, rather than three, ratings for each data point due to the distance between the sites.

MATERIALS AND METHODS

Experimental Design

A study was done at two locations in South Alabama, the Wiregrass Research and Extension Center in Headland (planted 18 May 2022) and the Gulf Coast Research and Extension Center in Fairhope (planted 19 May 2022). The experiment was designed as a split-split-split plot randomized complete block design with four replications and four 76.2 cm rows per plot. The main plots were consisted of 32 rows either without cover crops or with a cover crop mixture of crimson clover, radish, and rye. Sub-plots consisted of 16 rows and contained two peanut varieties, GA-12Y (field resistant to TSWV) and GA-06G (moderately field resistant, but historically high yielding). These varieties were selected due to their popularity amongst growers in Alabama. Sub-sub-plots consisted of eight rows, either treated or not treated with a herbicide spray mixture of Gramoxone (paraquat, 0.28 kg ai/ha), 2,4-DB (4-(2,4-dichlorophenoxy) butyric acid, 1.96 kg ai/ha), and Dual Magnum (s-metolachlor, 8.5 kg ai/ha). At the Headland location, 2,4-DB and Dual Magnum were applied at 27 days after planting (DAP), followed by Gramoxone at 36 DAP. At the Fairhope location, all three herbicides were applied at 28 DAP; however, due to miscommunication with the research station, all plots received this herbicide treatment. Finally, sub-sub-sub-plots consisted of four rows, either treated or not treated with an in-furrow application of Thimet (phorate, 1.68 kg ai/ha). Peanuts were not irrigated at either location and were grown with standard guidelines for fertilizers and fungicide applications as recommended by the Alabama Cooperative Extension System. No additional insecticides were applied.

Data Collection

Percent stand emergence was evaluated by randomly sampling the number of plants in the center two rows of each plot at 7 DAP. Vigor and thrips injury ratings were recorded on a whole plot scale at 7, 14, and 21 DAP. Vigor was rated on a 0 to 10 scale, with 0 being no vigor and 10 being maximum vigor, and is based on the overall health, growth, and fullness of the plants in the plot. Thrips injury was rated on a 0 to 5 scale, with 0 being no injury and 5 being complete absence of leaf tissue, measured based on the severity of thrips damage symptoms (silvery speckling, leaf crinkling, etc.) seen in the plot.

Fractional green canopy closure and virus incidence was recorded at 49, 56, and 63 DAP. FGCC was recorded both visually and with the Canopeo app in the center two rows of each plot to assess for growth stunting and general lack of vitality. Tomato spotted wilt virus incidence was recorded by counting the total number of row meter that contained plants exhibiting symptoms of TSWV (stunted growth, leaf chlorosis) in the center two rows of each plot. Finally, yield was taken approximately 150 DAP.

Statistical Analysis

Combined percent stand counts, seedling vigor, thrips injury ratings, Canopeo ratings, TSWV incidence and yield data were analyzed with a mixed model of variance (PROC GLIMMIX, SAS 9.4, SAS Institute Inc. Cary, NC). Variety, cover crop, herbicide, insecticide and their interactions were considered fixed effects and replication was considered random in the model. Degrees of freedom were estimated using the Kenward Rogers Method. Means and standard errors were calculated with PROC MEANS. Means were separated using LSMEANS and were considered significant at α=0.05. Unless indicated, two-, three- and four-way interactions were not significant and are not discussed.

Stand counts, seedling vigor, and thrips injury were assessed prior to herbicide treatment, thus only canopy closure, virus incidence, and yield were analyzed with the herbicide treatment effect. Due to the aforementioned miscommunication, which resulted in all plots receiving a herbicide treatment at the Fairhope location, only results from the Headland location are shown.

RESULTS

Early Season Results

No significant differences for stand counts were observed for variety (F = 2.21; df = 1, 56; P = 0.143), cover crop (F = 0.52; df = 1, 56; P = 0.473), or insecticide (F = 0.68; df = 1, 56; P = 0.414) treatments. However, there was a significant interaction of variety by cover crop (F = 7.42; df = 1, 56; P = 0.009), showing the GA-12Y variety planted in cover crop residue had significantly higher stand than GA-12Y planted without cover crop and GA-06G planted with cover crop, while cover crop residue did not impact stand for GA-06G (Table 1).

There was also an interaction of variety by cover crop (F = 16.95; df = 1, 181; P = <0.001) for seedling vigor ratings. GA-12Y planted with cover crop residue had significantly higher vigor than all other treatment combinations (Table 2). GA-06G without cover crop was significantly higher than GA-12Y planted without cover crop, and GA-06G with cover crop shared a group between these treatments (Table 2). There was no significant difference in insecticide (F = 0.47; df = 1, 183.3; P = 0.494) treatment.

Late Season Results

Variety (F = 12.06; df = 1, 183.2; P = <0.001) and insecticide treatment (F = 28.72; df = 1, 183.2; P = <0.001) significantly impact thrips injury ratings. The variety GA-06G had significantly lower thrips injury that GA-12Y (Table 3). Peanuts treated with Thimet had significantly less thrips injury than non-treated peanuts (Table 3). No significant effect of cover crop (F = 0.07; df = 1, 181.1; P = 0.797) was observed.

FGCC was significantly impacted by herbicide treatment (F = 4.52; df = 1, 108.7; P = 0.036), where peanuts treated with gramoxone had ≈3% lower average canopy coverage than those without herbicide treatment (Table 4). There was also an interaction of variety by cover crop (F = 4.74; df = 1, 108.7; P = 0.032), where GA-12Y planted in cover crop residue had significantly higher canopy coverage than other variety and cover crop treatment combinations (Table 5). Insecticide (F = 0.21; df = 1, 111; P = 0.648) did not impact canopy coverage.

Both variety (F = 15.57; df = 1, 112; P = <0.001) and cover crop (F = 17.88; df = 1, 112; P = <0.001) significantly impacted TSWV incidence. GA-12Y averaged 3.5% lower TSWV incidence than GA-06G, and peanuts with cover crop residue also had a significantly less TSWV incidence than those without (Table 4). There was no impact for herbicide (F = 0.18; df = 1, 112; P = 0.673) or insecticide (F = 3.62; df = 1, 112; P = 0.060) on TSWV ratings.

Variety (F = 23.57; df = 1, 48; P = <0.001) and cover crop (F = 73.98; df=1, 48; P = <0.001) were also significant impacts on yield. The variety GA-12Y yielded ≈361.1 kg/ha more than GA-06G (Table 4). Peanuts planted in cover crop residue yielded ≈639.8 kg/ha higher than plots that did not receive cover crop treatment (Table 4). There was no impact of herbicide (F = 1.83; df=1, 48; P = 0.182) or insecticide (F = 0.74; df=1, 48; P =0.393) for yield.

DISCUSSION

In our study, stand counts showed no significant differences in stand between cover crop, variety, or insecticide treatments. Stand averaged consistently ≈60-65% emergence across all treatments (≈12 plants per meter) (Table3). However, there was a significant interaction of cover crop by variety, in which GA-12Y planted in cover crop residue averaged 71.8% stand, which was over 10% higher stand than GA-12Y without cover crop and GA-06G with cover crop (Table 2). GA-06G planted without cover crop residue shared a group between these treatments, averaging 64.4% stand (Table 1). Faske et al. (2020) also found that GA-12Y averaged higher stand than GA-06G, though these differences were not significant, reflective of our main effect for stand (Table 3). Considering peanuts without cover crop treatment fell into the 60-65% emergence range that all treatments exhibited in the main effects (Table 3), cover crop residue seemingly benefited GA-12Y, but negatively impacted GA-06G, for seedling emergence.

A cover crop by variety interaction was also found for vigor, with GA-12Y planted in cover crop residue showed significantly high vigor, followed by GA-12Y and GA-06G planted without cover crop (Table 1). GA-06G planted with cover crop had the lowest vigor across treatments (Table 1). Given the slight differences (0.3/10), these differences may be a result of the trial design and are not likely biologically relevant.

Overall thrips injury ratings were low. Peanuts treated with Thimet had a significant, but minimal (0.2/5), reduction in thrips injury compared to non-treated peanuts, while GA-06G showed a significant, but minimal (0.1/5) reduction (Table 3). Cover crop use did not result in significant changes in thrips injury, as plots with and without cover crop residue each averaged the same rating (0.6) (Table). This slight separation in Thimet usage, lack of separation for cover crop treatment, and low overall thrips pressure may be correlated with the time this study was planted (18 May), which falls in the low-risk window for thrips injury according to Peanut Rx.

Herbicide treatment significantly impacted FGCC, with peanuts that received gramoxone treatment 27 DAP having significantly lower canopy closure than peanuts without this treatment (Table 4). This agrees with our hypothesis that herbicide may have a negative impact on plant health. There was also an interaction of cover crop by variety, where GA-12Y planted in cover crop residue had significantly higher canopy closure than GA-12Y without cover crop or either GA-06G treatment (Table 5). This finding is similar Plumblee et al. (2018), who reported faster canopy closure for GA-12Y compared to GA-06G. As was seen in interactions between variety and cover crop for percent stand and seedling vigor ratings (Table 2 and 3), cover crop residue continues to benefit GA-12Y, but not GA-06G. Although cover crop treatment reduced GA-06G canopy closure, like percent stand and seedling vigor, it did not result in significant improvement either.

Peanuts planted in cover crop residue had significantly lower TSWV incidence (Table 4). Additionally, and reflective of Peanut Rx guidelines on TSWV resistant varieties, GA-12Y had significantly lower TSWV incidence than GA-06G (Table 4). However, considering the lack of meaningful statistical separation relating to variety or cover crop earlier in the season, as well as the lower overall thrips injury, the reduction of virus incidence for variety and cover crop is presumed to be related to factors outside of thrips mitigation. As previously mentioned, utilizing cover crops can improve plant health, which may in turn improve plant defenses against TSWV.

Cover crop and variety treatments were found to have significant impacts on yield in this study. Plots planted in cover crop residue averaged ≈640 kg/ha more than plots without cover crop treatment, with the plant health advantages of cover crop use hypothesized to be the cause of this difference (source). GA-12Y had significantly higher yield than GA-06G by an average of ≈361 kg/ha. GA-12Y having higher average yield than GA-06G is consistent with the majority of 2022 Alabama peanut variety trials by Auburn University (Jordan 2023). While herbicide and insecticide treatments were not significant, peanuts treated with gramoxone were found to average ≈141 kg/ha lower than those without herbicide, while peanuts treated with insecticide averaged ≈64 kg/ha higher than those without (Table 4).

CONCLUSION

This study shows how a systematic approach to TSWV management can maximize yield potential in peanuts in the Wiregrass region of Southeast Alabama. Variety choice and cover crop usage were found to be the most important factors in maximizing yield, and significantly increased plant health for the GA-12Y variety. While Thimet treatment did significantly reduce thrips injury, the trials were planted in a low-risk window (mid-May), which likely resulted in too little thrips pressure for significant impacts to be made on yield. Considering this and a study by Prostko et al. (2002) which saw significant increases of TSWV incidence in herbicide-treated plots, future trials testing these variables should consider planting during a high-risk window to see more significant effects. The results of this study demonstrate that farmers should plant high yielding cultivars into cover crop residue to increase plant health and yield potential.

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TABLES

Table 1. Interaction of variety and cover crop treatments on percent stand (Mean ± SEM) for peanuts planted in Headland, AL (2022).

Variety	Cover Crop	Stand (%)
GA-12Y	No Cover	60.3 (0.6) b
GA-12Y	Cover	71.8 (0.6) a
GA-06G	No Cover	64.4 (0.7) ab
GA-06G	Cover	57.8 (0.7) b
P>F		*<0.01*

Means within a column followed by a common letter are not significantly different (FPLSD *P=0.05)*.

Table 2. Interaction of variety and cover crop treatments on seedling vigor (Mean ± SEM) for peanuts planted in Headland, AL (2022).

Variety	Cover Crop	Vigor (0-10)
GA-12Y	No Cover	7.6 (0.02) c
GA-12Y	Cover	7.9 (0.02) a
GA-06G	No Cover	7.7 (0.03) b
GA-06G	Cover	7.7 (0.1) bc
P>F		*<0.01*

Means within a column followed by a common letter are not significantly different (FPLSD *P=0.05).*

Table 3. Impact of peanut variety, cover crop, herbicide use, and at-plant insecticide on percent stand (Mean ± SEM), seedling vigor, and thrips injury for peanuts planted in Headland, AL (2022).

Variety	Cover Crop	Insecticide	Stand* (%)	Vigor* (0-10)	Thrips Injury (0-5)
GA-12Y	-	-	66.0 a	7.7 a	0.6 (0.02) a
GA-06G	-	-	61.1 a	7.7 a	0.5 (0.03) b
P>F			*0.143*	*0.363*	*<0.01*
-	No Cover	-	62.4 a	7.7 (0.02) b	0.6 a
-	Cover	-	64.8 a	7.8 (0.05) a	0.6 a
P>F			*0.473*	*<0.01*	*0.797*
-	-	No Thimet	64.9 a	7.7 a	0.5 (0.02) b
-	-	Thimet[1]	62.2 a	7.7 a	0.7 (0.03) a
P>F			*0.414*	*0.494*	*<0.01*

Means within a column followed by a common letter are not significantly different (FPLSD P=0.05).
[1]1.68 kg ai/ha (phorate).

Table 4. Impact of peanut variety, planting date and at-plant insecticide on fractional green canopy closure (FGCC) (Mean ± SEM), percent virus incidence, and yield (lbs/A) for peanuts planted in Headland, AL (2022-23).

Variety	Cover Crop	Herbicide	Insecticide	FGCC* (%)	Virus Incidence (%)	Yield (kg/ha)
GA-12Y	-	-	-	67.4 (0.9) a	10.6 (0.5) b	1372.2 (100.1) a
GA-06G	-	-	-	64.0 (0.8) b	14.1 (0.5) a	1011.1 (75.0) b
P>F				*0.037*	*<0.01*	*<0.01*
-	No Cover	-	-	62.2 (1.5) b	14.2 (0.6) a	871.72 (92.5) b
-	Cover	-	-	69.1 (1.3) a	10.5 (0.5) b	1511.5 (106.1) a
P>F				*<0.01*	*<0.01*	*<0.01*
-	-	No Herbicide	-	67.3 (1.4) a	12.2 a	1242.0 a
-	-	Herbicide[1]	-	64.0 (1.5) b	12.5 a	1141.3 a
P>F				*0.036*	*0.673*	*0.182*
-	-	-	No Thimet	65.3 a	13.2 a	1159.6 a
-	-	-	Thimet[2]	66.0 a	11.5 a	1223.7 a
P>F				*0.648*	*0.060*	*0.393*

Means within a column followed by a common letter are not significantly different (FPLSD *P=0.05)*.
[1]Gramoxone (paraquat, 0.28 kg ai/ha), 2,4-DB (4-(2,4-dichlorophenoxy) butyric acid, 1.96 kg ai/ha), and Dual Magnum (s-metolachlor, 8.5 kg ai/ha).
[2]1.68 kg ai/ha (phorate).
*Indicates significant interactions.

Table 5. Interaction of variety and cover crop treatments on fractional green canopy closure (FGCC) (Mean ± SEM) for peanuts planted in Headland, AL (2022).

Variety	Cover Crop	FGCC (%)
GA-12Y	No Cover	62.2 (1.3) b
GA-12Y	Cover	70.5 (1.1) a
GA-06G	No Cover	62.2 (1.1) b
GA-06G	Cover	65.7 (0.9) b
P>F		*0.032*

Means within a column followed by a common letter are not significantly different (FPLSD *P=0.05)*.

Chapter 4: Analysis of viral material extracted from four *Frankliniellas spp.* thrips populations collected from field trial peanuts and clover in 2022 and 2023

ABSTRACT

Thrips are the primary vector of tomato spotted wilt virus (TSWV), a devastating plant disease that causes high yield losses in various cash crops. While mechanical inoculation is possible, this type of transmission is not common in nature and is not suspected to be significant in agriculture. Since thrips and TSWV have a broad host range which includes many weed species and cash crops such as peanuts, tomato, and peppers, it is important to understand the ecology of the virus across the landscape. In this study, four populations of ~85 thrips were collected from peanut fields and nearby clover in 2022 and 2023. RNA was extracted from each population. Phusion PCR and gel electrophoresis then used to determine the presence or absence of TSWV. Nanodrop results indicated the RNA extraction was pure (260/280 ranged from 9.1 to 9.6). Phusion PCR was used to with TSWV-N-F and TSWV-N-R primers to isolate any existing N ORF regions from the RNA extraction, which were then compared to a previously cloned TSWV-N positive control for gel electrophoresis. Results showed all samples had matching bands with the positive control at 774 bp size, indicating TSWV presence in all four thrips populations.

INTRODUCTION

Tomato spotted wilt virus (TSWV) is a plant disease transmitted in a persistent and propagative manner, primarily by thrips species in the *Frankliniella* genus (ex. *Frankliniella fusca*, *F. occidentalis*, and *F. bispinosa*), though *Thrips setosus* and *T. tabacci* have also been noted as vectors (Sakimura 1963; Wijkamp et al. 1993; de Borbón et al. 1999; Tsuda et al. 1996; Nagata et al. 2004; Avila et al. 2006; Ohnishi et al. 2006; Rotenberg et al. 2015). Although *F.*

occidentalis is the more efficient vector, *F. fusca* is the most economically impactful species, as it has an earlier migration and is thus able to transmit the virus to more vulnerable seedling-stage plants (Todd et al. 1995; McPherson et al. 1999; Groves et al. 2001; Chaisuekul et al. 2003; Joost and Riley 2004; Riley et al. 2012).

Both thrips and TSWV have an incredibly broad host range, infesting and infecting not only a variety of cash crops, but also several weed species (Groves et al. 2001; Srinivasan et al. 2014). Weeds serve as a reservoir for thrips and TSWV, allowing both to persist in the environment surrounding agricultural fields, which makes it difficult to manage through tillage or crop rotations. Batuman et al. (2020) noted that while most weed species had low incidences of TSWV; however, others, such as rough-seeded buttercup (*Ranunculus muricatus*) had >85% TSWV infection rates. Even bridge crops, like radicchio, have been found to be TSWV reservoirs (Batuman et al. 2020).

Due to the intense ecological presence of thrips and TSWV, monitoring local thrips populations can provide insight into how prevalent the virus is in a given area or host plant. Timing can play a significant role in TSWV presence in thrips populations. Batuman et al. (2020) collected biweekly samples of thrips from March through July between 2008-2011 and found that thrips populations collected early in the season (March through May) mostly tested negative for TSWV (one positive per twenty samples each year), while thrips populations collected in June and July had higher TSWV incidence (one positive per eight samples), indicating that TSWV builds in thrips populations throughout the growing season. To test for the presence of TSWV in thrips populations, complimentary DNA (cDNA) synthesis must be performed on RNA extractions. cDNA synthesis is the process of synthesizing DNA from an

RNA template using reverse transcription, which can then be used in molecular applications such as PCR and RNA sequencing (Thermo Fisher).

In this study, our primary goal was to test for the presence or absence of TSWV in four thrips populations collected from field trial peanuts and nearby clover patches in 2022 and 2023 using cDNA synthesis, Phusion PCR, and gel electrophoresis. For cDNA synthesis, the S segment was targeted because it contains the viral nucleocapsid (N) protein (Ishibashi et al. 2017), which was then selected for amplification using Phusion PCR. The N gene was used for virus identification due to how highly conserved N genes are across different viruses, making them a reliable marker for identification for many different viruses, including influenza, HIV, and SARS-CoV-2 (Newcomb et al. 2009; Ding et al. 2020; Wu et al. 2023). Nucleocapsid genes are also well studied since they are necessary for replication and carry genetic material for viral gene transcription, making N genes a crucial target for many points of virus research, such as life cycles, diagnostics, and vaccine development (Wu et al. 2003). Our secondary goal is to have the extracted RNA sequenced to determine the presence of other animal and plant viruses within the thrips populations. However, because RNA sequencing is a highly time-consuming process, only the presence or absence of TSWV will be reported.

MATERIALS AND METHODS

Insect Collection

Adult thrips were collected by sampling from different host plants in May of 2022 and 2023 from Alabama Agricultural Experiment Stations in SW and SE Alabama (Table 1). A collection of 100 thrips was attempted from each host plant (peanut, cotton and clover) via aspiration. Thrips were kept in glass vials containing 90% ethanol in a -80°C freezer until ready for processing. In 2022, 85 tobacco thrips were collected from both peanuts and clover (170 total) across two locations; the Wiregrass Research & Extension Center located in Headland, AL and the Gulf Coast Research & Extension Center located in Fairhope, AL (Table 1). In 2023, 83 tobacco thrips were collected from peanuts and 83 *Frankliniela spp.* were collected from clover (166 total) from the Headland, AL location (Table 1). Although a collection from cotton was attempted, low thrips populations on seedlings prevented the collection. Thrips collections on clover were complete by sampling from multiple clover patches located within ~30 m of the peanut field. The peanut collection was made from the field from peanut varieties GA-12Y, GA-06G and AU-NPL 17 that were not treated with insecticides.

RNA Extraction and Sample Preparation

Eighty to eighty-five insects were moved from their original container into a sterile 1.7 mL tube containing 1000 µL of Trizol and ground thoroughly. The samples were then incubated for 3 minutes using a rotating mixer. Samples were spun down at 12,000 x g at 4°C for 15 minutes. Trizol was collected while carefully avoiding the insect debris and placed in a new sterile 1.7 mL tube. 200 µL of chloroform was added to the sample and shaken vigorously for 15 seconds before incubating at room temperature for 5 minutes. Samples were then spun down at 12,000 x g at 4°C for 10 minutes. The aqueous phase (top layer) was carefully collected and placed in a new sterile 1.7 mL tube with 500 µL of isopropanol. Samples were then shaken gently by hand and incubated at room temperature for 10 minutes. After incubation, the samples were spun down at 12,000 x g at 4°C for 10 minutes. The supernatant was removed, taking caution not to disturb the small white RNA pellet at the bottom. The pellet was washed with 500 µL of 80% ethanol and spun down at 7,500 x g at 4°C for 5 minutes. All ethanol was removed and after two minutes at room temperature the pellet was redissolved in 10 µL of molecular grade water. Sample concentrations were checked on the Nanodrop and stored in a -20°C freezer.

Diagnostics of TSWV in Thrips Samples

cDNA was synthesized from the RNA extracted from thrips collected on peanut and clover samples collected during 2022 and 2023 (P22, C22, P23, and C23) using the Verso cDNA Synthesis Kit (Thermo Scientific, catalog # - AB1453A), following the manufacturer's protocol. The DNA primer used was TSWV-S-R (AGAGCAATTGTGTCAA) which is specific to the S segment of TSWV. Phusion PCR (New England Biolabs, catalog # - M0530S) was performed following the manufacturer's recommendations using the cDNA samples at a 1:10 dilution as templates, with TSWV-N-F (CACCATGTCTAAGGTTAAGCTCACTAAGG) and TSWV-N-R (AGCAAGTTCTGTGAGTTTTGCCTG) as primers. Annealing temperature was set to 65°C and annealing time was set at 45 seconds. As a positive control, a previously cloned TSWV-N vector was used corresponding to strain MT2 (Martin et al., in review), TSWV-N-GFP-pIB, was used in a 1:100 dilution, and PCR mix with no template was used for the negative control. The ladder used was a 1 Kb Plus DNA Ladder (Invitrogen, Catalog # - 10787018). Samples were also sent for sequencing to confirm the presence of TSWV.

RESULTS

Results from the peanut 2022 RNA extraction was 10 µL with a concentration of 1,140 ng/µL and 260/280 of 1.95. Clover 2022 RNA extractions resulted in 10 µL with a concentration of 1,204 ng/µL with a 260/280 of 1.93. RNA extractions from 2023 peanut and clover resulted in 10 µL each with a concentration of 1,313 ng/µL and 1,105 ng/µL with 260/280's of 1.96 and 1.91, respectively.

Bands were obvious in the positive control, and all samples were identical to the positive control with clearly visible bands, although samples collected from peanuts had notably lighter bands in both years compared to samples collected from clover. The negative control produced no bands (except primer dimer at the bottom), working as intended. The bands from the positive control and samples match the expected size of the N ORF (~774 bp). This indicates that TSWV was present in all samples.

DISCUSSION

All four collected samples yielded RNA concentrations of 1,105 to 1,313 ng/µL. Although sample concentrations of 2.0 to 100 ng/µL are ideal for reproducibility, these concentrations still fit within the NanoDrop RNA detection range of 1.6 to 22,000 ng/µL with an error of ±2%, according to the NanoDrop One user guide (Thermo Fisher). The absorbance ratio 260/280 ranged from 1.91 to 1.96 across the four samples. The acceptable range for pure 260/280 ratios of RNA is of 1.9 to 2.2, with the ideal ratio being ~2.0 (Jones 2019). These results indicate that the RNA samples extracted from the collected thrips are pure.

The primer TSWV-S-R was used to synthesize cDNA from the RNA extraction samples, which was then used in Phusion PCR with TSWV-N-F and TSWV-N-R primers to create the TSWV-N-GFP-pIB template from the samples. This was compared to the positive control template (TSWV-N), and the negative control (PCR mix with no template) in a gel electrophoresis to determine presence of TSWV. The gel electrophoresis showed clearly visible bands across all samples, which matched the bands in the positive control lane. Compared to the DNA ladder, the band size of the samples and positive control were ~774 bp, matching the expected size of the TSWV nucleocapsid ORF. Although bands were visible in all four samples, the bands in samples from peanut thrips populations were much lighter than samples from clover thrips populations. This indicates that clover, as well as other weedy host plants, might serve as an early-season reservoir for TSWV which thrips may carry from weeds to peanuts. This is supported by research done by Srinivasan et al. (2014), which studied viruliferous thrips that developed on winter weed hosts of TSWV such as cudweed (*Gamochaeta falcata*) and chickweed (*Stellaria media* L.). Srinivasan et al. (2014) found that inoculating peanuts with viruliferous thrips collected from winter weed hostplants resulted in infection rates of 14.28 to 75%. Another study by Groves et al. (2002) observed the persistence of TSWV in perennial plants. It is known that TSWV cannot be transmitted by pollen or seed, but Groves et al. (2002) shows that the virus can persist in overwintering perennial plants at a rate of 70 to 80% after the first year and 40 to 42% after the second year. This shows that even if migrating thrips are not carrying TSWV, the virus inoculum can still be maintained in the environment by winter weed hostplants.

CONCLUSION

The lack of bands seen in the negative control, along with matching bands between the samples and positive control at the expected base pair size, indicates the RNA extraction and gel electrophoresis were successful; Tomato spotted wilt virus was present in all four thrips populations collected in 2022 and 2023 from peanuts and clover. The darker bands from clover thrips samples compared to peanut thrips samples suggest a higher TSWV concentration in nearby clover thrips populations early in the season, which may jump to peanuts as they emerge in mid to late May. Further research is needed to determine if thrips populations in nearby fallow could be a primary source of TSWV in peanut fields. If so, planting non-host borders such as corn could be an effective method of TSWV management worth exploring.

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FIGURES

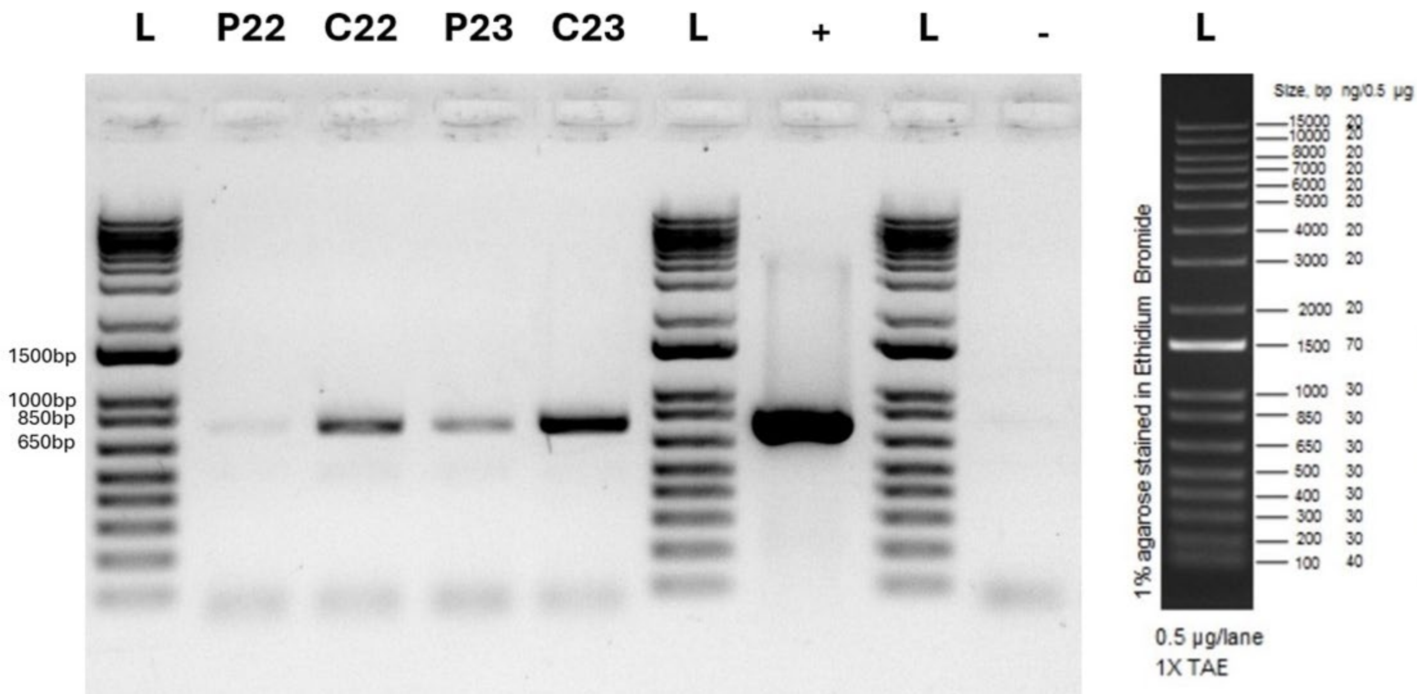

Figure 1: cDNA from thrips fed on peanut and clover 2022 and 2023 samples tested for TSWV-N.

Identification of the presence of Tomato spotted wilt virus nucleocapsid ORF through PCR. Lanes are as follows – L: 1kb plus DNA ladder, P22: cDNA from thrips collected off peanuts in 2022, C22: cDNA from thrips collected off clover in 2022, P23: cDNA from thrips collected off peanuts in 2023, C23: cDNA from thrips collected off clover in 2023, L: 1kb plus DNA ladder, +: positive control of TSWV-N-GFP-pIB, L: 1kb plus DNA ladder, -: negative control of PCR mix with no template.

TABLES

Table 1. Date, location, host plant, and number of thrips collected to be used in RNA extraction for TSWV identification and RNA sequencing in 2022 and 2023.

Year	Date	Location	Host Plant	Thrips Collected*
2022	May 4th	Headland, AL 31°21'19"N 85°19'19"W	Peanuts** and clover***	85 from each host plant
	May 5th	Fairhope, AL 30°32'42"N 87°52'55"W	Peanuts and clover	
2023	May 3rd	Headland, AL 31°21'32"N 85°19'11"W	Peanuts and clover	83 from each host plant

*Thrips collections were pooled into one sample per host plant, per year.
**Thrips collected from non-treated peanut varieties GA-12Y, GA-06G, and AU-NPL 17.
***Thrips collected from white clover growing in fallow within ~30 meters of the peanut research plots.