INFLUENCE OF PLANTING DATE, PLANT POPULATION, AND CULTIVAR ON MANAGEMENT OF SPOTTED WILT IN PEANUT (*Arachis hypogaea* L.)

By

JUSTIN L. MCKINNEY

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To my son, Garrett McKinney, who inspires me daily by always persevering through whatever life throws his way. I cherish every moment I get to spend with you and I will never stop believing, hoping, and praying for a complete healing of your body.

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TABLE OF CONTENTS

page

ACKNOWLEDGMENTS 4

LIST OF TABLES 7

LIST OF FIGURES 8

LIST OF ABBREVIATIONS 9

ABSTRACT 10

CHAPTER

1 OVERVIEW 12

2 LITERATURE REVIEW 15

Introduction 15
Planting Date 17
Plant Population (Seeding Density) 19
Cultivar Selection 21
Objectives 24

3 EFFECT OF PLANTING DATE, SEEDING DENSITY AND CULTIVAR RESPONSE IN MANAGEMENT OF SPOTTED WILT DISEASE OF PEANUT 25

Introduction 25
Materials and Methods 28
Experimental Design and Location 28
Pod Yield 30
Peanut Grades 31
Statistical Analysis 31
Results and Discussion 32
Planting Date and Seeding Density 32
Pod Yield 32
Grades 33
Plant Stand 33
Disease Assessment 34
Summary and Discussion 34

4 EFFECT OF CULTIVAR AND PLANTING DATE ON TSWV INFECTION IN PEANUT 40

Introduction 40
Materials and Methods 43

Experimental Design and Location 43
Disease Assessment 44
ELISA Sampling 45
Statistical Analysis 46
Results and Discussion 46
TSWV Infection 46
Disease Assessment 47
Seed Coat Symptomology 47
Summary and Discussion 48

LIST OF REFERENCES 60

BIOGRAPHICAL SKETCH 64

LIST OF TABLES

Table page

3-1 Probability values from ANOVA for effects of planting date, seeding densities, and cultivar on several traits averaged over three consecutive years, 2010-2012, in Marianna, Florida. 36

3-2 Variation in average pod yield, TSMK, rating scale 1 to 10, DIR 0 to 30, tap root crown counts, and emerged plant population over three years, 2010-2012, in Marianna, Florida. 36

3-3 Planting date effect on TSMK averaged over three years (2010-2012). 36

3-4 Seeding densities averaged over three years (2010-2012). 37

4-1 Probability values from ANOVA for Disease intensity rating, Rating scale, Seed coat symptoms, and TSWV infection on tap roots during, 2010-2012, in Marianna, Florida. 51

4-2 Various spotted wilt ratings averaged over three years, 2010-2012, in Marianna, Florida. 51

4-3 Correlation among 6 spotted wilt symptoms in four peanut genotypes averaged over three consecutive years, 2010-2012, in Marianna, Florida. 52

LIST OF FIGURES

Figure page

3-1 Yield test plots with low disease incidence of spotted wilt near Marianna, Florida in 2010. The experiment contained Florunner between every two plots to serve as susceptible host ‘spreader rows’ to encourage spotted wilt disease. 38

3-2 Interaction effect of cultivar x planting date on pod yield averaged over the three consecutive year (2010-2012) study near Marianna, Florida. 38

3-3 Interaction effect of planting date x seeding densities on pod yield averaged over the three consecutive year (2010-2012) study near Marianna, Florida. 39

4-1 TSWV infection results from ten randomly selected tap root crowns from four cultivars in Marianna, FL in 2010-2012. Blue bars represent negative ELISA results for TSWV infection and Maroon bars represent positive ELISA results for TSWV infection. 53

4-2 Four peanut seeds with A, C & D showing symptoms of TSWV while B showing little to no sign of symptoms. Photo taken by Justin McKinney. 54

4-3 Four peanut cultivars showing symptoms of TSWV on their seed coats. Florunner a susceptible cultivar (50 pts.) to TSWV, Georgia Green a moderately resistant cultivar (30 pts.) to TSWV, Florida-07 a moderate to resistant cultivar (10 pts.) to TSWV, and UF peanut breeding line highly resistant cultivar to TSWV infection. Photo taken by Justin McKinney. 55

4-4 Dried tap root crowns of four peanut cultivars collected from the field on all 13.1 seed m^{-1} plots during 2010-2012 in Marianna, FL. Photo taken by Justin McKinney. 56

4-5 Five prefilled Agdia sample bags with 4 negative (single red line) and 1 (double red lines) positive result for TSWV. Photo taken by Justin McKinney... 57

4-6 Example of plot number 91201 with 10 randomly selected tap roots tested for presence of TSWV. Photo taken by Justin McKinney. 58

4-7 A) Whole dried root crown, B) tap root sample weigh between 0.4 and 0.6 grams, C) sample process of tap root crown emulsified showing negative result for the presence of TSWV. Photos taken by Justin McKinney. 59

LIST OF ABBREVIATIONS

ANOVA	analysis of variance
CM	centimeters
DAP	days after planting
DIR	disease intensity rating
ELK	extra large kernels
HA	hectare
HSD	Honestly Significant Differences
KG	kilogram
LSK	loose shelled kernels
M	meters
OK	other kernels
SMK	sound mature kernels
SS	sound splits
T-K	Tukey-Kramer
TSMK	total sound mature kernels
TSWV	*Tomato spotted wilt virus*

Abstract of Thesis Presented to the Graduate School
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INFLUENCE OF PLANTING DATE, PLANT POPULATION, AND CULTIVAR ON MANAGEMENT OF SPOTTED WILT IN PEANUT (*Arachis hypogaea* L.)

By

Justin L. McKinney

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Spotted wilt, caused by *Tomato spotted wilt virus* (TSWV), is a major disease that has impacted peanut production for growers in the southeastern United States since the mid-1990s. Currently, there is no single control measure that manages spotted wilt in peanut sufficiently; however, collaborative research has identified several management factors that, when used together, can minimize losses due to the disease. Three of the major cultural practices used to reduce incidence and severity of spotted wilt in peanut are date of planting, seeding density, and cultivar selection. New peanut cultivars with improved resistance to spotted wilt have been developed in the Southeast peanut production areas, but even the most resistant cultivars are at risk for spotted wilt when planted in April at lower seeding densities. New peanut genotypes with superior resistance could reduce the risk of spotted wilt in situations of early planting and reduced plant stand. The effects of planting date, plant population and cultivar in management of spotted wilt were assessed in a field experiment over three consecutive years (2010-2012) near Marianna, Florida. Data collection included three measures of visual disease symptoms (two foliar and one seed), ImmunoStrip (ELISA) testing of root crowns for presence or absence of TSWV, pod yield, and grade. There was no effect of

planting date on spotted wilt symptoms or TSWV infection. Assessment of spotted wilt just prior to harvest indicated traces of the disease in all peanut cultivars with little variation among cultivars. However, ImmunoStrip results revealed that viral infection differed widely among cultivars. Viral infection was lower in cultivars Florida EP™ '113' and UFT-312 compared to Georgia Green and Florida-07. Foliar spotted wilt symptoms were highly correlated with each other (r=0.73), however, only symptoms on the seed were highly correlated with TSWV infection (r=0.77). These results indicate that foliar symptomology is not as reliable in assessing genotype resistance as is TSWV infection, especially in seasons when disease pressure is low as was the case during the three years of this study. It also indicates that seed inspection may be a good predictor of resistance for use in breeding programs because it is much less expensive than ImmunoStrip testing. New genotypes demonstrated higher TSMK than Florida-07, suggesting major improvements have been achieved in this very important trait. Results from these experiments illustrate that in years when negligible epidemics of spotted wilt occur, the impact of planting date and seeding density on spotted wilt are minimal. Cultivar resistance is the primary means of control and the cultivars Florida EP™ '113' and UFT-312 display a much higher level of resistance than is currently available. This level of resistance could possibly override other factors such as planting date and seeding density even in years with high disease pressure.

CHAPTER 1
OVERVIEW

The cultivated peanut (*Arachis hypogaea* L.) is one of the most important crops grown in the world. It is native to South America, but is now widely grown throughout much of the warm temperate areas of the world. An exact origin of the *Arachis* genus is not entirely clear, but some archeological data indicates it formed in the southwestern part of Mato Grosso do Sul, Brazil or northeast Paraguay in the gardens of primitive hunter and gatherer civilizations (Simpson et al., 2001). The most ancient species of the genus are still growing in that region today.

Peanut was introduced into the United States from Africa and the Caribbean Islands by Spanish traders (Coffelt et al., 1997). The first commercial production of peanut in the United States was thought to have been in Virginia, around 1844 (Coffelt et al., 1997). Even though records reveal some commercial production in the 1800's, peanut did not become a significant agronomic field crop for the United States until the Civil War. Once the peanut industry began developing its own machinery which improved planting, cultivating, harvesting, and shelling, peanut rapidly became an important crop for the United States. In the United States, peanuts are primarily used for peanut butter, cooking oils, snack foods (candy, boiled, roasted), and grain feed for animals, while elsewhere in the world they are mainly used for oil (Knauft et al., 1987).

In the United States, peanut is grown in three distinct regions; Southeast (Alabama, Florida, Georgia, and Mississippi), Southwest (New Mexico, Oklahoma, and Texas) and the Virginia-Carolina's (North Carolina, South Carolina, and Virginia). From 2007-2011, the United States grew and harvested an average of 495,060 hectares of peanut annually. During that time span, the average harvested pod yield was 3,695 kilograms per hectare, which resulted in an average of 1.845 trillion kilograms of domestic product annually during 2007-2011. Using the average price per kilogram ($0.419 dollars per kilogram) over that same time period, the total farm gate value[1] was slightly less than $942 million annually. The southeast region is responsible for approximately 70 percent of this total farm gate value; the southwest territory generates about 20 percent, and the Virginia-Carolina's creates nearly 10 percent (US Department of Agriculture National Agricultural Statistics Service USDA NASS 2013).

Today, peanut growers in the southeastern United States are faced with many disease problems that affect the peanut crop. In order to maximize profits, breeding programs in the Southeast have placed great emphasis on screening for disease resistant varieties. The peanut plant has to overcome many obstacles during the course of a growing season that are caused by both biotic (living organism) and abiotic (environmental limiting) agents. For the southeast region, biotic diseases are of primary concern and can be very costly to control. There are several diseases of major importance to peanut, but the disease spotted wilt, caused by *Tomato spotted wilt virus* (TSWV) is one of primary concern. Spotted wilt can cause severe damage to the peanut crop resulting in major economic losses. Some infected peanut plants are either severely stunted for the duration of their life, or die before reaching maturity. Symptoms of spotted wilt in peanut include stunting and mottling of the plant, concentric ring spot and chlorosis of the leaves, small and shriveled seeds, and discolored seed coats. The virus has been reported to infect over 650 plant species including more than 50 families in both monocotyledonous and dicotyledonous plants (Culbreath et al., 2003), which

[1] Value of the product when it leaves the farm without marketing or other variable cost considered

means it has an extensive host range. Currently, there is no single control measure that provides adequate management of spotted wilt in peanut (Culbreath et al., 2003). However, several cultural peanut practices have proven effective in minimizing losses due to the disease and include manipulating the planting date, obtaining a uniform plant population, and selecting a resistant cultivar.

CHAPTER 2
LITERATURE REVIEW

Introduction

Diseases caused by thrips-vectored *Tospoviruses* are serious problems in many of the world's agricultural ecosystems (Culbreath et al., 2003). The genus name is derived from *Tomato spotted wilt virus* (TSWV) within the family Bunyaviridae, which is made up of predominantly human and animal infecting viruses. The disease was initially described in Australia on tomato (*Lycopersicon esculentum* Miller) in 1915 (Brittlebank, 1919), and was first observed to be caused by a virus in 1930 (Samuel et al., 1930). The involvement of thrips in transmission of the causal agent was reported in 1927 (Pittman, 1927).

TSWV is transmitted or spread by several species of thrips, which are small winged insects that feed and reproduce on plant tissue (Culbreath et al., 2008). Thrips acquire the virus from an infected host plant in an immature larval stage (Culbreath et al., 2008). After the larva has acquired TSWV, it can transmit the virus to plants for the duration of its life (Culbreath et al., 2008). Adult thrips are capable of transmitting the virus to a plant, but are incapable of acquiring the virus from an infected plant (Kucharek et al., 1990). The virus has been found to be concentrated in the bud terminals where thrips larvae feed, but titer appears to be high in young leaves as well (Kresta et al., 1995). Three species of thrips (Thysanoptera) are the primary vectors for TSWV in agricultural production: tobacco thrips [*Frankliniella fusca (*Hinds)], western flower thrips [*Frankliniella occidentalis* (Pergande)] and onion thrips (*Thrips tabaci*). Other thrips species that vector TSWV are found in United States, but have not been associated as a significant vector of the virus in peanut (Culbreath et al., 2003). In peanut, tobacco thrips [*Frankliniella fusca (*Hinds)] and western flower thrips are the most common spreader vector of TSWV (Todd et al., 1996, Webb et al., 1997).

In general, the use of insecticides alone to control thrips has been ineffective in suppressing spotted wilt, and it has often been found that the severity of infections are independent of the thrips population (Culbreath et al., 2010).

Initially, TSWV was thought to be seed transmitted in peanut as well as vector transmitted by thrips, but no conclusive evidence found to confirm the seed transmission theory. Culbreath et al., 2003 stated that “there is no indication that seed transmission occurs in peanut.” Even though TSWV has been found in the pods and testae of both symptomatic and asymptomatic peanut plants, TSWV could not be identified in the cotyledons (Pappu et al., 1999). In grow-out studies, none of the seed from ELISA positive plants exhibited positive TSWV infection when assessed by ELISA the following season (Pappu et al., 1999). Therefore, planting infected seeds which may show TSWV symptomology on their seed coats from either symptomatic or asymptomatic plants does not increase the risk of spotted wilt incidence the following year.

The first observation of spotted wilt in the southeastern region of the United States was in Alabama in 1986 (Hagen and Weeks 1998). Since that time, epidemics of spotted wilt in peanut have become common and the disease is one of the most problematic in the Southeast (Culbreath et al., 1997). Spotted wilt is difficult to control because it affects a wide range of host plants such as tomato (*Solanum esculentum*), pepper (*Capsicum annuum*), peanut (*Arachis hypogaea* L.), and many others, thereby keeping the virus in the agricultural ecosystem year round. Major epidemics of the disease continue to limit pod yield and therefore require the use of proven cultural practices such as planting date, plant population, and cultivar selection.

There is no single management practice available that provides sufficient control of spotted wilt in peanut (Culbreath et al., 2003). In the southeastern US, an interdisciplinary and comprehensive research and extension group has been effective in providing management tools for the disease in peanut (Culbreath et al., 2003). Research has identified three essential management factors to minimize risk of spotted wilt; planting date, plant population, and cultivar selection. The most effective control methods utilize genetic resistance and cultural practices, which delay or slow development of spotted wilt epidemics (Culbreath et al., 2003).

Planting Date

Date of planting is an important factor affecting the risk of spotted wilt in peanut. The impact of planting date on spotted wilt was first noted in Texas. For production areas in southern Texas, peanut planted between May 5th and June 5th was less likely to have severe spotted wilt than those planted earlier or later (Black, 1990). Similar studies on planting date in the southeastern United States have suggested that planting in the first 2 weeks of May usually resulted in the lowest incidence of spotted wilt, while planting in mid-April or early June resulted in greater incidence of disease (Culbreath et al., 2008). Surveys of spotted wilt infestations in production fields in Georgia have supported these findings (Brown et al., 1996). However, the effects of planting date have not been as dependable as cultivar effects (Culbreath et al., 2008). The trend toward higher infestations of spotted wilt in April-planted peanuts compared to peanuts planted in early to mid-May has been consistent (Culbreath et al., 2008).

Explanations for the differing effects of planting date have been based on circumstantial evidence and remain speculative (Culbreath et al., 2003). A common explanation has been thrips populations and peanut susceptibility to infection are often variable across planting dates, but are at their highest in the early spring (Culbreath et al., 2010).

Several studies in Georgia indicated the greatest numbers of tobacco thrips (*Frankliniella fusca* Hinds) occurred in April-planted peanuts, whereas peanuts planted in May were subjected to fewer thrips (Culbreath et al., 2003). The population dynamics of thrips in non-crop plants or volunteer peanuts early in the season have been hypothesized as a reason for the effects since these plants may serve as reservoirs for spotted wilt (Culbreath et al., 1993). However, proof of this mechanism has not been reported (Culbreath et al., 2003). Wells et al. (2003) found differences between years in time of peak percentage of tobacco thrips which tested positive for nonstructural protein consistent with TSWV reproduction. These changes may be due to environmental conditions which affect other thrips populations that typically occur during that range of reasonable planting dates, host susceptibly and inoculation efficiency (Culbreath et al., 2003). Ambient air and soil temperatures often are much lower in mid-April than in mid-May in the southern United States. Therefore, soil temperature affects rate and uniformity of seed germination and seeding emergence, as well as subsequent plant populations and vigor (Culbreath et al., 2003). Temperature may also affect disease development in inoculated plants (Culbreath et al., 2003). Mandel et al. (2002) reported that fewer systemic infections occurred in field-resistant breeding lines, which indicated a slower rate of mechanical inoculation of spotted wilt at lower verses higher temperatures. Nevertheless, further studies are needed to clarify the reason for these shifts or fluctuations in the effects of planting date on TSWV in peanut.

Although management of planting date is a valuable instrument for suppressing spotted wilt, it does have limitations in practical application for large scale operations (Culbreath et al., 2003). Due to the sheer size and number of fields to be planted, limitations on equipment and labor, and the uncertainty of weather, most growers cannot plant all of their acreage in the “optimum” planting window (Culbreath et al., 2003). Flexibility in planting date is especially important for large farms and would be beneficial to the entire industry.

Optimum planting dates vary from year to year, but in general, avoiding early and late planting reduces the incidence and severity of spotted wilt (Olatinwo et al., 2008). In addition, utilizing planting date alone to minimize spotted wilt incidence may not be adequate as a sole cultural practice to prevent significant losses due to spotted wilt disease (Culbreath et al., 2003).

Plant Population (Seeding Density)

Achieving the optimum plant population is an important management factor that influences the severity of spotted wilt in peanut. Plant population can affect the amount of light interception, canopy closure, weed suppression, and either decrease or increase disease pressure. Spotted wilt is suppressed with higher plant populations. Apparently, infection rates of individual peanut plants are greater among sparse plant populations than with dense populations (Culbreath et al., 2003). Tobacco and western flower thrips prefer plants that are surrounded by bare ground over sites with canopy closure. Thus, any cultural practice that would shorten the period between sowing and canopy closure should decrease the incidence of spotted wilt (Wehtje et al., 1994). Even if the grower obtains a higher plant population per unit of land, this may not actually reduce the number of individual plants affected in a particular field, but possibly reduce the percentage of infected plants per square foot (Culbreath et al., 2010).

The effects of seeding density have been the primary means of evaluating plant population and the severity or incidence of spotted wilt of peanut in the southeastern United States. Results have indicated a decrease in the incidence of spotted wilt in peanut as seeding density increases (Wehtje et al., 1994). In Florida, spotted wilt incidence, pod yields, and grades were affected by in-row plant space modification among several genotypes (Gorbet and Shokes, 1994). Similar reductions in incidence of spotted wilt and an increase in pod yield of several cultivars were seen as seeding density increased (Branch et al., 2003). All of these reports demonstrate that the importance of high seeding density among different cultivars should result in high pod yields, and lower spotted wilt incidence.

The current recommendation in the Southeast is to plant all runner-type peanuts at 19.7 seed m^{-1}. This relatively high seeding density serves as a barricade against poor germination and slow emergence in hopes of actually obtaining a plant stand of 4 individual plants per 30.48 centimeter of row (Baldwin, 1997). Poor plant stands, along with inferior seed quality, insects, or challenging environmental conditions often result in decreased pod yield and low economic returns (Stermitzke et al., 2000). Often, growers contemplate replanting after poor germination, but the cost of peanut seed (Sorensen et al., 2007), normally exceeds the economic gain from replanting. Therefore, it is extremely important for a farmer to have a high quality seed source to minimize the chance of encountering these situations. To achieve a high plant, a grower must be able to obtain a high quality seed with good seedling vigor. Acceptable plant stands are also achieved through adequate soil moisture, soil temperature, and attaining the correct planting depth (Culbreath et al., 2010).

Increasing plant population will not always mean an increase in pod yield. For example, other diseases like Sclerotinia blight, caused by *Sclerotinia minor* Jagger in peanut intensify in severity and incidence with higher plant population compared to lower plant population in susceptible cultivars (Maas et al., 2006). Current extension recommendations for minimizing Sclerotinia blight suggest grower's plant resistant cultivars, avoid high seeding densities, and plant before June 15th. Even though some of the recommendations for reducing Sclerotinia blight are similar to those of spotted wilt, the lower seeding density recommended minimizing Sclerotinia blight the impact of seeding density is the opposite.

Additionally, peanut breeders in the Southeast have several advanced breeding lines with greater levels of resistance to spotted wilt that may allow lower seeding densities, especially when used in combination with other Peanut Rx (Culbreath et al., 2010) practices that aid in the suppression of spotted wilt (Culbreath et al., 2003).

Cultivar Selection

The most effective means of managing losses caused by spotted wilt in peanut is cultivar resistance. As with many viruses, peanut cultivars vary in their reaction to spotted wilt and so do the associated physiological responses (Rowland et al., 2005). Even though some cultivars have shown moderate levels of resistance, there are no known peanut cultivars with complete resistance or immunity (Culbreath et al., 2010). Symptoms cannot be correlated with differential responses to thrips vector so it is unlikely that resistance to thrips explains cultivar resistance observed. Research has proven that there is a differential tolerance level among cultivars to spotted wilt (Culbreath et al., 2000). Tolerance may be in some measure physiologically mediated (Rowland et al., 2005).

Utilizing cultivars with moderate levels of field resistance to spotted wilt is a leading tool in the integrated management tool developed for the disease, and as a result, resistance to spotted wilt is a major consideration in peanut production in the southeastern United States (Culbreath et al., 2000). Cultivar selection is a significant part of Peanut Rx which assigns a numerical risk value for each cultivar based on assessment from previous research. The index was originally developed in Georgia to assist growers and advisors in how to recognize and avoid high risk conditions for spotted wilt of peanut (Brown et al., 1999).

The peanut variety Southern Runner (Gorbet et al., 1987) released by University of Florida Peanut Breeding Program was the first peanut cultivar observed to have a moderate level of resistance to spotted wilt (Culbreath et al., 1992). After Southern Runner was observed with spotted wilt resistance, the development of new cultivars with greater levels of resistance to spotted wilt became a major objective for peanut breeding programs in the southeastern United States. Currently there is no peanut variety available that is completely immune to spotted wilt (Culbreath et al., 2010). However, new cultivars with better spotted wilt resistance than current cultivars have been developed, but many of these new cultivars have yet to be tested under the production practices recommended for reducing spotted wilt losses (Tillman et al., 2006).

The mechanism responsible for spotted wilt resistance in peanut has not been discovered (Culbreath et al., 2003). Several studies have investigated the possibility that the thrips vectors that reproduce and feed on the peanut have a lack of attractiveness to these highly resistant peanut lines (Culbreath et al., 2003) but this has not been confirmed. Reasons for the lack of correlation between results of field tests and mechanical inoculation tests have yet to be determined (Culbreath et al., 2003). However, a recent report from the USDA-ARS showed that the breeding line C11-2-39 has resistance to spotted wilt based on the mechanical inoculation results as well as field resistance (Mandal et al., 2002). One possible explanation might include differential reactions of the virus when it is vectored through thrips compared with inoculation (Culbreath et al., 2003). Regardless, it appears that new peanut cultivars with higher levels of resistance to spotted wilt have the most potential for improving the management of this challenging disease. Since research has demonstrated the importance of cultivar selection, most peanut breeding programs aim to produce lines with greater resistance to spotted wilt. Meanwhile, growers are encouraged to plant moderately resistant cultivars and utilize different combinations of the integrated management program to aid in minimizing losses to spotted wilt of peanut.

Major advancements in cultivar resistance have been achieved, but even the best cultivars (those with the highest level of resistance to spotted wilt) are still at significant risk for the disease when planted prior to May 1. Recently, during the 2009 growing season, the University of Florida's Peanut Breeding Program identified two lines with superior resistance to spotted wilt. These lines were tested under the name of Florida EP™ '113' (formally known as UFT-113) and UFT-312. They are derived from a *hirsuta* background and are related to the line described by Culbreath, et al., (2005). Studies to evaluate the performance of these new breeding lines are needed to determine their effects on planting date, planting population, and cultivar selection.

Objectives

Although the factors described above have been important in reducing the losses from spotted wilt in peanut, their implementation has caused major changes in peanut production in the southeastern United States including delayed planting, increased seeding density, and a major shift in cultivar selection. Therefore the objectives of this research are as follows:

1. To determine if new peanut genotypes (Florida EP^{TM} ‘113’ and UFT-312) could be planted in April with minimal risk of losses from spotted wilt.

2. To determine if new spotted wilt resistant genotypes (Florida EP^{TM} ‘113’ and UFT-312) could be planted at reduced seeding densities with minimal risk.

3. To determine if new peanut genotypes (Florida EP^{TM} ‘113’ and UFT-312) are resistant enough to spotted wilt to allow planting in April and at lower seeding densities.

4. To determine if Florida EP^{TM} ‘113’ and UFT-312 become infected by *Tomato spotted wilt virus* (TSWV).

CHAPTER 3
EFFECT OF PLANTING DATE, SEEDING DENSITY AND CULTIVAR RESPONSE IN MANAGEMENT OF SPOTTED WILT DISEASE OF PEANUT

Introduction

Spotted wilt, caused by Tomato spotted wilt virus (TSWV, genus Tospovirus, family Bunyaviridae), is a major disease that has impacted peanut production for growers in the southeastern United States since the mid-late 1990's. The disease was first described on peanut in the United States in 1971 in Texas (Halliwell and Philley 1974, Kucharek et al., 1990, Hagan and Weeks 1998) and has spread throughout all of the peanut producing regions of the United States (Rowland et al., 2005). TSWV has been reported to infect over 650 species of plants, including more than 50 families of both monocotyledonous and dicotyledonous plants (Culbreath et al., 2003), demonstrating its extensive host range. Some infected peanut plants are either severely stunted for the duration of their life, or die before reaching maturity. Spotted wilt symptoms in peanut are variable in degree and severity. Symptoms include stunting of the plant, mottling, concentric ring spots, and chlorosis of the leaflets; furthermore, the seed is small, shriveled, and can have a discolored and/or cracked seed coat. Symptoms can appear in as little as 30 days after planting and progress throughout the remainder of the growing season. In addition, asymptomatic[1] plants have been reported in previous field studies based on ELISA results (Culbreath et al. 1992). Incidence of asymptomatic infections has been reported to be as high as the incidence of symptomatic peanut plants (Culbreath et al. 2003).

[1] Plant that has the pathogen (TSWV) but does not express any foliar symptoms

The only known method of transmission of the virus is through certain species of thrips (singular or plural), which have previously acquired the virus by feeding on other infected plants (Culbreath et al., 2010). Three species of thrips (Thysanoptera) are the primary vectors for TSWV in agricultural production: tobacco thrips [*Frankliniella fusca (*Hinds)], western flower thrips [*Frankliniella occidentalis* (Pergande)] and onion thrips (*Thrips tabaci*). Other thrips species that vector TSWV are found in United States, but have not been associated as a significant vector of the virus in peanut (Culbreath et al., 2003). In peanut, tobacco thrips [*Frankliniella fusca (*Hinds)] and western flower thrips are the most common vectors of TSWV (Todd et al., 1996 and Webb et al., 1997).

Prior to spotted wilt in peanut, growers began planting their crop in mid-April at seeding density of 13.1 seed m^{-1} and would generally have planted a high percentage of their acres during this early spring time period. However, since the occurrence of spotted wilt, peanut growers are planting mainly in May at an increased seeding density of 19.7 seed m^{-1}.

Planting date manipulation is a primary control measure used to reduce spotted wilt incidence and severity. Planting in May versus April is believed to avoid high thrips populations during the month of April compared to lower populations in May. However, the effectiveness of this control measure can vary from year to year and as with all control measures for spotted wilt, it is preventative and its effect cannot be predicted prior to the growing season. Although management of planting date is a valuable instrument for suppressing spotted wilt, it does have limitations in practical application for large scale operations (Culbreath et al., 2003). Due to the sheer size and number of fields to be planted, limitations on equipment and labor, and the uncertainty of weather, most growers cannot plant all of their acreage in the “optimum” planting window (Culbreath et al., 2003). Furthermore, utilizing planting date alone to minimize spotted wilt incidence may not be adequate to prevent significant losses (Culbreath et al., 2003).

Another common method used to reduce risk of spotted wilt in peanut is seeding density. Results have demonstrated a decrease in the incidence of spotted wilt in peanut as seeding density increases (Wehtje et al., 1994). In Florida, spotted wilt incidence, pod yields, and grades were affected by in-row plant space modification among several genotypes (Gorbet and Shokes, 1994). Similar reductions in incidence of spotted wilt and an increase in pod yield of several cultivars were seen as seeding densities increased (Branch et al., 2003). These reports justify the use of high seeding density but they do not address the associated cost. Increased seed cost coupled with larger seeded cultivars, means that seed costs are high. Therefore, growers would benefit from reduced seeding density in order to minimize production cost and in return improve profitability. Development of resistant cultivars might allow planting during the month of April and/or at a lower seeding density, but current cultivars do not have sufficient resistance for that purpose.

Minimizing these and other production practices would allow peanut growers in the Southeast to return to pre-spotted wilt cultural practices, which could decrease buying point congestion and increase growers flexibility with other crop commodities. The objective of this study was to determine the effect of planting date and seeding density on spotted wilt symptoms and infection in two advanced peanut breeding lines with superior resistance to spotted wilt.

Materials and Methods

Experimental Design and Location

Field experiments were conducted at the North Florida Research and Education Center (NFREC), near Marianna, Florida for three consecutive years, 2010-2012. The research site was chosen because of a history of severe spotted wilt epidemics. Management of experiments utilized commercial peanut cultural practices following current UF extension guidelines for peanut in the region. Soil preparation utilized conventional tillage consisting of offset disking, level harrowing, deep moldboard plowing, then level harrowing with two field cultivations just prior to planting (early spring). All plots were irrigated using an overhead center pivot as needed. The soil type was a Chipola loamy sand (Loamy, kaolinitic, thermic Arenic Kanhapludults). Prior to peanut plot establishment, fields were planted in maize (*Zea mays* L.) followed by cotton (*Gossypium hirsutum* L.). No insecticides were applied either foliar or in-furrow in order to maximize thrips-vector pressure on peanut plants.

The experimental design was a randomized complete block with a split-split plot treatment arrangement and three replications. Factorial treatments consisted of three planting dates (main plots), two seeding densities (subplots), and four cultivars (sub-subplots). Planting dates corresponded to three different risk levels for spotted wilt (30pts, 15pts, & 5pts) according to the Peanut Rx (Culbreath et al., 2010), which were separated by 2 week intervals. Plots were planted on 16 April, 30 April, and 14 May in 2010; on 15 April, 29 April, and 13 May in 2011; on 13 April, 27 April, and 11 May in 2012. Seeding densities corresponded to two different risk levels (15pts & 5pts) according to the Peanut Rx (Culbreath et al., 2010). Seeding densities were 13.1 and 19.7 seed m^{-1} of row and plant populations were determined in all plots by counting emerged plants (14 to 21 DAP) and individual tap roots immediately after each digging date following plot inversion. Genotypes tested were Florida-07 (10pt. risk), Georgia Green (30pt. risk), and two advanced breeding lines from the UF Peanut Breeding Program known as Florida EP^{TM} '113' (Tillman and Gorbet, 2012) and UFT-312. Peanut plots consisted of two 4.6 m long rows spaced 91 cm apart.

Disease Assessment

Prior to digging, plants in each plot were evaluated for spotted wilt disease using two visual ratings: disease intensity rating (DIR) and a 1 to 10 scale. The DIR represents a combination of incidence and severity that was calculated by counting the number of foci (1/2 to 1 foot sections of diseased row) of severely diseased plants for each individual plot (Culbreath et al., 1997). This method was originally adapted from a similar method used for incidence of southern stem rot in peanut (Rodriguez et al., 1975). There were a maximum number of 30 foci possible in each plot. Severity of spotted wilt was also evaluated on a rating scale of 1 to 10 where each value represents a percentage of plants severely diseased in the plot. In this trial, 1 = 10% or less, 2 = 11-20%, 3 = 21-30%, 4 = 31-40%, 5 = 41-50%, 6 = 51-60%, 7 = 61-70%, 8 = 71-80%, 9 = 81-90%, and 10 = 91-100%.

For mid-April planting dates, spotted wilt was evaluated at 97 DAP, 110 DAP, 123 DAP, and 138 DAP in 2010; 95 DAP, 108 DAP, and 129 DAP in 2011; 95 DAP, 108 DAP, 126 DAP, and 136 DAP in 2012. In late-April planting dates, spotted wilt disease was evaluated at 83 DAP, 97 DAP, 110 DAP, 123 DAP, and 138 DAP in 2010; 81 DAP, 95 DAP, 108 DAP, and 129 in 2011; 80 DAP, 95 DAP, 108 DAP, 126 DAP, and 136 DAP in 2012. Mid-May planting dates, spotted wilt was evaluated in all plots at 70 DAP, 83 DAP, 97 DAP, 110 DAP, 123 DAP, and 138 DAP in 2010; 68 DAP, 81 DAP, 95 DAP, 108 DAP, and 129 DAP in 2011; 80 DAP, 95 DAP, 108 DAP, 126 DAP, and 136 DAP in 2012.

Pod Yield

Peanut plots were dug according to maturity class with a standard two row conventional peanut digger-shaker-inverter manufactured by KMC[2]. Maturity was determined visually and by the hull scrape method (Williams and Drexler 1981) based on samples from adjacent border rows that were established on the first planting date (mid-April) in all three years. Digging of the subsequent planting dates was separated by 2 week intervals to maintain consistency over the study. In 2010, Florida EP™ '113', UFT-312, Florida-07, and Georgia Green were dug and inverted on 2 September, 17 September, and 1 October for the three respective planting dates. In 2011, Florida EP™ '113', UFT-312, Florida-07, and Georgia Green were dug and inverted on 31 August, 1 September, and 26 September for the three respective planting dates. In 2012, Florida EP™ '113', UFT-312, Florida-07, and Georgia Green were dug and inverted on 31 August, 14 September, and 28 September for the three respective planting dates.

Inverted plants were allowed to dry in the field for 3 to 4 days before harvesting with small stationary peanut threshing equipment. Once pod samples were collected in the field, they were placed in forced-air drying wagons and allowed to dry at 35°C for 12 to 18 hours until 10.5 percent moisture was obtained based on a standard grain meter calibrated to measure moisture of peanuts. After curing, samples were cleaned with de-

[2] Kelley Manufacturing Corporation, Tifton, GA 31793

stemmer saws removing foreign material which was not removed during the threshing process, and weighed for pod yield.

Peanut Grades

Each plot was graded following the USDA procedures for peanut. In addition to de-stemming and foreign material removal, sub-samples were further cleaned by hand before grading to remove all loose shelled kernels (LSK) and other non-related foreign matter (i.e. damage caused by small plot threshers) not normally seen by mechanically picked peanuts. Percent of LSK and other foreign related material was not recorded in this trial. Grading samples consisted of a two hundred gram pod sample derived as described above. To estimate pod maturity, 20 random 2-cell pods were selected and maturity was recorded by inspecting the inside of the hull for color (white, brown, or black) and pressure points located within the hull. Pods were sized to determine the percentage of the sample classified as virginia pods. Subsequently, pods were shelled and the seeds were classified. The extra-large kernels (shelled), medium kernels (shelled), number one kernels (shelled), were summed to calculate sound mature kernels (SMK). Sound splits (SS) and other kernels (OK) were also shelled measurements collected. Final pod grades were analyzed and presented as percent of total sound mature kernels (TSMK), which is the sum SMK plus SS.

Statistical Analysis

All data collected was subjected to analysis of variance (ANOVA). Data were analyzed using the Mixed Procedure of SAS (SAS v.9.3; SAS Institute, Cary NC) with planting date, seeding density, cultivar, and all their interactions as fixed effects, with block nested within planting date and all interactions with year and replication considered random effects. Differences in treatment means were considered significant at the $p \leq 0.05$ level using Tukey-Kramer Honestly Significant Differences (HSD) Multiple Comparison method adjustment for protection.

Results and Discussion

Planting Date and Seeding Density

Mid-April, late-April and mid-May planting dates did not affect pod yield, spotted wilt ratings or TSWV incidence. Seeding densities of 13.1 and 19.7 seed m^{-1} did not affect pod yield, grading characteristics such as total sound mature kernels, or visual spotted wilt disease symptoms.

Pod Yield

Pod yield was not affected by interactions year by planting date, year by seeding rate, or year by cultivar; therefore, data was not analyzed separately by year. Pod yield varied among cultivars, cultivar x planting date, and planting x seeding density. Average pod yields were 6,733, 5,480, 5,215, and 5,130 kg ha^{-1} (S.E. 530), for Florida-07, Florida EP^{TM} '113', Georgia Green, and UFT-312, respectively (Table 3-2). Florida-07 pod yield was higher than Florida EP^{TM} '113', Georgia Green and UFT-312 indicating its superior yield potential in moderate to low disease environments.

Pod yield varied among cultivars when planted in mid-April, but not in late-April or mid-May (cultivar x planting date interaction) (Figure 3-2). Florida-07 yields were higher than Florida EP^{TM} '113', Georgia Green, and UFT-312; however, no differences were found among Florida EP^{TM} '113', Georgia Green, and UFT-312. Although there was cultivar x planting date interaction, rank changes were minimal indicating that pod yield of most cultivars respond similarly across planting dates.

Pod yield was lower in mid-April compare to mid-May when planted at 13.1 seed m^{-1} seeding density, but they were the same at 19.7 seed m^{-1} seeding density (planting date x seeding density interaction) (Figure 3-3). These results suggest that genotypes with higher field resistance to spotted wilt respond better to lower seeding density when planted in April. Additional work on plant populations is needed to determine the optimal density since higher pod yields were obtained with the lower seeding density in this present study.

Grades

TSMK varied among cultivars (Table 3-2) with 78.1%, 77.5%, 76.3% and 74.7% (S.E. 0.82%) for Florida EP^{TM} '113', Georgia Green, UFT-312 and Florida-07, respectively. TSMK also varied among planting dates (Table 3-3) with 77.6%, 76.7%, and 75.7% (S.E. 0.81%) for mid-May, late-April and mid-April, respectively. Higher TSMK in mid-May plantings could also have been a result of more even pod maturity since plants are not stressed by cool temperatures as are those planted in April.

Plant Stand

Tap root counts varied between seeding densities (Table 3-4) with means of 122 roots per plot and 89 roots per plot (S.E. 5) for seeding densities of 19.7 and 13.1 seed m^{-1}, respectively. Cultivars did not vary in tap root counts with 110 roots per plot, 107 roots per plot, 106 roots per plot, and 100 roots per plot (S.E. 7) for UFT-312, Georgia Green, Florida-07, and Florida EP^{TM} '113', respectively. Emerged plant stand counts differed between the two seeding densities (Table 3-4) with 141 plants per plot and 100 plants per plot (S.E. 4) for seeding densities of 19.7 and 13.1 seed m^{-1}, respectively. Emerged plants were not different among cultivars with 127, 119, 119 and 116 (S.E. 5) for Georgia Green, UFT-312, Florida-07, and Florida EP^{TM} '113', respectively. Neither emerged counts nor tap root counts averaged over both seeding densities differed among Florida-07, Georgia Green, Florida EP^{TM} '113' and UFT-312. Results suggest that cultivars did not differ in germination or in field emergence. However, tap root counts seem to be more reliable estimates of final plant stand compared to emergence counts especially in the 19.7 seed m^{-1} density because it is hard to distinguish between individual seedlings at such a young age and double counts likely occurred.

Disease Assessment

Final foliar disease assessment ratings, which were taken just prior to harvest from both DIR 0 to 30 and the 1 to 10 scale indicated differences among the genotypes. Georgia Green had more spotted wilt than Florida-07, Florida EP^{TM} ‘113’ and UFT-312 with 2.4, 1.4, 1.2, and 1.0 (S.E. 0.10) on the 1 to 10 scale. Similar results were obtained from the DIR with Georgia Green being higher than Florida-07, Florida EP^{TM} ‘113’ and UFT-312 with 3.2, 1.4, 1.2, and 0.4 (S.E. 0.38). Foliar symptoms were more prevalent in Georgia Green, than Florida-07, Florida EP^{TM} ‘113’, and UFT-312. Results demonstrated the low disease pressure conditions during the three years of this study. The influence of planting date and seeding density were not major factors in decreasing visual symptoms of spotted wilt in this study.

Summary and Discussion

Planting date, seeding density and cultivar selection are important factors used to reduce risk of losses to spotted wilt in peanut. The most critical factor is genetic resistance (Tillman et al., 2006). Results in this present study validate the importance of cultivar in managing spotted wilt disease. Although spotted wilt epidemics occurred in all three years of the field experiment, the disease was less severe than in previous seasons. Results from our trials do not reflect historical patterns of spotted wilt epidemics on standard cultivars Georgia Green and Florida-07 when planted in mid to late April at 13.1 seed m^{-1}. Explanations for this phenomenon are unknown at this point; however, through ELISA testing, we know that the pathogen (TSWV) was present in the field during all three years of this study (see Chapter 4). In addition, thrips feeding was observed in all plots in every year between 30 and 60 DAP, but thrips were not sampled.

In conclusion, Florida-07 and Georgia Green pod yields were higher than expected on April planting dates at seeding density of 13.1 seed m^{-1}, which is an indication of their yield potential in moderate to low disease environments. Lower yield potential of the breeding lines may be a result of lower harvest index, but they were competitive with Georgia Green, a cultivar that dominated production in the southeastern U.S. for about 10 years. Florida EP™ ‘113’ and UFT-312 showed promising resistance to spotted wilt in the field and more so than the most resistant cultivars reported to date. The two breeding lines may not be desirable to peanut growers in the southeast because of their lower yields, but they would make excellent parenteral lines for introgression of resistance to spotted wilt.

Table 3-1. Probability values from ANOVA for effects of planting date, seeding densities, and cultivar on several traits averaged over three consecutive years, 2010-2012, in Marianna, Florida.

		TSMK[1]	DIR[2] 0-30	Rating[3] 1-10	Tap root	Emerged plant	Pod yield
Source	df	p	p	p	p	p	p
Cultivar (C)	3	0.0016	0.0040	0.0001	0.7108	0.3700	<.0001
Planting Date (P)	2	0.0425	0.9262	0.7424	0.7847	0.7116	0.2374
Seeding Density (D)	1	0.2983	0.3999	0.9596	0.0118	0.0034	0.6676
C x P	6	0.3463	0.2556	0.8791	0.1536	0.2107	0.0029
C x D	3	0.7142	0.1007	0.9112	0.1780	0.0003	0.8702
P x D	2	0.2234	0.8502	0.2496	0.0325	0.6198	0.0134
C x P x D	6	0.1412	0.8224	0.7682	0.6079	0.8313	0.1553

[1] Total sound mature kernel (TSMK) taken during the grading process
[2] Disease intensity rating 0 to 30 (0= no disease and 30= all plants diseased or dead in plot)
[3] Rating scale 1 to 10 (1= little to no disease and 10= all plants diseased or dead in plot)

Table 3-2. Variation in average pod yield, TSMK, rating scale 1 to 10, DIR 0 to 30, tap root crown counts, and emerged plant population over three years, 2010-2012, in Marianna, Florida.

Cultivar	kg/ha[1]	TSMK avg[2]	Rating[3] 1-10	DIR[4] 0-30	Tap root	Emerged plant
Florida-07	6,733 a	74.7 c	1.4 b	1.4 a	106 a	119 a
Florida EP™ '113'	5,480 b	78.1 a	1.2 b	1.2 b	100 a	116 a
Georgia Green	5,215 b	77.5 ab	2.4 a	3.2 b	107 a	127 a
UFT-312	5,130 b	76.3 bc	1.0 b	0.4 b	110 a	119 a
LSD[5] $_{(0.05)}$	530	0.82	0.10	0.38	7	5

[1] Adjusted least square means based on kilogram per hectare
[2] Adjusted least square means based on 200 gram samples
[3] Rating scale 1 to 10 (1= little to no disease and 10= all plants diseased or dead in plot)
[4] Disease intensity rating 0 to 30 (0= no disease and 30= all plants diseased or dead in plot)
[5] Means separated using T-K HSD multiple comparison for protection in each column. Means within a column with the same letter do not differ at P<.05.

Table 3-3. Planting date effect on TSMK averaged over three years (2010-2012).

	Total sound mature kernels (TSMK) %	
Planting Date	TSMK avg[1]	Group[2]
Mid-April	75.7	B
Late-April	76.7	AB
Mid-May	77.6	A
LSD[3] $_{(0.05)}$	0.81	

[1] Adjusted least square means based on 200 gram samples
[2] Means within a column with the same letter do not differ at P<.05
[3] Means separated using T-K HSD multiple comparison for protection in each column.

Table 3-4. Seeding densities averaged over three years (2010-2012).

	Seeding populations	
Density	Emerged count[1]	Tap root count[1]
19.7 seed m^{-1}	141 a	122 a
13.1 seed m^{-1}	100 b	89 b
$LSD^{2}_{(0.05)}$	4	5

[1]Means within a column with the same letter do not differ at P<.05
[2]Means separated using T-K HSD multiple comparison for protection in each column.

Figure 3-1. Yield test plots with low disease incidence of spotted wilt near Marianna, Florida in 2010. The experiment contained Florunner between every two plots to serve as susceptible host ‘spreader rows’ to encourage spotted wilt disease.

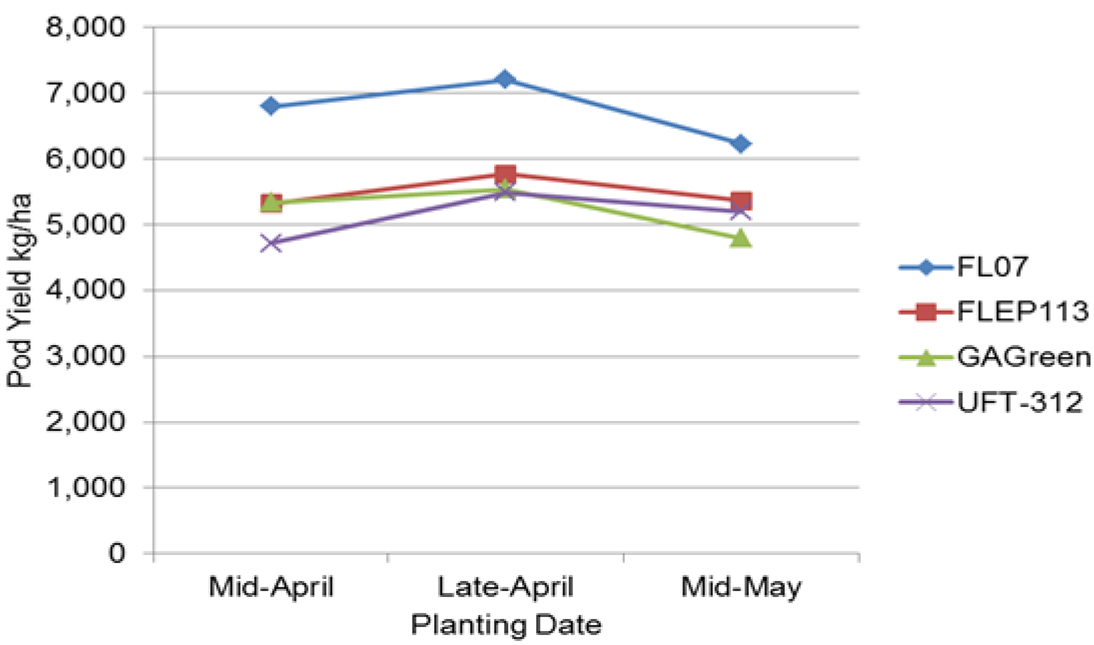

Figure 3-2. Interaction effect of cultivar x planting date on pod yield averaged over the three consecutive year (2010-2012) study near Marianna, Florida.

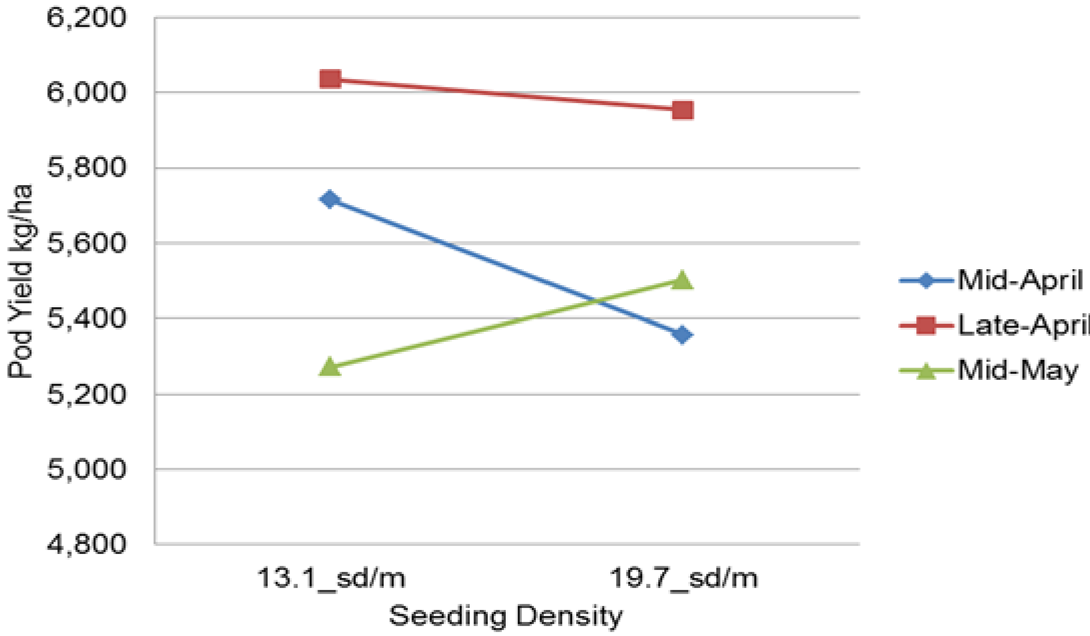

Figure 3-3. Interaction effect of planting date x seeding densities on pod yield averaged over the three consecutive year (2010-2012) study near Marianna, Florida.

CHAPTER 4
EFFECT OF CULTIVAR AND PLANTING DATE ON TSWV INFECTION IN PEANUT

Introduction

Spotted wilt, caused by *Tomato spotted wilt virus* (TSWV, genus *Tospovirus*, family *Bunyaviridae*), is a major disease that has impacted peanut production for growers in the southeastern United States since the mid-late 1990's. The disease was first described in the United States in 1971 on peanuts in Texas (Halliwell and Philley 1974, Kucharek et al., 1990, Hagan and Weeks 1998) and has spread throughout all of the peanut producing regions of the United States (Rowland et al., 2005). Spotted wilt of peanut can cause serious yield reductions resulting in large economic losses for growers (Tillman et al., 2006). The industry's greatest loss occurred in 1997 in Georgia alone, spotted wilt caused an estimated $40 million in monetary losses on peanut (Bertrand, 1998). The disease continues to be a yield reducing factor in peanuts in the state of Georgia (Pappu et al., 1999), and other peanut producing states within the southeastern United States.

The only known method of transmission of the virus is through certain species of thrips (singular or plural), which have previously acquired the virus by feeding on other infected plants (Culbreath et al., 2010). TSWV has been reported to infect over 650 species of plants, including more than 50 families in both monocotyledonous and dicotyledonous plants (Culbreath et al., 2003), suggesting it has an extensive host range. Some infected peanut plants are either severely stunted for the duration of their life, or die before reaching maturity. Disease symptoms in peanut from spotted wilt are variable in degree and severity. Symptoms include stunting of plant, mottling, concentric ring spots, and chlorosis of the leaflets; furthermore, the seed is small, shriveled, and can have discolored and/or cracked seed coats. Spotted wilt symptoms can appear in as little as 30 DAP and progress throughout the remainder of the growing season.

In addition, asymptomatic[1] plants have been reported in previous field research studies (Culbreath et al. 1992); based on ELISA results when no foliar symptoms are noticed. Incidence of asymptomatic infections has been reported to be as high as the incidence of symptomatic peanut plants (Culbreath et al. 2003). Therefore, the use of ELISA testing for detecting the presence or absence of TSWV may be the clearest way of delineating new resistant types from other genotypes. The problems with ELISA testing are high cost for the peanut breeding program and time constraints given the sheer number of breeding plots.

Two major control methods used to reduce epidemics of spotted wilt by suppressing severity and incidence on peanut are planting date and cultivar selection. Presently, no peanut cultivars are available to the growers that can manage spotted wilt alone; therefore, other major production practices have to be used in combination with each other in order to reduce spotted wilt severity. The three most commonly grown varieties in the southern United States are Florida-07, Georgia-06G, and Tifguard. The dominant variety is Georgia-06G with over 75 percent of the peanut acreage planted with this cultivar. Culbreath et al., (2013) reported field resistance in Florida-07, Georgia-06G, and Tifguard was sufficient to allow planting them at reduced seeding densities as compared to Georgia Green. However, these cultivars are still at some risk of spotted wilt disease if they are planted in mid-April with the past (prior to spotted wilt)

[1] Plant that has the pathogen (TSWV) but does not express any foliar symptoms

extension recommended seeding density 13.1 seed m^{-1}. Therefore, breeding efforts have continued in the areas of improved spotted wilt resistance so farmers could potentially return to mid-April planting with reduced seeding densities.

The primary objective of this research was to further investigate two new cultivars Florida EP™ '113' and UFT-312 under various field conditions in order to characterize their infection to TSWV compared to the commercial cultivars Florida-07 and Georgia Green. The effect of planting date and the importance of cultivar selection in management of spotted wilt disease of peanut is well documented. However, the potential impacts of new, highly resistant genotypes under traditional or historical peanut production practices have not been evaluated and need to be described to determine if the breeding efforts have made significant progress on spotted wilt resistance to allow farmers to plant at seeding densities of 13.1 seed m^{-1} during mid-April. This combination of planting date and seeding density was very common and the extension recommendation prior to the onset of spotted wilt. If new genotypes prove to have resistance sufficient to obviate planting date and seeding density as important management factors for spotted wilt, growers could reduce production costs by reducing seeding density and spread planting to solve planting and harvest logistics caused by a narrow planting window. These problems have plagued farmers since the mid-1990s when spotted wilt became a common problem to all growers in the southeast. Studies were conducted to compare two new peanut cultivars with commercial standards relative to pod yield, market pod grading characteristics, and spotted wilt ratings. Cultivars Georgia Green (Branch, 1996) and Florida-07 (Gorbet and Tillman, 2007) were chosen based on their variable levels of field resistance to spotted wilt to compare to two new spotted wilt resistant cultivars tested as Florida EP™ '113' (Tillman and Gorbet 2012) and UFT-312.

Materials and Methods

Experimental Design and Location

Studies were conducted at the North Florida Research and Education Center (NFREC), near Marianna, Florida for three consecutive years (2010-2012). The research site was chosen based on past epidemics and increased pressure of spotted wilt disease on peanuts. Management of experiments utilized commercial peanut production l practices following current UF extension guidelines for peanut. Soil preparation utilized conventional tillage consisting of offset disking, level harrowing, deep moldboard plowing, then level harrowing with two field cultivations just prior to planting (early spring). All plots were irrigated using an overhead center pivot as needed. The soil type was a Chipola loamy sand (Loamy, kaolinitic, thermic Arenic Kanhapludults). Prior to peanut plot establishment, fields were planted in maize (*Zea mays* L.) followed by cotton (*Gossypium hirsutm* L.). No insecticide applications were applied either foliar or in-furrow in order to maximize thrips-vector pressure on peanut plants.

The experimental design was a randomized complete block with a split-split plot treatment arrangement and three replications. Factorial treatments consisted of three planting dates (whole plots), two seeding densities (subplots), and four cultivars (sub-subplots). Planting dates corresponded to three different risk levels (30pts, 15pts, & 5pts) according to the Peanut Rx (Culbreath et al., 2010), which were separated by 2 week intervals. Plots were planted on 16 April, 30 April, and 14 May in 2010; on 15 April, 29 April, and 13 May in 2011; on 13 April, 27 April, and 11 May in 2012. Seeding densities corresponded to two different risk levels (15pts & 5pts) according to the Peanut Rx (Culbreath et al., 2010). Seeding densities were 13.1 and 19.7 seed m^{-1} and plant populations were determined in all plots by counting emerged plants (14 to 21 DAP) and individual tap roots immediately after each digging date following plot inversion. Genotypes tested were Florida-07 (10pt risk), Georgia Green (30pt risk), and two advanced breeding lines from the UF Peanut Breeding Program known as Florida EP™ '113' (Tillman and Gorbet, 2012) and UFT-312. Peanut plots were established on single rows spaced 91 centimeters (cm) apart and rows were 4.6 meters (m) long with two rows in each plot.

Disease Assessment

Prior to digging, plants in each plot were evaluated for spotted wilt disease using two visual ratings: disease intensity rating (DIR) and a 1 to 10 scale. The DIR represents a combination of incidence and severity that was calculated by counting the number of foci (1/2 to 1 foot sections of row) of severely diseased plants for each individual plot (Culbreath et al., 1997). This method was originally adapted from a similar method used for incidence of southern stem rot in peanut by (Rodriguez et al., 1975). There were a maximum number of 30 foci possible in each plot. Severity of spotted wilt was also evaluated on a rating scale of 1 to 10 where each value represents a percentage of plants severely diseased in the plot. In this trail, 1 = 10% or less, 2 = 11-20%, 3 = 21-30%, 4 = 31-40%, 5 = 41-50%, 6 = 51-60%, 7 = 61-70%, 8 = 71-80%, 9 = 81-90% and 10 = 91-100%. In addition to foliar symptoms of the canopy, seed coat symptoms caused by TSWV were inspected visually by randomly selecting 50 seeds out of the SMK. Then were weighed separately and rated. Ratings consisted of seed coat discoloration (red, pink, purple, brown), constriction, shriveling, and cracking

(Figure 4-2). Average seed weight and number of seeds showing symptomology were recorded so data could be used to develop a correlation analysis.

ELISA Sampling

Plants were sampled immediately after digging while the plant tissue was still fresh. Tap root crowns were collected after population counts were finalized and placed in brown paper bags and allowed to dry using circular fans in the lab (Figure 4-4). Viral detection was assessed using the ImmunoStrip Kit (ISK) (Agdia Inc., Elkhart, IN, USA) specifically designed for Tomato spotted wilt virus (TSWV) in plant tissue. ISK assay kit tests were used for testing the frequency of viral infection on individual peanut plants following manufacturer's directions (Figure 4-5). Antibodies were stored at 4-6 ºC (not allowed to freeze) until all root crowns were collected from the field and allowed to dry. Plant samples between 0.4 to 0.6 grams (Figure 4-7b) of root crown tissue were placed in a sample extract pouch, which were prefilled with SEB1 (sample extract buffer # 1) buffer solution containing 3 milliliters per bag. Root crowns were cleaned by hand during the removal of the all the lateral roots from the main tap root. Each extraction pouch was opened using standard office scissors, crowns were placed between mesh screens, bags were sealed briefly with fingers, and then tissue became emulsified by hammering with a mallet (Figure 4-7c). Once solution settled, test strips were inserted into the channel portion (no mesh) of the bag and allowed to set for one hour in an upright position, so fluid did not escape from the extract bag. Results developed in as little as 5 minutes but were not recorded until an hour passed in order to allow lower titer samples time to develop (Figure 4-6). Any test strip reading that indicated an invalid result was discarded. A total of 10 randomly selected root crowns were tested from all 3 planting dates, 3 replications from only the 13.1 seed m^{-1} plots.

Statistical Analysis

Data were subjected to analysis of variance (ANOVA) using the Mixed Procedure of SAS (SAS v.9.3; SAS Institute, Cary NC) with planting date, cultivar, and planting date x cultivar interaction considered as fixed effects, with block nested within planting date and all interactions with year and replication considered random effects. Differences in treatment means were evaluated using Tukey-Kramer Honestly Significant Differences (HSD) Multiple Comparison method adjustment for protection. Probability was set at $P<0.05$ and considered different if less than 0.05. Seeding density differences were not characterized through ELISA testing in this study because of time constraints, equipment and labor limitations. In addition, to analysis of variance, a correlation analysis was conducting using PROC CORR of SAS (SAS v.9.3; SAS Institute, Cary NC) to further examine the relationship among visual spotted wilt ratings and TSWV infection.

Results and Discussion

TSWV Infection

Planting date had no effect on TSWV infection frequency, DIR, TSWV rating, or seed coat symptoms (Table 4-1). TSWV infections varied among cultivars ($p=0.0001$). Incidence of TSWV infections averaged ($n=270$) for each cultivar over three planting dates with three replication over three consecutive years study were 67, 44, 10, and 4 percent (S.E. 7.0), for Georgia Green, Florida-07, Florida EP^{TM} '113' and UFT-312, respectively (Figure 4-1). TSWV infection in Georgia Green was higher than Florida-07, Florida EP^{TM} '113' and UFT-312 indicating it was the most susceptible cultivar. Florida-07 had lower TSWV infection than Georgia Green, but higher than Florida EP^{TM} '113' and UFT-312 and represented a cultivar with moderate TSWV infection. Infection of

TSWV in Florida EP^{TM} '113' and UFT-312 TSWV was similar but lower than Florida-07 and Georgia Green. The two advanced breeding lines were highly resistant to TSWV infections and therefore, showed little to no disease symptoms.

Disease Assessment

Final foliar disease assessment ratings, which were taken just prior to harvest from both DIR and the 1 to 10 scale indicated differences among the genotypes. Georgia Green had more spotted wilt than Florida-07, Florida EP^{TM} '113' and UFT-312 with 2.4, 1.4, 1.2, and 1.0 (S.E. 0.10) on the 1 to 10 rating scale. Similar results were obtained from the DIR with Georgia Green being higher than Florida-07, Florida EP^{TM} '113' and UFT-312 with 3.2, 1.4, 1.2, and 0.4 (S.E. 0.38) respectively. Seed coat symptoms taken during the grading process indicated differences among the cultivars. Georgia Green had the most seed coat symptoms following by Florida-07 which was lower than Georgia Green but higher than Florida EP^{TM} '113' and UFT-312 with 15.7, 7.0, 2.8, and 1.5 (S.E. 0.72) symptomatic seeds out of 50 evaluated. Foliar symptoms were more prevalent in Georgia Green, than Florida-07, Florida EP^{TM} '113', and UFT-312. However, foliar symptoms were not highly correlated with TSWV infection as were seed coat symptoms (Table 4-3). Nonetheless, results show that disease pressure was low and that the influence of planting date was minimal.

Seed Coat Symptomology

To date, this is the first study in peanut to describe the important trait of seed coat symptomology and its relationship with the viral pathogen TSWV. Seed quality has always been a major factor in peanut processing. One of the primarily methods that the shelling industry uses to maintain high seed quality is to remove all the dark, purple to red spotted or blemished kernels. This process has been traditionally accomplished by manual selection. New technological advancements have equipped modern shelling facilities with color sorting machines (ScanMaster^{TM}) which automatically reject kernels reflecting certain shades of light inconsistent with desirable seed coat color parameters (Manual 1998). Therefore, cultivars susceptible to high incidence of seed coat symptoms may be at a disadvantage in the market. This creates the need for peanut breeding programs to develop new cultivars that are more resistant to seed coat blemishes associated with spotted wilt. In this present study, seed coat symptomology is the only consistent visual disease symptom that serves as a good predictor of infection by TSWV. These findings have huge implications on both growers and breeders. TSWV infection causes reduced pod yields and could cause rejection of seeds with blemished seed coats. Developing cultivars with resistance to both foliar and seed coat symptoms of spotted wilt could minimize the impact on both.

Summary and Discussion

In general, spotted wilt epidemics were low in all treatments and followed this pattern throughout the three consecutive (2010-2012) year field study. ELISA assessment indicates that cultivar was the most significant factor in reducing TSWV infection. Peanut genotypes Florida EP^{TM} ‘113’ and UFT-312 had very low levels of TSWV which make them good candidates for both large and small scale growers who may want to start planting a high percentage of their peanut acreage in the month of April at a reduced seeding density of 13.1 seed m^{-1}.

According to a correlation analysis (Table 4-5), symptomology based on the DIR and the 1-10 scale were highly correlated with each other (r=0.73); however, there was a poorer relationship between foliar symptoms and actual TSWV infection with DIR (r=0.42) and 1-10 scale (r=0.55). These results indicate that foliar symptomology is not as reliable in assessing genotype resistance as is TSWV infection, especially in seasons when disease pressure is low. The strongest correlation observed was between seed coat symptoms (visual assessment) and TSWV infection (r=.77). Seed coat symptoms of spotted wilt were evaluated as part of the grading process during the winter season. Even though visual assessments from both foliar ratings of symptomatic plants varied among cultivars, they did not correlate with TSWV presence or absence in the root crown. Georgia Green had the highest incidence of TSWV infection compared to Florida-07, Florida EP^{TM} '113' and UFT-312 even though 44% of Florida-07 plants were infected with TSWV. This suggests that seed coat symptomology may be a better means of assessing TSWV resistant lines than current foliar evaluation methods commonly used in peanut breeding programs in the United States.

Evaluations of spotted wilt severity based exclusively on visible foliar symptoms may underestimate the actual presence of TSWV, since asymptomatic plants are common. In years with minimal phenotypic symptomology or when asymptomatic plants are present, such as in this study, foliar symptoms are not as reliable as seed coat symptoms in identifying resistant genotypes such that breeding programs may be inadvertently advancing and releasing cultivars with unacceptable susceptibility to spotted wilt. Seed coat symptomology was the only visual disease symptom that served as a good predictor for the presence or absence of TSWV and is less expensive than ELISA testing. Breeding programs cannot afford to misdiagnose escape for spotted wilt resistance. Utilizing seed symptomology could help to eliminate susceptible genotypes from the breeding populations and perhaps avert release of a spotted wilt susceptible cultivar.

Even though foliar symptomology was minimal, this study revealed through ELISA testing that TSWV is still in our agricultural ecosystem (Figure 4-1) and is therefore capable of causing disease. The reason for the lack of spotted wilt disease on peanut during those seasons remains unknown, but the disease is known to vary from season to season. Nevertheless, these results confirm the importance of cultivar selection in management of spotted wilt. Florida EP^{TM} ‘113’ and UFT-312 have exceptional resistance to spotted wilt apparently because they are less likely to become infected by TSWV than other standard cultivars. Other studies have reported lower levels of TSWV infection with advanced breeding material, but never as low as Florida EP^{TM} ‘113’ and UFT-312 as demonstrated in this research. Data suggest Florida EP^{TM} ‘113’ and UFT-312 have low enough infection levels to TSWV that if called upon, they could be planted in high risk situation without severe loss due to spotted wilt. In fact, similar TSWV infection levels in future cultivars may help growers negate major production factors that have constrained the industry since spotted wilt became an issue in the mid-1990s.

Table 4-1. Probability values from ANOVA for Disease intensity rating, Rating scale, Seed coat symptoms, and TSWV infection on tap roots during, 2010-2012, in Marianna, Florida.

		DIR[1] 0-30	Rating[2] 1-10	Seed coat[3] symptoms	TSWV[4] infection
Source	df	p	p	p	p
Cultivar (C)	3	0.0040	0.0001	<.0001	0.0001
Planting Date (P)	2	0.9262	0.7424	0.9241	0.1773
Seeding Density (D)	1	0.3999	0.9596	0.8491	N/A
C x P	6	0.2556	0.8791	0.5594	0.3957
C x D	3	0.1007	0.9112	0.4884	N/A
P x D	2	0.8502	0.2496	0.3112	N/A
C x P x D	6	0.8224	0.7682	0.1664	N/A

[1] Disease intensity rating 0 to 30 (0= no disease and 30= all plants diseased or dead in plot)
[2] Rating scale 1 to 10 (1= little to no disease and 10= all plants diseased or dead in plot)
[3] Seed coat symptoms taken during the grading process [50 random (SMK)]
[4] TSWV infection frequency using tap root crowns from all 13.1 seed m^{-1} plots

Table 4-2. Various spotted wilt ratings averaged over three years, 2010-2012, in Marianna, Florida.

	Spotted wilt severity		
Cultivar	Rating [1] 1-10	DIR [2] 0-30	Seed Coat[3] No. out of 50
Georgia Green	2.4 a	3.2 a	15.7 a
Florida-07	1.4 b	1.4 b	7.0 b
Florida EP™ ‘113’	1.2 b	1.2 b	2.8 c
UFT-312	1.0 b	0.4 b	1.5 c
LSD[4].$_{(0.05)}$	0.10	0.38	0.72

[1] Rating scale 1 to 10 (1= little to no disease and 10= all plants diseased or dead in plot)
[2] Disease intensity rating 0 to 30 (0= no disease and 30= all plants diseased or dead in plot)
[3] Number of seeds with symptoms of spotted wilt out of 50 random (SMK)
[4] Means separated using T-K HSD multiple comparison for protection in each column
Means within a column with the same letter do not differ at P<.05

Table 4-3. Correlation among 6 spotted wilt symptoms in four peanut genotypes averaged over three consecutive years, 2010-2012, in Marianna, Florida.

	DIR Disease intensity rating	Final TSWV Rating Scale 1 to 10	Seed coat symptomology	Pod Yield	TSMK Total sound mature kernels
Final DIR Disease intensity rating	---				
Final TSWV Rating Scale 1 to 10	0.73**	---			
Seed coat symptomology	0.57**	0.70**	---		
Pod Yield	-0.27**	-0.17**	-0.08	---	
TSMK Total sound mature kernels	0.13	0.10	0.01	-0.35**	---
ELISA Positive ImmunoStrip	0.42**	0.54**	0.77**	-0.22*	-0.20

*,** Significant at the 0.05 and 0.01 levels of probability, respectively.

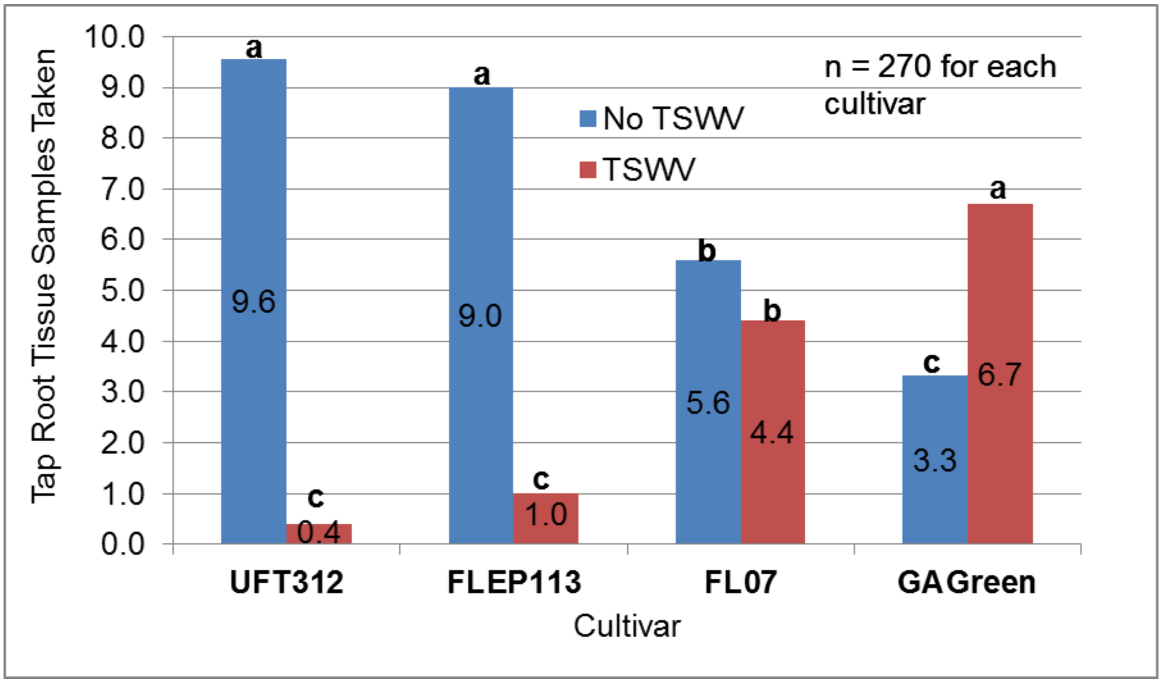

Figure 4-1. TSWV infection results from ten randomly selected tap root crowns from four cultivars in Marianna, FL in 2010-2012. Blue bars represent negative ELISA results for TSWV infection and Maroon bars represent positive ELISA results for TSWV infection

Figure 4-2. Four peanut seeds with A, C & D showing symptoms of TSWV while B showing little to no sign of symptoms. Photo taken by Justin McKinney.

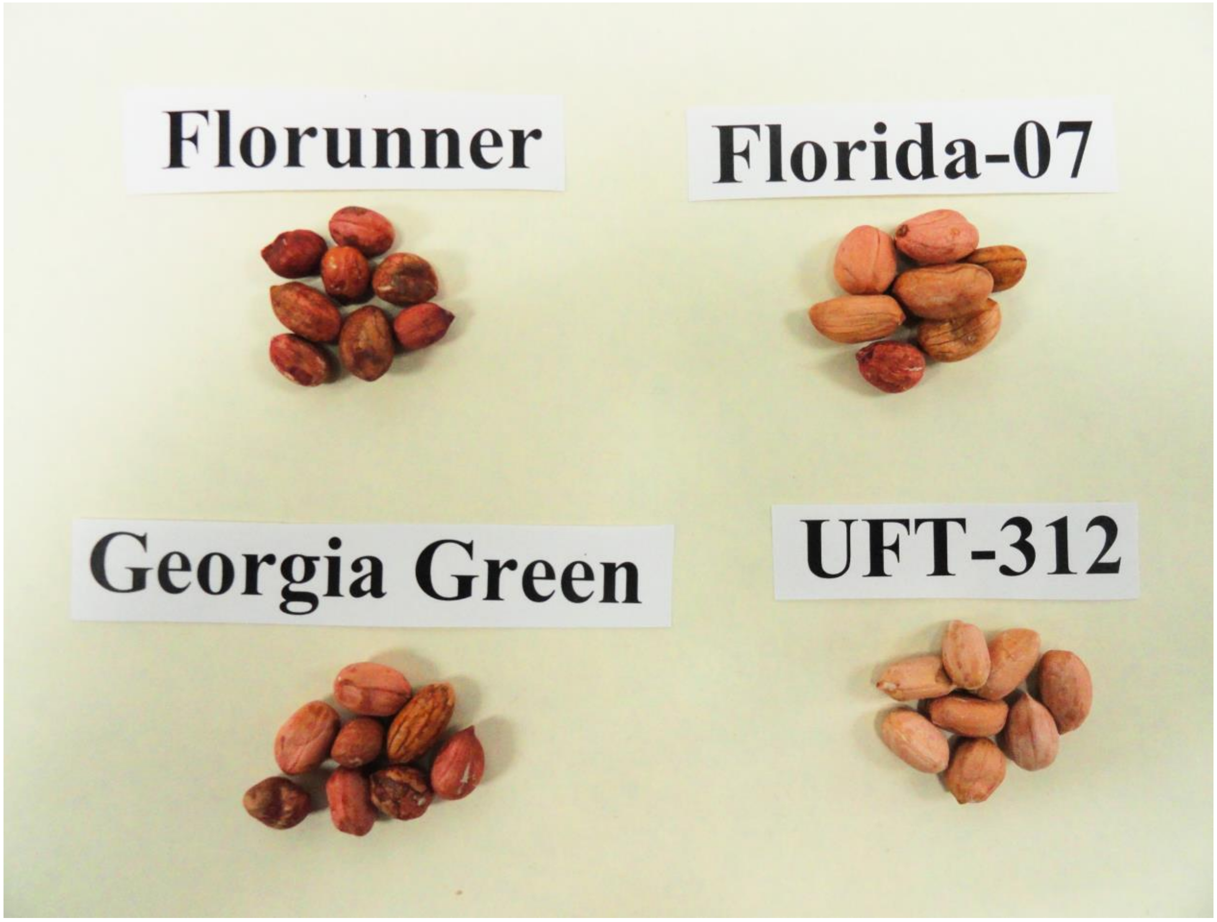

Figure 4-3. Four peanut cultivars showing symptoms of TSWV on their seed coats. Florunner a susceptible cultivar (50 pts.)[1] to TSWV, Georgia Green a moderately resistant cultivar (30 pts.) to TSWV, Florida-07 a moderate to resistant cultivar (10 pts.) to TSWV, and UF peanut breeding line highly resistant cultivar to TSWV infection. Photo taken by Justin McKinney.

[1] According to Peanut Risk Index in 2010 'Peanut Rx.'

Figure 4-4. Dried tap root crowns of four peanut cultivars collected from the field on all 13.1 seed m^{-1} plots during 2010-2012 in Marianna, FL. Photo taken by Justin McKinney.

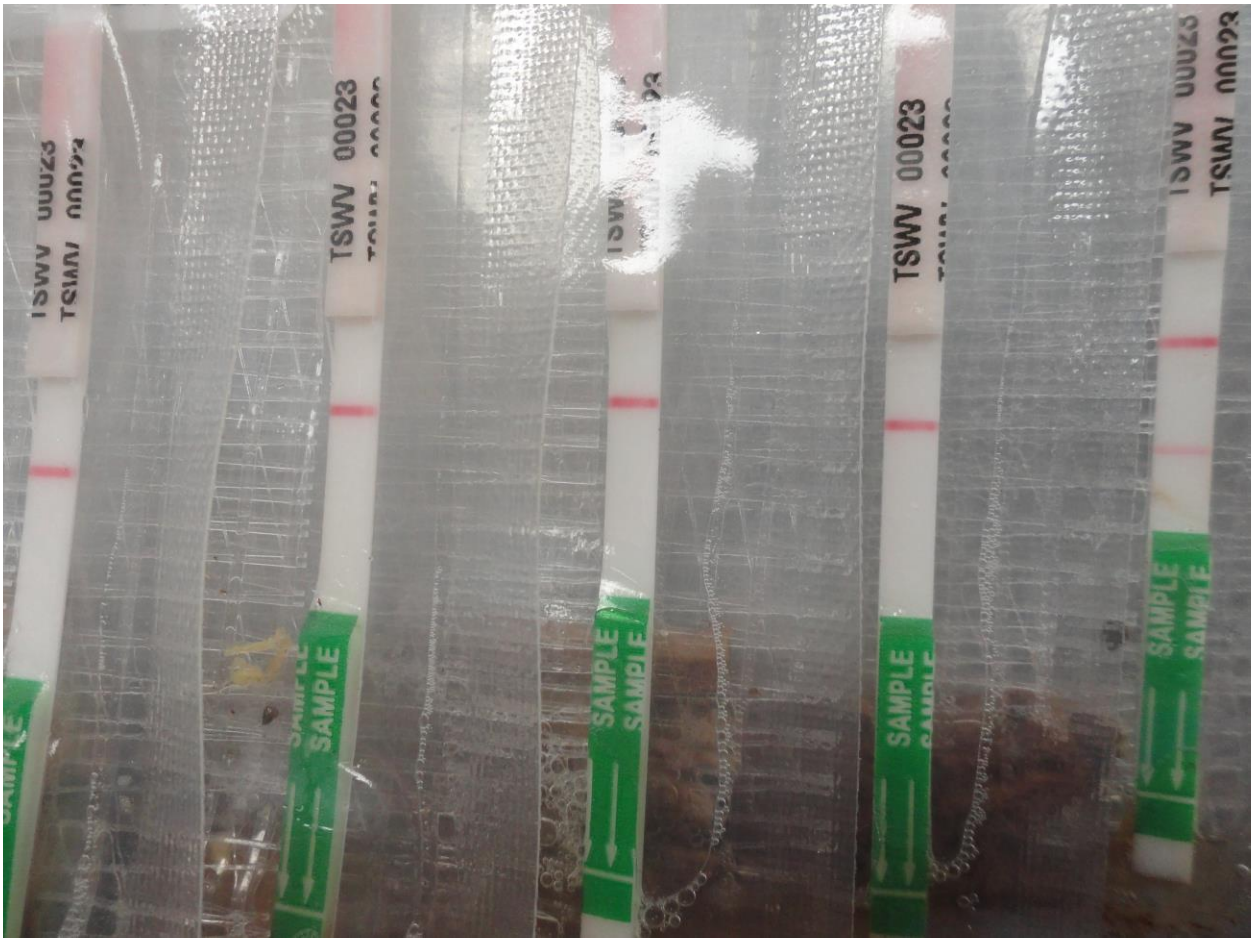

Figure 4-5. Five prefilled Agdia sample bags with 4 negative (single red line) and 1 (double red lines) positive result for TSWV. Photo taken by Justin McKinney.

Figure 4-6. Example of plot number 91201 with 10 randomly selected tap roots tested for presence of TSWV. Photo taken by Justin McKinney.

A B C

Figure 4-7. A) Whole dried root crown, B) tap root sample weigh between 0.4 and 0.6 grams, C) sample process of tap root crown emulsified showing negative result for the presence of TSWV. Photos taken by Justin McKinney.

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BIOGRAPHICAL SKETCH

Justin L. McKinney is a devout Christian who was born in Wauchula, Florida. He is the son of Mr. and Mrs. Gerald Lee McKinney of Ona, Florida. Raised in Hardee County, he graduated from Hardee Senior High School in 1997. He received an Associate of Arts degree with an emphasis on accounting from South Florida Community College in December 1999. He entered the University of Florida in January 2000 to pursue a Bachelor of Science degree in the major of food and resource economics with a minor in sales, and an emphasis in agribusiness management. Upon graduating in May 2002, he accepted a Biologist position at the University of Florida's Plant Science Research and Education Unit. In 2005 he transferred into a Senior Biologist position within the Agronomy Department where he has worked mainly for the University of Florida Peanut Breeding Program. Since that time, he has been promoted to Research Coordinator within the Agronomy Department, where he continues to serve primarily the peanut breeding program in Citra and assists other Agronomy Faculty with their research needs. Following the completion of his Master of Science degree in agronomy, he plans to continue his career within the University of Florida system.