USE OF UNMANNED AERIAL SYSTEMS FOR ASSESSING TOMATO SPOTTED WILT AND LEAF SPOT DISEASES IN PEANUT MAPPING POPULATIONS

by

SARA ELIZABETH PELHAM

(Under the Direction of Albert Culbreath)

ABSTRACT

Tomato spotted wilt virus (TSWV) and late leaf spot (LLS), caused by *Cercosporidium personatum*, are important diseases affecting peanuts (*Arachis hypogaea* L.). One of the most promising ways to combat these diseases is with resistant cultivars created through the development of recombinant inbred lines allowing for the discovery of resistance genes. These mapping populations can be very large making phenotyping time consuming and difficult; the objectives of this research aim to increase the efficiency of phenotyping methods. Field experiments were conducted to compare genotypes from multiple mapping populations for resistance to the two diseases as well as evaluate the use of unmanned aerial systems (UASs) and their ability to increase efficiency when phenotyping TSWV and LLS. Juvenile plants were also evaluated for resistance to LLS. Results indicated that some populations showed more resistance to disease than other and UASs proved to be helpful in the phenotyping of mapping populations. Parental lines evaluated were also susceptible to infection by *C. personatum* at a very early age.

INDEX WORDS: Tomato spotted wilt virus, late leaf spot, *Cercosporidium personatum*, *Arachis hypogaea* L., field resistance, recombinant inbred line, unmanned aerial system, multispectral imaging, vegetative index, juvenile resistance

USE OF UNMANNED AERIAL VEHICLES FOR ASSESSING TOMATO SPOTTED WILT AND LEAF SPOT DISEASES IN PEANUT MAPPING POPULATIONS

by

SARA ELIZABETH PELHAM

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SARA ELIZABETH PELHAM

Major Professor:	Albert Culbreath
Committee:	Tim Brenneman Scott Monfort

Electronic Version Approved:

Suzanne Barbour
Dean of the Graduate School
The University of Georgia
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DEDICATION

For my parents who have always stood by my side and never stopped encouraging me.

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CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

Peanut history, production, and uses

Cultivated peanut, *Arachis hypogaea* L., a legume related to the pea and bean, are thought to have originated around 3500 years ago in South America (47). There are two possible origins: Peru and Brazil. Pottery found by the Europeans has been found in both areas that pay tribute to peanuts. Tribes in Brazil also created a drink out of ground peanuts and maize. Incans in Peru used peanuts during sacrificial ceremonies and thought that by placing them in tombs it would aid the mummy in their spirit life (92).

In the 1700's, Africans were the first people to introduce peanuts into North America and in the early 1800's peanuts became a commercial crop. In the United States peanut production began in Virginia where it was grown for oil, food, and as a substitute for cocoa. Since they were difficult to grow and harvest they were grown primarily for the poor and as a food for livestock. They gained popularity in the late 1800's when P.T. Barnum's circus wagons began to sell them as a snack and soon after street vendors followed suit. Even though their popularity was growing, due to lack of harvest equipment, hand harvesting meant they were sold with stems and leaves attached (92).

In the early 1900's, demand once again hit a splurge because new equipment was developed making peanut production quicker and easier (92). At the suggestions of Dr. George Washington Carver, peanuts became an important cash crop in the United States around 1915

when cotton took a dramatic hit in production due to the introduction of boll weevils (63, 92). For a short period, peanut production even rivaled cotton production in the south (92).

Peanuts can be used for a variety of products. Some of the main uses are peanut flour, peanut oil, roasted peanuts, and peanut butter (129). Peanut butter is the most common use for peanuts; contrary to popular belief, George Washington Carver did not invent peanut butter but he did invent many other uses for peanuts. The recipe was patented by Dr. John Harvey Kellogg in 1895 and the patent for a peanut-butter-making machine followed shortly in 1903 by Dr. Ambrose Straub (93).

There are four types of peanuts grown in the United States. The runner, the type most widely grown throughout Georgia, Alabama, and Florida since the introduction of Florunner in the 1970's, is mostly used to produce peanut butter (16, 129). Runners, popular for their attractive kernel size, account for nearly 80 percent of the total peanut production in the United States. Another type, Virginias, are primarily used for roasting and account for nearly 15 percent of total US peanut production. The other two types, Spanish and Valencia, account for the other 5 percent of peanuts grown in the United States (129).

Today the United States is the world's third largest producer after China and India with these three-combined producing about 70% of the world's total peanut production (17). The United States accounts for approximately 1.25 million metric tons of the 29 million metric tons produced throughout the whole world and this number is on the rise (129). In the U.S. seven states are responsible for 99% of the nation's total production with Georgia ranking number one with 56% of the total production (49).

Peanut production in Georgia is a $1.3 billion industry with 75 out of 159 counties having peanuts planted. These were planted by approximately 3,400 farmers on 785,000 acres. The average yield in 2015 of peanuts in Georgia was 4,470 pounds per acre. This is the leading yielding average in the United States. In 2006 the average yield was 2,780 pounds per acre, resulting in almost a doubling of the average yield in the past 10 years. Production in Georgia has continuously been on the rise. Production has increased by nearly a million tons in the last 10 years from 799,250 in 2006 to 1,736,595 in 2015 (49).

Tomato Spotted Wilt Virus

Tomato Spotted Wilt Virus (TSWV) is a *Tospovirus* in the family Bunyaviridae which infects over 1000 species and was first described on tomato in Australia in 1915 (13, 100). It was not until 1930 that the disease was reported to be of viral etiology (118). There are more than a dozen viruses that belong in the genus *Tospovirus* and throughout the years occurrence has been continuously spreading (122). In recent years TSWV has been reported in the Dominican Republic on peppers and tomatoes (83), on gerbera and chrysanthemum in Venezuela (83), in Zimbabwe on butternut squash (69), and *Stevia rebaudiana* in North Carolina (74). Across the world, TSWV is the most damaging and prevalent member of the *Tospovirus* genus (91, 122, 142). In 1927, it was discovered that thrips were the vector for TSWV (102). All tospoviruses are transmitted by thrips and the virus can replicate in both the thrips vector and the plant host (122).

TSWV of peanut was first reported in Brazil in 1941 (27). Since then reports on peanuts have come out of South Africa (43, 72), Australia (64, 115), India (23, 46, 51, 112), and the United States (62). In the United States, the virus was first reported affecting peanuts in 1971 in Texas with yield reductions approaching 95 percent during several epidemics throughout the

next 20 years (9, 10, 62). This yield loss can be attributed to the death of the plant as well as a reduction in pod number and size; kernels can also be affected by decreased number, discoloration, or malformation (123). It was not until 1989 to the mid-1990s that TSWV became a problem in Georgia (29, 35, 38) and since the early 1990s, TSWV has been one of the most important diseases in the southeastern U.S. (32). In 1997, approximately 12 percent of the peanut crop in Georgia was lost to TSWV with losses in crop value estimated at over $40 million. Since 2006, yield losses have improved with them staying at three percent or less (32). TSWV has caused a dramatic shift since the mid 1990's in cultural practices, planting dates, and cultivars in the southeastern United States (136).

Symptoms

TSWV can be characterized by many different symptoms. The most common of these are concentric ringspots, various patterns of chlorosis on leaflets, and stunting of all above ground plant parts (27, 37). TSWV can also cause pods and kernels to be small or misshapen (38). A reddish discoloration and cracking of the seed coat is also common (62). Symptoms can either be found on the entire plant or can be found on just one or a few leaflets. If symptoms get severe enough TSWV can cause the entire plant to die. Yield can be greatly reduced since the number of pods the plant produces and kernel size are reduced by TSWV. These effects have also been correlated with time of infection; plants showing symptoms early in the season typically yield less than those that do not display symptoms until later in the season (36). A disease known as peanut yellowing in Texas (87) is associated with chlorosis and wilting of peanut plants. This disease can accompany typical above-ground symptoms of TSWV (30).

Roots of plants affected by TSWV generally develop necrosis, which can result in death of the entire plant (30). There may be other pathogens involved besides TSWV in the ultimate

death of the peanut roots (30, 87). Even though TSWV seems to increase the development of peanut yellowing syndrome Mitchell (87) discovered that infection by TSWV was not essential for plants to develop the disease. When a plant is infected with TSWV it may not always show symptoms, therefore being asymptomatic. The infection can be confirmed with immunoassays of the root tissue. Culbreath et al. (36) reported incidence of asymptomatic infections as high as that of disease incidence based on visible foliar symptoms.

Epidemiology

In peanuts the only significant spread of TSWV has been shown to be through thrips (117, 138, 142). There are 10 species of thrips that are known to transmit TSWV to peanuts, but two are predominate in the Southeastern United States. The two thrips are the tobacco thrip, *Frankliniella fusca* (Hinds), and the western flower thrip, *Frankliniella occidentalis* (Pergande) with *F. fusca* being the predominant vector due to its ability to reproduce more efficiently on peanut than *F. occidentalis* (138). These two species have been discovered to overwinter in weeds in the immediate area as well as volunteer peanuts (32).

The virus is spread by immature thrips feeding on infected host plants (50, 53). From there the virus is maintained in gut of the thrip throughout its life waiting to be spread to uninfected host plants. This allows the virus to thrive for long periods of time and infect many new hosts. Shrestha et al. (125) also showed that thrips density, susceptibility of host plant stage, and mode of TSWV inoculation, thrips mediated or mechanically inoculated, all play a major role in *Tospovirus* transmission. A main finding in this study showed that with an increased thrips population the severity was shown to increase (125).

Management

Since 1997, the main management practice for combating TSWV has been to combine resistant cultivars with cultural methods that have been shown to suppress epidemics (14, 32, 34). Many practices that are in use today that favor epidemics of TSWV are the use of susceptible cultivars, early planting dates, use of single row patterns, and conventional tillage (32). The use of neonicotinoid insecticides, such as Imidacloporid, seem to show no decrease in the incidence of TSWV in peanuts (137). On the other hand, organophosphates, such as phosphate, have been shown to suppress populations of thrips (2, 33, 139). Plant density within the row can also have a large impact on TSWV epidemics; higher plant populations result in plant being less vulnerable to losses to the disease (11, 32, 34, 57, 70). Increasing the number of plants in a field does not appear to reduce the number of infected plants but likely reduces the percentage of plants that become infected. Greater plant density may allow for compensation for yield loss of diseased plants by more healthy plants (32). Planting dates in mid-May have proven to reduce the risk of infection by TSWV due to the low populations of thrips at that time (136). Three commonly used cultivars, 'Georgia-06G' (12), 'Florida-07' (60), and 'Tifguard' (67), have been known to have some resistance to TSWV (28, 33). Culbreath et al. (40) showed that incidence of TSWV in all three of these cultivars decreased with increasing seeding rates and subsequent increased plant population.

Late Leaf spot

Leaf spots are the most serious foliar diseases of peanut in the world (45, 96, 127, 132) with yield losses ranging from 10 percent to 80 percent (85, 86). Late leaf spot, caused by *Cercosporidium personatum* (Berk. & M. A. Curtis) Deighton, was the main foliar disease affecting the Southeastern United States in the 1980s and early 1990s (127). Over the years, late

leaf spot has been predominating in Florida and has increased its prevalence in Georgia (16). Late leaf spot occurs wherever peanuts are grown (68, 128) with yield losses of 50% - 70% in West Africa and up to 50% worldwide (144).

Symptoms

Late leaf spot symptoms can appear on any above ground parts of the peanut. This includes leaves, petioles, stipules, stems and pegs in the later stages of disease (133, 134). At first spots develop on the upper surface of lower leaves as small necrotic pinhead size spots and develop into large light to dark brown or black circular spots. These spots can range anywhere from 1 to 10 mm in diameter (140). Biomass and yield can be greatly affected when these spots coalesce. The loss is a result of defoliation of the plant. Late leaf spot spores are generally formed on the lower surface giving it a rough appearance, while the upper surface of the leaf is smooth (141).

Epidemiology

Since there are several secondary cycles in each season, leaf spot can increase rapidly under favorable conditions. Environmental conditions that favor the onset of disease are warm temperatures and long periods of high humidity or leaf wetness. When adequate moisture is present and temperatures are high, leaf spot infections may occur in a relatively short period (15). Information on the developmental stages of the plant and leaf spot disease progression is limited (141).

Management

In the mid-1980's, the main management strategy for managing late leaf spot was to breed for genetic resistance (73). Some sources of resistance were found quickly in cultivated

peanuts (1, 133) as well as in wild *Arachis* species (1). However, resistance in cultivated peanut was easier to manipulate and implement (18). Although no source of complete resistance has been found (1), Gorbet et al. (56), Pixley (103), and Watson (149) all found partial resistance to late leaf spot which all reduced the severity of the disease. Partial resistance is typically a function of multiple components of resistance that contribute additively to a reduction in the rate of epidemic progress (99). Chiteka et al. (18), Cook (25), Subrahmanyam et al. (133), Walls et al. (145), and Watson et al. (149) were all influential in describing the components of resistance to late leaf spot in the 1980s. The components that are most commonly associated with genetic resistance are latent period, spore production, and lesion size (18-20, 145). A positive genetic relationship between late leaf spot lesion size and the quantity of secondary spores produced has been confirmed while both were negatively correlated with latent period (19).

Since the pathogen only attacks peanuts, rotations with any other crop can help to reduce disease. A fungicide application is usually required for leaf spot control as well. Applications are usually set on a 14-day calendar schedule, or according to a weather-based leaf spot advisory. Some fungicides that control late leaf spot include: chlorothalonil, tebuconazole, and propiconazole (124). In the United States, chemical control for late leaf spot is estimated at $26.8 million in 2009 for Georgia alone (151). The advisory is a safe way to minimize the use of fungicides by spraying only when weather conditions favor disease (124).

Recombinant Inbred Lines

Recombinant inbred lines (RILs) have become of great use in peanut breeding and pathology. The most common use of RILs in peanut breeding is for trait mapping through the use of phenotyping and the identification of quantitative trait loci, or QTLs (71). Some of the QTLs that have been identified in peanut are TSWV resistance, early leaf spot resistance, late

leaf spot resistance, rust resistance, flavonoid content, DPPH radical scavenging activity, and total phenolic content (71, 75, 88).

Four recombinant inbred line populations that are popular for current phenotyping and QTL research are: S, T, 1799, and 1801. Two of the populations, referred to as S population and T population were developed by Baozhu Guo at the USDA Coastal Plains Experiment Station in Tifton, GA using a single seed descent method (71). These two populations were developed for phenotyping of oil content and three oil quality traits, oleic acid, linoleic acid, and their ratio (98). The other two populations (1799 and 1801) were developed by Corley Holbrook at the USDA Coastal Plains Experiment Station in Tifton, GA (66). These two populations, along with six other recombinant inbred lines, were created for marker-assisted selection for six diseases, including TSWV and leaf spot pathogens, as well as oleic acid content (66).

The S population, consisting of 352 individuals, is derived from the crosses SunOleic 97R (55) and F NC94022-1-2-1-1-b3-B (referred to as NC94022) (108). The female parent, SunOleic 97R, is a runner market-type with high oleic acid content and is susceptible to TSWV. This genotype is a selection from the cross between a BC_4F_5 selection of a cross of SunOleic 95R, known as F435-2-2-E-2-l-b4-E-b2-b3-1-E, and Sunrunner (F519-9) (55). F435-2-2-E-2-l-b4-E-b2-b3-1-E is a high oleic line while Sunrunner is a runner market-type line (55, 98). The male parent, NC94022 is known to have a high level of field resistance to TSWV (31). It was developed from a cross between N91026E, an early maturing Virginia-type line with moderate susceptibility to TSWV, and a tan-seeded component selected from PI 576638, a *hirsute* botanical-type line from Mexico (6).

The T population, which consisted of 248 individuals, is created from a cross between Tifrunner (65) and GT-C20. The female parent, Tifrunner, is a late maturing runner market-type

cultivar with a high level of resistance to TSWV and moderate resistance to leaf spot pathogens (66). Tifrunner is derived from a cross of F439-16-10-3 and PI 203396 (65). The male parent, GT-C20, is a spanish-type breeding line with high susceptibility to TSWV and leaf spot pathogens (146).

The 1799 population is a cross between Tifrunner (65) and NC 3033 (8). The female parent, Tifrunner has a high level of resistance to TSWV and moderate resistance to leaf spot pathogens (66). The male parent, NC 3033, is a small-seeded virginia-type line (8). It has been characterized as being highly susceptible to both TSWV and leaf spot pathogens (66). NC 3033 is the result of a cross between Ga207 and A48 (8).

The 1801 population is derived from a cross of Florida-07 (60) and SPT 06-06 (135). The female parent, Florida-07, has normal resistance to TSWV and moderate resistance to leaf spot pathogens (66). Florida-07 is the result of a cross between an early-maturing, high-oleic breeding line 89XOL14-11-1-1-1-b2-B and the leaf spot resistant cultivar C-99R (58, 60). The male parent, GP-NC WS 16, referred to as SPT 06-06 is derived from a three-way cross by the modified pedigree method of inbreeding in the early generation segregating populations (135). The three lines used were C-99R (UF 94320) (58), DP-1 (UF 97318) (59), and GP-NC WS 12 (131). SPT 06-06 has been shown to have high resistance to TSWV and leaf spot pathogens (135).

Imaging Techniques for Disease

Correct disease identification is the basis for every management strategy, making it one of the most basic and important activities in agriculture. However visual identification has many problems associated with it. Visual identification can either be biased, wrong due to optical

illusions, or simply wrong. On the other hand, laboratory analysis can often be very time consuming and expensive. Therefore, it is important to develop automatic methods that take bias and error out of the equation. These automatic methods can identify diseases in a rapid and reliable way because they rely on digital images allowing for fast analysis. However, there is no perfect technique and automatic methods can have problems associated with them (4).

So far, literature has focused mainly on automatic methods for disease detection in the visible and near-infrared bands (3). These bands can either be analyzed separately or together with the help of a multispectral image (4). Visible light images are becoming more popular because they are easier to capture since the imaging system is cheap and compact. Multispectral cameras are generally very pricey, but they are also known for allowing more information than a normal image (4).

Many of the studies in literature focus on detecting a single disease at a time (5, 95, 104, 105, 155, 157). This can be a problem due to other issues affecting the plant such as other diseases, nutritional problems (97, 114, 152), and pests (21, 76, 126) which can mimic disease symptoms. Advancements have been made in trying to discriminate between different diseases, but, so far, methods can only distinguish between a few diseases at a time (101, 107, 119). Studies have shown this to be unrealistic in real world applications (4).

There are also some other problems that can affect image analysis. One of these is that the background of the image often contains objects other than the area or interest, such as soil or other plants. Another problem is that the sunlight can affect the color of the plant or shadows depending on the time the image was taken. Also, most symptoms do not have boundaries that are well defined making it hard to differentiate between diseased and healthy tissue. Some

diseases can have very different characteristics based on where it is found on the plant or even the stage of development. Finally, many diseases have very similar characteristics making them hard to differentiate between (4).

These challenges are not only relevant for disease identification but also disease severity measurements. The only difference is that accurately outlining the symptoms is much more important in severity measurements. Correct disease identification is an extremely important first step in making severity measurements (4).

Unmanned Aerial Vehicles

The use of unmanned aerial vehicles (UAVs) in agriculture has greatly increased over the past decade due to their wide range of uses. UAVs can deliver information of high spatial and temporal resolution in non-destructive ways (143). The main reason for implementing UAVs is to deliver near-real-time information for crop and soil properties which is important since farmers need to know these properties in a timely fashion to implement production decisions (79, 106, 154).

Precision Agriculture

UAVs fall under the category of precision agriculture which suggests that the agricultural management can be performed with a degree of precision. Precision agriculture has become popular in recent years because it can be used to cut down on the amount of chemicals used, reducing costs and possible environmental harm (154). In order for variable rate technology to be useful accurate maps of nutrient levels, crop growth, weeds, insect populations, disease presence, and many other elements are necessary (90). This information can then be laid out spatially to more precisely manage the needs of the crop (26, 113). Since this information is

continuously changing to get the most accurate information data must be taken in short time intervals allowing near-real-time response to unfavorable field conditions (154). This is becoming harder and costlier to accomplish because of growing farm sizes (109). One method to combat this has been to use satellite and aerial images to monitor crop stressors and growth as well as to predict yield (130, 147). Satellite images are far from ideal due to poor revisiting times and coarse spatial resolutions (90, 130). Even more, manned aerial vehicles have high operational costs as well as high complexity and long turnaround time in delivery of data (7, 90, 110, 154).

Precision agriculture has multiple stages: data collection, field variability mapping, decision making, and management practice (154). Research has shown that remote sensing platforms could be useful in the first three of those stages (130, 147). Especially in the collection of data to be used for decision making, suggesting that field variability could be mapped using remotely sensed imagery (154)

History

It is documented that the Agricultural Adjustment Administration of the United States used aerial imagery to measure crop lands in the 1930s (89). But it was not until the 1950s that remote sensing using geospatial techniques was implemented in agriculture (24). Remote sensing is based on the idea that differences in crop growth can be detected through variations within the spectral responses (147). More importantly, that changes in reflectance can detect the presence of a pathogen, such as a fungal foliar disease, before symptoms appear (81, 82).

Remote Sensing Aerial Platforms and Analysis

Some of the most commonly used aerial remote sensing platforms are satellites, airplanes, helicopters, and balloons. These platforms can either be equipped with optical, near infrared, or multi-spectral sensors. Data derived from images taken on board an aerial platform can show biomass, Leaf Area Index (LAI), disease, water stress, lodging, and Normalized Differential Vegetation Index (NDVI) which can be useful in many aspects of crop production (154).

Uses for aerial remote sensing platform include monitoring and mapping of soil properties (41, 54), pest management (78), water analysis (44, 80), and weed control monitoring (61, 78, 120). Remote sensing has been used in many crops such as canola, corn, cotton, sorghum, and wheat (80, 121, 147, 153, 156). Aerial photography has been used in yield mapping to identifying field variation (52), but most images used in these studies are either airborne hyperspectral (41, 52, 80), satellite hyperspectral (111), or satellite multi-spectral (22, 42, 54). The use of UAVs for remote sensing and detection of diseases is an area that has yet to be explored.

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CHAPTER II

PHENOTYPING OF RECOMBINANT INBRED LINES OF PEANUT FOR RESISTANCE TO TOMATO SPOTTED WILT VIRUS AND *CERCOSPORIDIUM PERSONATUM* [1]

[1] Pelham, S. E., Holbrook, C. C., Guo, B., Chu, Y., Ozias-Akins, P., and Culbreath, A. K. 2017. To be submitted to *Crop Science*.

Abstract

Recombinant inbred line (RIL) populations of peanut (*Arachis hypogaea* L.) are important in the development of markers for resistance to several diseases, including Tomato spotted wilt virus (TSWV) and late leaf spot (*Cercosporidium personatum*). Susceptibility to both diseases within populations has been characterized, but populations have not been compared within the same trial. In 2015 and 2016, field trials were conducted to determine the effect of 18 RILs from each of four mapping populations (designated S, T, 1799, and 1801 populations). Based on ranked results for early and late leaf spot combined from previous trials, the 6 RILs with the highest, 6 RILS with the lowest scores for disease severity, and 6 RILs nearest the population mean were included from each population. Parental lines from all populations were also included. Area under the disease progress curve (AUDPC) values were calculated from multiple assessments for both TSWV and LLS and were converted to standardized area under the disease progress curves (SAUDPC) to compare across years.

TSWV ratings were highest for the T population. The S population had the highest SAUDPC for late leaf spot and mean SAUDPC values were similar for T, 1799, and 1801 populations. SAUDPC of SPT 06-06, a parental line for population 1801, was lower than for any other parent for TSWV and was the second lowest for late leaf spot. Florida-07 and Tifrunner proved to have resistance to both diseases as well. Results indicate the populations differ for both TSWV incidence and late leaf spot severity, and highest levels of late leaf spot resistance in individual RILs may not come from the most resistant parent to TSWV. Results also indicate that levels of resistance can be obtained in individual lines that are better than that of either parent. Populations in this experiment showed transgressive segregation both towards resistance and susceptibility for both TSWV and late leaf spot.

Introduction

Across the world, tomato spotted wilt tospovirus (TSWV), in the family Bunyavirdae, infects over 1000 species and is the most damaging and prevalent member of the Tospovirus genus (6, 32, 36, 42, 55). In the United States, the virus was first reported affecting peanuts in 1971 in Texas with yield reductions approaching 95 percent during several epidemics throughout the next 20 years (4, 5, 20). This yield loss can be attributed to plant death as well as a reduction in pod number and size; kernels can also be affected by decreased number, discoloration, or malformation (40). TSWV has caused a dramatic shift since the mid 1990's in cultural practices, planting dates, and cultivars in the southeastern United States (54).

Late leaf spot is one of the most serious foliar diseases of peanut in the world (15, 33, 46, 51). This disease, caused by the pathogen *Cercosporidium personatum* (Berk. & M. A. Curtis) Deighton (teleomorph = *Mycosphaerella berkeleyi* Jenk) (48), has become predominate in Florida and has increased its prevalence in Georgia in previous years (7). Late leaf spot occurs wherever peanuts are grown (22, 45) with yield losses up to 50% worldwide (56).

The development and use of resistant cultivars is one of the most desirable ways to manage both TSWV and late leaf spot (13). Currently, peanut breeding programs focus on extensive field screening to look for sources of resistance. This screening requires considerable space and a relatively large number of plants. Marker assisted selection is useful in the development and implementation of peanut cultivars with nematode resistance (44, 45). There are no markers currently available for resistance to TSWV or late leaf spot. Selecting for resistance to these diseases through the help of molecular markers could significantly increase the usefulness of breeding programs (26).

Four recombinant inbred line (RIL) populations were used for this study: S, T, 1799, and 1801. Two of the populations, referred to as S population and T population were developed by Baozhu Guo at the USDA Coastal Plains Experiment Station in Tifton, GA using a single seed descent method (25). These two populations were developed for phenotyping of oil content and three oil quality traits, oleic acid, linoleic acid, and their ratio (34). The other two populations (1799 and 1801) were developed by Corley Holbrook at the USDA Coastal Plains Experiment Station in Tifton, GA (22). These two populations, along with six other recombinant inbred lines, were created for marker-assisted selection for six diseases, including TSWV and late leaf spot pathogens, as well as oleic acid content (22).

The S population, consisting of 352 individual lines, is derived from the crosses SunOleic 97R (16) and F NC94022-1-2-1-1-b3-B (referred to as NC94022) (38). The female parent, SunOleic 97R, is a runner market-type with high oleic acid content and is highly susceptible to TSWV. This genotype is a selection from the cross between a BC4F5 selection of a cross of SunOleic 95R, tested initially as F435-2-2-E-2-l-b4-E-b2-b3-1-E, and Sunrunner (F519-9) (16). F435-2-2-E-2-l-b4-E-b2-b3-1-E is a high oleic line while Sunrunner is a runner market-type cultivar (16, 34). The male parent, NC94022 has a high level of field resistance to TSWV (10). It was developed from a cross between N91026E, an early maturing virginia-type line with moderate susceptibility to TSWV, and a tan-seeded component selected from PI 576638, a hirsuta botanical-type line from Mexico (2).

The T population, which consists of 248 lines, was developed from a cross between Tifrunner (21) and GT-C20. The female parent, Tifrunner, is a late maturing runner market-type cultivar with a high level of resistance to TSWV and moderate resistance to late leaf spot (22).

Tifrunner is derived from a cross of F439-16-10-3 and PI 203396 (21). The male parent, GT-C20, is a Spanish-type breeding line with high susceptibility to TSWV and late leaf spot (58).

The 1799 population was developed from a cross between Tifrunner (21) and NC 3033 (3). The female parent, Tifrunner has a high level of resistance to TSWV and moderate resistance to late leaf spot (22). The male parent, NC 3033, is a small-seeded virginia-type line (3). It has been characterized as being highly susceptible to both TSWV and late leaf spot (22). NC 3033 is the result of a cross between Ga207 and A48 (3).

The 1801 population is derived from a cross of Florida-07 (18) and SPT 06-06 (52). The female parent, Florida-07, has moderate to high levels of field resistance to TSWV and moderate resistance to late leaf spot (22). Florida-07 is the result of a cross between an early-maturing, high-oleic breeding line 89XOL14-11-1-1-1-b2-B and the late leaf spot resistant cultivar C99R (18, 19). The male parent, GP-NC WS 16, referred to as SPT 06-06 is derived from a three-way cross by the modified pedigree method of inbreeding in the early generation segregating populations (49). The three lines used were C-99R (UF 94320) (19), DP-1 (UF 97318) (17), and GP-NC WS 12 (49). SPT 06-06 has a high resistance to TSWV and late leaf spot (52).

With this knowledge, we decided to conduct an experiment using these populations. The objective of this study was to compare field susceptibility of 18 recombinant inbred lines from mapping populations that have never been compared for resistance to TSWV and late leaf spot.

Materials and Methods

Field Setup

Field experiments were conducted at the University of Georgia Coastal Plain Experiment Station, Lang Farm, Tifton, GA in 2015 and 2016. Fields were previously planted to cotton (*Gossypium hirsutum* L.) and corn (*Zea mays* L.) in 2015 and 2016, respectively, and both were

grown using conventional tillage. Peanuts have been grown in these fields previously, and disease history of fields included severe epidemics of TSWV and leaf spot in previous peanut crops.

The experimental design was a randomized complete block design with three replicates of each genotype. Plots consisted of two 1.5 m long rows spaced 0.91 m apart. The planting density was 18 seed/m. Each plot was bordered on one side by the susceptible cultivar TUFRunner 511 (53) to increase overall incidence of TSWV and late leaf spot in all entries.

To maximize the potential for late leaf spot in the test the experiment was plated later in the season. The field was planted on June 2nd in 2015 and June 1st in 2016. No fungicides were applied. Herbicides, insecticides, and fertilizers were applied following recommendations of the University of Georgia (31, 37).

Selection of genotypes

There was a total of 16 genotypes chosen from each of the previously described mapping populations: 1799, 1801, T, and S. Based on ranked results for both early and late leaf spot combined from previous trials, the 6 RILs with the highest diseases severity, 6 RILS with the lowest disease severity, and 6 RILs nearest the population mean for previous leaf spot ratings were included from each population. Parental lines from all populations were also included (Table 2.1). Since two of the populations have a common parent there were only seven parental lines tested. This resulted in a total of 79 genotypes being compared.

Evaluation of TSWV epidemics

Naturally occurring thrips populations were relied upon for TSWV epidemics. Although asymptomatic infections of TSWV have been shown to occur in peanuts (11) all discussion about TSWV incidence in this experiment refers to symptomatic plants. Symptoms of TSWV were

rated on a continuous percentage-based scale from 0 to 10 where 0 = no disease and 10 = all plants severely diseased. Plants with any of the five typical foliar symptoms of TSWV, as described in the literature, on one leaflet or more were considered symptomatic (12, 13, 30). Symptoms included concentric ringspots, "oak-leaf" patterns of chlorosis, bronzing of leaves, stunting and distortion, and necrosis of leaves in the terminal bud (14). TSWV evaluations in all plots were taken at 29, 64, and 87 DAP in 2015 and 40, 47, and 57 DAP in 2016.

Evaluation of late leaf spot epidemics

Naturally occurring inoculum of late leaf spot was relied on to initiate epidemics. Disease severity for late leaf spot was assessed using the Florida 1 to 10 scale system, where 1 = no disease, 2 = very few lesions (none on upper canopy), 3 = few lesions (very few on upper canopy), 4 = some lesions with more on upper canopy than for rank of 3 and slight defoliation noticeable, 5 = lesions noticeable even on upper canopy with noticeable defoliation, 6 = lesions numerous and very evident on upper canopy with significant defoliation (50%+), 7 = lesions numerous on upper canopy with much more defoliation (75%+), 8 = Upper canopy covered with lesions with high defoliation (90%+), 9 = very few leaves remaining and those covered with lesions (some plants completely defoliated), and 10 = plants dead (9). Late leaf spot was evaluated in all plots at 87, 99, 108, 116, 122, and 134 DAP in 2015 and 90, 104, 113, 121, 127, and 135 DAP in 2016.

Statistical analysis of epidemics

Both TSWV and late leaf spot were analyzed individually using RStudio (R Core Team, Vienna Austria). In both years, area under the disease progress curve (AUDPC) was computed for each plot using disease ratings as described by Shaner and Finney (39). Since the intervals between evaluations and number of evaluations differed for the two years, standardized area

under the disease progress curves (SAUDPC) were calculated by dividing AUDPC by the total time in days that the epidemic was monitored (27). Data was analyzed across years. Fisher's protected least significant difference (LSD) values were calculated for comparison of genotypes (50). Linear regressions were calculated using SigmaPlot 13.0 (Systat Software, Inc., San Jose, CA 95131 USA) to examine the relationship between final incidence of TSWV and SAUDPC across genotypes (14). Differences referred to in the text are significant at $P \leq 0.05$ unless otherwise indicated.

Results

Evaluation of TSWV epidemic progress

TSWV epidemics in both years were not significantly different from each other resulting in the ability to combine across years. SAUDPCs of all the genotypes show differences between some genotypes and their TSWV resistant parent (Fig. 2.1). Both the 1799 and the S population, had at least one genotype with a SAUDPC that was less than that of its more resistant parent. The S population had the most genotypes that were lower with 8 out of the 18 genotypes from this population being less than its resistant parent, NC 94022. Tifrunner, the more resistant parent for both the 1799 population and the T population, had two genotypes that had lower disease ratings. These were 704 and 712 from the 1799 population. Results were similar for final incidence ratings (Fig. 2.2). The S population once again had the most genotypes with lower TSWV ratings than its resistant parent, NC 94022, with 3 in total. The 1799 population was the only other population with significantly better genotypes. These were 596, 600, and 704 which surpassed Tifrunner in terms of resistance. There is a positive correlation between SAUDPC and final incidence as seen in Figure 2.3.

To analyze the populations against each other, the genotypes were broken out into their original categories: lowest initial leaf spot ratings (low treatment), leaf spot ratings closest to the population mean (mean treatment), and highest initial leaf spot ratings (high treatment) (Fig. 2.4). The 1801 population was the most resistant population to TSWV in the low treatment with a mean estimate of overall TSWV SAUDPC of 1.40 (Fig. 2.4a). The 1799 population was close behind with 1.42 as its estimate of overall TSWV SAUDPC. For final incidence, these two populations had the lowest estimates for overall TSWV final incidence but their ranking was reversed (Fig. 2.4d). The 1801 population and the S population were ranked number one and two respectively for the mean treatment for both SAUDPC (Fig. 2.4b) and final incidence (Fig. 2.4e). The S population had a slightly lower estimate of overall TSWV final incidence than the other three populations for SAUDPC in the high treatment (Fig. 2.4c). It was also the best population for final incidence in the high treatment (Fig. 2.4f). The T population had the highest SAUDPC for TSWV.

When looking at the parental lines together, Florida-07 had the lowest SAUDPC for TSWV followed by SPT 06-06 (Fig. 2.5a). This was reversed for final incidence (Fig. 2.5b). The two worst lines were GT-C20 and SunOleic 97R. SunOleic 97R had the highest SAUDPC and GT-C20 had the highest final incidence.

Evaluation of late leaf spot epidemic progress

Late leaf spot epidemics began later in 2016 due to a lack of rain in the beginning of the season. However, the epidemics in both years were not significantly different so they could be analyzed across years. When analyzing the SAUDPCs of all the genotypes only two populations had genotypes that were better than their resistant parent (Fig. 2.6), and in both cases the resistant parent was Tifrunner. The 1799 population had three genotypes that are significantly

better (596, 607, and 608), and the T population had one (T11). Results were very similar for the final severity ratings (Fig. 2.7). Once again, all genotypes that had less disease than their resistant parent, Tifrunner, came from either the 1799 population or the T population. The 1799 population had five genotypes (596, 607, 626, 691, and 757), and the T population had three (11, T21 and T70) that were better than Tifrunner. There is a strong positive correlation between SAUDPC and final incidence as seen in Figure 2.8.

Once again, to analyze the populations against each other the genotypes were broken out into their original categories: low initial leaf spot ratings, leaf spot ratings closest to the population mean, and high initial leaf spot ratings (Fig. 2.9). The T population was the best population in terms of late leaf spot resistance in the low treatment for both SAUDPC (Fig. 2.9a) and final severity (Fig. 2.9d). The 1799 population had the highest overall late leaf spot SAUDPC estimate in the low treatment (Fig. 2.9a). The T population and the 1799 population had almost the similar SAUDPC values (Fig. 2.9b) and final incidence (Fig. 2.9e) for the mean treatment but the T population was slightly lower. SAUDPC was lowest for the 1801 population with the 1799 population only slightly higher (Fig. 2.9b). For final incidence, these two populations had the lowest late leaf spot overall final incidence estimates in the same ranking (Fig. 2.9d). Overall, the S population was less resistant to leaf spot.

Among the parental lines, SPT 06-06 had the lowest SAUDPC (Fig. 2.10a) and final incidence (Fig. 2.10b) for late leaf spot. This was closely followed by Tifrunner. The two parental lines with highest SAUDPC were GT-C20 and NC3033, although for final severity, Florida-07 and SunOleic 97R were the highest. Figure 2.11 shows the correlation between SAUDPC and final incidence for the two diseases. Each set of data has a weak correlation

coefficient showing that there is not a strong relationship between SAUDPC or final ratings for the two diseases.

Discussion

Research has shown frequently that crosses between two plant genotypes with varying levels of resistance or even susceptibility can result in offspring with a significantly higher or lower level of resistance compared to that of both parents (24, 57). This is known as transgressive segregation which suggests that both parents carry genes for the trait of interest, but those genes are located at different loci. This results in alleles for resistance from both parents ending up together in some of the offspring plants leading to a higher level of resistance than in the parents (1).

Populations in this experiment showed transgressive segregation both toward resistance and susceptibility. Tifrunner has multiple genotypes associated with it that had disease scores for TSWV and late leaf spot that were better than itself, both from the 1799 population and the T population. The S population also had genotypes that were better than its resistant parent NC94022 for TSWV. SPT 06-06 shows a high level of resistance but some genotypes from Tifrunner show similar levels of resistance for both TSWV and late leaf spot. This could suggest that there are different mechanisms of resistance in those two genotypes. SPT 06-06 is an interspecific breeding line with *Arachis cardenasii* in its breeding line. This could be the source of the different mechanisms since Tifrunner has *A. hypogaea* in its pedigree. These are important genotypes to research more to find QTLs for disease resistance associated with TSWV and late leaf spot. Most mapping projects focus on one population to identify QTLs for resistance to a single disease (22). This research helps to reiterate the fact that it is important to

identify mechanisms of resistance in multiple population due to the fact that the mechanisms can be different.

When looking at TSWV and leaf spot resistance together for breeding purposes it is important to look at the overall performance of populations and genotypes against each other. For TSWV and late leaf spot, the best performing population, based on estimates, was the 1801 population, with the resistant parent SPT 06-06. Although none of the genotypes that were significantly better than their resistant parent came from this population, the genotypes tended to perform better than all other genotypes. This further helps to demonstrate that there are multiple mechanisms of resistance throughout the populations that should be considered.

When looking at the genotypes split into treatments (Table 2.1) for both TSWV (Fig. 2.4) and late leaf spot (Fig. 2.9) when can see that the high treatment had the lowest final disease rating values and the low treatment had the highest final disease ratings. This can be attributed to the fact that the treatments were determined based off of previous disease ratings for both early and late leaf spot. These ratings do not seem to be predicative of TSWV ratings or late leaf spot ratings alone. These populations have also never been ranked against each other. A genotype that was ranked as low disease in one population could be ranked as high disease if it was in another population. This reiterates the fact that it is important to compare the populations to determine the most resistant and susceptible genotypes for diseases for further evaluation.

The linear regression from Figure 2.11 shows that there is not a strong relationship between the SAUDPC values for each disease or the final ratings for each disease. Therefore, it is evident that selecting for resistance to one disease does not guarantee resistance to the other disease. However, Figures 3 & 8 do show strong relationships between the SAUDPC and the final rating for both of these diseases. This suggests that instead of multiple ratings throughout

the year being needed one rating at the end of the growing season is sufficient. The research done here can lead to better peanut breeding programs for resistance to the two most economically devastating diseases. This can be done through the use of conventional breeding lines using genotypes from the 1801 population or through the use of all the breeding lines to screen for QTLs.

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Table 2.1. Recombinant inbred lines selected for comparison for TSWV and late leaf spot

Treatment[a]	S Population	T Population	1799 Population	1801 Population
Highest Severity Ratings				
	S17	T11	596	952
	S24	T49	607	954
	S51	T70	608	980
	S119	T113	626	1028
	S128	T148	712	1036
	S329	T219	757	1040
Mean Severity Ratings				
	S4	T17	600	943
	S75	T21	602	946
	S102	T71	643	981
	S276	T106	691	982
	S316	T119	693	1001
	S344	T142	713	1008
Lowest Severity Ratings				
	S98	T20	660	917
	S179	T22	663	924
	S197	T48	700	971
	S223	T133	704	1012
	S338	T145	708	1042
	S347	T158	724	1075
Parental Lines				
	NC94022	GT-C20	NC3033	Florida-07
	SunOleic 97R	Tifrunner	Tifrunner	SPT 06-06

[a]Severity ratings are based from previous trials with combined ratings for early leaf spot (*Cercospora arachidicola* Hori) and late leaf spot (*Cercosporidium personatum* (Berk. & M. A. Curtis) Deighton)

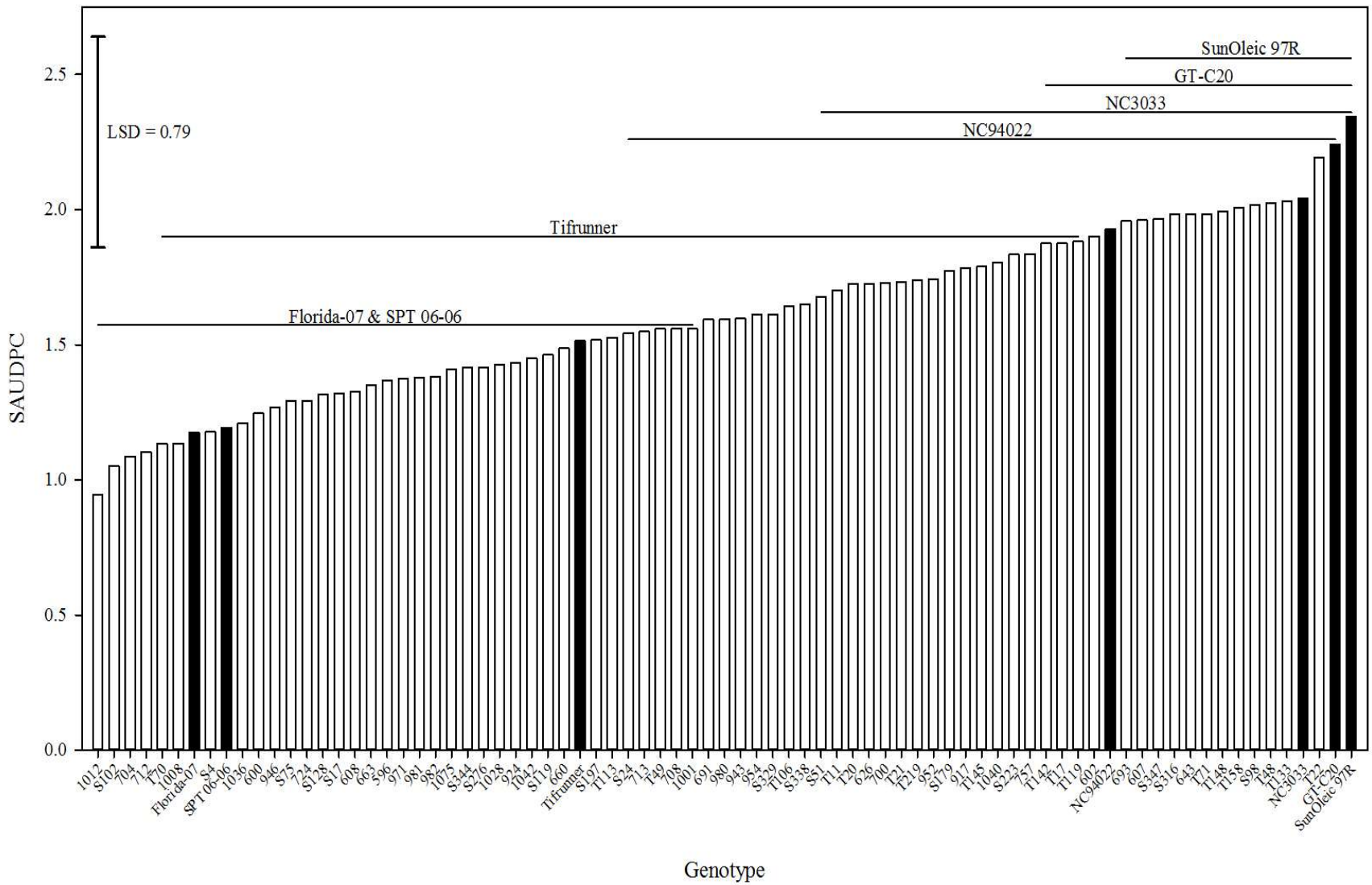

Fig. 2.1. Effect of peanut genotype on TSWV SAUDPC, calculated from 3 assessments in both 2015 and 2016 using a percentage based scale. Genotypes under the same bar do not differ from the specified parental line.

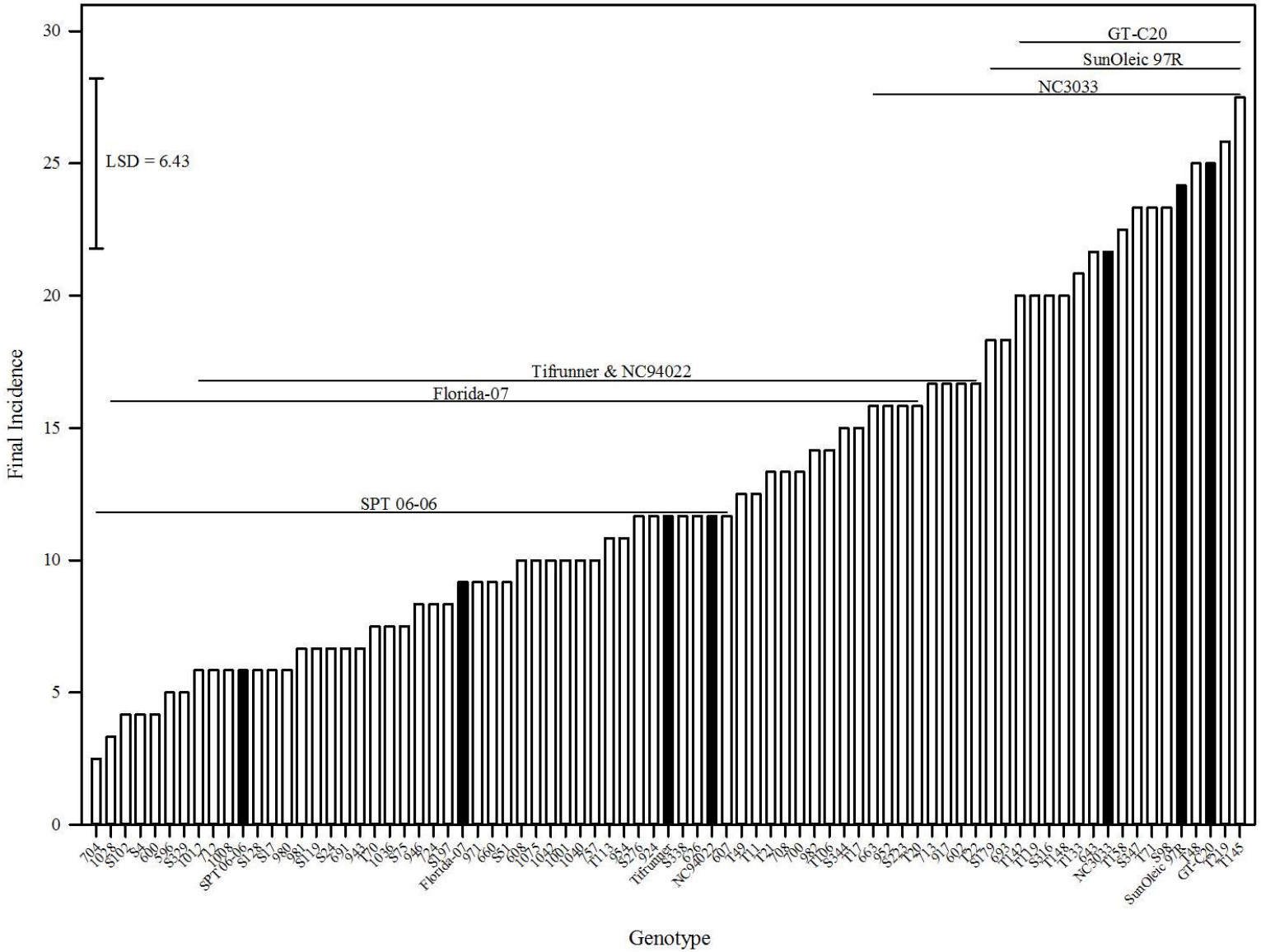

Fig. 2.2. Effect of peanut genotype on TSWV final incidence. Rating taken as a percentage of plot showing symptoms. Genotypes under the same bar do not differ from the specified parental line.

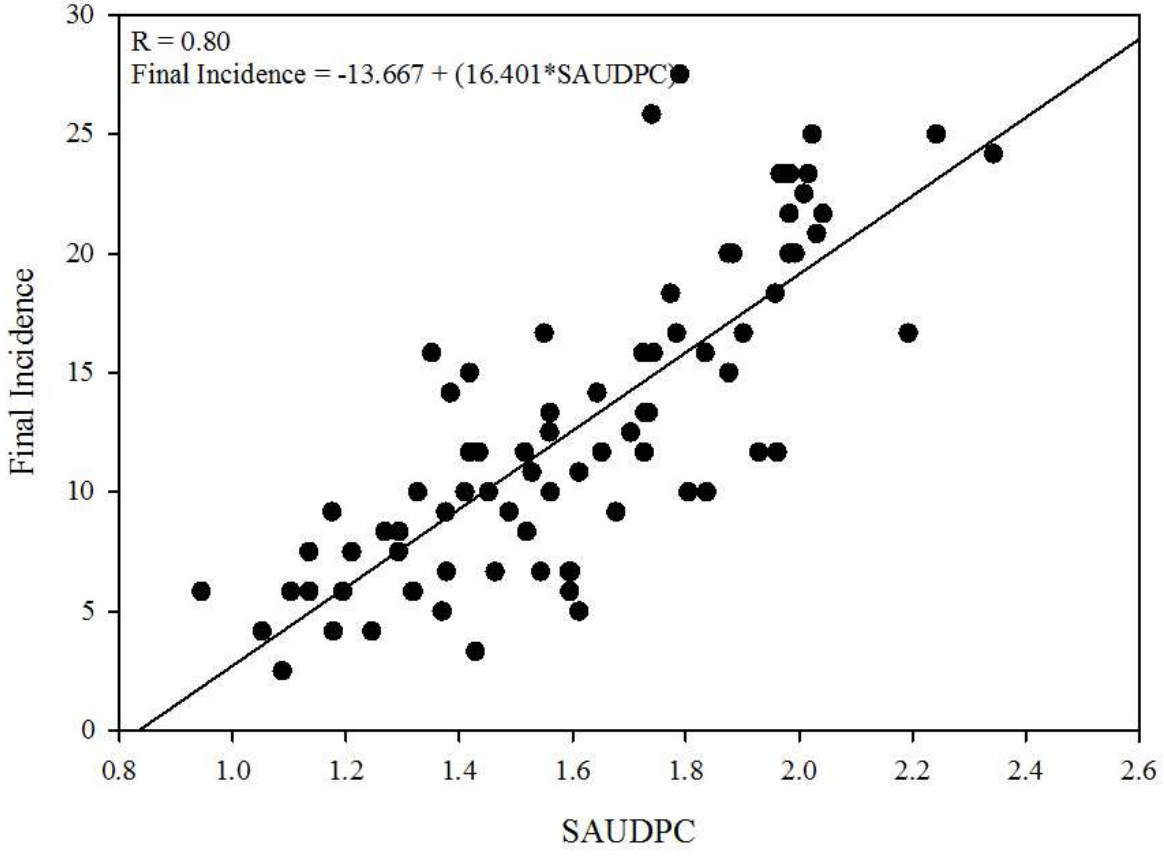

Fig. 2.3. Linear regression between SAUDPC and final incidence for TSWV ($P < 0.001$).

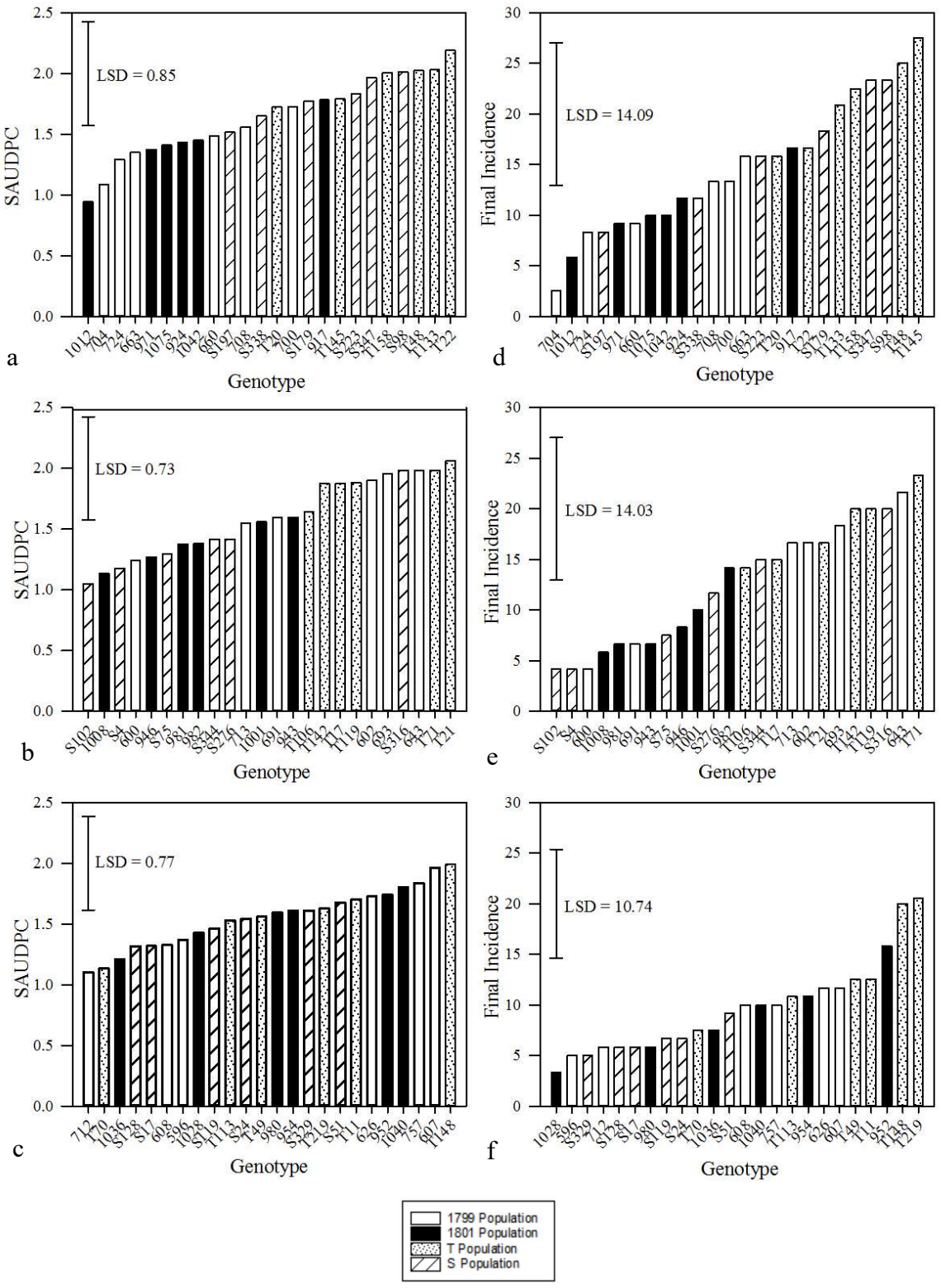

Fig. 2.4. Effect of peanut genotypes on TSWV SAUDPC and final incidence broken into three treatments: lowest predetermined leaf spot severity ratings (a, d), mean predetermined leaf spot severity ratings (b, e), and highest predetermined leaf spot severity ratings (c, f).

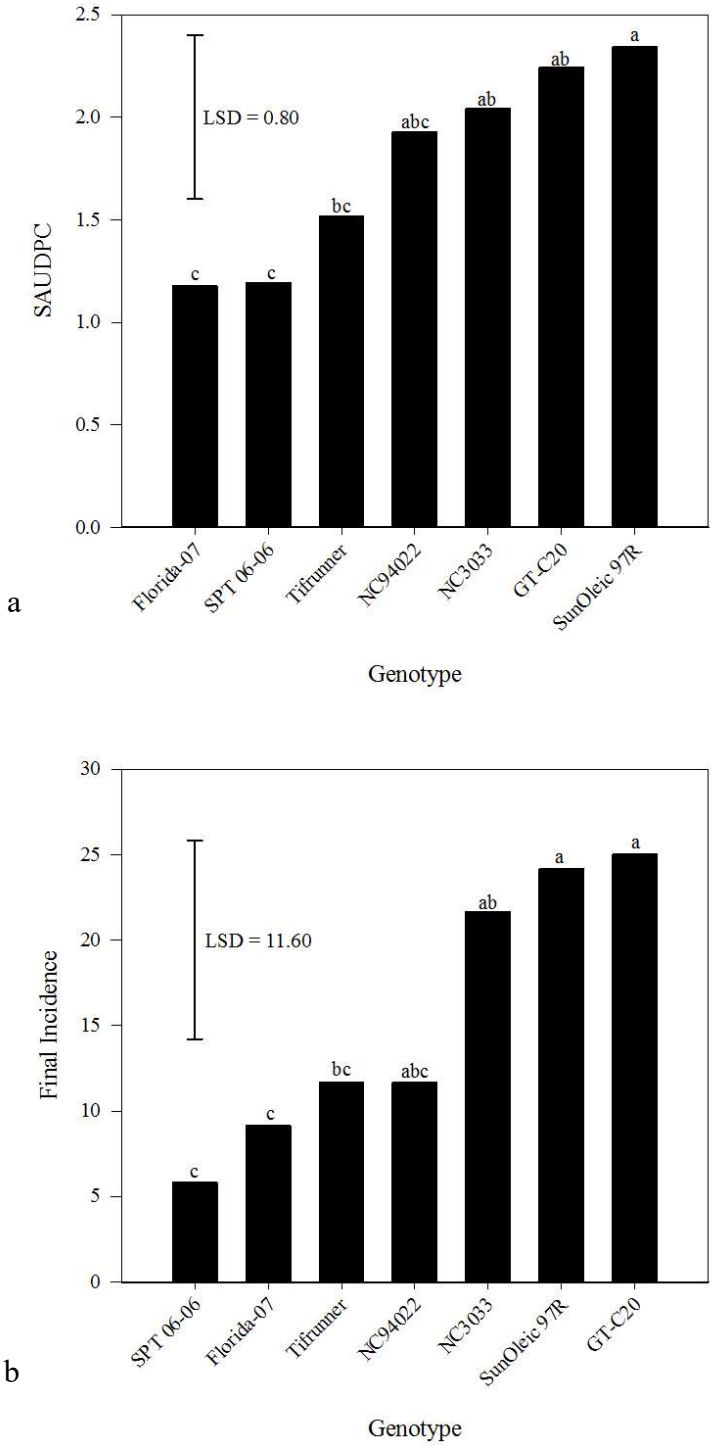

Fig. 2.5. Effect of parental lines on TSWV SAUDPC (a) and final incidence (b).

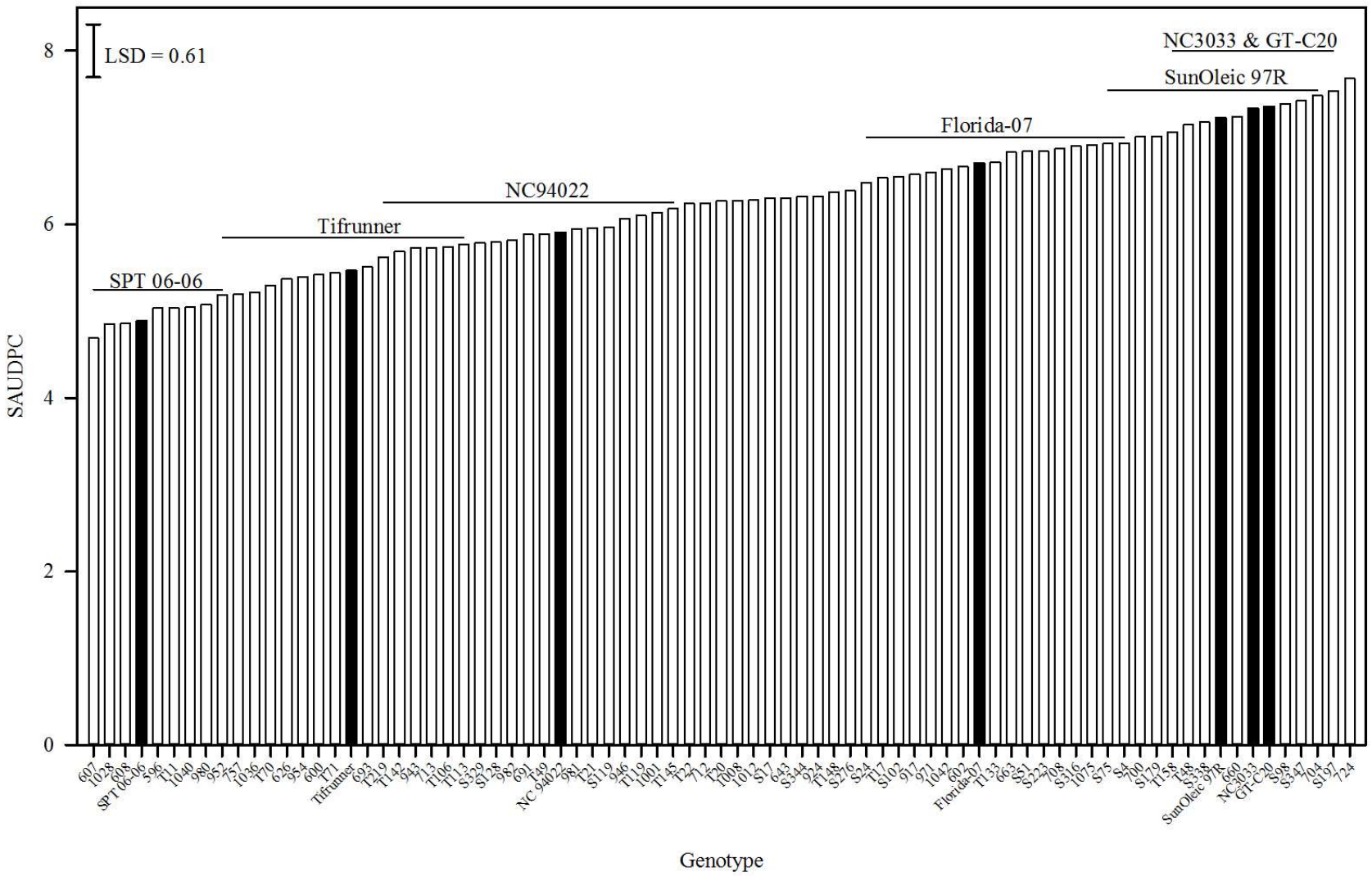

Fig. 2.6. Effect of peanut genotype on late leaf spot SAUDPC, calculated from 6 assessments in both 2015 and 2016 using the Florida 1-10 scale. Genotypes under the same bar do not differ from the specified parental line.

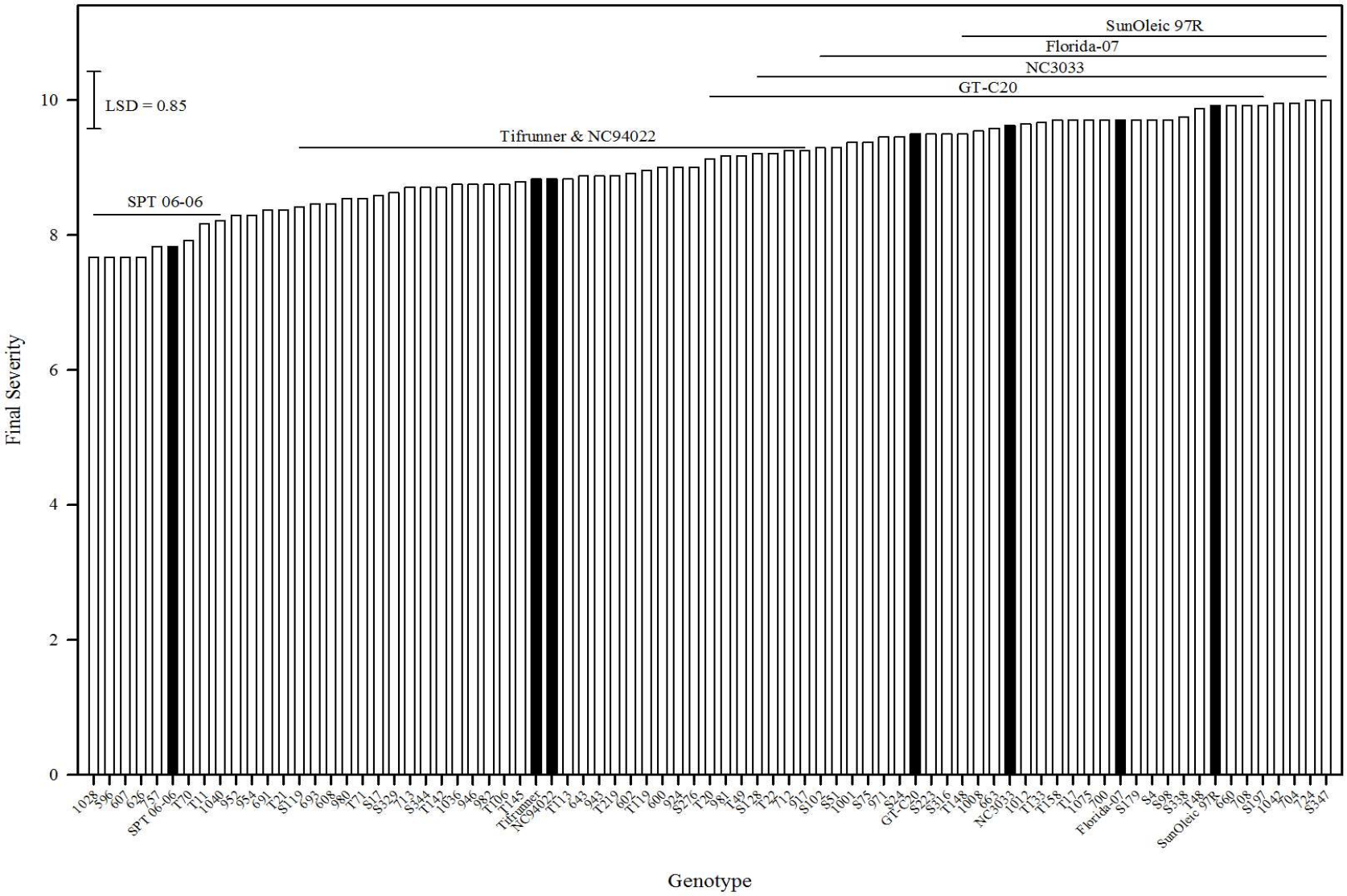

Fig. 2.7. Effect of peanut genotype on late leaf spot final severity based on the Florida 1-10 scale. Genotypes under the same bar do not differ from the specified parental line.

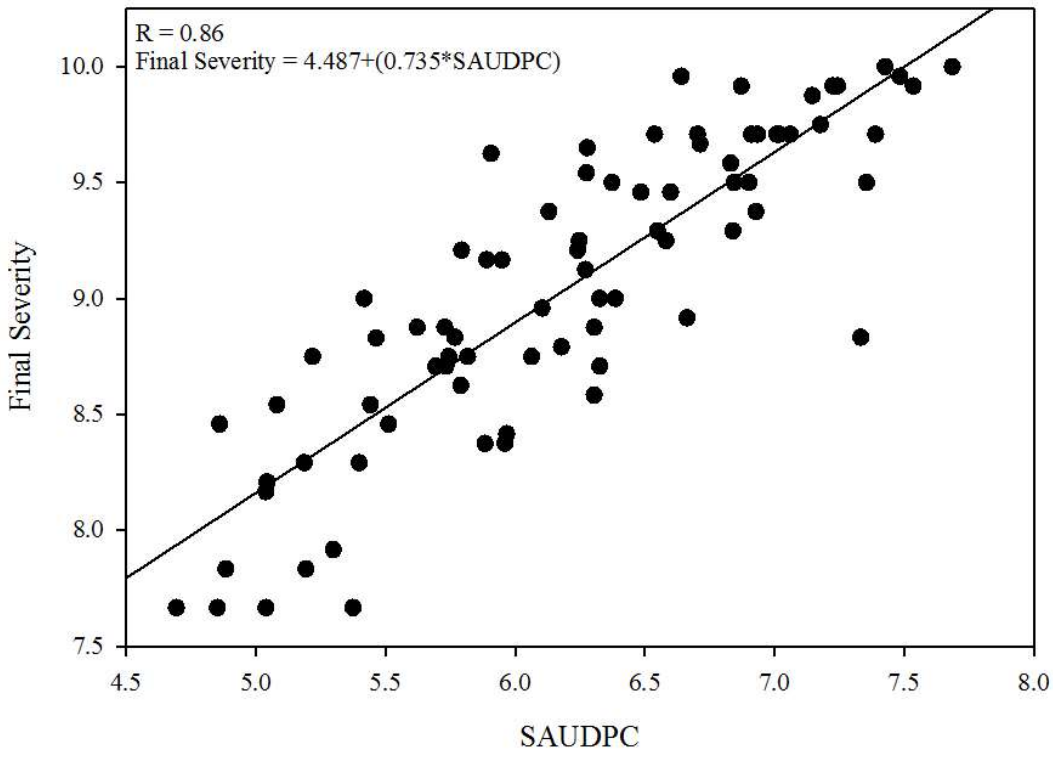

Fig. 2.8. Linear regression between SAUDPC and final severity for late leaf spot ($P < 0.001$).

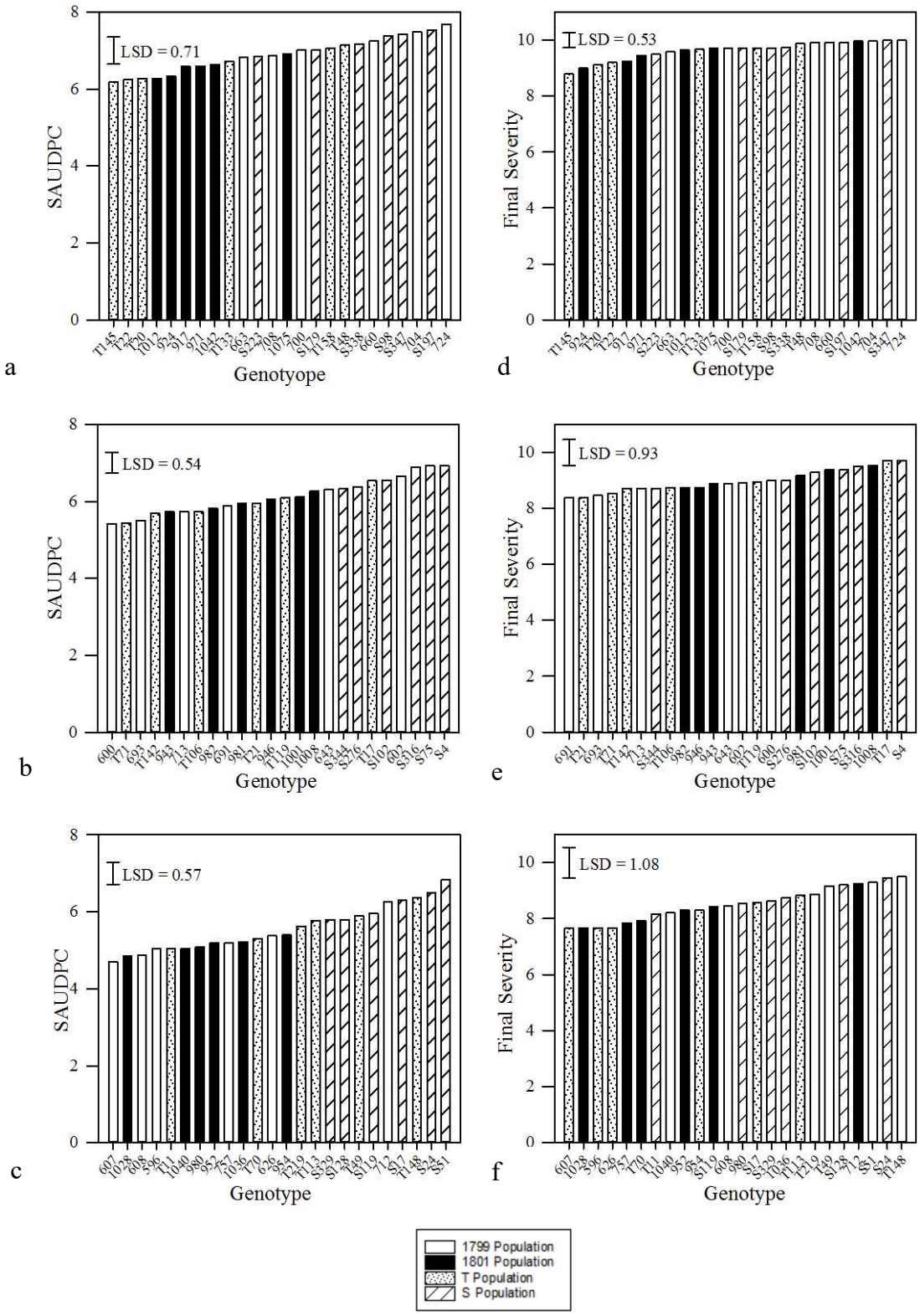

Fig. 2.9. Effect of peanut genotypes on late leaf spot SAUDPC and final incidence broken into three treatments: lowest predetermined leaf spot severity ratings (a, d), mean predetermined leaf spot severity ratings (b, e), and highest predetermined leaf spot severity ratings (c, f).

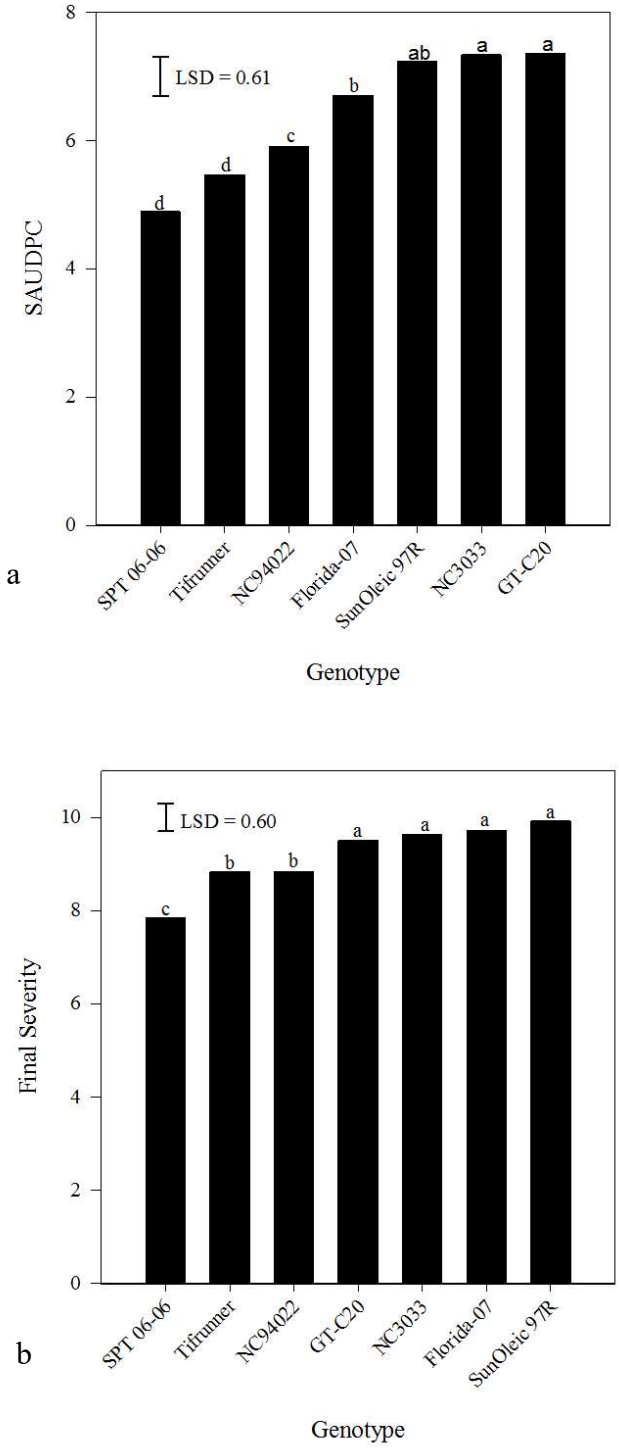

Fig. 2.10. Effect of parental lines on late leaf spot SAUDPC (a) and final incidence (b).

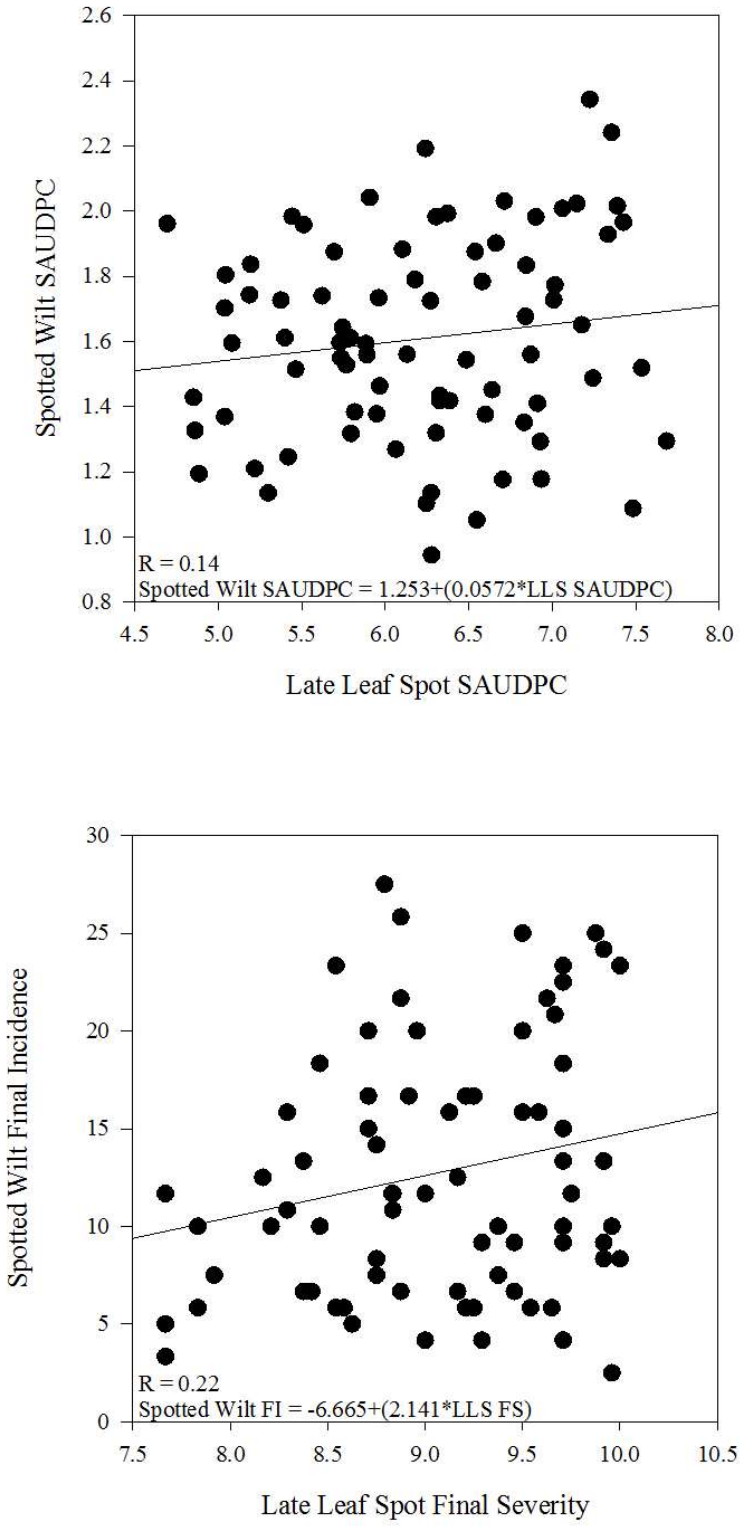

Fig. 2.11. Linear regression of late leaf spot SAUDPC versus TSWV SAUDPC ($P = 0.217$) (a) and late leaf spot final severity versus TSWV final incidence ($P = 0.054$) (b).

CHAPTER III

DEVELOPMENT OF A TECHNIQUE FOR ASSESSING TOMATO SPOTTED WILT VIRUS IN PEANUT (*ARACHIS HYPOGAEA* L.) USING AN UNMANNED AERIAL SYSTEM [2]

[2] Pelham, S. E., Monfort, S., Xu, R., Li, C., Holbrook, C. C., Godoy, I., and Culbreath, A. K. 2017. To be submitted to *Plant Disease*.

Abstract

Recombinant inbred line (RIL) populations of peanut (*Arachis hypogaea* L.) are being used to develop markers for resistance to several diseases, including Tomato spotted wilt virus (TSWV). In efforts to develop molecular markers to assist in selection for resistance to TSWV, several populations have been developed from parents with varying levels of resistance. Breeding programs are faced with the challenge of phenotyping large numbers of genotypes in a timely manner with a limited number of people. Unmanned aerial systems (UAS) may have potential to help overcome this challenge. Through the use of a UAS, imaging techniques, and ground truthing we developed an analysis method to quickly and easily assess TSWV incidence in the field. This experiment utilized 16 Brazilian genotypes with high susceptibility to TSWV and four cultivars currently used in the U.S. with some resistance to TSWV. With adequate ground truthing, TSWV can be differentiated from other diseases in the field by the color and the visible stunting of the plant. By creating an equation in Matlab version R 2016b (MathWorks, Natick, MA 01760 USA) to identify yellow pixels, known as the greenness equation, we can rate the incidence of the disease in many small plots in a relatively short amount of time. Matlab greenness equation values had varying levels of correlation with field disease incidence ratings. Analyses later in the growing season with more canopy width tended to be better correlated than analyses from earlier in the season. Although correlations were not strong between visual and aerial ratings, aerial ratings were able to separate the most resistant genotypes from the most susceptible genotypes. This technique can greatly increase the efficiency of breeding programs to screen large recombinant inbreed lines of peanut to TSWV.

Introduction

The use of unmanned aerial systems (UAS) in agriculture has greatly increased over the past decade. There are three main components in an UAS that are commonly identified. These are the unmanned aerial vehicle (UAV), the ground control station, and the communication data link. There are other components that are identified as critical in the use of an UAS. These can include but are not limited to imaging sensors, autopilots, and navigational systems (4). UAS have a wide range of uses and in combination with powerful data analysis methods can deliver information of high spatial and temporal resolution in non-destructive ways (29). A major reason for implementing UAS is to deliver near-real-time information for crop and soil properties which is important since growers need to know these properties in a timely fashion to implement production decisions (15, 20, 34).

Uses for UASs include monitoring and mapping of soil properties (5, 10), pest management (14), water analysis (8, 16), and weed control monitoring (11, 13, 23). Remote sensing, the broad term for any system that uses aerial platforms or satellites from which to collect data (31), has been used in many crops such as canola, corn, cotton, sorghum, and wheat (16, 24, 31, 33, 35). Visible light images taken in this manner have been used in yield mapping to identify field variation (9). However, most aerial images used in these studies are either airborne hyperspectral (6, 9, 16), satellite hyperspectral (22), or satellite multi-spectral (3, 7, 10). These methods allow for the use of vegetative indices to be applied to the images that can be used to assess the health of the crop (32). The use of UAVs for remote sensing and detection of diseases is an area that has a wide range of possibilities.

The development and use of resistant cultivars is one of the most desirable ways to manage peanut diseases (5). One of the most important of these diseases is Tomato spotted wilt

virus (TSWV), a *tospovirus* in the family Bunyavirdae (25). In 1971, the virus was reported affecting peanuts for the first time in the United States in Texas (12). Throughout the next 20 years, yield reductions of up to 95 percent occurred during severe epidemics (1, 2). This yield loss can be attributed to death of the plant as well as a reduction in pod number and size; kernels can also be affected by decreased number, discoloration, or malformation (26). TSWV has caused a dramatic shift since the mid 1990's in cultural practices, planting dates, and cultivars in the southeastern United States (28).

Selecting for resistance to TSWV through the help of molecular markers could significantly increase the usefulness of breeding programs (17). Field screening, however, requires considerable space and a relatively large number of plants. Disease severity and incidence ratings for large populations take massive amounts of time and are not feasible for multiple times throughout the season. This does not allow for the full potential of the population to be explored. The development of automated methods that rely on digital images for analysis could be useful in identifying and rating TSWV in a reliable and rapid way. Therefore, the objective of this study was to develop aerial imaging techniques using a UAS for assessing TSWV incidence in field evaluations of peanut genotypes and determine the relationship between assessment made with aerial imaging and visual ratings.

Materials and Methods

Field Setup

A field experiment was carried out in 2015 and 2016 at the Coastal Plain Experiment Station, Lang Farm and Rigdon Farm respectively. The trials included 16 peanut genotypes from Brazil and four commercial lines currently used in the United States (Table 3.1). The field was planted on 12 May, 2015 and 17 April, 2016. Plots consisted of two 6 m long rows spaced 0.91

m apart. The planting density was 18 seed/m. Experiment design was a randomized complete block design with four replicates (Fig. 3.1). Regular fungicide and herbicide applications were made from recommendations by the University of Georgia (18, 21) No insecticides were applied for control of tobacco thrips (*Franklinella fusca*) the primary vector of TSWV in peanut in Georgia (27). The purpose of this study was to determine if the Brazilian lines, which have never been screened for resistance to TSWV had field resistance to the virus.

Visual Data Collection

Visual assessments of TSWV incidence were made by counting the number of 0.3-m portions of row containing severely stunted, chlorotic, wilted or dead plants for each plot and converting that number to a percentage of total row length (5). In 2015 ratings were taken at 59, 72, 85, 102, and 125 days after planting. In 2016 the ratings were taken 54, 65, 83, 92, and 118 days after planting.

Aerial Data Collection

Video of the field was taken with a GoPro HERO4 (GoPro, San Mateo, 94402 USA) camera mounted on a DJI Phantom 2 quadcopter (DJI, Shenzhen, China) (Fig. 3.2). Assessments were from an altitude of approximately 8 meters and at a speed less than 5 m/s. In 2015 aerial assessment were made 85 and 124 days after planting (DAP). In 2016 aerial assessments were made 58, 63, 68, 90, 92, and 107 DAP. Table 3 shows the days after planting of the visual and aerial ratings for each analysis.

Image Pre-Processing

After the video was acquired, the video was edited to obtain an image of each plot. This was done by playing the video in Windows Media Player (Microsoft, Redmond, WA 98052 USA) and using Snipping Tool (Microsoft, Redmond, WA 98052 USA) to capture images of the

plots. The image of each plot was cropped further and rotated in Photos (Microsoft, Redmond, WA 98052 USA) to remove any surrounding plots from the image.

Image Processing

Images were processed using MATLAB version R 2016b (MathWorks, Natick, MA 01760 USA) to acquire TSWV incidence ratings. Using the visible light image of each plot, the red, green, and blue channels were separated and a greenness equation was created to identify the yellow pixels in the image using the following code:

```
r = (:, :, 1);
g = (:, :, 2);
b = (:, :, 3).
```

The following greenness equation was applied to the image:

```
greenness = 2*double(g)-double(r)-double(b).
```

To reduce interference from the soil, a mask was created which only showed greenness pixel values over 60:

```
mask = greenness > 60.
```

This value was determined after trial and error. An average of the pixel values in the mask was then taken and recorded giving the TSWV rating for that plot without soil. The following code was used to obtain the value:

```
mean(greenness(mask)).
```

This was repeated for all aerial ratings and plots. Figure 3.3 shows the process.

Statistical Analysis

Linear regression was performed for each aerial rating and its corresponding visual rating using SigmaPlot 13.0 (Systat Software, Inc., San Jose, CA 95131 USA). Analysis of variance

(ANOVA) was performed using RStudio (R Core Team, Vienna Austria) to separate the genotypes by each rating technique.

Results

In 2015, greenness values were significantly correlated with both visual ratings and aerial ratings. Figure 3.4 shows the linear regression for the two aerial analyses. The first analysis (Fig. 3.4a), with visual and aerial rating at 85 DAP, has a R value of 0.43 and the second analysis (Fig. 3.4b), with a visual rating at 125 DAP and an aerial rating at 124 DAP, has a R value of 0.67.

Data collected in 2016 shows correlations between visual ratings and aerial ratings as well. The first analysis (Fig. 3.5a) in 2016 was done with a visual rating that was taken at 54 DAP and the aerial rating was taken at 58 DAP. This analysis, with four days between the ratings, has an R value of 0.21. The second one (Fig. 3.5b) has an R value of 0.13 and has nine days between the visual and aerial ratings. Figure 3.5c shows a higher R value of 0.68 with only two days between the data collections. The last analysis (Fig. 3.5d), with both visual and aerial ratings, at 92 DAP has a R value of 0.53.

For aerial flights that did not have a visual rating taken within ten days three analyses were done. These three analyses consisted of the visual rating taken before the aerial rating, the visual rating taken after the rating, and an average of the two visual ratings. This can be seen in Figure 3.7. For the aerial rating taken at 68 DAP the analysis was regressed back to visual ratings taken 54 DAP (Fig. 3.7a), the average of visual ratings taken 54 and 83 DAP (Fig. 3.7b), and visual ratings taken 83 DAP (Fig. 3.7c). The regressions have R values of 0.13, 0.16, and 0.17 respectively. The same process was used for the aerial rating taken at 107 DAP. This video was analyzed using the visual ratings taken 92 DAP (Fig. 3.7d), the average of the visual ratings

taken at 92 and 118 DAP (Fig. 3.7e), and the visual rating taken at 118 DAP (Fig. 3.7f). These regressions have an R value of 0.48, 0.51, and 0.52 respectively.

Figure 3.9 shows the final visual rating (Fig. 3.9a) and final aerial rating (Fig. 3.9b) in 2015. Overall, the two graphs are very similar. The four genotypes with the lowest visual ratings are the same genotypes with the lowest aerial ratings. This is also the same for the other end of the spectrum. The four genotypes with the highest final rating only varied by one genotype between the visual rating and the aerial rating. This trend was continued into 2016 where the rankings between the two types of ratings were not different (Fig. 3.10). Tifguard, Georgia-06G, and Georgia-12Y were all in the bottom three genotypes for both the visual and the aerial ratings. SunOleic 97R as well as IACOL4 were also ranked similarly in both ratings.

Discussion

Results varied greatly depending on the maturity stage of the plant. Overall there appeared to be a linear relationship between visual ratings and aerial ratings that grew stronger as the plant matured. Figures 3.4 and 3.5 both show that an aerial rating taken later in the growing season results in a stronger relationship with the visual rating. This is likely due to the increase in canopy as the growing season progresses. Figure 3.6 can be used to demonstrate this. The figure shows the highest and lowest aerial ratings for the analyses in Figure 3.5. The two analyses from early in the season at 54 DAP (Fig. 3.6a & b) and 63 DAP (Fig.3.6c & d) show very little canopy width. However, the two analyses from later in the season at 90 DAP (Fig. 3.6e & f) and 92 DAP (Fig.3.6g & h) have a much greater canopy area. Due to this the analyses have a much higher R value demonstrating a stronger linear relationship.

It is also evident in figure 3.6 that the color of the plants early in the season are very similar. If there is not much difference in color the greenness equation would not be able to

differentiate between healthy and diseased tissue. This may play a role in the low correlations early in the season if there are no noticeable color differences early in the season. This suggests that ratings with greater differences in healthy versus diseased tissue, or later in the season, are the best option for rating TSWV severity aerially.

The time of day that the analysis is taken can also affect the correlation with visual ratings. The aerial rating at 90 DAP and 92 DAP were taken at different times in the day. The aerial rating at 90 DAP was taken in the early morning and the aerial rating taken at 92 DAP was taken close to solar noon. The aerial rating taken in the morning yields a better correlation with visual ratings than the aerial rating taken at solar noon. This could be due to the amount of sunlight present or the angle of the sun. Too much sunlight may wash out the color of the plants in the image. Also, the angle of the sun can create shadows that could skew the aerial ratings. Both of these factors, time of day and angle of the sun, play a major role in the ability to be able to accurately rate aerially for diseases. This is an area that more research needs to be done on for TSWV in peanut.

R values in Figure 3.7 show once again that early season aerial ratings have a weaker linear relationship with the visual ratings. Averaging two visual ratings to compare to an aerial rating taken in between them does not result in a stronger relationship (Fig. 3.7). In both cases, the latest visual rating had the highest R value (Fig 3.7c & f). This helps to reiterate that later analyses result in better linear relationships. Figure 3.8 once again shows the highest and lowest aerial ratings for aerial ratings 68 DAP and 107 DAP showing the canopy area available for these analyses.

In 2015 and 2016 rankings of the genotypes of the final visual and aerial rating were very similar (Fig. 3.9 & 3.10). Even though linear regressions between visual and aerial ratings were

not very strong, the aerial ratings were still able to separate the most resistant and most susceptible genotypes. This suggests that aerial ratings taken late in the growing season can be used to separate genotypes in a field evaluation for TSWV.

Two major advantages to aerial ratings are the collection of permanent data and the decrease in the amount of time it takes to collect and analyze the data. Field ratings can take an enormous amount of time depending on the size of the field and the amount of genotypes in the field. An aerial flight can be conducted in a fraction of the time. That data is then permanent and can be revisited at any time. If there is a discrepancy in the ratings the reason can then be investigated since there is a permanent image of the field. Another major advantage is the standardization of aerial ratings. Field ratings are done by a human and they can be biased. An aerial rating conducted by a computer program shows no bias. This can help to eliminate the possibility of human error in disease ratings.

Previous studies have shown that final incidence ratings and area under the disease progress curves for TSWV share a strong relationship (19). This suggests that many disease ratings are not necessary throughout the season, and one late season rating could be sufficient. Since aerial ratings taken later in the season can differentiate genotypes for TSWV suceptibility this can greatly cut down on the amount of time needed to evaluate genotypes for TSWV. This method of evaluation could improve the efficiency of breeding programs to screen large populations of peanut to TSWV.

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Table 3.1. Genotypes selected for evaluation and their origin.

Plot Number	Genotype	Origin
1	IAC113	Brazil
2	IAC127	Brazil
3	IAC137	Brazil
4	IAC147	Brazil
5	IAC322	Brazil
6	IAC503	Brazil
7	IAC505	Brazil
8	IAC506	Brazil
9	IAC573	Brazil
10	IAC599	Brazil
11	IAC825	Brazil
12	IAC886	Brazil
13	IAC8008	Brazil
14	IACOL3	Brazil
15	IACOL4	Brazil
16	GNL	Brazil
17	SunOleic 97R	United States
18	Tifguard	United States
19	Gerogia-06G	United States
20	Gerogia-12Y	United States

Table 3.2. Visual and aerial ratings taken in 2015 and 2016 and the difference, in days, between the two.

Year	Analysis	Visual Rating (DAP)	Aerial Rating (DAP)	Difference (days)
2015	1	85	85	0
	2	125	124	-1
2016	3	54	58	+4
	4	54	63	+9
	5	54	68	+14
	6	Average of 54 & 83	68	NA
	7	83	68	-15
	8	92	90	-2
	9	92	92	0
	10	92	107	-15
	11	Average of 92 & 118	107	NA
	12	118	107	-11

a b

Fig. 3.1. Field experiment layout in 2015 at 85 DAP (a) and 2016 at 90 DAP (b).

Fig. 3.2. GoPro HERO4 camera mounted on a DJI Phantom 2 quadcopter.

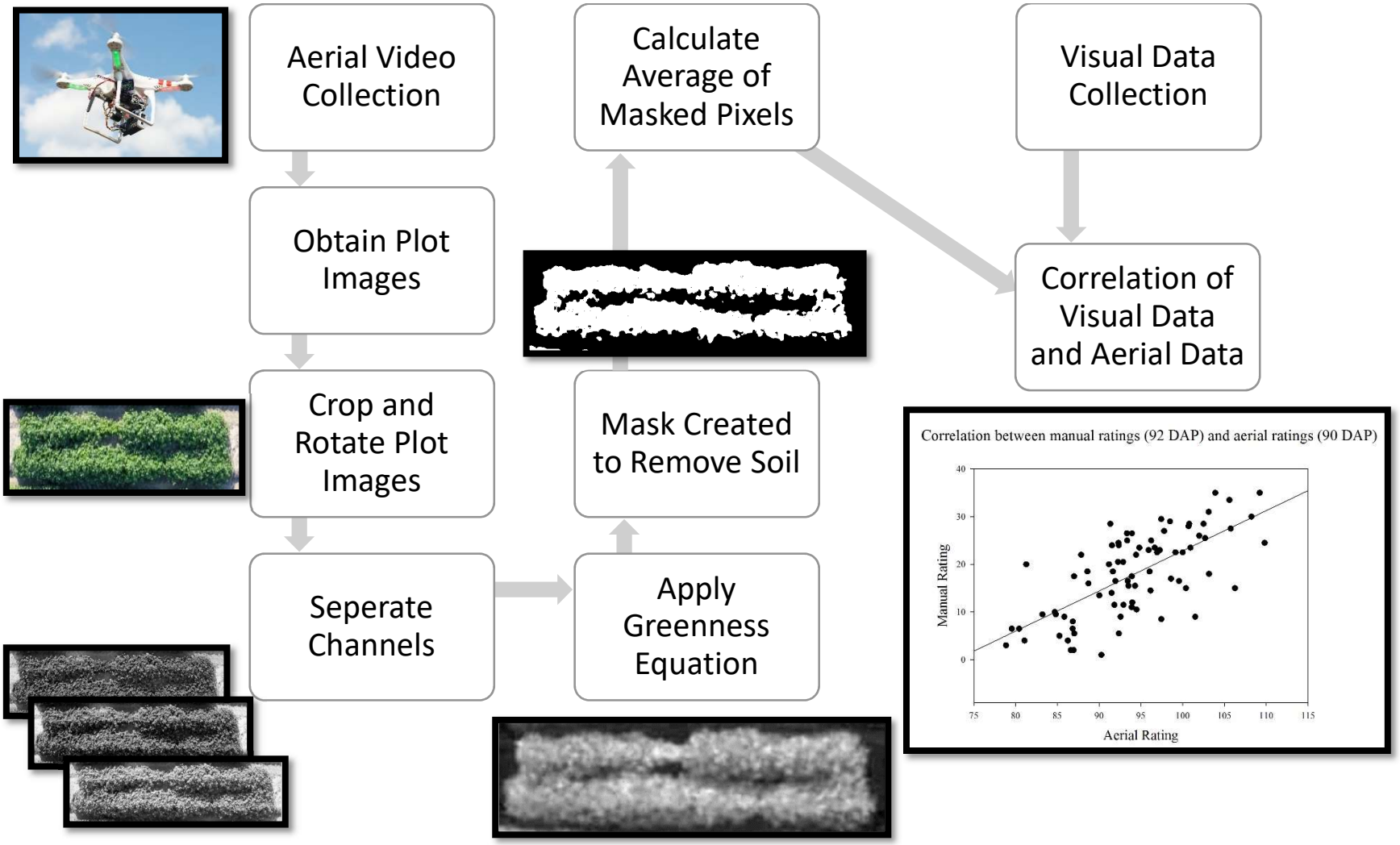

Fig. 3.3. Flow chart of method for aerial analysis of TSWV.

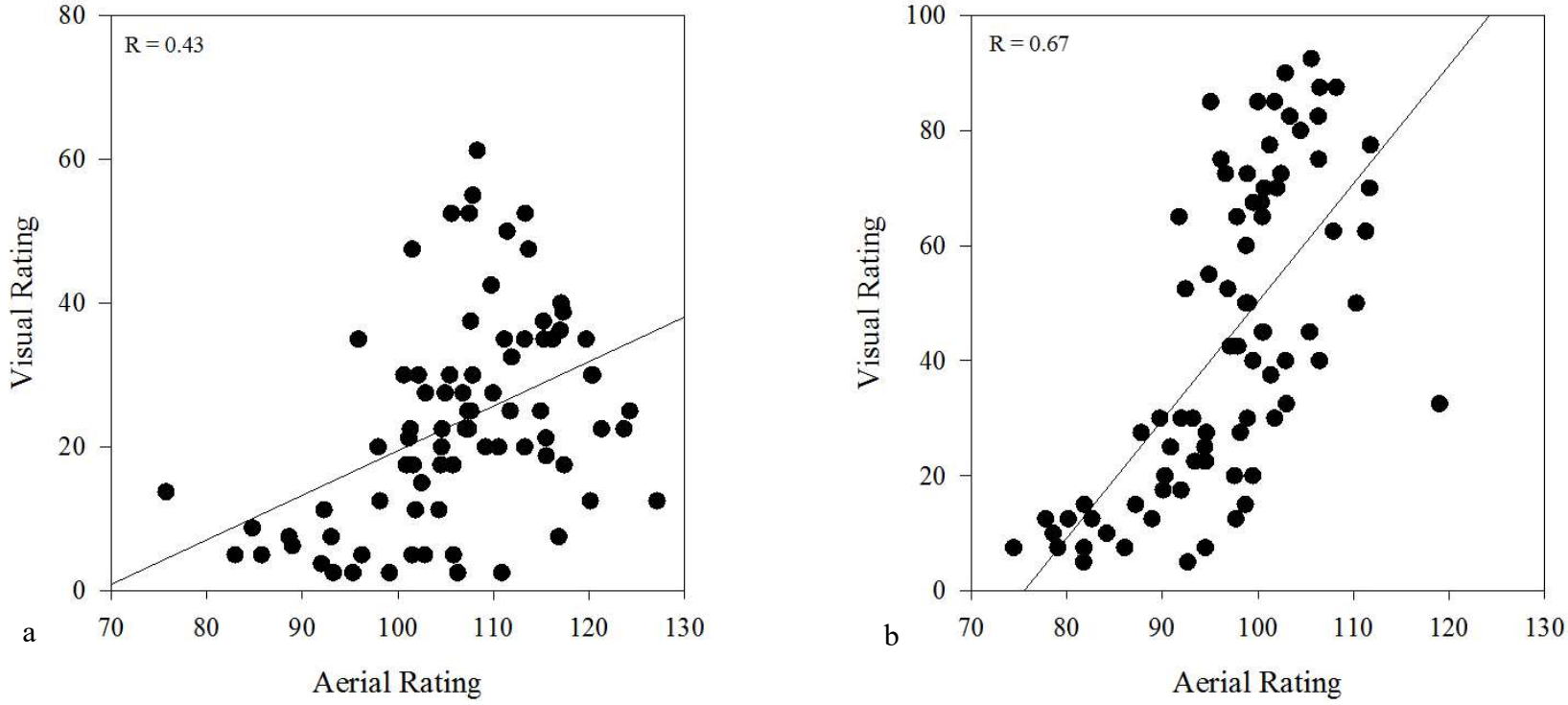

Fig. 3.4. Linear regression of visual ratings versus aerial ratings in 2015. Visual ratings at 85 DAP versus aerial ratings at 85 DAP (a) and visual ratings at 125 DAP versus aerial ratings at 124 DAP (b). Regression equations are as follows: (a) visual rating = -17.0 + (0.248 * aerial rating), $P < 0.001$; (b) visual rating = -155.047 + (2.053 * aerial rating), $P < 0.001$.

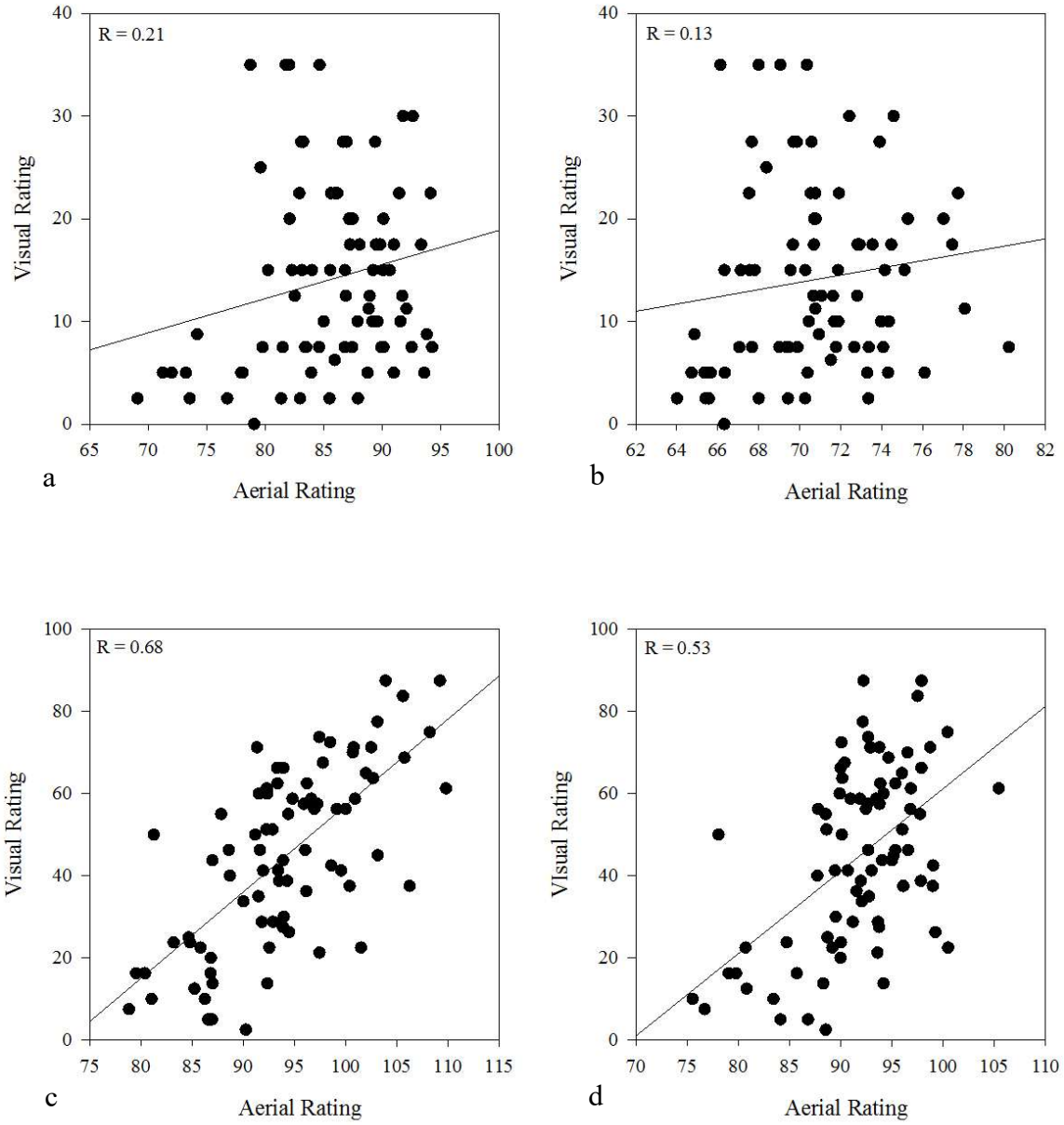

Fig. 3.5. Linear regression of visual ratings versus aerial ratings with less than 10 days separating in 2016. Visual ratings at 54 DAP versus aerial ratings at 58 DAP (a), visual ratings at 54 DAP versus aerial ratings at 63 DAP (b), visual ratings at 92 DAP versus aerial ratings at 90 DAP (c), and visual ratings at 92 DAP versus aerial ratings at 92 DAP (d). Regression equations are as follows: (a) visual rating = -14.43 + (0.333 * aerial rating), $P = 0.062$; (b) visual rating = -10.90 + (0.353 * aerial rating), $P = 0.234$; (c) visual rating = -153.48 + (2.11 * aerial rating), $P < 0.001$; (d) visual rating = -139.69 + (2.00 * aerial rating), $P < 0.001$.

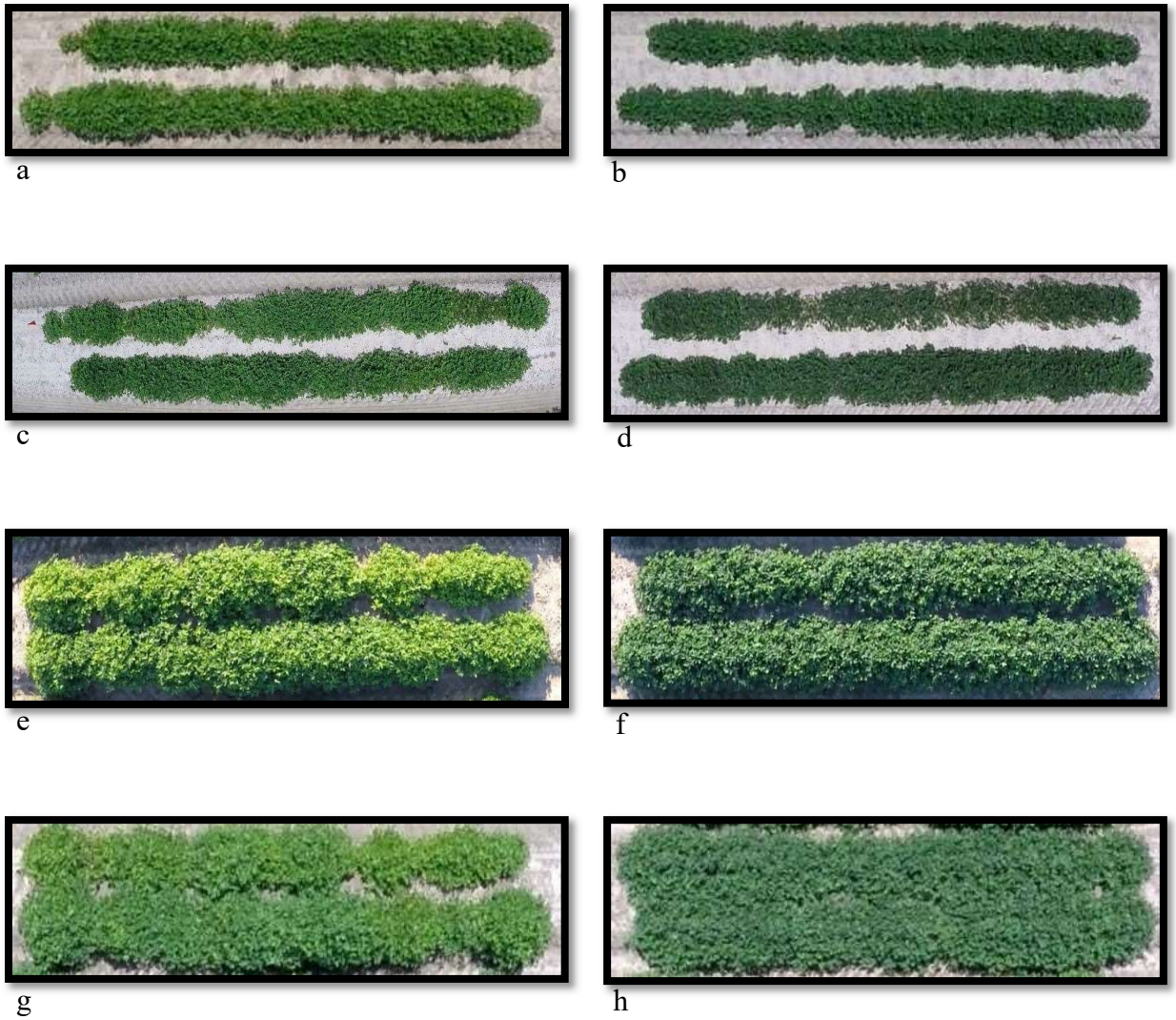

Fig. 3.6. Individual plot images for the lowest and highest aerial ratings at 58 DAP (a,b), 63 DAP (c,d), 90 DAP (e,f), and 92 DAP (g,h) in 2016.

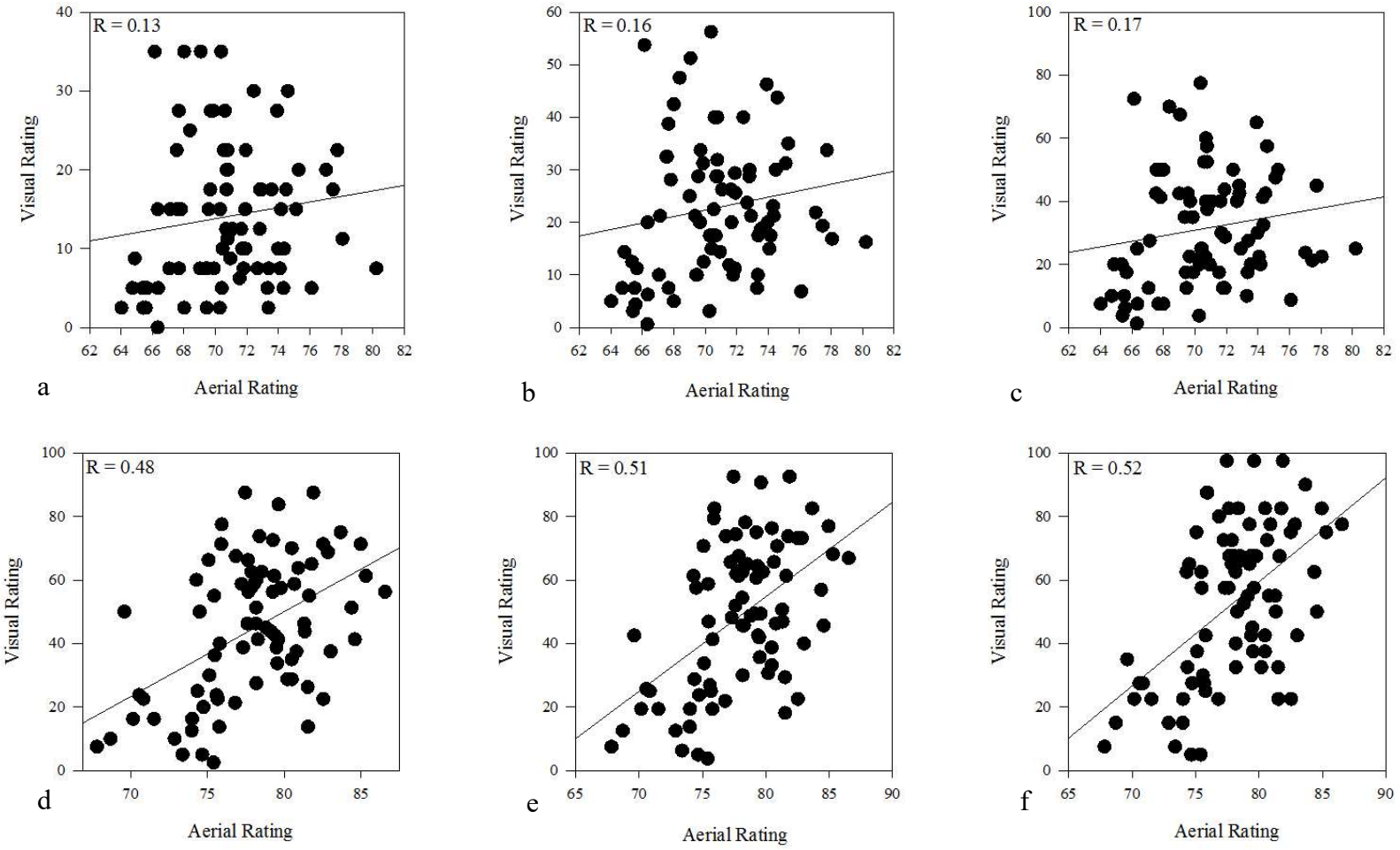

Fig. 3.7. Correlations for aerial ratings versus visual ratings with more than 10 days separating in 2016. Aerial ratings for 68 DAP versus visual ratings at 54 DAP (a), 83 DAP (c), and an average of both visual ratings (b) and aerial ratings for 107 DAP versus 92 DAP (d), 118 DAP (f). and an average of both visual ratings (e). Regression equations are as follow: (a) visual rating = -10.90 + (0.35 * aerial rating), $P = 0.234$; (b) visual rating = -20.79 + (0.62 * aerial rating), $P = 0.148$, (c) visual rating = -30.683 + (0.88 * aerial rating), $P = 0.141$; (d) visual rating = -164.47 + (2.68 * aerial rating), $P < 0.001$; (e) visual rating = -183.63 + (2.98 * aerial rating), $P < 0.001$; (f) visual rating = -202.80 + (3.278 * aerial rating), $P < 0.001$.

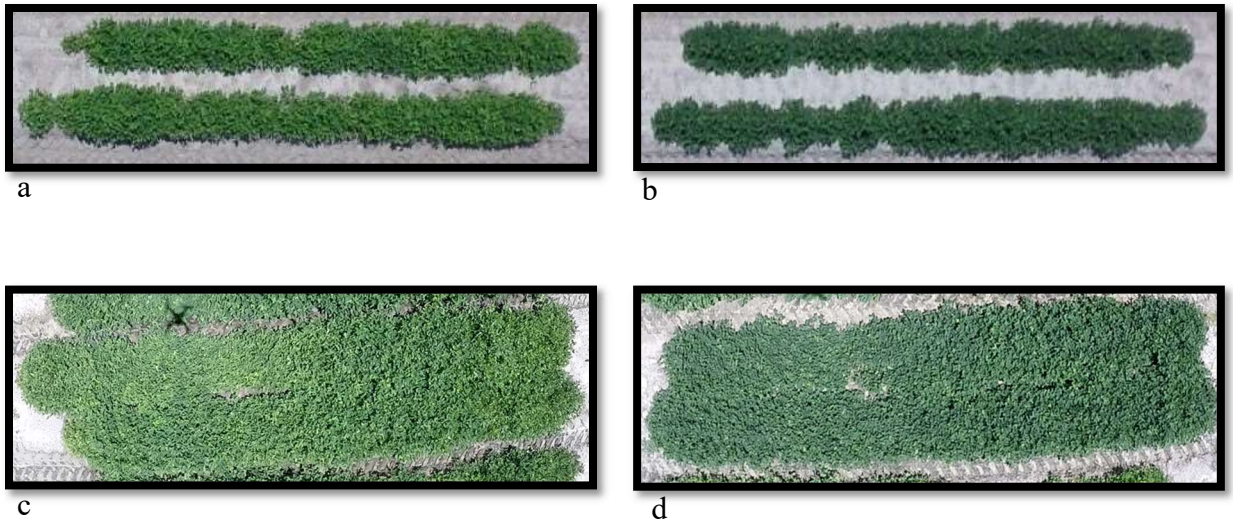

Fig. 3.8. Individual plot images for the lowest and highest aerial ratings at 68 DAP (a,b) and 107 DAP (c,d) in 2016.

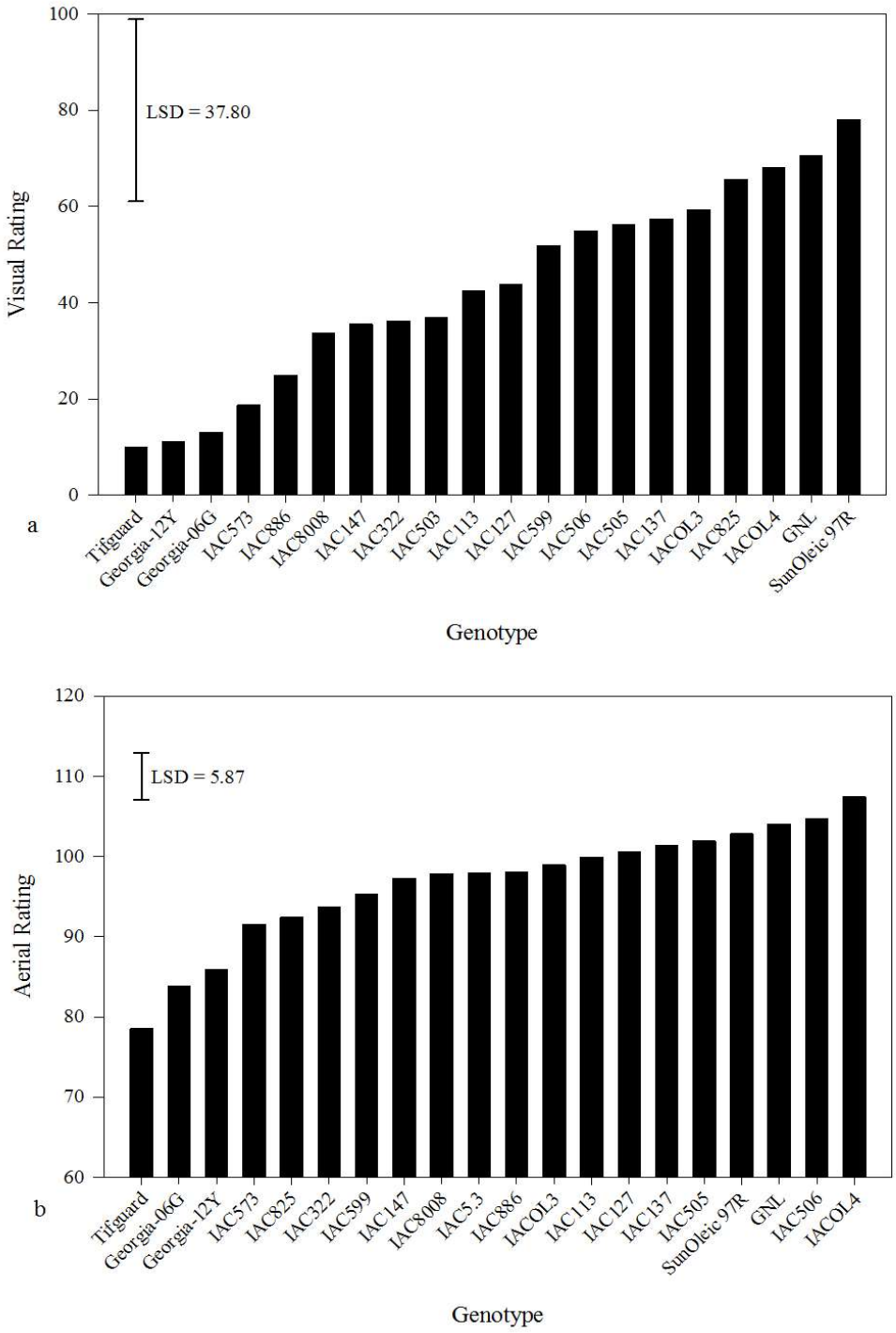

Fig. 3.9. Separation of genotypes in 2015 by visual ratings at 125 DAP (a) and aerial ratings at 124 DAP (b). Visual ratings are based on percentage of the plot showing symptoms of TSWV. Aerial ratings are an average of pixel values after the greenness equation is applied. Aerial rating values start at 60 due to the mask created to eliminate soil.

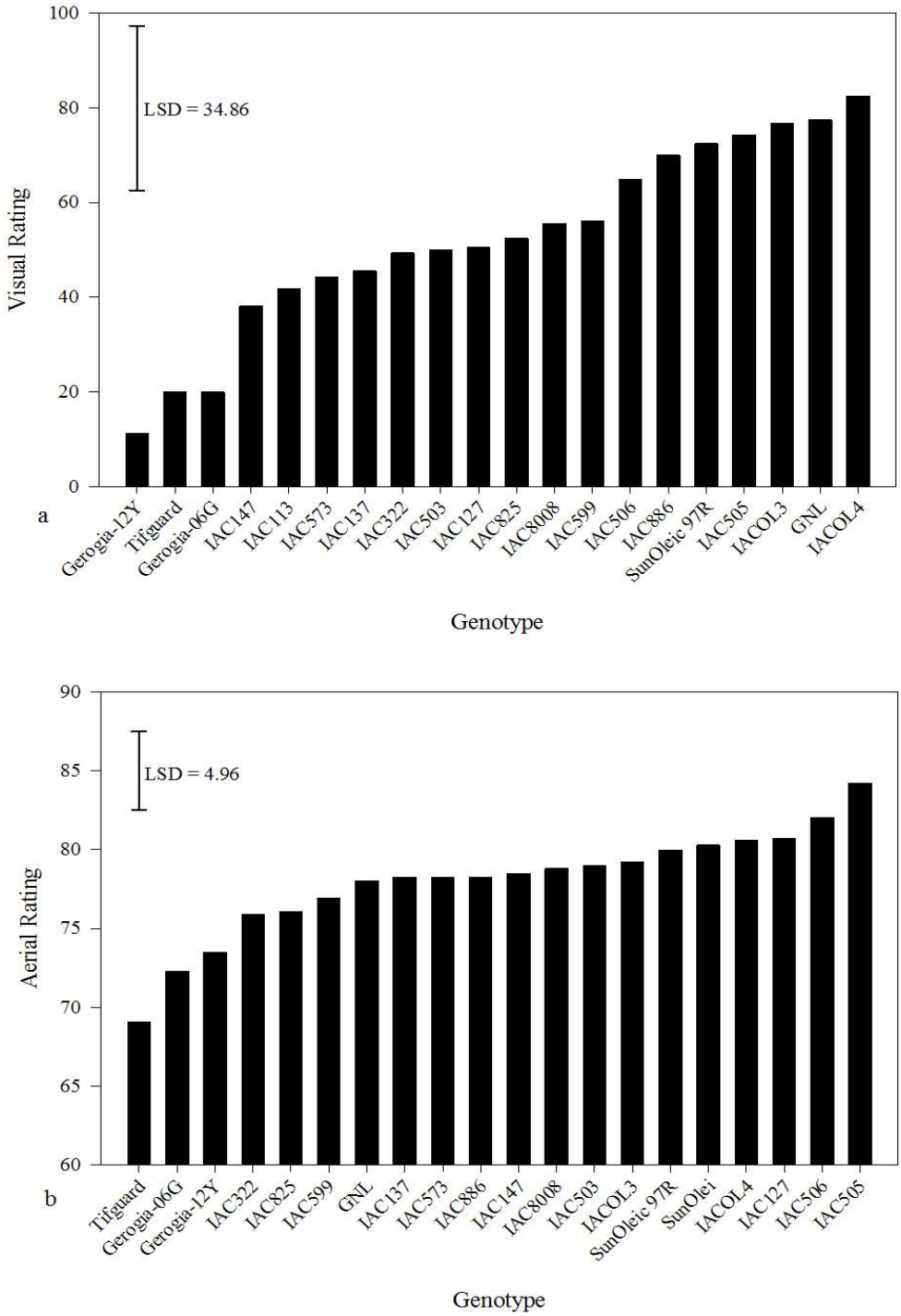

Fig. 3.10. Separation of genotypes in 2016 by visual ratings at 118 DAP (a) and aerial ratings at 107 DAP (b). Visual ratings are based on percentage of the plot showing symptoms of TSWV. Aerial ratings are an average of pixel values after the greenness equation is applied. Aerial rating values start at 60 due to the mask created to eliminate soil.

CHAPTER IV

DEVELOPMENT OF A TECHNIQUE FOR ASSESSING LATE LEAF SPOT (*CERCOSPORIDIUM PERSONATUM*) USING AN UNMANNED AERIAL SYSTEM IN LARGE MAPPING POPULATIONS OF PEANUT (*ARACHIS HYPOGAEA* L.) [3]

[3] Pelham, S. E., Monfort, S., Liakos, B., Holbrook, C. C., Guo, B., Chu, Y., Ozias-Akins, P., and Culbreath, A. K. 2017. To be submitted to *Plant Disease*.

Abstract

Recombinant inbred line (RIL) populations of peanut (*Arachis hypogaea* L.) are being used to develop markers for resistance to several diseases, including late leaf spot, caused by *Cercosporidium personatum*. In efforts to develop molecular markers to assist in selection for resistance to late leaf spot, several populations have been developed from parents with varying levels of resistance. Breeding programs are faced with the challenge of phenotyping large numbers of genotypes in a timely manner with a limited number of people. Unmanned aerial systems (UAS) may have potential to help overcome this challenge. Through the use of a UAS, imaging techniques, and ground truthing we developed an analysis method to quickly and easily assess late leaf spot severity in the field. This experiment utilized 72 recombinant inbreed lines (RIL) with varying levels of susceptibility to late leaf spot and seven parental lines. With adequate ground truthing, late leaf spot can be differentiated from other diseases in the field by the necrotic lesions on all above ground plant parts and defoliation. By testing 25 vegetative indices (VIs) in ArcMap (ESRI, Redlands, CA 92373 USA) we could determine the VI with the strongest correlation to visual ratings. Ratings at two weeks from harvest had the strongest correlations for all VIs. Results suggest that through the use of a UAS equipped with a multispectral camera a rating using the ratio vegetative index two weeks from harvest could greatly increase the efficiency of peanut breeding programs.

Introduction

The use of unmanned aerial systems (UASs) in agriculture has greatly increased greatly since the demonstration of their potential as a high-resolution remote sensing tool almost a decade ago through the use of early research type prototype sensors (1, 40). There are three main components in an UAS that are commonly identified. These are the unmanned aerial

vehicle (UAV), the ground control station, and the communication data link. There are other components that are identified as critical in the use of an UAS. These can include but are not limited to imaging sensors, autopilots, and navigational systems (11). UASs have a wide range of uses and in combination with powerful data analysis methods can deliver information of high spatial and temporal resolution in non-destructive ways (56). A major reason for implementing UASs is to deliver near-real-time information for crop and soil properties which is important since growers need to know these properties in a timely fashion to implement production decisions (30, 38, 61).

Uses for UASs include monitoring and mapping of soil properties (14, 20), pest management (28), water analysis (17, 31), and weed control monitoring (21, 27, 42). Remote sensing, the broad term for any system that uses aerial platforms or satellites from which to collect data (59), has been used in many crops such as canola, corn, cotton, sorghum, and wheat (31, 43, 58, 60, 62). Visible light images taken in this manner have been used in yield mapping to identify field variation (19). However, most aerial images used in these studies are either airborne hyperspectral (14, 19, 31), satellite hyperspectral (39), or satellite multi-spectral (9, 16, 20). Systems like this involve optical sensors that use light from the sun to illuminate a surface, then record the reflectance of that light in a number of wavelengths. These wavelengths can range from visible light through to the near-infrared and shortwave infrared to the thermal infrared part of the electromagnetic spectrum. A majority of the systems in use are multispectral, measuring reflectance in three to 10 bands. Satellite systems tend to have wide spectral bands, whereas airborne systems use narrow spectral bands. The most advanced systems are hyperspectral, with 126-224 very narrow spectral bands. Both of these methods allow for the use of vegetative indices to be applied to the images that can be used to assess the health of the crop

(59). The use of UAVs for remote sensing and detection of diseases is an area that has a wide range of possibilities.

The development and use of resistant cultivars is one of the most desirable ways to manage peanut diseases (12). Late leaf spot is one of the most serious foliar diseases of peanut in the world (18, 35, 44, 49). This disease, caused by the pathogen *Cercosporidium personatum* (Berk. & M. A. Curtis) Deighton (teleomorph = *Mycosphaerella berkeleyi* Jenk) (45), has become predominate in Florida and has increased its prevalence in Georgia in previous years (6). Late leaf spot occurs wherever peanuts are grown (25; 45) with yield losses up to 50% worldwide (57).

Late leaf spot symptoms can appear on any above ground parts of the peanut. This includes leaves, petioles, stipules, stems and pegs in the later stages of disease (50, 51). At first spots develop on the upper surface of lower leaves as small necrotic pinhead size spots and develop into large light to dark brown or black circular spots. These spots can range anywhere from 1 to 10 mm in diameter (55). Biomass and yield can be greatly affected when these spots coalesce. The loss is a result of defoliation of the plant.

Selecting for resistance to late leaf spot through the help of molecular markers could significantly increase the usefulness of breeding programs (32). Field screening, however, requires considerable space and a relatively large number of plants. Disease severity and incidence ratings for large populations take massive amounts of time, making it difficult to do multiple ratings throughout the season. This does not allow the full potential of the population to be explored. The development of automated methods that rely on multispectral images for analysis could be useful in identifying and rating late leaf spot in a reliable and rapid way.

Multispectral images have been used to detect plant diseases with varying levels of success in recent years. Calderon et al. (5) found significant differences between asymptomatic opium poppy plants and symptomatic opium poppy plants infected with downy mildew using the normalized difference vegetation index (NDVI) and the green/red ratio. Downy mildew in opium poppy initially produces small, chlorotic to light-yellow leaf lesions which progress to large necrotic areas in leaves or death of the entire leaf similar to the effect of late leaf spot on peanut plants (29, 37). The potential of UASs in the early detection of potato blight was shown by Nebiker et al. (40) when they detected an infestation using NDVI. A strong correlation was also found between NDVI values and grapevine leaf stripe disease symptoms, and discrimination between symptomatic and asymptomatic plants. The strong relationship between foliar symptoms and alterations of photosynthetic activity has been the driving force between the adoption of aerial techniques to assess diseases (15).

The red edge region which is located at the red-near infrared (680 and 780 nm) transition in leaf reflectance has been shown to have high information content for vegetation spectra as well (10). The abrupt change between 680 and 780 nm caused by the combined effects of strong absorption in the red wavelengths and high reflectance in the NIR wavelengths is due to leaf internal scattering (24). A shifting in the red edge slope and wavelength of maximum slope towards a longer wavelength could be due to the increasing amount of chlorophyll, otherwise the red edge position will shift towards a shorter wavelengths (4, 9). This band has been thought to be beneficial in estimating changes in foliar chlorophyll content and also as an indicator of vegetative stress (9, 13, 24, 47). With a lack of research being done in the area in peanut diseases the following objective was created. The objective of this study was to test vegetative indices to develop aerial imaging techniques using a UAS for assessing late leaf spot severity in

field evaluations of peanut genotypes and determine the relationship between assessments made with aerial imaging and visual ratings.

Materials and Methods

Field Setup

A field experiment was conducted at the University of Georgia Coastal Plain Experiment Station, Lang Farm, Tifton, GA in 2016. The field was previously planted to corn (*Zea mays* L.) grown using conventional tillage. Peanuts have been grown in this field previously, and disease history of the field includes severe epidemics of late leaf spot in previous peanut crops.

The experimental design was a randomized complete block design with three replicates of each genotype. Plots consisted of two 1.5 m long rows spaced 0.91 m apart. The planting density was 18 seed/m. Each plot was bordered on one side by the susceptible cultivar TUFRunner 511 (52) to increase overall incidence of TSWV and late leaf spot in all entries.

To maximize the potential for late leaf spot in the test the experiment was plated later in the season than usual (June 1st).. No fungicides were applied. Herbicides, insecticides, and fertilizers were applied following recommendations of the University of Georgia (34).

Selection of genotypes

There were a total of 16 genotypes chosen from each of the following mapping populations: 1799, 1801, T, and S. Based on ranked results from previous trials, the 6 RILs with the highest, 6 RILS with the lowest, and 6 RILs nearest the population mean for previous leaf spot ratings were included from each population. Parental lines from all populations were also included (Table 4.1). Since two of the populations have a common parent there were only seven parental lines tested instead of eight. This resulted in a total of 79 genotypes being tested in the field.

Visual Data Collection

Naturally occurring inoculum of leaf spot was relied on to create a natural epidemic. Disease severity for leaf spot was assessed using the Florida 1 to 10 scale system, where 1 = no disease, 2 = very few lesions (none on upper canopy), 3 = few lesions (very few on upper canopy), 4 = some lesions with more on upper canopy than for rank of 3 and slight defoliation noticeable, 5 = lesions noticeable even on upper canopy with noticeable defoliation, 6 = lesions numerous and very evident on upper canopy with significant defoliation (50%+), 7 = lesions numerous on upper canopy with much more defoliation (75%+), 8 = Upper canopy covered with lesions with high defoliation (90%+), 9 = very few leaves remaining and those covered with lesions (some plants completely defoliated), and 10 = plants dead (7). Late leaf spot was evaluated in all plots at 90, 104, 113, 121, and 135 DAP.

Aerial Data Collection

Multispectral images of the field were taken with a MicaSense RedEdge multispectral camera (MicaSense, Seattle, WA 98103 USA) mounted on a DJI Phantom 2 quadcopter (DJI, Shenzhen, China) (Fig. 4.1). The MicaSense Rededge camera has five spectral bands blue (B) (480 nm), green (G) (560 nm), red (R) (670 nm), red edge (RE) (720 nm), and near infrared (NIR) (840 nm). Images of a calibration pad were taken with the multispectral camera before and after flight image collection for image correction. Assessments were from an altitude of approximately 50 meters and at a speed less than 5 m/s. Aerial assessments were made 83, 106, 113, 121, and 135

Image Pre-Processing

After the images were acquired, they were uploaded to the MicaSense ATLAS data processing system. The online service aligned and stitched the images together. The images were

downloaded as a five-layered GEOTIFF. Using ArcMap 10.4.1(ESRI, Redlands, CA 92373 USA) the soil was removed for each layer of the GEOTIFF. Plots were then isolated from other plots, borders, and weeds.

Image Processing

The multispectral images were then used to produce vegetative index (VI) values for each plot. There was a total of 25 VIs that were chosen for the study (Table 4.2). Most of the VIs chosen were adapted from Patrick et al. (35). These VI are either well known and used in precision agriculture or a variation of a well-known VI that replaces the red band with the red edge band. The red edge region, which is located at the red-near infrared (680 and 780 nm) transition in the leaf reflectance, has been shown to have high information content for vegetation spectra (10). Two others, Soil-Adjusted Vegetation Index (SAVI) and Optimized Soil-Adjusted Vegetation Index (OSAVI), were added that correct for any soil that was left over (48). This process can be seen in figure 4.2

Statistical Analysis

Pearson's correlation coefficients were obtained for each VI and its corresponding visual rating using SigmaPlot 13.0 (Systat Software, Inc., San Jose, CA 95131 USA). Correlation plots were also made for certain VIs using SigmaPlot 13.0.

Results

Table 4.2 shows the vegetative indices and their corresponding Pearson's correlation coefficients for each rating date. The ratio VI has the strongest r value, 0.537, for the final rating. The Normalized Red VI had the second strongest correlation for this rating. The correlation for NDVI increased as the growing season progressed and ultimately was the 4th best VI on the last rating date. The Woebbecke Index had the weakest correlation for the last rating.

As shown in the table, correlations at 121 DAP were higher than correlations at 135 DAP. Looking at the correlation plots for the ratio VI the progression of the correlation is well seen (Fig. 4.3).

Discussion

Results indicated that the ratio vegetative index had the strongest correlation with late leaf spot ratings using the Florida 1-10 scale. This VI is a ratio of the NIR band and the red band. This is in line with literature which states that the spectral reflectance of green vegetation in the red band is the most sensitive to leaf chlorophyll and pigment contents while the NIR band in most sensitive to biomass (2, 3, 26, 51, 53). It has also been found that stressed plants, generally, have lower absorption of red light and higher absorption of NIR radiation (22, 23, 33). Since late leaf spot causes necrotic lesions on all of the above ground plant parts and ultimately kills the plant this makes sense. The weakest correlations came from the excess green minus excess red VI which does not utilize the NIR or the red band. Therefore, this VI is not using the bands that are related to chlorophyll and biomass.

The strength of the correlation for NDVI, one of the most common VIs in precision agriculture, to the visual ratings increased as the growing season progressed. This is line with the literature that has found that NDVI correlations increase as the season progresses (40). This study also showed that different genotypes of barley vary in correlations with NDVI. They found that NDVI correlates very well with some while it does not correlate at all with others. This could cause some of the variations in our study. There are 79 different genotypes in the study all with varying plant colors which can affect the NDVI values.

When examining the correlation graphs for the ratio VI it is easy to see the progression of the correlation as the season goes. This progression can be attributed to a couple of reasons.

Firstly, this test was conducted to measure both Tomato spotted wilt virus (TSWV) and late leaf spot. TSWV was evaluated early in the season before late leaf spot was present. Due to this, it is probable that there is TSWV present in the plant for the earlier ratings. This could cause some changes in values for the VIs. Secondly, the Florida 1-10 scale is based on the progression of the disease through the plant. The first 5 values on the scale correspond with increasing incidence of lesions, with very little defoliation. These two factors are important for vegetative indices. The correlations started to increase once the majority of the plots in the field reached a four on the scale. Values for the first visual rating were mainly one, two, and three which with few or no lesions in the upper canopy, could account for the weak correlation. Correlations were also strongest for the rating taken at 121 DAP. The lack of correlation for the final rating could be lower due to the large amount of defoliation in most of the plots by that late in the season. Due to this, correlations were run again by converting the Florida 1-10 to a scale based on percent defoliation. There was not a significant difference between the correlations from both scales.

When examining the graph of the ratio VI at 121 DAP it was discovered that there is a tight cluster of three outliers in the top right corner. Upon investigation, it was found that all three of the data points are from the genotype SPT 06-06. This may be attributed to its physiological properties. When examining this genotype in the field it has a slightly brighter green color than the surrounding plots. Nebiker et al. (40) showed that different genotypes of barley had varying NDVI values. Results indicated that some genotypes correlated very strongly with NDVI values while some genotypes correlated very weakly. The correlation values were run again without SPT 06-06 (Table 4.3). Correlation coefficients increase slightly without this genotype. The ratio VI still has the strongest correlation. It is evident that physiological

properties of the genotype affect values for VIs but the extent of this relationship is not yet known.

It was also discovered that some plots on the southwestern edge of the field were shaded by large pine trees during some of the flights. To correct for this those plots were taken out of all rating dates and the correlations were run again. The new correlations (Table 4.4) were slightly higher than the original correlations (Table 4.2) but not as high as the correlations without SPT 06-06 (Table 4.3). The rankings of the VIs also changed with this exclusion of the shaded plots. The Normalized Red VI that uses RE instead of R was ranked as the best followed by the Normalized Red VI and the Ratio VI was ranked third. This shows that cloud cover and shadows may affect VI values but physiological differences between genotypes has a greater effect.

Two major advantages to aerial ratings are the collection of permanent data and the decrease in the amount of time it takes to collect and analyze the data. Field ratings can take an enormous amount of time depending on the size of the field and the number of genotypes in the field. An aerial flight can be conducted in a fraction of the time. That data is then permanent and can be revisited at any time. If there is a discrepancy in the ratings, the reason can then be investigated since there is a permanent image of the field. Another major advantage is the standardization of aerial ratings. Field ratings are done by a human and they can be biased. An aerial rating conducted by a computer program should be less likely to have bias. This can help to eliminate the possibility of human error in disease ratings.

Results suggest that through the use of a UAS equipped with a multispectral camera a rating using the ratio vegetative index two weeks from harvest could greatly aid in evaluation of

large populations for field response to leaf spot diseases in trials where there is a range of levels of defoliation caused by the diseases.

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Table 4.1. Recombinant inbred lines selected for comparison.

S Population	T Population	1799 Population	1801 Population
S4	T11	596	917
S17	T17	600	924
S24	T20	602	943
S51	T21	607	946
S75	T22	608	952
S98	T48	626	954
S102	T49	643	971
S119	T70	660	980
S128	T71	663	981
S179	T106	691	982
S197	T113	693	1001
S223	T119	700	1008
S276	T133	704	1012
S316	T142	708	1028
S329	T145	712	1036
S338	T148	713	1040
S344	T158	724	1042
S347	T219	757	1075
SunOleic 97R	GT-C20	Tifrunner	SPT 06-06
NC94022	Tifrunner	NC3033	Florida-07

Table 4.2. Vegetative Indices, their formula, and the corresponding Pearson's correlation coefficient for visual versus aerial ratings.

	Visual Rating[a]	90 DAP	104 DAP	121 DAP	135 DAP
	Aerial Rating	83 DAP	106 DAP	121 DAP	135 DAP
Vegetative Index[b]	Formula[c]	R	R	R	R
Ratio VI	NIR/R	0.323	0.496	0.597	0.537
Normalized Red	R/(NIR+R+G)	0.303	0.400	0.600	0.527
Normalized Red – Partly RE	R/(NIR+RE+G)	0.295	0.402	0.597	0.527
Normalized Difference VI	(NIR-R)/(NIR+R)	0.302	0.370	0.583	0.522
Optimized Soil Adjusted VI	(NIR-R)/(NIR+R+0.16)	0.302	0.370	0.583	0.522
Difference VI	NIR-R	0.397	0.511	0.606	0.519
Normalized Near Infrared	NIR/(NIR+R+G)	0.324	0.523	0.578	0.505
Normalized Pigment Chlorophyll Index - RE	(RE-B)/(RE+B)	0.241	0.316	0.584	0.479
Normalized Excess Blue	(1.4*B-G)/(1.4*B+G)	0.217	0.301	0.603	0.461
Green Difference VI	NIR-G	0.398	0.525	0.584	0.457
Difference VI - RE	NIR-RE	0.412	0.505	0.569	0.425
Green VI - RE	(G-RE)/(G+RE)	0.217	0.342	0.532	0.388
Green Ratio VI	NIR/G	0.307	0.490	0.541	0.382
Normalized Green - RE	G/(NIR+RE+G)	0.307	0.456	0.532	0.382
Green Normalized Difference VI	(NIR-G)/(NIR+G)	0.318	0.472	0.528	0.364
Normalized Near Infrared - RE	NIR/(NIR+RE+G)	0.337	0.498	0.533	0.340
Excess Red - RE	1.4*RE-G	0.237	0.330	0.444	0.336
Excess Green Minus Excess Red	(|2*G-RE-B|)-(1.4*RE-G)	0.165	0.189	0.348	0.327
Normalized Difference VI - RE	(NIR-RE)/(NIR+RE)	0.347	0.499	0.521	0.284
Optimized Soil Adjusted VI - RE	(NIR-RE)/(NIR+RE+0.16)	0.347	0.499	0.521	0.284
Ratio VI - RE	NIR/RE	0.337	0.462	0.520	0.283
Excess Green - RE	|2*G-RE-B|	0.221	0.381	0.488	0.244
Normalized Green	G/(NIR+R+G)	0.314	0.439	0.470	0.179
Normalized Red - RE	RE/(NIR+RE+G)	0.350	0.450	0.493	0.176
Woebbecke Index	(G-B)/(RE-G)	0.174	0.290	0.576	0.055

[a]Ratings for late leaf spot (*Cercosporidium personatum* (Berk. & M. A. Curtis)) were taken based on the Florida 1-10 scale (7).

[b]VI = Vegetation Index, RE = Red edge band replaces red band

[c]B = Blue band (480 nm), G = Green band (560 nm), R = Red band (670 nm), NIR = Near infrared band (670 nm), RE = Red edge band (720 nm)

Table 4.3. Vegetative Indices, their formula, and the corresponding Pearson's correlation coefficient for visual versus aerial ratings without SPT 06-06.

	Visual Rating[a]	90 DAP	104 DAP	121 DAP	135 DAP
	Aerial Rating	83 DAP	106 DAP	121 DAP	135 DAP
Vegetative Index[b]	Formula[c]	R	R	R	R
Ratio VI	NIR/R	0.333	0.548	0.688	0.599
Normalized Red	R/(NIR+R+G)	0.310	0.428	0.658	0.571
Normalized Red – Partly RE	R/(NIR+RE+G)	0.301	0.428	0.651	0.568
Normalized Difference VI	(NIR-R)/(NIR+R)	0.309	0.397	0.641	0.566
Optimized Soil Adjusted VI	(NIR-R)/(NIR+R+0.16)	0.309	0.397	0.641	0.566
Difference VI	NIR-R	0.409	0.574	0.675	0.565
Normalized Near Infrared	NIR/(NIR+R+G)	0.333	0.601	0.641	0.550
Normalized Pigment Chlorophyll Index - RE	(RE-B)/(RE+B)	0.248	0.338	0.636	0.517
Green Difference VI	NIR-G	0.410	0.592	0.657	0.502
Normalized Excess Blue	(1.4*B-G)/(1.4*B+G)	0.223	0.316	0.652	0.499
Difference VI - RE	NIR-RE	0.425	0.568	0.647	0.474
Green Ratio VI	NIR/G	0.317	0.545	0.622	0.426
Green VI - RE	(G-RE)/(G+RE)	0.224	0.371	0.592	0.420
Normalized Green - RE	G/(NIR+RE+G)	0.316	0.500	0.594	0.418
Green Normalized Difference VI	(NIR-G)/(NIR+G)	0.327	0.532	0.591	0.402
Normalized Near Infrared - RE	NIR/(NIR+RE+G)	0.348	0.567	0.603	0.379
Excess Red - RE	1.4*RE-G	0.243	0.360	0.495	0.363
Excess Green Minus Excess Red	(|2*G-RE-B|)-(1.4*RE-G)	0.166	0.205	0.375	0.349
Normalized Difference VI - RE	(NIR-RE)/(NIR+RE)	0.358	0.557	0.592	0.318
Ratio VI - RE	NIR/RE	0.349	0.511	0.598	0.318
Optimized Soil Adjusted VI - RE	(NIR-RE)/(NIR+RE+0.16)	0.358	0.557	0.592	0.318
Excess Green - RE	|2*G-RE-B|	0.228	0.418	0.571	0.270
Normalized Red - RE	RE/(NIR+RE+G)	0.361	0.493	0.568	0.202
Normalized Green	G/(NIR+R+G)	0.324	0.477	0.541	0.200
Woebbecke Index	(G-B)/(RE-G)	0.180	0.318	0.603	0.052

[a]Ratings for late leaf spot (*Cercosporidium personatum* (Berk. & M. A. Curtis)) were taken based on the Florida 1-10 scale (7).
[b]VI = Vegetation Index, RE = Red edge band replaces red band

[c]B = Blue band (480 nm), G = Green band (560 nm), R = Red band (670 nm), NIR = Near infrared band (670 nm), RE = Red edge band (720 nm)

Table 4.4. Vegetative Indices, their formula, and the corresponding Pearson's correlation coefficient for visual versus aerial ratings without shaded plots.

	Visual Rating[a]	90 DAP	104 DAP	121 DAP	135 DAP
	Aerial Rating	83 DAP	106 DAP	121 DAP	135 DAP
Vegetative Index[b]	Formula[c]	R	R	R	R
Normalized Red – Partly RE	R/(NIR+RE+G)	0.324	0.421	0.627	0.575
Normalized Red	R/(NIR+R+G)	0.333	0.422	0.628	0.574
Ratio VI	NIR/R	0.352	0.529	0.619	0.572
Normalized Difference VI	(NIR-R)/(NIR+R)	0.332	0.397	0.611	0.568
Optimized Soil Adjusted VI	(NIR-R)/(NIR+R+0.16)	0.332	0.397	0.611	0.568
Difference VI	NIR-R	0.395	0.541	0.644	0.552
Normalized Near Infrared	NIR/(NIR+R+G)	0.352	0.550	0.605	0.549
Normalized Pigment Chlorophyll Index - RE	(RE-B)/(RE+B)	0.252	0.322	0.618	0.523
Normalized Excess Blue	(1.4*B-G)/(1.4*B+G)	0.226	0.306	0.642	0.502
Green Difference VI	NIR-G	0.399	0.557	0.624	0.483
Difference VI - RE	NIR-RE	0.419	0.533	0.607	0.452
Green Ratio VI	NIR/G	0.331	0.517	0.564	0.413
Green VI - RE	(G-RE)/(G+RE)	0.220	0.353	0.557	0.413
Normalized Green - RE	G/(NIR+RE+G)	0.331	0.481	0.557	0.410
Green Normalized Difference VI	(NIR-G)/(NIR+G)	0.343	0.499	0.554	0.397
Normalized Near Infrared - RE	NIR/(NIR+RE+G)	0.360	0.525	0.560	0.380
Excess Red - RE	1.4*RE-G	0.217	0.336	0.475	0.344
Excess Green Minus Excess Red	(|2*G-RE-B|)-(1.4*RE-G)	0.140	0.182	0.374	0.338
Ratio VI - RE	NIR/RE	0.357	0.491	0.546	0.334
Normalized Difference VI - RE	(NIR-RE)/(NIR+RE)	0.369	0.526	0.546	0.332
Optimized Soil Adjusted VI - RE	(NIR-RE)/(NIR+RE+0.16)	0.369	0.526	0.549	0.332
Excess Green - RE	|2*G-RE-B|	0.208	0.398	0.516	0.229
Normalized Red - RE	RE/(NIR+RE+G)	0.371	0.476	0.520	0.227
Normalized Green	G/(NIR+R+G)	0.338	0.464	0.495	0.192
Woebbecke Index	(G-B)/(RE-G)	0.168	0.304	0.626	0.097

[a]Ratings for late leaf spot (*Cercosporidium personatum* (Berk. & M. A. Curtis)) were taken based on the Florida 1-10 scale (7).
[b]VI = Vegetation Index, RE = Red edge band replaces red band

[c]B = Blue band (480 nm), G = Green band (560 nm), R = Red band (670 nm), NIR = Near infrared band (670 nm), RE = Red edge band (720 nm)

Fig. 4.1. MicaSense RedEdge multispectral camera mounted on DJI Phantom 2 quadcopter.

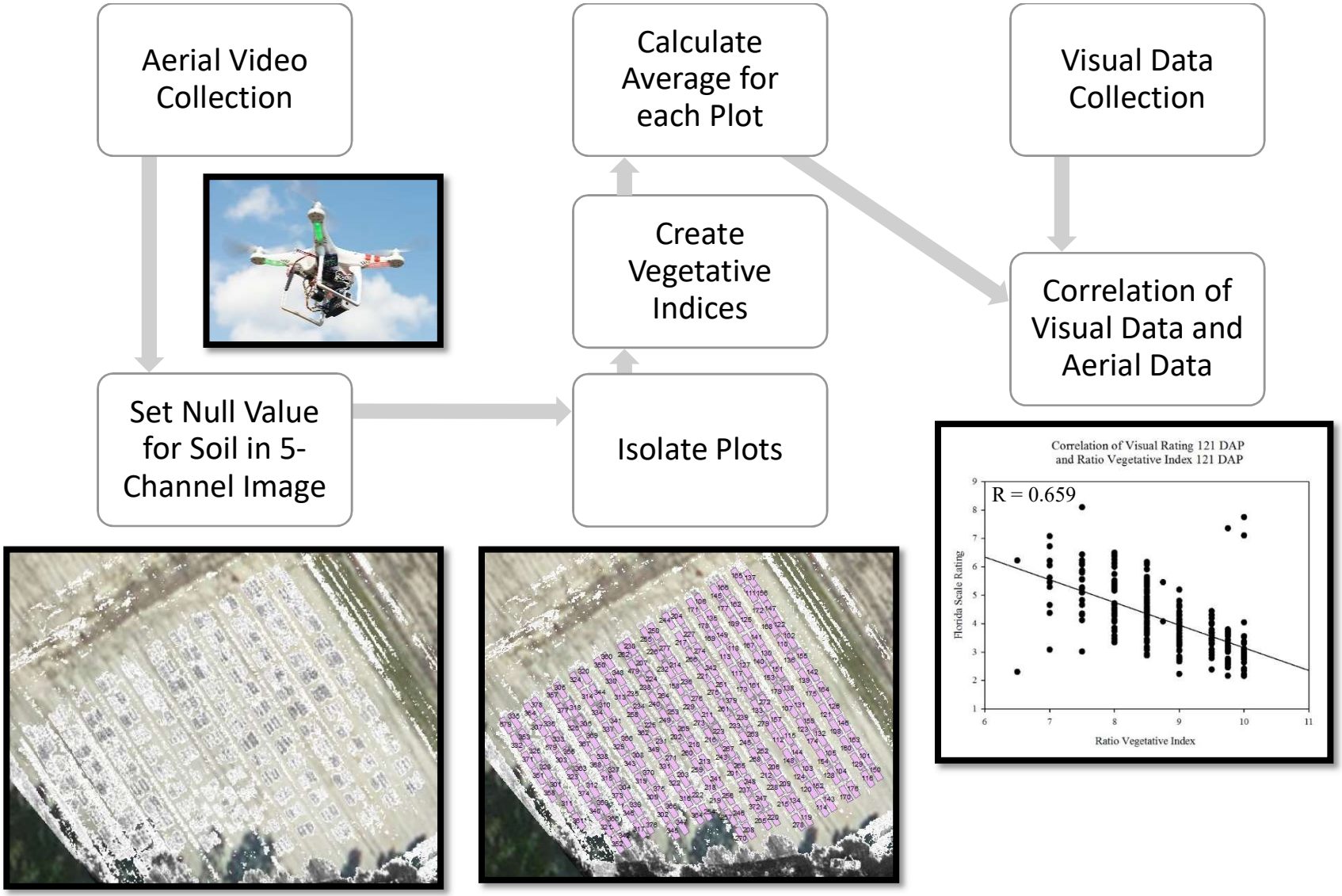

Fig. 4.2. Flow chart of method for aerial analysis of late leaf spot.

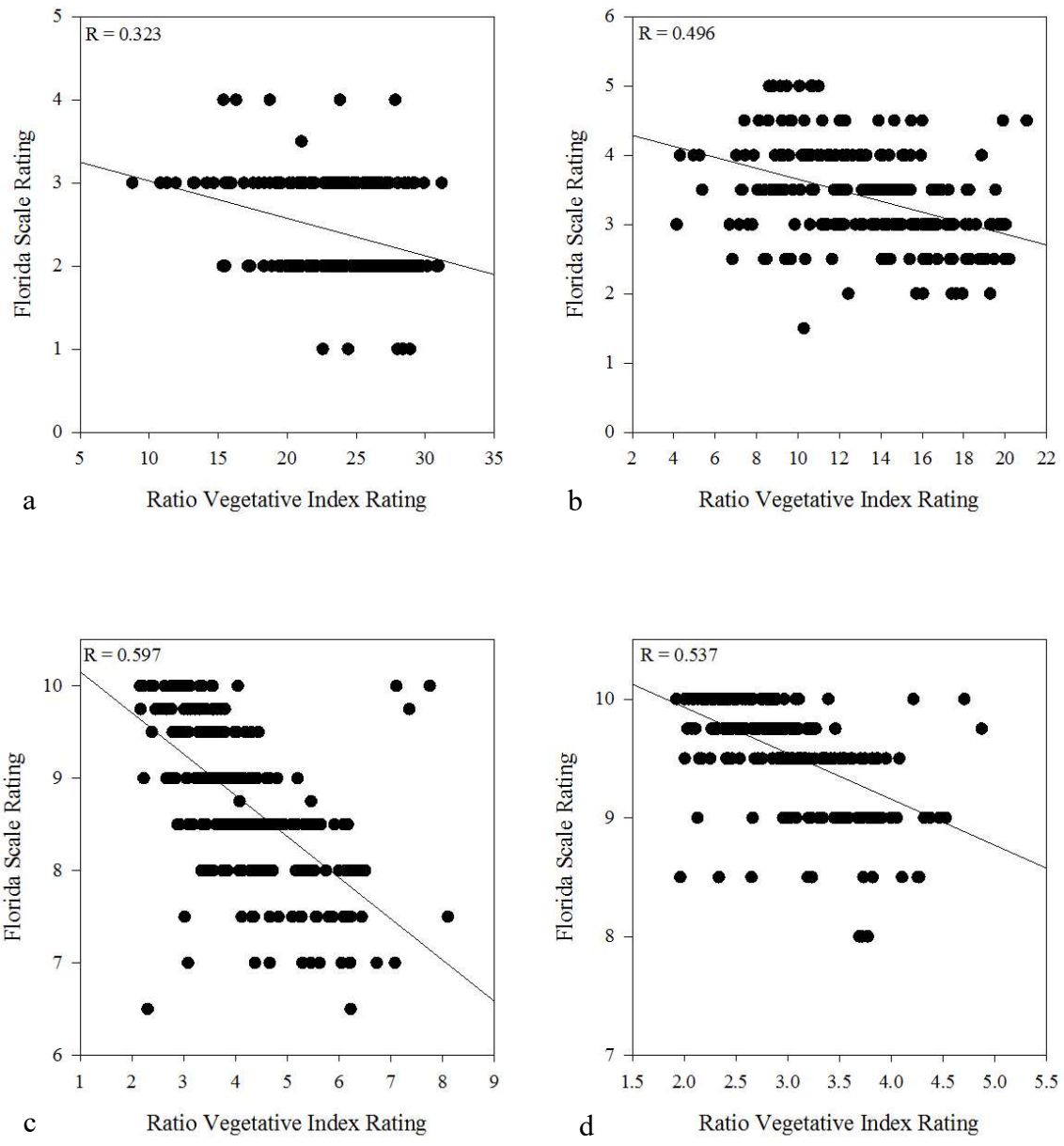

Fig. 4.3. Correlations between visual rating and ratio vegetative index rating at a) 90 and 83 DAP ($P < 0.001$), b) 104 DAP ($P < 0.001$), c) 121 DAP ($P < 0.001$), and d) 135 DAP ($P < 0.001$).

CHAPTER V

EVALUATION OF POPULATION PARENTAL LINES OF PEANUT (*ARACHIS HYPOGAEA* L.) FOR JUVENILE RESISTANCE TO LATE LEAF SPOT (*CERCOSPORIDIUM PERSONATUM*)

[3] Pelham, S. E., Holbrook, C. C., and Culbreath, A. K. 2017. To be submitted to *Peanut Science*.

Abstract

Cultivated peanut (*Arachis hypogaea* L.), an important oil and food crop because of its high levels of oil, protein, and fiber, is plagued by diseases. One of the most common is late leaf spot (*Cercosporidium personatum*). This fungal disease can defoliate and kill the plant causing a significant reduction in yield if not managed. Resistant cultivars have become the most desirable ways to manage this disease. Research in this area has led to the question of whether juvenile plants may possess a resistance to this disease which could lead to the development of resistant cultivars if identified in the genome. The objective of this study was to compare field susceptibility of parental lines of many mapping populations at the juvenile growth stage to late leaf spot pathogens. While it is apparent from the current study that there is no complete resistance to late leaf spot in juvenile plants, there are varying levels of incidence in the juveniles. This could be the result of partial resistance in the juveniles which can differ from any resistance the mature plant may possess.

Introduction

Cultivated peanut (*Arachis hypogaea* L.) is an important oil and food crop because of its high levels of oil, protein, and fiber (31). Peanut seeds can be used for human consumption in many ways while the vines can be a nutritious animal feed making it popular throughout the world (23, 25). Currently, the United States is the world's third largest producer of peanuts accounting for approximately 1.25 million metric tons of the 29 million metric tons produced (10, 38). Similar to all other economically important crops peanut is susceptible to many diseases caused by nematodes, bacteria, viruses, and fungi (36). Among the fungal diseases is late leaf spot, caused by *Cercosporidium personatum* (Berk. & M. A. Curtis), which is one of the most economically important fungal diseases worldwide (23, 37). In the United States, most

peanuts are produced in the southeastern states where weather conditions are often favorable for leaf spot epidemics (21). Late leaf spot is characterized by dark brown to black spots on any above ground part of the plant (40, 41). The spores are usually found on the lower surface giving it a rough appearance, while the upper surface of the leaf is smooth (44). To combat this disease multiple fungicide applications are needed throughout the season which can be costly and select for resistance (27).

One of the most desirable ways to manage late leaf spot has been the development of resistant cultivars (16). Partial resistance to late leaf spot is available and was discovered by Pixley (28), Gorbet et al. (18), and Watson (46). Earlier breeding programs focused on selecting genotypes with high levels of different components of rate-reducing resistance, allowing incorporation of these into desirable commercial cultivars (11). This method requires a large amount of field space and an extensive amount of time to phenotype for the disease throughout the growing season (21). In more recent years, the creation of large recombinant inbred line (RIL) populations in order to develop and use genetic markers for marker-assisted selection (MAS) has become a primary objective in peanut breeding programs (20). MAS has proven to be useful in the development of nematode resistant peanut cultivars (34, 35) and in the development of high-oleic cultivars (12, 13, 33).

Juvenile resistance to late leaf spot has been a topic of debate in the peanut breeding community. Cook (9) conducted a greenhouse study that showed that peanut plants became less susceptible to rust as they aged. The conclusion was that this was related to a decrease in wettability. In contrast, Power (29) reported that peanut leaves of the same age from plants at different maturity stages differed significantly in susceptibility to rust. The results stated that younger leaves were the most resistant to the pathogen. Resistance like this that breaks down as

the plant ages has also been reported in other pathosystems; some of these include anthracnose in lentil (*Lens culinaris* ssp. *culinaris* Medik.), (45), turnip mosaic virus in Chinese cabbage (*Brassica rapa*) (47), and spot blotch in barley (*Hordeum vulgare* L.) (3). This resistance can also work inversely. Balmelli et al. (1) found that by selecting for *Eucalyptus globulus* with a short juvenile period or by selecting a high proportion of adult foliage in the second year, resistance to diseases such as *Mycospaerella* leaf disease and *Eucalyptus* rust could be achieved. This selection method does protect from pathogens that do not affect adult foliage, but does not protect against other pathogens that do. Orians et al. (26) found that faster growing hybrid willow seedlings had greater resistance to diverse plant enemies. This is because they reach a size threshold where they could produce higher concentrations of defenses or greater concentrations of deterrent volatiles. With this knowledge, the objective of this study was to compare field susceptibility of parental lines of many mapping populations at the juvenile growth stage to late leaf spot pathogens and relate juvenile susceptibility to susceptibility in mature plants.

Materials and Methods

Field Setup

Three field experiments were conducted in 2015 and 2016. In 2015 and 2016 experiments were conducted at the at The University of Georgia Attapulgus Research Farm, Attapulgus (Decatur County) and in 2016 a third experiment was conducted at the University of Georgia Coastal Plain Experiment Station, Rigdon Farm, Tifton, GA. Peanuts had been grown in these fields previously and disease history of fields included severe epidemics of late leaf spot in previous peanut crops.

The experimental design in all cases was a randomized complete block design with three replicates of each genotype. Plots consisted of two 2.5 m long rows spaced 0.91 m apart. The planting density was 18 seeds/ft. No fungicides were applied and herbicides, insecticides, and fertilizers were applied following recommendations of the University of Georgia.

Epidemic onset

Each plot was bordered on both sides by the susceptible cultivar TUFRunner 511 (43) to increase overall incidence of leaf spot in all entries. The border was planted on 4 June 2015 and 13 June 2016 at the Attapulgus Research Farm. At the Rigdon Farm, Tifton, GA the borders were planted on 25 May 2016.

Selection of genotypes

A total of 16 genotypes were chosen for the study because they are parents in one or more of the RIL populations currently being used in peanut breeding programs. The genotypes, and their characteristics, selected for the experiment are listed in Table 5.1.

To ensure that juvenile plants were exposed to natural amounts of natural inoculum the genotypes were not planted until very late in the season. Planting dates were 21 September 2015 and 9 September 2016 at Attapulgus and 12 September 2016 at Tifton. At the time the parental lines were planted, border rows had severe epidemics of late leaf spot with defoliation greater than or equal to 75 percent. This method has been used in fungicide testing of in-furrow versus early-season banded applications (15).

Evaluation of late leaf spot incidence in the field

In the field leaf spot incidence ratings were linear, based on a percentage of leaflets with one or more lesions. For example, 0 equates to no leaflets with one or more lesions and 10 equals all leaflets have one or more lesions. In 2015 two leaf spot ratings were taken and in 2016

five ratings were taken at each location. Incidence was assessed at 30 and 39 days after planting (DAP) in 2015. In 2016 incidence was assessed at 19, 24, 28, 31, and 38 DAP at Attapulgus and 22, 30, 36, 42, and 50 at Tifton.

Statistical analysis of late leaf spot incidence

In 2016, area under the disease progress curve (AUDPC) was computed for each plot using incidence as described by Shaner and Finney (32). Since the intervals between evaluations differed for the tests, standardized area under the disease progress curves (SAUDPC) were calculated by dividing AUDPC by the total time in days that the epidemic was monitored (22). Data was combined across locations. Fisher's protected lease significant difference (LSD) values were calculated for comparison of genotypes (39). Differences referred to in the text are significant at $P \leq 0.05$ unless otherwise indicated.

Evaluation of late leaf spot incidence in the lab

In 2016, samples were taken from both fields for evaluation of late leaf spot incidence in the lab. This was done by selecting 12 genotypes from each field based on incidence ratings from the second to last rating date. The four with the highest leaf spot incidence, the four with the lowest leaf spot incidence, and four chosen randomly were used (Table 5.2). On the last rating date, five plants were randomly chosen out of each replication and brought back to the lab. Number of leaflets with one or more lesion were counted for all leaflets and that was divided by total number of leaflets to get a value for each plant that indicated percentage of the leaflets with one or more lesion. Linear regression models were run for the percent of the leaflets infected versus the final incidence rating. Differences referred to in the text are significant at $P \leq 0.05$ unless otherwise indicated.

Results

Final incidence ratings for the two years show that disease pressure was greatest for 2016 (Fig. 5.1). In 2015 and 2016 Georgia Valencia had the highest final incidence values of all the genotypes. New Mexico Valencia A had the second highest final incidence in 2015 while GT-C20 had the second highest final incidence in 2016. The genotypes with the lowest final incidence were different for both years. In 2015 the genotype with the lowest incidence was NC 3033 followed by N08082. In 2016 the genotype with the lowest incidence was Georgia 06G followed by Georgia Green.

In 2016 Georgia Valencia had the highest SAUDPC across both locations (Fig. 5.2). Georgia Valencia is closely followed by GT-C20. There were two Valencia type genotypes and two Spanish type genotypes in the study. The Valencia type genotypes were Georgia Valencia and NM Valencia A and the Spanish type genotypes were GT-C20 and OLin. These genotypes all had the highest SAUDPCs. The SAUDPC scores for the genotypes in the lower end of the graph are all very similar with Georgia 06G being the most resistant to leaf spot as a juvenile.

Disease progress curves for the genotypes in 2016 with the highest SAUDPC, lowest SAUDPC and two others are shown in Figure 28 and Figure 29 for Attapulgus and Tifton respectively. In 2016 the disease progress curves in Attapulgus follow a specific pattern (Fig. 5.3). There is a large increase in incidence between the ratings at 24 DAP and 28 DAP. There is then a decrease at 31 DAP and another increase at 38 DAP. The trends for the disease progress curves at Rigdon Farm in 2016 do not follow a specific pattern (Fig. 5.4). Very few of the genotypes had a decrease in incidence level at any point in the rating. An exception for this was SPT 06-06 which has a final incidence level that is less than its incidence rating at 35 DAP.

Percentage of the plant with one or more lesions on the leaves can be seen in Figure 5.5. The average percent infected for each genotype was slightly higher at Attapulgus than Tifton for all genotypes. As with the SAUDPC and final incidence values Georgia Valencia had the highest percent of the plant infected for both trials. Genotypes with the highest percent effected were the same across both locations. After Georgia Valencia the ranking was NM Valencia A, OLin, and GT-C20. At Attapulgus the genotype with the lowest percentage of the plant infected was Georgia-06G and at Tifton it was Tifrunner. The rankings for the genotypes with low percentage of the plant infected all differ across locations.

The correlations between percent of the leaflets with one or more lesion and final incidence of the plot are very different for the two locations (Fig. 5.6). For Attapulgus (Fig. 5.6a), the linear regression results in an R value of 0.35 that is only slightly significant with a *P* value of 0.04. The linear regression for Rigdon Farm (Fig. 5.6b) however has an R value of 0.62 and is highly significant with a *P* value < 0.001.

Discussion

Results indicate that all genotypes evaluated in this study are susceptible to infection by *C. personatum* at a very early stage. All genotypes evaluated had a final incidence greater than 10 percent (Fig. 5.1). Final incidence values ranged from 12 to 77 percent suggesting that there are sources of partial resistance to the pathogen. This supports the findings by Zhou et al. (48) that late leaf spot resistance in peanut is controlled by several major QTLs and many minor QTLs.

Results also suggest that the market type of the genotype plays a role in the level of susceptibility the genotype has to *C. personatum*. The Valencia and Spanish type genotypes had high levels of susceptibility to late leaf spot while the Virginia and Runner type genotypes were

the most resistant to late leaf spot. These results suggest that there could be a relationship between market type and resistance to late leaf spot. Historically, it has been shown that Valencia and Spanish type genotypes are more susceptible to late leaf spot.

Overall, final incidence values for the juveniles (Fig. 5.1) supported the literature on season long disease evaluations for late leaf spot (Table 5.1). However, there were a couple of genotypes that did not. Georgia-06G has been shown to be moderately susceptible to late leaf spot but it had the lowest final incidence values outperforming the highly resistant SPT 06-06. Also, NC 3033 has been shown to be highly susceptible in the literature but had the forth lowest final incidence. This suggests that juvenile ratings of late leaf spot are not indicative of season long results for late leaf spot.

Individual disease progress curves from experiments in 2016 show variations in the development of the epidemic as well (Fig. 5.3 & 5.4). The trials at Attapulgus show a decrease in the incidence around 30 days after planting. This is most likely related to the growth habit of the plant. The plant is putting on more vegetation at this stage and the symptoms are not being seen on this new vegetation. At Tifton, this decrease in incidence was not seen. The spacing of the ratings most likely were not conducive for showing this effect. SPT 06-06 however did have a final incidence that was less than the rating at 36 DAP. This suggests a latent resistance to late leaf spot that is being expressed in the plant. This supports the literature that this genotype is highly resistant to late leaf spot (42).

Figure 5.4 helps to realize that at both locations in 2016 the amount of leaves on the plant with one or more lesion are very similar. Visual incidence ratings, which were not as closely grouped for each genotype, could vary for a couple of reasons. Personal judgment of percentages can vary greatly between locations and over time. Also, symptoms too small to be

seen in the field can be seen when looking at the leaves up close in the lab. The relationship between values for percent of the leaves showing one or more lesion and final incidence values vary between locations (Fig. 5.5). Tifton has a very strong correlation while Attapulgus has only a slight correlation. This could be due to the fact that just because a plant has a high amount of infected leaves does not mean that the whole plot has that same level of infection. Most of the plants in that plot could be healthy.

The results of this study can be beneficial to peanut breeding programs. The results show that there is no juvenile resistance to late leaf spot that could be implemented in mature genotypes. Also, juvenile incidence values are not reflective of final incidence values in season long evaluations to late leaf spot.

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Table 5.1. Market class, late leaf spot resistance, and lineages for genotypes selected.

Genotype	Market Class	Late Leaf Spot Resistance[ab]	Lineage	Reference
C76-16	Runner	U	Unknown	
Florida-07	Runner	S	89xOL14-11-1-1-1-b2-B X C-99R	Gorbet and Tillman, 2009
Florunner	Runner	S	Florispan X Early Runner	Norden et al., 1969
Georgia Green	Runner	S	Southern Runner X Sunbelt Runner	Branch, 1996
Georgia Valencia	Valencia	HS	Georgia Red X UF85179	Branch, 2001
Georgia-06G	Runner	MS	Georgia Green X C-99R	Branch, 2007
Gerogia-09B	Runner	S	Georgia Green X Georgia Green x GA 94022	Branch, 2009
Georgia-12Y	Runner	MS	Georgia-09B X Georganic	Branch, 2013
GT-YY20 (GT-C20)	Spanish	HS	Unknown	Qin et al., 2012
N08082olJCT (N08082)	Virginia	U	Unknown	
NC 3033	Virginia	HS	Georgia 207-7 X A48	Beute et al., 1976
F NC94022-1-2-1-1-b3-B (NC94022)	Virginia	MS	N91026E X PI 576638	Culbreath et al., 2005
NM Valencia A	Valencia	S	Unknown	
OLin	Spanish	S	Tamspan 90 X UF435-1	Simpson et al., 2003
SPT 06-06	Runner	HR	C-99R (UF 94320) X DP-1 (UF 97318) X GP-NC WS 12	Tallury et al., 2013
Tifrunner	Runner	MR	F439-16-10-3 X PI 203396	Holbrook and Culbreath, 2007

[a]R = Resistant, MR = Moderately Resistant, MS = Moderately Susceptible, S = Susceptible, HS = Highly Susceptible, U = Unknown
[b]Late leaf spot [*Cercosporidium personatum* (Berk. & M. A. Curtis)]
Table modified from Holbrook et al. 2013

Table 5.2. Genotypes chosen for lab incidence ratings and their ranking.

Ranking	Attapulgus	Rigdon
Highest Disease	Florunner	Georgia Valencia
	Georgia Valencia	GT-C20
	GT-C20	NM Valencia A
	OLin	OLin
Randomly Selected	C76-16	Florunner
	Georgia-12Y	Georgia-12Y
	NC94022	N08082
	NM Valencia A	NC94022
Lowest Disease	Georgia-06G	Georgia-06G
	Georgia Green	Georgia Green
	N08082	SPT 06-06
	SPT 06-06	Tifrunner

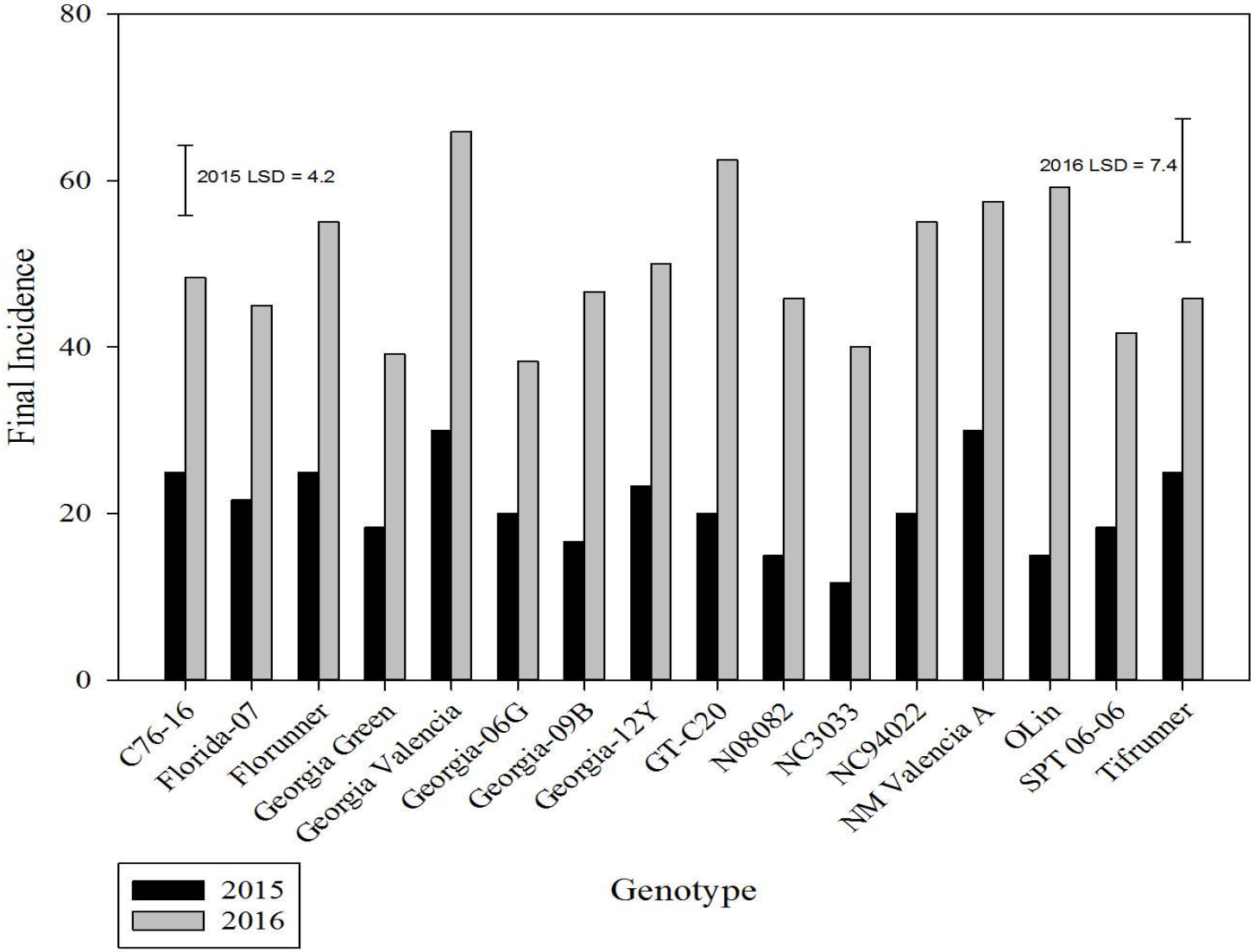

Fig. 5.1. Effect of peanut genotype on late leaf spot final incidence across years. Data is pooled across location.

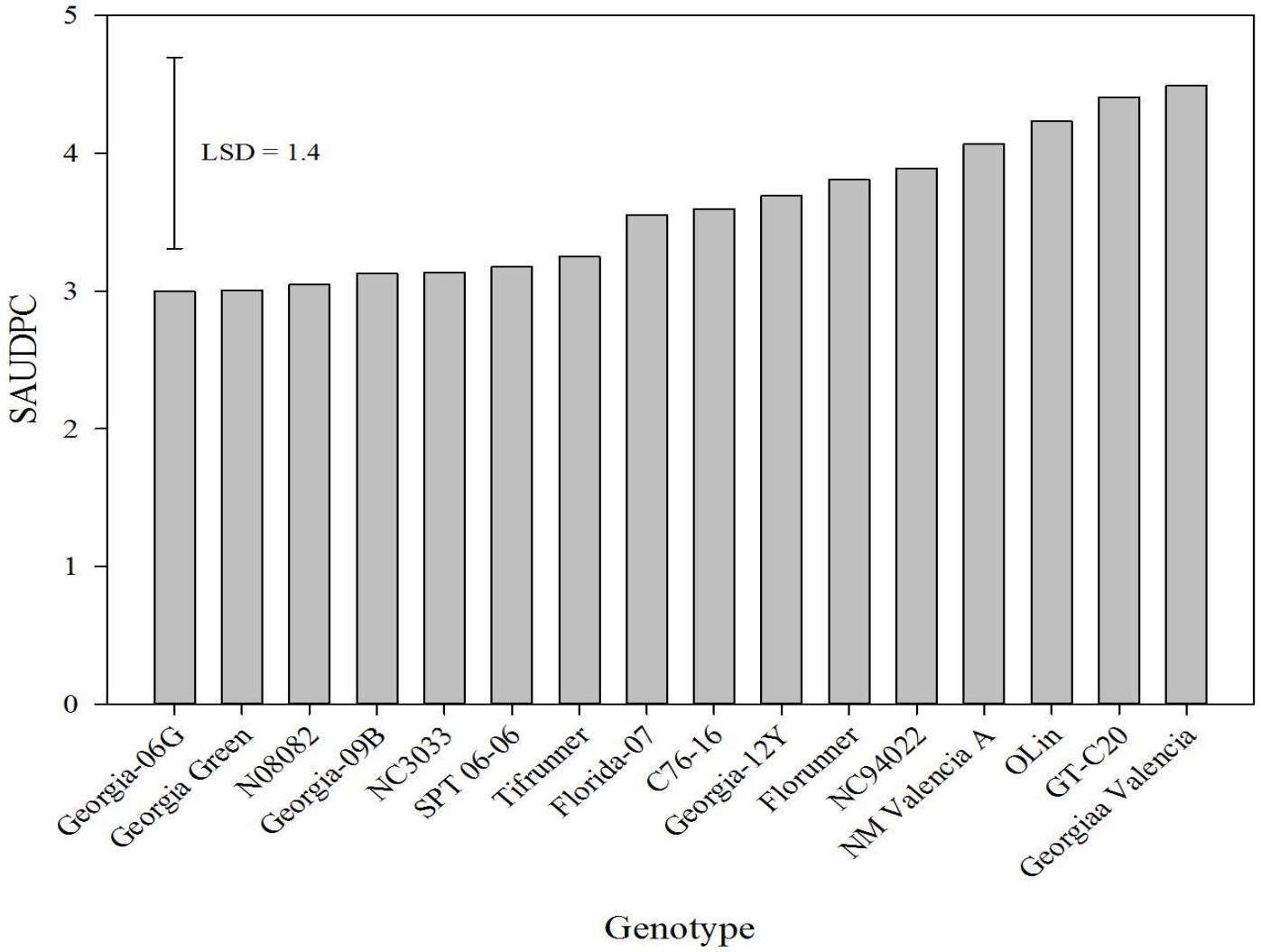

Fig. 52. Effect of peanut genotype on late leaf spot SAUDPC in 2016. Data is pooled across locations.

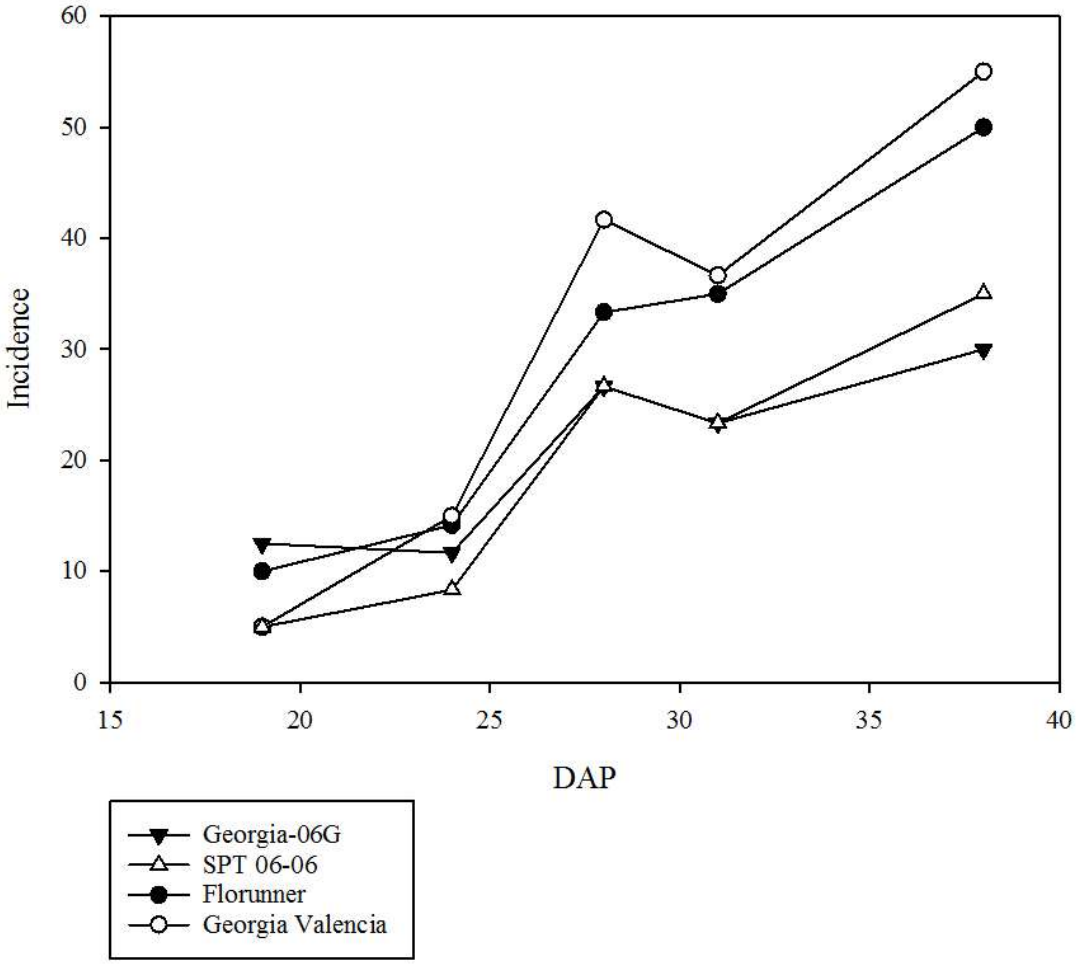

Fig. 5.3. Disease progress curves for four genotypes at Attapulgus in 2016.

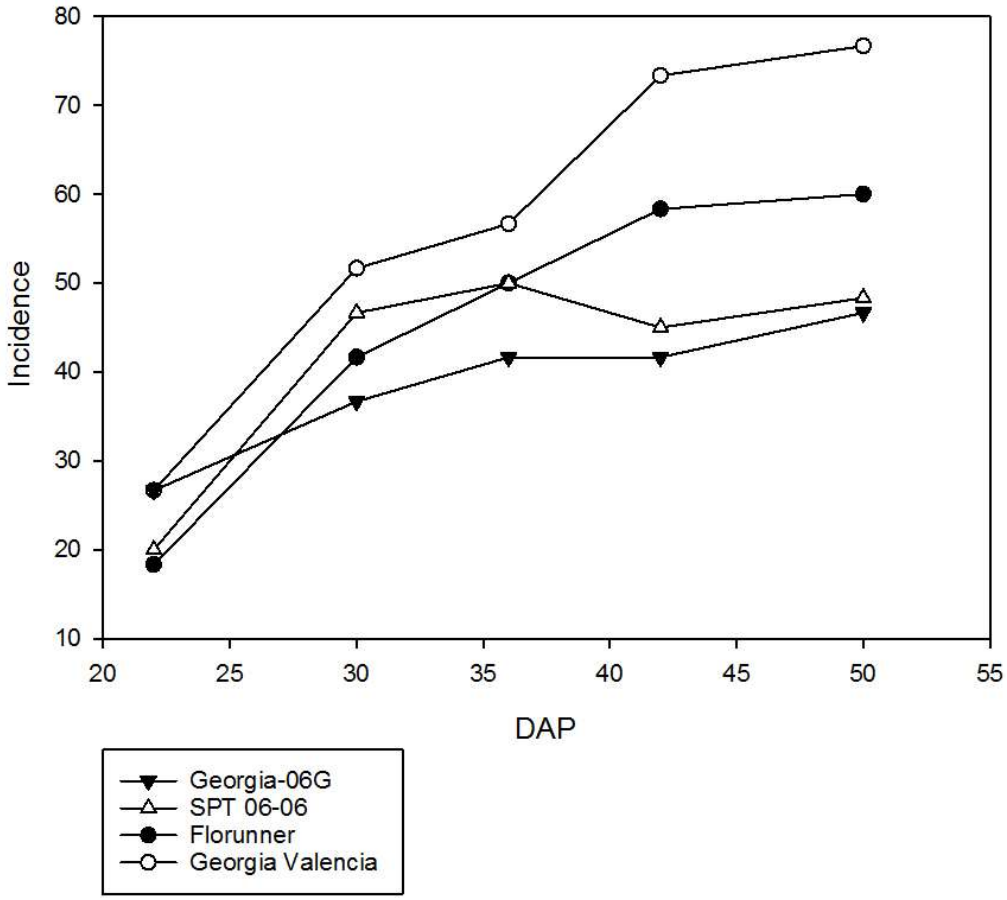

Fig. 5.4. Disease progress curves for four genotypes at Tifton in 2016.

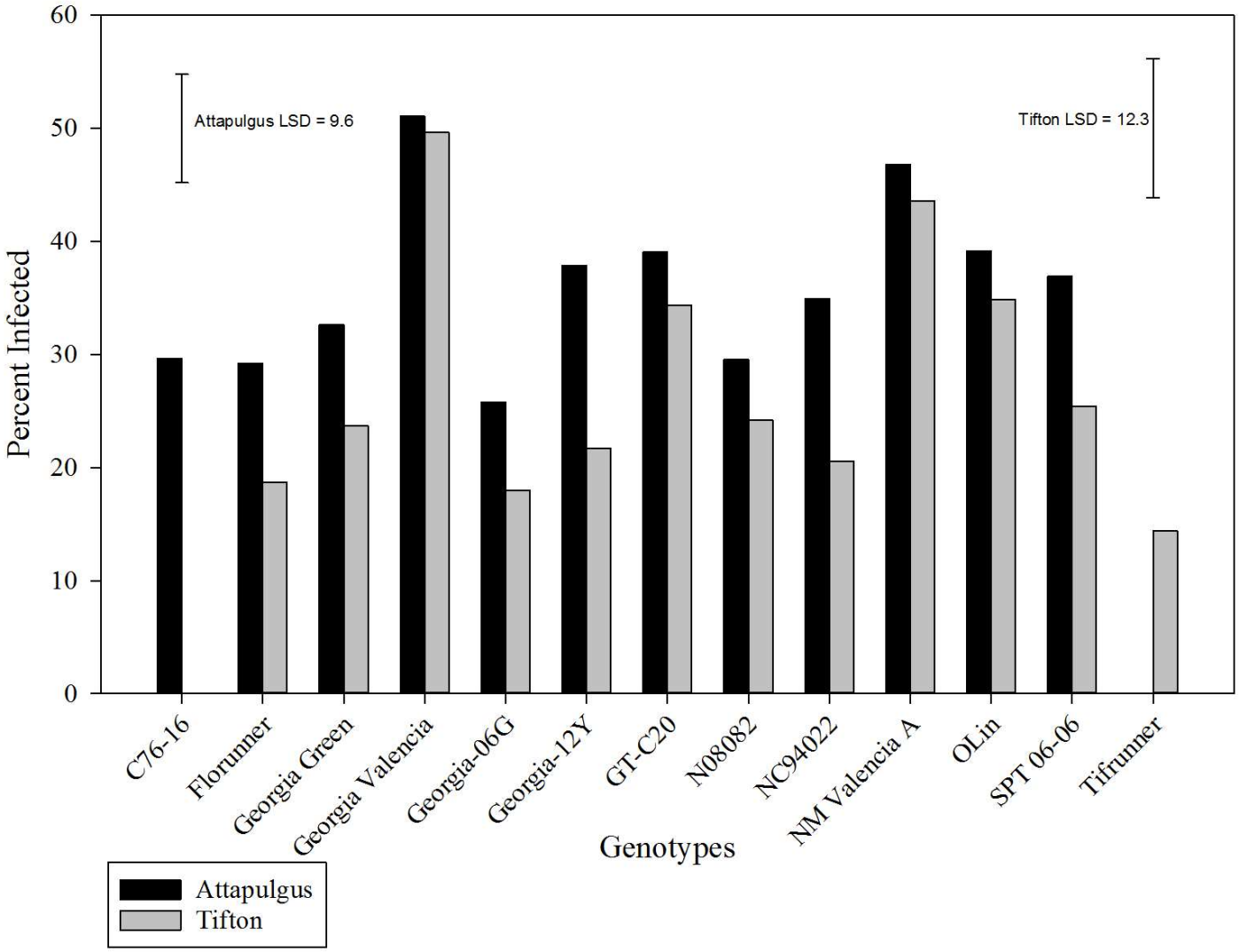

Fig. 5.5. Effect of peanut genotype on percentage of plant with one or more lesion caused by late leaf spot.

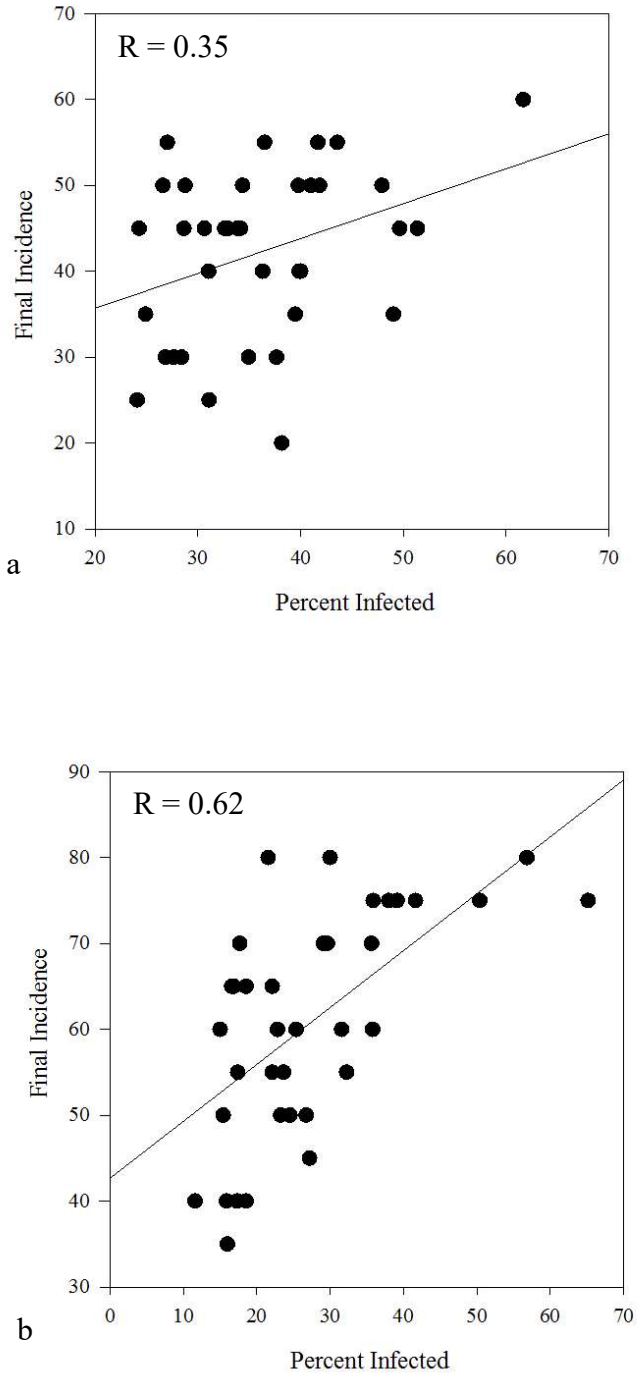

Fig. 5.6. Correlation of percent of plant with one or more lesion versus final incidence in plots at a) Attapulgus in 2016 (final incidence = 27.57 + (0.406 * percent infected), $P = 0.035$) and b) Tifton in 2016 (final incidence = 42.654 + (0.663 * percent infected), $P < 0.001$).

CHAPTER VI

CONCLUSIONS

Tomato spotted wilt virus (TSWV) and late leaf spot, caused by *Cercosporidium personatum*, are important diseases affecting peanuts (*Arachis hypogaea* L.). One of the most promising ways to combat these diseases is with resistant cultivars created through the development of recombinant inbred lines allowing for the discovery of resistance genes. These mapping populations can be very large making phenotyping time consuming and difficult; the objectives of this research aimed to increase the efficiency of phenotyping methods.

Field experiments were conducted to compare genotypes from multiple mapping populations for resistance to the two diseases. Results indicated that TSWV ratings were highest for the T population. The S population had the highest SAUDPC for late leaf spot and mean SAUDPC values were similar for T, 1799, and 1801 populations. SAUDPC of SPT 06-06, a parental line for population 1801, was lower than for any other parent for TSWV and was the second lowest for late leaf spot. Florida-07 and Tifrunner proved to have resistance to both diseases as well. Results indicate the populations differ for both TSWV incidence and late leaf spot severity, and highest levels of late leaf spot resistance in individual RILs may not come from the most resistant parent to TSWV. Results also indicate that levels of resistance can be obtained in individual lines that are better than that of either parent. Populations in this experiment showed transgressive segregation both towards resistance and susceptibility for both TSWV and late leaf spot.

Field experiments were also conducted to the use of unmanned aerial systems (UASs) and their ability to increase efficiency when phenotyping TSWV and LLS. With adequate ground truthing, it was found that TSWV can be differentiated from other diseases in the field by the color and the visible stunting of the plant. By creating an equation in Matlab version R 2016b (MathWorks, Natick, MA 01760 USA) to identify yellow pixels, known as the greenness equation, we can rate the incidence of the disease in many small plots in a relatively short amount of time. Matlab greenness equation values had varying levels of correlation with field disease incidence ratings. Analyses later in the growing season with more canopy width tended to be better correlated than analyses from earlier in the season. Although correlations were not strong between visual and aerial ratings, aerial ratings were able to separate the most resistant genotypes from the most susceptible genotypes. This technique can greatly increase the efficiency of breeding programs to screen large recombinant inbreed lines of peanut to TSWV. Results from a second study also indicated that with adequate ground truthing, late leaf spot can be differentiated from other diseases in the field by the necrotic lesions on all above ground plant parts and defoliation. By testing 25 vegetative indices (VIs) in ArcMap (ESRI, Redlands, CA 92373 USA) we could determine the VI with the strongest correlation to visual ratings. Ratings at two weeks from harvest had the strongest correlations for all VIs. Results suggest that through the use of a UAS equipped with a multispectral camera a rating using the ratio vegetative index two weeks from harvest could greatly increase the efficiency of peanut breeding programs.

Juvenile plants were also evaluated for resistance to LLS. While it is apparent from the current study that there is no complete resistance to late leaf spot in juvenile plants, there are varying levels of incidence in the juveniles. This could be the result of partial resistance in the

juveniles which can differ from any resistance the mature plant may possess. Parental lines evaluated were also susceptible to infection by *C. personatum* at a very early age.