COMPARISON OF EARLY AND LATE LEAF SPOT PATHOGEN PRE-PENETRATION INFECTION AND DISEASE PROGRESS IN DIFFERENT PEANUT GENOTYPES

by

FATIMA FLORIE MAY A. SILVA

(Under the Direction of Albert K. Culbreath)

ABSTRACT

Peanut (*Arachis hypogaea* L.) is a vital global crop, particularly in tropical and subtropical regions. However, its production is severely impacted by early leaf spot (ELS), caused by *Passalora arachidicola* (PA), and late leaf spot (LLS), caused by *Nothopassalora personata* (NP), leading to significant yield losses and management costs. This study evaluates peanut genotypes with resistance to ELS and LLS, comparing them to susceptible cultivars. It explores both pre-infection resistance components, including reduced conidial adhesion and shorter germ tube length, and post-infection components, such as lower lesion incidence, reduced disease severity, and delayed sporulation. Results suggest that genotypes with resistance from *Arachis cardenasii*, *Arachis stenosperma*, and *Arachis batizocoi* exhibited delayed pre-infection and post-infection processes for PA and NP, while susceptible cultivars exhibited faster infection processes. These findings enhance our understanding of the genetic basis of resistance to leaf spot pathogens and lay the groundwork for future research.

INDEX WORDS: Early leaf spot, Late leaf spot, *Passalora arachidicola*, *Nothopassalora personata*, pre-infection, post-infection, resistance mechanisms, *Arachis cardenasii*, *Arachis stenosperma, Arachis batizocoi*

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DEDICATION

This thesis is humbly dedicated to my late parents, Emma and Wally. Their boundless love, sacrifices, and unwavering faith in me have made everything possible. Without their gentle guidance and endless support, I would not be who I am today, nor would I have had the strength to complete this journey. Their memory lives on in everything I do, inspiring me each day to be worthy of the love they so selflessly gave.

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CHAPTER 1

INTRODUCTION

Peanut (*Arachis hypogaea* L.), also known as groundnut, is a member of the legume family and serves as an important global food and oilseed crop, sustaining millions of people (Njoroge, 2018; Yu, 2023; Zhang et al., 2023). Peanuts are cultivated in tropical and subtropical regions across Asia, Africa, and the Americas (FAO, 2010). As of 2024, the leading global producers of peanuts include China, India, Nigeria, and the United States (USDA-FAS, 2024). In the U.S., Georgia accounts for approximately 55% of national production, followed by Texas and Alabama at 10% each, and Florida at 9% (USDA-FAS, 2024). Peanut diseases constitute a major limiting factor to peanut production annually, and management of multiple diseases is a primary concern for producers (Thiessen and Woodward, 2012). Early leaf spot (ELS) and late leaf spot (LLS) are among the most economically significant diseases affecting peanuts, causing substantial yield losses and increased control costs (Anco et al., 2020; Thiessen and Woodward, 2012). ELS is caused by *Passalora arachidicola* (Hori) U. Braun syn. *Cercospora arachidicola* Hori, and LLS is caused by *Nothopassalora personata* (Berk. & M.A. Curtis) U. Braun, C. Nakash., Videira & Crous syn. *Cercosporidium personatum* (Berk. & M.A. Curtis) Deighton (Anco et al., 2020; Mycobank, 2024; York et al., 1995).

Due to the economic impact of leaf spot diseases, scientists have remained dedicated to developing effective strategies to mitigate the associated losses (Kankam et al., 2022). Recommended control strategies for growers involve an integrated approach,

including the use of more resistant cultivars, cultural practices, and optimal use of available fungicides (Cantonwine et al., 2006; Culbreath et al., 2002b; Kemerait et al., 2012). However, challenges such as increased production costs, variable fungicide effectiveness under different environmental conditions, fungicide resistance, and weather-related delays in fungicide applications, continue to lead to significant yield losses (Little et al., 2021; McDonald et al., 1985; Woodward et al., 2014; Wynne et al., 1991). A promising solution to reduce fungicide use is the cultivation of peanut varieties with enhanced resistance and tolerance to leaf spot, achieved through breeding efforts (Chu et al., 2019; Culbreath et al., 1992; Dang et al., 2021; Woodward et al., 2008). Recent releases of peanut cultivars with varying degrees of resistance to leaf spot include AU-PNL 17 (Chen et al., 2017), Georgia-12Y (Branch, 2012), and Georgia-14N (Branch, 2014), as noted in the Peanut Rx Guide (Peanut Rx, 2024; Kaur et al., 2024). Despite these advancements, Georgia-06G, a cultivar more susceptible to leaf spot, still constitutes most of the peanut acreage in Alabama and Georgia (Kaur et al., 2024). No cultivar offers complete resistance to both leaf spot pathogens, and multiple applications of fungicides are required to maintain adequate control in most fields (Culbreath, et al., 2002a; 2002b; Kaur et al., 2024; Phipps & Powell, 1984).

Considerable effort has been devoted to identifying sources of resistance to leaf spot diseases. In recent years, intensive screening of germplasm has led to the identification of several sources of resistance to both ELS and LLS (Dang et al., 2021; Denwar et al., 2021; Chu et al., 2019; Gonzales et al., 2023; Lamon et a., 2021; Waliyar et al., 1993; Wynne et al., 1991). Wild peanut species hold potential for broadening the genetic base of cultivated peanuts. In recent years, there has been substantial emphasis on

screening these wild species for resistance to various diseases, with some exhibiting high levels of resistance (Chu et al., 2019; Stalker, 2017; Subrahmanyam, 1985a; 1985b; Wynne et al., 1991). Furthermore, detailed cytogenetic studies and molecular phylogenies have greatly enhanced the understanding of the relationships between cultivated and wild species (Bertioli et al., 2021; Leal-Bertioli et al., 2024; Moretzsohn et al., 2013). A well-established "pipeline" has been developed for producing induced allotetraploid hybrids that are sexually compatible with cultivated peanuts (Bertioli et al., 2021; de Paula et al., 2017; Favero et al., 2006; Favero et al., 2015; Leal-Bertioli et al., 2015; Leal-Bertoli et al., 2017).

Several induced allotetraploids derived from wild peanut species have been generated and evaluated, demonstrating strong disease resistance (Bertioli et al., 2019; Bertioli et al., 2021; Chu et al., 2021; Favero et al., 2006; Gao et al., 2021; Gonzales et al., 2023; Leal-Bertioli et al., 2017; Levinson et al., 2021; Simpson et al., 1993). There is no standardized method for evaluating leaf spot resistance across different peanut genotypes. Researchers utilize various criteria for evaluations based on their specific objectives (McDonald et al., 1985; Waliyar, 1989). Leaf spot resistance in peanuts is quantitatively inherited and influenced by multiple factors (Anderson et al., 1991; Chu et al., 2019; Clevenger et al., 2018; Kornegay et al., 1980; Lamon et al., 2021; Waliyar et al., 1989). A comprehensive evaluation of leaf spot resistance requires assessing various disease criteria, combining controlled methods like detached leaf assays and greenhouse inoculations with natural field experiments (Gonzales et al., 2023; Leal-Bertioli et al., 2009; Levinson et al., 2021; McDonald et al., 1985; Waliyar et al., 1989). In greenhouse or laboratory settings, screening by inoculating potted plants or detached leaves is

effective when minimizing environmental interactions and excluding other foliar pathogens (Melouk, 1978; McDonald et al., 1985). The detached leaf assay is more cost-effective, and requires less space, pathogen, and host material (Favero et al., 2004; Guimaraes et al., 2017; Lamon, 2019; Leal-Bertioli et al., 2009; Sharma et al., 2005).

In addition to greenhouse, detached leaf assays, and field resistance assessments confirming delayed disease progression in resistant genotypes, early studies revealed that larger stomatal apertures in peanuts increase susceptibility to leaf spot pathogens (D'Cruz & Upadhyaya, 1961; Gibbons & Bailey, 1967; Hemingway, 1957; Waliyar et al., 1989). These findings highlight the importance of stomatal traits in disease susceptibility and their relevance in resistance breeding programs. Additionally, in susceptible genotypes, the *P. arachidicola* (syn. *Cercospora arachidicola*) germ tube exhibit directed growth toward stomata, facilitating infection, a behavior not observed in resistant genotypes (Abdou, 1974; Alderman & Beute, 1986; Waliyar et al., 1989). Despite these early insights, germ tube growth patterns in resistant genotypes remain understudied, representing a gap in understanding resistance mechanisms.

In summary, peanut leaf spot resistance is evaluated using various epidemiological and physiological components. Assessments of disease resistance in peanut genotypes are conducted in both controlled environments and field conditions. The controlled settings enable efficient screening of large numbers of lines and the isolation of specific resistance traits but may not fully capture the environmental complexity encountered in field trials. The field evaluation is essential for understanding genotype-by-environment interactions and provides a comprehensive view of resistance under natural conditions. Advancing promising genotypes from controlled settings to

field trials is crucial for assessing resistance to both early and late leaf spot in real-world conditions, which is key to developing stable and durable resistance.

The main objective of this study is to conduct a thorough field evaluation of peanut genotypes that have shown promising resistance to early and late leaf spot diseases. The study will include previously identified resistant lines, advanced resistant breeding lines, and susceptible cultivars. In addition to evaluating various field resistance components, the study will also investigate the infection process of early and late leaf spot pathogens in selected resistant and susceptible genotypes. While previous research has focused on post-infection stages, this study will also explore pre-penetration infection dynamics by examining conidial adhesion and germ tube growth on the leaf surface in both resistant and susceptible genotypes. The specific objectives are to (1) assess the effect of peanut genotype on early infection stages by *Passalora arachidicola* (PA) and *Nothopassalora personata* (NP), focusing on conidial adhesion and germ tube elongation, and (2) to determine the effect of peanut genotype in the disease progression of early and late leaf spot by analyzing field resistance components. This study will offer understanding of resistance mechanisms by examining both pre- and post- infection stages of disease, offering a more detailed perspective on how peanut genotypes interact with PA and NP. These insights could inform the development of cultivars with improved resistance to both early and late diseases stages, supporting more effective and sustainable disease management in peanut breeding programs.

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CHAPTER 2

LITERATURE REVIEW

Peanut Origin, Global spread and Genetic Challenges

The peanut (*Arachis hypogaea* L.), a key species in the *Arachis* genus, originated in the Cerrado region of South America, with cultivation in Peru's Zaña Valley dates back approximately 8,500 years (Hammons et al., 2016). The spread of peanuts beyond South America occurred through global trade routes, including the transatlantic slave trade, although the specific details of its introduction to the southeastern United States remain undocumented (Simpson et al., 2001). Peanuts, which produce fruit underground, belong to the *Arachis* genus, which consists of 80 species grouped into nine sections (Bertioli et al., 2011; Fernández and Krapovickas, 1994; Krapovickas and Gregory, 1994; Lavia, 1999; Valls and Simpson, 2005). While wild species are diploid, cultivated peanuts are tetraploid (AABB genome), originating from *Arachis duranensis* and *Arachis ipaensis* (Bertioli et al., 2011; Fávero et al., 2006; Fernández and Krapovickas, 1994; Husted, 1936; Smartt et al., 1978). The narrow genetic base within cultivated peanuts presents significant challenges for breeding, particularly developing disease-resistant varieties. Additionally, ploidy barriers complicate the use of wild relatives in breeding programs (Bertioli et al., 2011). However, advancements in genetic research are improving the effectiveness of peanut breeding, providing solutions to overcome these limitations (Bertioli et al., 2011; Fávero et al., 2006; Garcia et al., 1995; Khedikar et al., 2010; Nagy et al., 2010; Simpson et al., 1993; Stalker et al. 1979). Peanuts are a vital

global food and oilseed crop, crucial for food security, and sustains millions of people, particularly in tropical and subtropical region across Asia, Africa, and the Americas (FAO, 2010; Njoroge, 2018; Valentine, 2016; Zhang et al., 2023). In 2024, global peanut production is projected at 51, 314,000 metric tons (MT), led by China at 19,000,000 MT (37%), followed by India at 7,100,000 MT (14%) and Nigeria at 4,300,000 MT (8%) (USDA-FAS, 2024). The United States is a significant player, producing 2,922,000 MT (6%) and contributing approximately 80% of the total output in the global peanut marker ((USDA-FAS, 2024).

Peanut leaf spot disease

Early leaf spot (ELS) and late leaf spot (LLS) are important foliar diseases affecting peanut (*Arachis hypogaea* L.). ELS is caused by *Passalora arachidicola* (Hori) U. Braun syn. *Cercospora arachidicola* Hori, and, while LLS is caused by *Nothopassalora personata* (Berk. & M.A. Curtis) U. Braun, C. Nakash., Videira & Crous syn. *Cercosporidium personatum* (Berk. & M.A. Curtis) Deighton (Anco et al., 2020; Mycobank, 2024; Shokes & Culbreath, 1997; York et al., 1995). These diseases can cause substantial yield losses, reaching up to 80 % in severe cases, even when fungicides are applied (Knauft et al., 1986; 1988; Shokes & Culbreath, 1997). These leaf spot diseases pose a major limitation to peanut production, making their management a primary concern for growers (Theissen & Woordward, 2012). In the Southern U.S., ELS and LLS are among the most significant foliar diseases impacting peanut yields, alongside peanut rust (Porter et al., 1982).

Symptom, Development and Spread of Early and Late Leaf Spot

P. arachidicola (Hori) U. Braun syn. *C. arachidicola* exhibit slow growth on certain media, producing conidia that measure 37-108 µm in length. The optimal germination occurs at 20-30°C and high humidity, sporulation under both continuous light and continuous darkness (Alderman & Beute, 1986; Mims et al., 1989). After germination, the pathogen exhibits stomatal tropism to penetrate host tissues within 3 to 5 days (Abdou et al., 1974; Alderman & Beute, 1986; Johnson & Cantonwine, 2013). *P. arachidicola* is classified as a necrotroph (perthotroph), killing host cells before absorbing nutrients (Mims et al., 1989). *Nothopassalora personata* (Berk. & M.A. Curtis) U. Braun, C. Nakash., Videira & Crous syn. *Cercosporidium personatum* (Berk. & M.A. Curtis) Deighton exhibits distinct sporulation patterns, requiring light for conidial production, with conidia measuring 20-70 µm in length (Abdou, 1974; Jenkins 1938; Mims et al., 1989; Woodroof, 1933). Its infection process is characterized by either stomatal penetration or direct penetration through the epidermis, with symptoms appearing 10-14 days post-infection under optimal conditions (Shokes & Culbreath, 1997). *N. personata* is a hemibiotroph, forming intracellular haustoria that remain intact and functional even in dying host cells, suggesting nutrient absorption continues post-host cell death (Mims et al., 1989).

Both pathogens thrive in similar environmental conditions, including high humidity and moderate temperatures, and primarily spread through conidia that overwinter in infected peanut residues (Hemingway, 1954; Shokes & Culbreath, 1997). While other inoculum sources, such as mycelial fragments, ascospores, and chlamydospores, have been proposed, they are rarely observed (Hemingway, 1954; Jackson & Bell, 1969; Shanta, 1960; Shokes & Culbreath, 1997). In tropical regions, volunteer peanuts can perpetuate

pathogen presence between growing seasons (McDonald et al., 1985), but freezing temperatures in the southern United States limits this potential (Shokes & Culbreath, 1997). Peanut residue in soil remains a critical inoculum source, with viability influenced by environmental factors such as temperature, moisture, and burial depth (Nuesry, 1981; Rao et al., 1993; Wolf, 1914;). The initial leaf spot symptoms appear as chlorotic specks that develop into circular lesions ranging from 1 to 10 mm in diameter (Shokes & Culbreath, 1997). ELS lesions usually appear as lighter brown on the underside of leaves compared to the darker LLS lesions (Shokes & Culbreath, 1997). ELS symptoms also formed light-brown to black subcircular spots, typically with a yellow halo, while LLS forms dark brown to black lesions, which may or may not form halo, and lesion can also appear on other plant parts such as petioles and stems during sever outbreaks (Shokes & Culbreath, 1997; Nutter & Shokes, 1995) The sporulation patterns of ELS and LLS differ, with ELS primarily sporulating on the adaxial (upper) surface of leaves, while LLS sporulate on the abaxial (lower) surface (McDonald et al., 1985). The spread of these diseases is facilitated by wind, water, insects and human activity through the movement of infected residues (Shokes & Culbreath, 1997). Both pathogens thrive in conditions of extended leaf wetness, high relative humidity, and temperatures exceeding 19°C (Alderman & Beute, 1987; Shokes & Culbreath, 1997). The optimal conditions for ELS development include temperatures between 16 and 25 °C and relative humidity above 90% (Nutter & Shokes, 1995; Shokes & Culbreath, 1997), while LLS develops at temperatures ranging from 20 to 26°C (Shokes & Culbreath, 1997).

Peanut Leaf Spot Management

The use of fungicides has become a critical strategy in managing leaf spot diseases in crops, particularly peanuts. Among the available fungicides, chlorothalonil has been the primary active ingredient for controlling leaf spot infections, providing effective protection against these damaging pathogens (Culbreath et al., 2006). In addition to chlorothalonil, fungicides such as tebuconazole and propiconazole have also been utilized for managing leaf spot (Brenneman & Murphy, 1991; Culbreath et al., 1995). While these chemical treatments are effective in managing leaf spot diseases, they pose significant challenges, particularly for small-scale farmers. The high costs associated with purchasing and applying these fungicides make their use impractical for many growers (Shokes & Culbreath, 1997). To address these challenges, researchers and agricultural experts recommend an integrated pest management approach, which aims to enhance disease control while reducing reliance on fungicides (Cantonwine et al., 2006; Culbreath et al., 2002; Kemerait et al., 2012). Despite the efficacy of these control strategies, several challenges remain. Rising production costs, variable fungicides effectiveness under different environmental conditions, and difficulties small scale farmers face in implementing these strategies can lead to significant yield losses (Little et al., 2021; McDonald et al., 1985; Woodward et al., 2014; Wynne et al., 1991). A promising solution to these challenges is the development of peanut varieties with enhance resistance to leaf spot diseases. Such advancements can significantly reduce the need for fungicide applications, ultimately leading to more sustainable farming practices. These improvements have been achieved through targeted breeding efforts aimed at

enhancing genetic resistance of peanut cultivars against leaf spot pathogens (Chu et al., 2019; Culbreath et al., 1992; Dang et al., 2021; Woodward et al., 2008).

Resistance to Leaf Spot Diseases in Peanut

Breeding programs aimed at enhancing resistance to peanut leaf spot diseases began in the 1980s in the United States, focusing on polygenic traits such as reduced sporulation and smaller lesion size (Gill, 2013). Genetic resistance to leaf spot pathogens is known to be partial and quantitative, reducing disease severity rather than conferring complete immunity (Sehgal et al., 2016). Quantitative trait loci (QTL) mapping has been instrumental in identifying loci associated with resistance. QTLs for early leaf spot (ELS) resistance have been mapped to chromosome 3, while those for late leaf spot (LLS) have been located on chromosome 5 (Chu et al., 2019). Cultivating peanut varieties with genetic resistance can reduce fungicide applications by two to three treatments per growing season (Cantonwine et al., 2006). Despite the advantages of genetic resistance, resistance to ELS and LLS is typically partial and polygenic, influenced by environmental stress factors as well as yield traits (Sehgal et al., 2016). Resistance has been identified in both cultivated peanut varieties and wild *Arachis* species, with wild relatives often exhibiting higher levels of resistance (Abdou et al., 1974; Chalal & Sandhu, 1972; Hassan & Beute, 1977; Monasterios de La Torre, 1980; Sowell et al., 1976; Subrahmanyam et al., 1982). The relatively low levels of resistance in cultivated peanuts have prompted breeders to explore new sources of resistance form wild species (Subrahmanyam et al., 1985). These efforts are essential for developing more resilient peanut cultivars capable of reducing dependence on chemical treatments and improving crop sustainability.

Phenotyping for Resistance to Leaf Spot Pathogens in Peanuts

Partial resistance to leaf spot diseases in peanuts involves multiple components that collectively slow disease progression (Abdou et al., 1974; Parlevliet, 1979). Research in the 1980s identified several key resistance components for early leaf spot (ELS) (Foster et al., 1980; Green & Wynne, 1986; Melouk & Banks, 1984; Ricker et al., 1985) and late leaf spot (LLS) (Chiteka et al., 1988; Cook, 1981; Subrahmanyam et al., 1982; Walls et al., 1985; Watson et al., 1998). However, a standardized method for evaluating leaf spot resistance across peanut genotypes has not yet been established. Researchers employ different criteria based on specific objectives (McDonal et al., 1985; Waliyar, 1989), using controlled approaches such as detached leaf assays and greenhouse inoculations alongside field experiments (Gonzales et al., 2023; Leal-Bertioli et al., 2009; Levinson et al., 2021; McDonald et al., 1985; Waliyar et al., 1989). The detached leaf method, initially developed for ELS (Melouk & Banks, 1978), has since been adapted for LLS and other foliar diseases (Nevill, 1982; Subrahmanyam et al., 1995). While a variety of methodologies are employed, field assessments remain crucial since resistant plant introductions may underperform outside controlled environments (Hassan & Beute, 1977). Techniques used to assess leaf spot severity in field trials include the defoliation ratio (Hassan & Beute, 1977), lesion count (Chiteka et al., 1988; Hassan & Beute, 1977), leaf area infected (Foster et al., 1980), percent necrotic area per leaf (Chiteka et al., 1988), and visual estimations based on the Florida scale (Chiteka et al., 1988). The Florida scale, established by Chiteka (1988), remains widely accepted for evaluating lesion count and defoliation, Additionally, Culbreath et al. (1992) suggested assessing stem lesions in field trials, noting a correlation with late leaf spot severity. However,

further research is needed to investigate differences in susceptibility to stem lesions among peanut genotypes. Phenotyping for resistance has predominantly focused on post-infection factors. Abdou et al. (1974) suggested that resistance to LLS could manifest before infection by inhibiting conidial germination, but studies on pre- and during-infection resistance mechanisms are limited. Research on fungal colonization, particularly hyphal development, remains insufficient (Abdou et al., 1974), highlighting the need for further investigation into infection process to improve understanding of resistance mechanisms. Resistance stability may vary across growing regions due to environmental interactions and pathogen variability (Chiteka et al., 1997; Shew et al., 1988; Waliyar et al., 1993).

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CHAPTER 3

EFFECT OF PEANUT GENOTYPE ON EARLY INFECTION STAGES BY *Passalora arachidicola* AND *Nothopassalora personata*, FOCUSING ON CONIDIAL ADHESION AND GERM TUBE ELONGATION

INTRODUCTION

Peanut (*Arachis hypogaea* L.) is an economically and nutritionally vital crop, serving as a key source of protein, oil and other essential nutrients. However, its production is severely impacted by fungal pathogens, particularly *Passalora arachidicola* (Hori) U. Braun and *Nothopassalora personata* (Berk. & M.A. Curtis) U. Braun, C. Nakash., Videira & Crous (Anco et al., 2020; Mycobank, 2024; York et al., 1995). These pathogens pose a significant threat to peanut yields, necessitating robust disease management strategies that include the development and identification of resistant genotypes. Resistance to leaf spot diseases has been evaluated using various methodologies, including detached leaf assays, greenhouse inoculations, and field trials. These methods provided unique insights into resistance, allowing for a thorough assessment across different research contexts (McDonald et al., 1985; Subrahmanyam et al., 1980; 1985b; Waliyar et al, 1989). The common metrics in field evaluations include the percentage of leaves with lesions, the number of lesions per leaf, and the percentage of defoliation, often assessed using the Florida scale (Anderson et al., 1991; Chiteka et al., 1988a; 1988b; Foster et al., 1980; Hassan & Beute, 1977; McDonald et al., 1985).

These approaches are crucial for quantifying disease severity and identifying genotypes with field resistance to leaf spot. Stalker (2017) had demonstrated the desirable traits in wild *Arachis* species for crop improvement, and high level of resistance has been identified in this species for many peanut pathogens and insects (Stalker, 2017; Stalker & Moss, 1987). Notably, both *A. cardenasii* and *A. stenosperma* have resistance to *P. arachidicola* and *N. personata* (Abdou et al., 1974; Kolawole 1976; Nigam et al., 1991; Sharief et al., 1978; Subrahmanyam et al., 1980, 1985a; Stalker 1991; 2017; Stalker et al., 2002a, 2002b). These wild *Arachis* species are key sources of resistance for breeding programs aimed at improving cultivated peanuts.

The evaluation methods previously described primarily emphasize post-infection resistance mechanisms, which analyze how well plants suppress disease progression after infection. In contrast, pre-infection resistance mechanisms, though equally critical to understanding overall disease resistance, remain relatively understudied. Abdou (1974) demonstrated that highly susceptible peanut shows rapid conidial germination and penetration by *P. arachidicola* (syn. *Cercospora arachidicola*), while resistant plants exhibit delayed processes. Similarly, *A. stenosperma* delays germ tube development after inoculation with *N. personata* (syn. *Cercosporidium personatum*), highlighting the important role of early resistance mechanisms (Leal-Bertioli et al., 2010). Resistance to both early and late leaf spots is linked to specific genomic regions and quantitative trait loci (QTLs) (Chu et al., 2019). Key resistance mechanisms identified by Nobile et al. (2008) during the initial phase of infection include the hypersensitive response (HR), activation of signaling pathways, and production of antimicrobial compounds. Dang et al. (2021) found differential expression of 36 R-genes, primarily receptor-like kinases

(RLKs) and receptor-like proteins (RLPs), which trigger defense responses such as the production of reactive oxygen species (ROS).

Although these findings confirm the involvement of early-stage resistance mechanisms to *P. arachidicola* and *N. personata*, research on pre-infection resistance components remains limited, particularly in understanding how these processes vary among genotypes with differing resistance levels. This study aims to address this gap by investigating the pre-infection components in selected genotypes, focusing on conidial adhesion and germ tube length in response to both pathogens.

MATERIALS AND METHODS

The laboratory experiment was conducted to assess the effect of peanut genotype on the early infection stages by *P. arachidicola* and *N. personata*. It involved the use of light microscopy to examine pathogen development on the leaf surface, utilizing a controlled detached leaf assay and preparing leaf tissue for microscopy analysis. See Appendix Figure 1 for a simplified flowchart of the methodology.

Peanut Genotypes. The peanut genotypes used in the laboratory experiment were provided by Dr. Soraya Bertioli and Dr. David Bertioli of the UGA Athens Campus, and Dr. Corley Holbrook of USDA-ARS, Tifton. These genotypes included cultivars without resistance segments, along with cultivars and breeding lines with wild introgressions, as summarized in Appendix Table 1.1.

Experimental locations. The selected genotypes for the laboratory experiment were planted at the Plant Pathology Greenhouse, University of Georgia, Tifton, Georgia. The seeds were sown in small pots containing a soil mixture of 4 parts sand, 2 parts field

soil, 2 parts ProMix, and 1 part perlite. Leaf samples were collected once the second or third fully expanded, healthy leaves from the apex were observed. These leaves were washed with tap water followed by sterile water and then placed in 100 mm x 15 mm petri dishes lined with wet cotton pads moistened with sterile water. The laboratory experiments were carried out in the Natural Products Laboratory, Room 201, at the University of Georgia, Tifton, Georgia. The experimental set-up was maintained at a laboratory temperature of 20 $\pm$ 2 °C, with all working areas and containers properly disinfested with 70% ethanol throughout the experiment and incubation process.

Experimental design. The experiment was conducted as two trials, both using a completely randomized design (CRD) as all samples were incubated under similar conditions. For each genotype, three leaves were inoculated and subjected to three different treatments: inoculation with *P. arachidicola* (PA), *N. personata* (NP), and a mock control. Georgia-06G (Branch, 2007), the predominant genotype in U.S. production systems (UGA Extension, 2019), is susceptible to leaf spot diseases (Kaur et al., 2024). This makes it a good control for comparison against genotypes containing wild resistance segments.

Inoculum preparation. Sporulating lesions of PA were collected from leaves at Black Shank Farm, while NP lesions were collected from Lang Farm. The isolates used for the experiments were obtained from infected leaves exhibiting approximately 50% lesion coverage, with no signs of secondary pathogens or other symptoms. The leaf samples were examined under a dissecting microscope. The sporulating lesions were gently tapped with a sterilized needle and transferred to V 8 Juice Agar (V8), which consists of V8 juice, calcium carbonate, and agar. The cultures were incubated for 10 to

14 days, or until sporulating fungal growth was observed. The fungal cultures grown in V8 medium were carefully scraped and transferred to 1.5 ml microcentrifuge tubes containing sterile water with 0.005% Tween-20. The resulting spore suspension was then transferred to potato dextrose agar (PDA) plates and incubated for additional 10 days to serve as the inoculum source. After incubation, fungal cultures on PDA were again scraped and transferred to 1.5 ml microcentrifuge tubes with sterile water containing 0.005% Tween-20. The spore suspension was calibrated to $1x10^5$ conidia/ml using a hemocytometer before being used for leaf inoculation. The inoculation of experiments for PA and NP were conducted separately to prevent cross-contamination and reduce the potential for confusion.

Leaf Inoculation. The three prepared leaves were inoculated following the detached leaf assay method adapted from Leal-Bertioli et al. (2009) and Levinson et al. (2021). A light application of the conidial suspension of $1x10^5$ conidia/ml was brushed onto the adaxial surface of the leaves for PA and onto the abaxial surface for NP. The difference in leaf surface inoculation was based on the typical sporulation pattern observed in the field (see Appendix Figure 2). After inoculation, the leaves were air-dried for 10 minutes at room temperature and then incubated in disinfested containers kept at the laboratory temperature of 20 ± 2 °C. A set of uninoculated leaves from each genotype was prepared as a mock control.

Leaf fixing and clearing. In each test for measuring conidial adhesion and germ tube length, six small leaf sections, approximately 1cm x 1cm, were arbitrarily collected from the three inoculated leaves for each genotype. The modified leaf fixation and clearing method involved soaking the leaves in a fixing solution composed of 70% ethanol and

acetic acid in a 1:2 v:v ratio. During the clearing process, the leaves were subjected to 2 or 3 successive exchanges of 70% ethanol until they were fully cleared. For the conidial adhesion test, six leaf sections were soaked in the fixing solutions for designated periods of 12, 48, and 96 hours after inoculation (HAI). Similarly, six leaf sections were fixed for 12, 48, and 96 HAI for the conidial germ tube length test. Following these fixation periods, the leaves were transferred to the clearing ethanol solutions for further processing.

Data collection. After the chlorophyll was almost completely removed from the leaf sections and leaf tissue appeared nearly white, the sections were mounted on microscope slides with a diluted lactophenol cotton blue solution. The mounted sections were then examined using a light microscope. For the conidial adhesion test, the entire 1cm x 1cm sections were examined under a light microscope at 400X magnification to count the conidia (see Appendix Figure 3). For the conidial germ tube length test, the microscope was calibrated using ocular and stage micrometers prior to measuring the lengths of the conidial germ tubes in micrometers (μm) (see Appendix Figure 4). In the conidial adhesion test, six leaf sections were examined, while in the germ tube length assessment, fifteen conidia were measured from each of the six fixed and cleared leaf sections. Images displaying visible germ tubes were processed using ImageJ (Schneider et al. 2012) to quantify germ tube lengths.

Data analysis. The mean conidial count and mean conidial germ tube length at various time points were plotted to visualize the data distribution for each genotype. Residual plots including histograms and Q-Q plots for each dataset were employed to assess the assumptions of variance and normality to determine the suitability of the data for linear regression analysis (See Appendix Figure 5 for plots).

Statistical analysis. Analysis was performed using RStudio statistical software (R version 4.2.2, 2022) (RStudio Team 2020). The employed RStudio packages were "car", "carData", "ggplot2", "ggpubr" and "agricolae". A linear mixed model (LMM) was applied to the data from the two trials using a completely randomized design (CRD) for the analysis. The LMM was specified as follows:

$$Y_{ij} = \beta_0 + \beta_1 \text{Genotype}_i + \beta_2 \text{Trial}_j + \beta_3 \left(\text{Genotype}_i \text{ x Trial}_j\right) + \epsilon_{ij}$$

where Y_{ij} represents the response variable for genotype *i* and trial *j*, β_0 is the overall mean, and β_1 and β_2 are the fixed effects for genotype and trial, respectively, β_3 represents the interaction effect between genotype and trial, and ϵ_{ij} is the error term associated with the response. Statistical significance in all tests was decided at a p-value of 0.05.

ANOVA: Analysis was carried out using Analysis of Variance (ANOVA) to evaluate the significance of the interaction effects between genotype and trial. If the interaction effects were not significant, they were not further analyzed. But if significant interactions were detected, separate analyses were conducted for each trial.

Tukey's HSD: Differences among the least square means of conidial adhesion and conidial germ tube length for each genotype were assessed using Tukey's Honest Significant Difference (HSD) test. The post-hoc analysis was conducted following ANOVA to determine which specific genotype means for the conidial adhesion and conidial germ tube length differed significantly from one another.

Correlation Analysis: Pearson's correlation coefficient was calculated to examine the relationships among PA and NP conidial adhesion and conidial germ tube length for each genotype at the final observation time (96HAI).

Supplementary data. The experiments included an initial optimization experiment and non-duplicated trial, which differed in observation time and peanut genotypes to the duplicated trial. The initial optimization experiment aimed to optimize the methodology, including inoculation, leaf processing, and data collection. The non-duplicated trial included additional breeding lines to gather preliminary data on these genotypes. The genotypes used in each experiment are listed in Appendix Table 1.2.

In the initial optimization experiment, only the inoculation of PA was performed, and data collected included conidial count per cm^2 at 12 hours after inoculation (HAI), as well as conidial germ tube length assessments at 48 and 96 HAI. In the non-duplicated trial, both PA and NP were inoculated, with data collected on conidial count per cm^2 at 12, 48, and 96 HAI, and conidial germ tube length assessed at 48, and 96 HAI.

Data were analyzed using a linear regression model (LM) that assessed the effect of genotype on conidial adhesion and conidial germ tube length. The LM was specified as follows:

$$Y_i = \beta_0 + \beta_1 \text{Genotype}_i + \epsilon_i$$

where Y_i represents the response variable for genotype *I*, β_0 is the overall mean, and β_1 is the coefficient representing the effect of genotype, and ϵ_i is the error term. Statistical significance in all tests was decided at a p-value of 0.05. Analysis of Variance (ANOVA), Tukey's Honest Significance Difference (HSD) test was used to compare the least square means. All statistical analyses were performed RStudio statistical software (R version 4.2.2, 2022) (RStudio Team 2020).

RESULTS

Conidial adhesion for *Passalora arachidicola*

The initial optimization and non-duplicate trial showed a significant effect of genotype on *P. arachidicola* conidial adhesion ($P < 0.001$) (Table 1). At 12HAI, in the initial optimization, slight but significant differences in the mean conidial counts were observed among genotypes (Table 2.1). Across the observation time, the non-duplicated trial showed that the susceptible cultivars Georgia-06G and TUFRunner'511' had mean counts ranging from 10.83 to 19.00 conidia/cm^2, while genotypes with resistance segments had mean counts ranging from 0.67 to 3.17 conidia/cm^2 (Table 2.2). The genotypes derived from *A. stenosperma* and *A. batizocoi,* RBS-158_B_10, RBS-95_C9, RBS-170_A had mean counts ranging from 0.00 to 1.00 conidia/cm^2 (Table 2.2). Meanwhile, *A. cardenasii*-derived genotypes such as IAC 322, TBI-S11, and CB-7 had mean counts between 0.50 to 3.17 conidia/cm^2 (Table 2.2).

Both the initial and non-duplicated trial were consistent with the results from duplicated trial, where genotype had a significant effect on *P. arachidicola* conidial adhesion ($P < 0.001$). A significant genotype x trial (G x T) interaction was observed at 12HAI ($P < 0.05$) (Table 3.1). In these trials, the susceptible cultivar Georgia-06G exhibited mean counts ranging from 11.0 to 18.6 conidia/cm^2 (Table 4.1). RBS-158_B10 had mean counts ranging from 0.42 to 0.75 conidia/cm^2, CB-7 from 0.83 to 0.92 conidia/cm^2, TBI-S11 from 1.50 to 2.75 conidia/cm^2, and IAC 322 from 2.92 to 4.58 conidia/cm^2 (Table 4.1). At 12HAI, a significant trial effect was observed, with Georgia-06G with mean counts of 11.17 and 10.83 conidia/cm^2, while genotypes with resistance segments had 0.00 and 5.33 conidia/cm^2 (Table 4.3).

Conidial adhesion for *Nothopassalora personata*

The non-duplicated trial showed the significant effect of genotype on NP conidial adhesion ($P < 0.001$) (Table 1). In this experiment, across observation time, the susceptible cultivars Georgia-06G and TUFRunner'511' had mean conidial counts ranging from 8.50 to 9.17 conidia/cm^2 while genotypes with resistance segments ranged from 0.50 to 6.00 conidia/cm^2 (Table 2.2). The genotypes derived from *A. stenosperma* and *A. batizocoi,* RBS-158_B_10, RBS-95_C9, RBS-170_A had mean conidial counts ranging from 0.50 to 4.00 conidia/cm^2 (Table 2.2). While *A. cardenasii*-derived genotypes such as IAC 322, TBI-S11, and CB-7 had conidial counts between 0.50 to 6.00 conidia/cm^2 (Table 2.2).

The duplicated trial confirmed the significant effect of genotype on *N. personata* (NP) conidial adhesion ($P < 0.001$) (Table 3.2). At 48HAI and 96HAI, the susceptible cultivar Georgia-06G had mean counts of 9.08 and 9.92 conidia/cm (Table 4.1). RBS-158_B10 had mean counts of 4.50 and 4.33 conidia/cm^2, CB-7 had 0.58 and 0.50 conidia/cm^2, TBI-S11 had 2.17 and 2.25 conidia/cm^2, and IAC 322 had 4.25 and 6.17 conidia/cm^2 (Table 4.1).

Conidial germ tube length for *Passalora arachidicola*

The initial optimization and non-duplicated trial showed the significant effect of genotype on *P. arachidicola* germ tube length ($P < 0.001$) (Table 1). In the initial optimization experiment, in the susceptible cultivar Georgia-06G, PA exhibited mean germ tube length of 123.8 μm at 48HAI and 212.9 μm at 96HAI, while genotypes with resistance segments exhibited length ranging from 21.63 μm to 70.35 μm at 48HAI and 40.52 μm to 140.37 μm (Table 2.1). Across observation time, in the non-duplicated trial,

the susceptible cultivar Georgia-06G and TUFRunner'511' had PA mean germ tube length, ranging from 101.19 μm to 235.03 μm (Table 2.3). The genotypes derived from *A. stenosperma* and *A. batizocoi,* RBS-95_C9, RBS-158_B_10, and RBS-170_A, had mean length ranging from 37.1 to 79.3 μm (Table 2.3). While *A. cardenasii*-derived genotypes such as IAC 322, TBI-S11, and CB-7 had mean length ranging from 21.39 to 85.16 μm (Table 2.3).

The duplicated trial confirmed the significant effect of genotype *P. arachidicola* (PA) germ tube length ($P < 0.001$) (Table 2). A significant genotype x trial (G x T) interaction was observed at 48HAI ($P < 0.01$) (Table 4.1). In these trials, the susceptible cultivar Georgia-06G exhibited mean germ tube length of 106.5 μm at 48HAI and 198.9 μm at 96HAI (Table 4.2). RBS-158_B10 had mean lengths of 37.58 μm at 48Hai and 54.85 μm at 96HAI, CB-7 had 21.39 μm at 48HAI and 53.55 μm at 96HAI, TBI-S11 had 19.16 μm at 48HAI and 47.44 μm at 96HAI, and IAC 322 had 61.31 μm at 48HAI and 88.42 μm at 96HAI (Table 4.2). At 48HAI, a significant trial effect was observed, with Georgia-06G with mean length of 111.70 μm in Trial 1 and 101.19 μm in Trial 2, while genotypes with resistance segments ranges had 16.83 to 53.02 μm in Trial 1 and 21.39 to 69.61 μm in Trial 2 (Table 4.3).

Conidial germ tube length for *Nothopassalora personata*

The non-duplicated trial showed the significant effect of genotype on *N. personata* germ tube length ($P < 0.001$) (Table 1). Across observation time, the susceptible cultivars Georgia-06G and TUFRunner'511' had NP mean germ tube length, ranging from 64.96 to 113.24 μm (Table 2.3). The genotype derived from *A. stenosperma* and *A. batizocoi,* RBS-95_C9, RBS-158_B_10, and RBS-170_A, had mean length

ranging from 21.58 to 36.57 μm (Table 2.3). While *A. cardenasii*-derived genotypes such as IAC 322, TBI-S11, and CB-7 had mean length ranging from 1.52to 31.65 μm (Table 2.3).

The duplicated trial confirmed the significant effect of genotype *N. personata* germ tube length ($P < 0.001$) (Table 4.2). In these trials, the susceptible cultivar Georgia-06G exhibited mean germ tube length of 70.64 μm at 48HAI and 95.50 μm at 96HAI (Table 4.2). RBS-158_B10 had mean lengths of 35.05 μm at 48HAI and 40.99 μm at 96HAI, CB-7 had 4.92 μm at 48HAI and 12.05 μm at 96HAI, TBI-S11 had 3.11 μm at 48HAI and 10.54 μm at 96HAI, and IAC 322 had 24.67 μm at 48HAI and 36.86 μm at 96HAI (Table 4.2).

Relationship of conidial adhesion and conidial germ tube length for PA and NP

The study examined the relationship between conidial adhesion (count/cm^2) and germ tube length (μm) for *P. arachidicola* (PA) and *N. personata* (NP), in peanut genotypes with different resistance sources (Table 5). The susceptible cultivar Georgia-06G showed no significant correlations between conidial adhesion and germ tube length ($P > 0.05$). IAC 322, with *A. cardenasii* resistance, had a significant positive correlation between PA adhesion and NP germ tube length ($R = 0.58$, $P < 0.05$) and a moderately negative correlation between PA adhesion and PA germ tube length ($R = -0.52$, $P = 0.08$). CB-7 and TBI-S11, both with *A. cardenasii* resistance, showed no significant correlations ($P > 0.05$). TBI-S11 had a moderate positive correlation between PA and NP adhesion ($R = 0.56$, $P = 0.06$). RBS-158_B_10, with *A. stenosperma* and *A. barizocoi* resistance, had weak, non-significant correlations ($P > 0.05$). Pooled data across all

genotypes showed significant and strong correlations between PA and NP adhesion (R = 70, $P < 0.001$), and between NP adhesion and NP germ tube length (R = 0.62, $P < 0.001$).

DISCUSSION

This study provides new information on how peanut genotypes may influence the early infection stages of *Passalora arachidicola* (PA) and *Nothopassalora personata* (NP), in their effects on conidial adhesion and germ tube length. The results show that conidial adhesion in both pathogens is strongly affected by peanut genotype, with resistant genotypes displaying lower frequency of adhesion and, consequently, reduced infection potential. Genotypes with resistance derived from *A. stenosperma* and *A. batizocoi* (RBS-158_B10) and from *A. cardenasii* (IAC 322, CB-7, and TBI-S11) exhibited significantly lower conidial counts and shorter germ tube lengths than the susceptible cultivar Georgia-06G for both pathogens. These findings were consistent across all experiments, including initial optimization, non-duplicated, and duplicated trials.

For *P. arachidicola,* RBS-158_B10, carrying resistance from *A. stenosperma* and *A. batizocoi*, showed the lowest conidial counts, while CB-7, TBI-S11, and IAC 322 (all with *A. cardenasii* resistance) had slightly higher counts. In contrast, for *N. personata*, CB-7 and TBI-S11 displayed lower conidial counts, while RBS-158_B10 had slightly higher counts. Germ tube length also emerged as a critical factor in resistance to these leaf spot pathogens. The longer germ tube lengths observed in Georgia-06G for both PA and NP suggest that its lack of resistance allows the pathogens to grow and establish more readily. Conversely, shorter germ tubes in resistant genotypes indicate more

effective resistance mechanisms that inhibit pathogen development. For both PA and NP, CB-7 and TBI-S11 consistently exhibited shorter germ tube lengths compared to other resistant genotypes. Although these shorter lengths suggest resistance, the resistance appears to be partial, as it does not completely prevent pathogen growth. This study did not address whether slower germ tube growth prevented infection or slowed infection compared to the standard cultivars. The differences observed between the susceptible Georgia-06G and the resistant genotypes, as well as the varied responses to PA and NP, highlight the range of resistance levels based on peanut genetic background. These findings support previous studies, which demonstrated restricted conidial development and penetration in resistant genotypes (Leal-Bertioli et al., 2010; Abdou et al., 1974). This restriction may be linked to resistance genes active in the early stages of pathogen infection, such as those identified by Nobile et al. (2008) against in *N. personata*.
The relationship between conidial adhesion and germ tube length further suggests that these early infection stages are interconnected. Significant correlations observed across all genotypes between PA and NP adhesion and germ tube length suggest that conidial adhesion and germ tube elongation are associated and critical for disease progression. The confirmed differences in disease progression between susceptible and resistant genotypes in this study, as observed in detached leaf assays and previous fieldwork (Gobin,a 1983; Melouk, 1978; Johnson, 1986; Hassan & Beute, 1977; Melouk & Bank, 1984), reinforce the role of genetic resistance in influencing both pre- and post-infection stages of pathogen development.

These results indicate that peanut genotypes affect both conidial adhesion and germ tube development of both leaf spot pathogens and suggests that these effects may be

involved in the resistance mechanisms of lines derived from *Arachis cardenasii* and *A. stenosperma*. Furthermore, the method developed for characterizing these effects may be useful in elucidating effects of additional genotypes and different sources of resistance to these important foliar pathogens.

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Table 1. Analyzing the effect of genotype on conidial adhesion and conidial germ tube length in the initial optimization experiment (inoculated with PA) and non-duplicated trial experiment (inoculated with PA and NP).

Variable[a]	HAI[b]	Linear mixed model[cf]				Source of variation	ANOVA[df]			Tukey's HSD[ef]		
		R^2	DF	RSE	p-value		DF	f-value	p-value	DF	q-value	MSD
Initial optimization												
PA adhesion	12	0.62	24	0.86	<0.001	Genotype	7	5.64	<0.001	24	4.68	2.01
PA germ tube length	48	0.62	72	25.38	<0.001		7	16.52	<0.001	72	4.41	35.44
	96	0.70	72	33.75	<0.001		7	24.14	<0.001	72	4.41	47.12
Non-duplicated trial												
PA adhesion	12	0.93	40	1.34	<0.001	Genotype	7	78.67	<0.001	40	4.52	2.47
	24	0.93	40	1.76	<0.001		7	76.20	<0.001	40	4.52	3.24
	48	0.93	40	2.30	<0.001		7	72.22	<0.001	40	4.52	4.25
PA germ tube length	48	0.64	112	26.42	<0.001		7	27.95	<0.001	112	4.37	29.80
	96	0.67	112	51.26	<0.001		7	31.79	<0.001	112	4.37	57.82
NP adhesion	48	0.70	40	2.07	<0.001		7	13.11	<0.001	40	4.52	3.82
	96	0.79	40	1.92	<0.001		7	21.64	<0.001	40	4.52	3.54
PA germ tube length	48	0.60	112	21.52	<0.001		7	23.51	<0.001	112	4.37	24.27
	96	0.72	112	22.19	<0.001		7	40.90	<0.001	112	4.37	25.03

[a] Data for conidial count (count/cm²) and germ tube length (μm) for *P. arachidicola* (PA) and *N. personata* (NP).

[b] hours after inoculation.

[c] Analyzes genotype-variable relationships, accounting for both fixed and random effects. Metrics: R^2, DF, RSE, p-value.

[d] Asses whether genotypes significantly affect variables. Metrics: DF, f-value, p-value.

[e] Identifies significantly different groups. Metrics: DF, q-value, MSD.

[f] Metrics: R^2 – coefficient of determination, indicating model fit; DF – degrees of freedom, representing sample size; RSE – residual standard error. measuring model prediction error; p-value – tests statistical significance ($p < 0.05$ is significant); f-value – higher values indicate more significant differences between groups; q-value – higher values indicate larger, more significant group differences; MSD – minimum significant difference between group means.

Table 2.1. Least square means of conidial adhesion and conidial germ tube length for *Passalora arachidicola* (PA) in the initial optimization experiment.

Genotype	Wild *Arachis* resistance source	PA adhesion[ac] 12HAI		PA germ tube length[bc] 48HAI		96HAI	
Georgia-06G	None	2.50	A	123.82	A	212.92	A
IAC 322	*A. cardenasii*	0.00	B	70.35	B	140.37	B
CB-1	*A. cardenasii*	0.50	AB	32.96	C	73.95	CD
CB-2	*A. cardenasii*	0.50	AB	47.79	BC	102.18	BC
CB-7	*A. cardenasii*	1.25	AB	33.27	C	128.21	B
TBI-S11	*A. cardenasii*	0.50	AB	37.42	BC	76.64	CD
BBI-S25	*A. cardenasii*	0.00	B	42.33	BC	99.57	BC
RBS-158B10	*A. stenosperma/ A. batizocoi*	2.50	A	21.63	C	40.52	D

[a] Least square means of conidial adhesion (count/cm²) for PA at 12 HAI.

[b] Least square means of conidial germ tube length (μm) for PA at 48, and 96 HAI.

[c] Genotypes are grouped based on Tukey's HSD test, with letters indicating significant differences. Groups with the same letter are not significantly different.

Table 2.2. Least square means of conidial adhesion for *Passalora arachidicola* (PA) and *Nothopassalora personata* (NP) in the non-duplicated trial experiment.

Genotype	Wild *Arachis* resistance source	PA adhesion[ac]						NP adhesion[bc]			
		12HAI		48HAI		96HAI		48HAI		96HAI	
TUFRunner'511'	None	11.00	A	14.50	A	19.00	A	8.50	A	8.83	A
Georgia-06G	None	10.83	A	14.17	A	18.17	A	8.67	A	9.17	A
IAC 322	*A. cardenasii*	0.50	B	1.50	B	3.17	B	3.67	B	6.00	AB
CB7	*A. cardenasii*	0.50	B	0.67	B	1.00	B	0.50	B	0.17	D
TBI-S11	*A. cardenasii*	0.67	B	1.83	B	2.00	B	1.83	B	1.67	CD
RBS-95C9	*A. cardenasii*	0.33	B	0.33	B	0.67	B	4.00	B	1.50	CD
RBS-158B10	*A. stenosperma/ A. batizocoi*	0.00	B	0.17	B	0.67	B	3.50	B	4.00	BC
RBS-170A	*A. stenosperma/ A. batizocoi*	0.67	B	0.67	B	1.00	B	1.50	B	0.50	CD

[a] Least square means of conidial adhesion (count/cm²) for PA at 12, 48, and 96 HAI.

[b] Least square means of conidial adhesion (count/cm²) for NP at 48, and 96 HAI.

[c] Genotypes are grouped based on Tukey's HSD test, with letters indicating significant differences. Groups with the same letter are not significantly different.

Table 2.3. Least square means of conidial germ tube length for *Passalora arachidicola* (PA) and *Nothopassalora personata* (NP) in the non-duplicated trial genotypes experiment.

Genotype	Wild *Arachis* resistance source	PA germ tube length[ac]				NP germ tube length[bc]			
		48HAI		96HAI		48HAI		96HAI	
TUFRunner'511'	None	118.86	A	235.03	A	73.95	A	113.24	A
Georgia-06G	None	101.19	A	199.75	A	64.96	A	85.90	B
IAC 322	*A. cardenasii*	69.61	B	85.16	B	20.17	BC	31.65	CD
CB7	*A. cardenasii*	21.39	D	47.71	B	1.90	C	8.39	D
TBI-S11	*A. cardenasii*	21.49	D	39.81	B	1.52	C	10.43	D
RBS-95C9	*A. cardenasii*	41.58	BCD	79.26	B	17.85	BC	32.32	CD
RBS-158B10	*A. stenosperma/ A. batizocoi*	53.67	BC	68.99	B	33.71	B	33.41	CD
RBS-170A	*A. stenosperma/ A. batizocoi*	37.12	CD	46.82	B	21.58	BC	36.57	C

[a] Least square means of conidial adhesion conidial germ tube length (μm) for PA at 48, and 96 HAI.
[b] Least square means of conidial adhesion conidial germ tube length (μm) for NP at 48, and 96 HAI.
[c] Genotypes are grouped based on Tukey's HSD test, with letters indicating significant differences. Groups with the same letter are not significantly different.

Table 3.1. Analyzing the effect of genotype on *Passalora arachidicola* (PA) conidial adhesion and conidial germ tube length in the replicated genotypes experiment.

Variable[a]	HAI[b]	Linear mixed model[cg]				Sources of variation[d]	ANOVA[eg]			Tukey's HSD[fg]		
		R^2	DF	RSE	p-value		DF	f-value	p-value	DF	q-value	MSD
PA adhesion	12	0.87	50	1.70	<0.001	G	4	80.10	<0.001	50	4.00	1.96
						G x T	4	3.51	<0.05	s	s	s
						G in T1	4	26.33	<0.001	25	4.15	3.48
						G in T2	4	83.35	<0.001	25	4.15	2.12
	48	0.88	50	2.02	<0.001	G	4	93.23	<0.001	50	4.00	2.34
						G x T	4	0.82	0.52	ns	ns	ns
	96	0.92	50	2.22	<0.001	G	4	135.97	<0.001	50	4.00	2.56
						G x T	4	0.87	0.49	ns	ns	ns
PA germ tube length	48	0.71	140	22.32	<0.001	G	4	78.81	<0.001	140	3.91	15.93
						G x T	4	4.05	<0.01	s	s	s
						G in T1	4	63.07	<0.001	70	3.96	19.97
						G in T2	4	27.99	<0.001	70	3.96	25.35
	96	0.63	140	45.39	<0.001	G	4	59.00	<0.001	140	3.91	32.39
						G x T	4	1.12	0.35	ns	ns	ns

[a] Data for conidial count (count/cm²) and germ tube length (µm) for PA.
[b] Hours after inoculation.
[c] Analyzes genotype-variable relationships, accounting for both fixed and random effects. Metrics: R^2, DF, RSE, p-value.
[d] Considers genotype (G) and Genotype x Trial (G x T) interaction effect.
[e] Assesses whether genotypes and interaction with trial significantly affect variables. Metrics: DF, f-value, p-value.
[f] Identifies significantly different groups. Metrics: DF, q-value, MSD.
[g] Metrics: R^2 – coefficient of determination, indicating model fit; DF – degrees of freedom, representing sample size; RSE – residual standard error, measuring model prediction error; p-value – tests statistical significance ($p < 0.05$ is significant); f-value – higher values indicate more significant differences between groups; q-value – higher values indicate larger, more significant group differences; MSD –minimum significant difference between group means.

Table 3.2. Analyzing the effect of genotype on *Nothopassalora personata* (NP) conidial adhesion and conidial germ tube length in the replicated genotypes experiment.

Variable[a]	HAI[b]	Linear mixed model[cg]				Source of variation[d]	ANOVA[eg]			Tukey's HSD[fg]		
		R^2	DF	RSE	p-value		DF	f-value	p-value	DF	q-value	MSD
NP adhesion	48	0.77	50	1.77	<0.001	G	4	39.24	<0.001	50	4.00	2.05
						G x T	4	0.44	0.78	ns	ns	ns
	96	0.77	50	1.99	<0.001	G	4	40.11	<0.001	50	4.00	2.30
						G x T	4	0.16	0.96	ns	ns	ns
NP germ tube length	48	0.66	140	18.50	<0.001	G	4	66.43	<0.001	140	3.91	13.20
						G x T	4	0.31	0.87	ns	ns	ns
	96	0.77	140	17.93	<0.001	G	4	110.45	<0.001	140	3.91	12.80
						G x T	4	1.24	0.30	ns	ns	ns

[a] Data for conidial count (count/cm²) and germ tube length (μm) for NP.
[b] Hours after inoculation.
[c] Analyzes genotype-variable relationships, accounting for both fixed and random effects. Metrics: R^2, DF, RSE, p-value.
[d] Considers genotype (G) and Genotype x Trial (G x T) interaction effect.
[e] Assesses whether genotypes and interaction with trial significantly affect variables. Metrics: DF, f-value, p-value.
[f] Identifies significantly different groups. Metrics: DF, q-value, MSD.
[g] Metrics: R^2 – coefficient of determination, indicating model fit; DF – degrees of freedom, representing sample size; RSE – residual standard error, measuring model prediction error; p-value – tests statistical significance ($p < 0.05$ is significant); f-value – higher values indicate more significant differences between groups.; q-value – higher values indicate larger, more significant group differences; MSD –minimum significant difference between group means.

Table 4.1. Least square means of conidial adhesion for *Passalora arachidicola* (PA) and *Nothopassalora personata* (NP) in the replicated genotypes experiment.

Genotype	Wild *Arachis* resistance source	PA adhesion[ac]						NP adhesion[bc]			
		12HAI		48HAI		96HAI		48HAI		96HAI	
Georgia-06G	None	11.00	A	13.92	A	18.58	A	9.08	A	9.92	A
IAC 322	*A. cardenasii*	2.92	B	2.67	B	4.58	B	4.25	B	6.17	B
CB7	*A. cardenasii*	0.83	C	0.83	B	0.92	C	0.58	C	0.50	D
TBI-S11	*A. cardenasii*	1.50	BC	1.92	B	2.75	BC	2.17	C	2.25	CD
RBS-158B10	*A. stenosperma/ A. batizocoi*	0.42	C	0.42	B	0.75	C	4.50	B	4.33	BC

[a] Least square means of conidial adhesion (count/cm^2) for PA at 12, 48, and 96 HAI.
[b] Least square means of conidial adhesion (count/cm^2) for NP at 48, and 96 HAI.
[c] Genotypes are grouped based on Tukey's HSD test, with letters indicating significant differences. Groups with the same letter are not significantly different.

Table 4.2. Least square means of conidial germ tube length for *Passalora arachidicola* (PA) and *Nothopassalora personata* (NP) in the duplicated experiment, with groupings based on Tukey's HSD.

Genotype	Wild *Arachis* resistance source	PA germ tube length[ac]				NP germ tube length[bc]			
		48HAI		96HAI		48HAI		96HAI	
Georgia-06G	None	106.45	A	198.92	A	70.64	A	95.50	A
IAC 322	*cardenasii*	61.31	B	88.42	B	24.67	B	36.86	B
CB7	*A. cardenasii*	21.39	D	53.55	C	4.92	C	12.05	C
TBI-S11	*A. cardenasii*	19.16	D	47.44	C	3.11	C	10.54	C
RBS-158B10	*A. stenosperma/ A. A. batizocoi*	37.58	C	54.85	C	35.05	B	40.99	B

[a] Least square means of conidial germ tube length (µm) for PA at 48, and 96 HAI.
[b] Least square means of conidial germ tube length (µm) for NP at 48, and 96 HAI.
[c] Genotypes are grouped based on Tukey's HSD test, with letters indicating significant differences. Groups with the same letter are not significantly different.

Table 4.3. Least square means of conidial adhesion and conidial germination length for *Passalora arachidicola* (PA) in the duplicated experiment, with significant trial effect.

Genotype	Wild *Arachis* resistance source	PA adhesion[ac]				PA germ tube length[bc]			
		Trial 1		Trial 2		Trial 1		Trial 2	
Georgia-06G	None	11.17	A	10.83	A	111.70	A	101.19	A
IAC 322	*A. cardenasii*	5.33	B	0.50	B	53.02	B	69.61	B
CB7	*A. cardenasii*	1.17	C	0.50	B	21.39	C	21.39	C
TBI-S11	*A. cardenasii*	2.33	BC	0.67	B	16.83	C	21.49	C
RBS-158B10	*A. stenosperma/ A. batizocoi*	0.83	C	0.00	B	21.49	C	53.67	B

[a] Least square means of conidial adhesion (count/cm^2) for PA at 12 HAI in Trial 1 and 2.
[b] Least square means of conidial germ tube length (μm) for PA at 48 HAI in Trial 1 and 2.
[c] Genotypes are grouped based on Tukey's HSD test, with letters indicating significant differences. Groups with the same letter are not significantly different.

Table 5. Pearson's correlation analysis for the linear relationships between *Passalora arachidicola* (PA) and *Nothopassalora personata* (NP) in the replicated genotypes experiment.

Genotype	Wild *Arachis* resistance source	Correlation coefficient (R)[a] (p-value [b])					
		PAad - PAgt	PAad – NPad	PAad – NPgt	PAgt – NPad	PAgt – NPgt	Npad – NPgt
Georgia-06G	None	-0.09 0.78	-0.31 0.33	-0.28 0.37	-0.35 0.26	0.02 0.93	-0.39 0.21
IAC 322	*A. cardenasii*	-0.52 0.08	-0.20 0.53	0.58 <0.05	0.41 0.18	-0.01 0.94	-0.41 0.19
CB7	*A. cardenasii*	-0.18 0.58	0.07 0.82	0.32 0.31	-0.23 0.47	-0.14 0.45	-0.33 0.29
TBI-S11	*A. cardenasii*	0.47 0.12	0.56 0.06	0.53 0.08	0.35 0.27	0.21 0.26	0.37 0.23
RBS-158B10	*A. stenosperma/ A. batizocoi*	-0.27 0.40	0.33 0.30	0.11 0.72	0.26 0.42	-0.33 0.08	-0.22 0.49
Pooled		0.31 <0.05	0.70 <0.001	0.28 <0.05	0.24 0.06	0.62 <0.001	0.23 0.07

[a] Measures the strength and direction of the relationship between conidial adhesion (ad) (count/cm²) and conidial germ tube length (gt) (µm) at 96 HAI for PA and NP. Negative values indicate an inverse relationship, while positive values indicate a direct relationship.
[b] Statistical significance of the correlation ($p < 0.05$ is significant).

CHAPTER 4

EFFECT OF PEANUT GENOTYPE ON DISEASE PROGRESS OF EARLY AND LATE LEAF SPOT: FIELD RESISTANCE COMPONENTS INCLUDING LESION INCIDENCE, DISEASE SEVERITY, LEVELS OF DEFOLIATION AND SPORULATION[1]

[1]Silva, F. F. M. A., Culbreath, A. K., Leal-Bertioli, S. C. M., Cantonwine, E. G., and Kemerait, R. C. 2024. To be submitted to *Plant Disease*.

INTRODUCTION

Early and late leaf spot (ELS and LLS), caused by *Passalora arachidicola* (Hori) U. Braun and *Nothopassalora personata* (Berk. & M.A. Curtis) U. Braun, C. Nakash., Videira & Crous, are significant foliar diseases impacting peanut crops globally (Mycobank, 2024; Shokes & Culbreath, 1997). Under conducive conditions, these diseases can progress rapidly in the absence of effective management practices, leading to severe defoliation and yield losses of up to 70% due to the reduced photosynthetic area, impaired plant health (Backman & Crawford, 1984; McDonald et al., 1985), reduced peg integrity, and pod shed. Although fungicides are commonly employed in the U.S., their associated costs underscore the need for breeding programs focused on resistance (Abdou et al., 1974). Resistance has been identified in wild *Arachis* species and successfully incorporated into breeding lines through crosses with allotetraploids, which demonstrate promising resistance in both field and laboratory trials (Godoy et al, 2022; Stalker, 1983, 2017; Subrahmanyam et al., 1985; Wynne et al., 1991).

Evaluation of effects of peanut genotypes on leaf spot diseases during the 1970s and 1980s utilized various methodologies to quantify resistance mechanisms and assess disease progression. Foster (1980) found that resistance to *Cercospora arachidicola* effectively reduced infection rates, with wild species like *Arachis batizocoi* exhibiting limited sporulation due to small lesion sizes and early leaf abscission. Gobina et al. (1983) emphasized sporulation as a critical criterion for screening peanut genotypes, introducing a detached leaf culture technique that correlated well with field assessments and highlighting the importance of necrotic lesion area and conidia density. Similarly, Melouk and Banks (1978) developed a detached leaf screening method for evaluating

lesion counts and defoliation rates 3 to 6 weeks post-inoculation, although he acknowledged that greenhouse results might not fully reflect field performance. Hassan and Beute (1977) identified defoliation ratios as reliable indicators of disease impact, while Johnson et al. (1986) found significant correlations between resistance components and disease progression, particularly regarding sporulation per lesion. Melouk and Banks (1984) further confirmed the importance of sporulation in resistance evaluations by developing a leaf spot reaction index to assess disease severity. Chiteka et al. (1988) investigated resistance components for late leaf spot, creating a field appearance score designed to rapidly assess overall leaf spot resistance (Chiteka et al., 1988a; 1988b). This score correlated with longer latent periods, reduced sporulation, and smaller lesion diameters, revealing significant variability among genotypes and underscoring the value of greenhouse evaluations for ranking genotypes before field testing.

In Georgia, the Florida scale (1-10) has become the predominant method for rating leaf spot (LS) disease since its introduction in 1988, primarily due to its efficiency and expediency (Chiteka et al., 1988; 1988b). This scale evaluates multiple LS resistance components simultaneously, including lesion and defoliation severity per plot. For values of 4 and greater, the scale is primarily based on levels of defoliation. For values less than four, the scale is more subjective, rating based on subjective categories of the number of lesions and location in the canopy (Chiteka et al., 1988a; 1988b). Despite earlier research highlighting the importance of additional resistance components, evaluations have largely relied on the Florida scale (Cantonwine et al., 2006; 2007a, 2007b; 2008; Chu et al., 2021; Denwar et al., 2021; Holbrook et al., 2021; Jordan et al., 2019), limiting the exploration of factors such as sporulation, lesion incidence, and stem lesion impact in

newly developed genotypes. While field assessments of other components have been lacking, evaluations of lesions and sporulating lesions are conducted using the detached leaf method (Bertioli et al., 2021b; Favero et al., 2009; Gonzales et al., 2023; Lamon et al., 2020). However, there have been few studies examining how components measured in detached leaf assays correlate with related studies in the field.

Despite previous studies emphasizing the importance of additional components, there is a significant gap in understanding the full scope of resistance mechanisms and their impact on disease progression in the field. Additionally, traits such as stem lesion severity have received minimal attention, with limited reporting from Culbreath (1991) and Navia Gine (2012). Understanding stem lesion severity might help characterize better the relationship between leaf spot severity and yield through increased plant stress and loss of peg integrity.

Therefore, this study aims to bridge these knowledge gaps by evaluating additional disease components across different locations. Specifically, this study seeks to determine whether previously identified resistant genotypes also suppress lesser-studied factors, such as lesion incidence, sporulation, and stem lesions, beyond conventional field ratings such as the Florida 1-10 scale. This expanded assessment may provide a more comprehensive understanding of resistance consistency and genotype performance across multiple disease parameters.

MATERIALS AND METHODS

Field evaluation of different peanut genotypes to early and late leaf spot

Peanut Genotypes. The peanut genotypes used in the field trials were provided by Dr. Albert Culbreath of the UGA Tifton Campus, Dr. Soraya Bertioli and Dr. David Bertioli of the UGA Athens Campus, and Dr. Corley Holbrook of USDA-ARS, Tifton. These genotypes included cultivars without resistance segments, along with cultivars and breeding lines with wild introgressions, as summarized in Appendix Table 2.1.

Field locations. Field trials were conducted in at the Rigdon and Black Shank farms in 2022, and at the Lang and Black Shank farms in 2023 at the University of Georgia Coastal Plain Experiment Station, Tifton, Georgia. Soil type in all farms was a Tifton loamy sand. The fields were planted to cotton (*Gossypium hirsutum*) the previous year. The trials were previously reported to have high leaf spot incidence in previous years when peanut was planted. Adjacent field sites were primarily planted to peanut and cotton.

Experimental design. All field trials in all locations used a randomized complete block design (RCBD) with four replications. The different genotypes were randomized across four 5-ft replications, each planted with 12 seeds. In 2023, TUFRunner'511' was planted as susceptible border rows but not in 2022.

Data Collection. All plants in each plot were monitored weekly from planting until the appearance of the first leaf spot symptoms. Lesion incidence (LI) was assessed in both years by visually estimating the percentage of leaflets throughout the canopy with at least one lesion at 90, 98, 106, and 114 days after planting (DAP). In 2022, fewer ratings were conducted due to the researcher's unavailability during part if the growing

season. In 2023, even though the researcher was available for a longer period, the same number of ratings was performed to ensure consistency for comparative analysis between two years.

In 2023, early leaf spot (ELS) and late leaf spot (LLS) were monitored separately based on sporulation for differentiation. The evaluation also considered instances where symptoms and severity of each were difficult to separate. The evaluation of different genotypes for leaf spot disease was based on severity assessed using the Florida 1-10 scale (FS); levels of defoliation (LD), rated on a 0-5 scale; and stem lesion severity (STL), evaluated by estimating the percentage of lesion severity on 10 arbitrarily selected stems per plot at 148 DAP, the final rating day. Severity ratings using Florida 1-10 scale and levels of defoliation scale were recorded at 113, 120, 127, 133, 141, and 148 DAP. Additionally, lesion incidence was estimated on each of those dates, following the same method as in the 2022 trials.

The evaluation of ELS and LLS based on sporulation utilized the following measures: days from planting until the first noticeable sporulating lesions (DSL); incidence of sporulating lesions (SLI), rated on a 0-5 scale for the percentage of leaflets with at least one sporulating lesion observed throughout the canopy; and sporulation degree (SD), rated on a 0-3 scale based on the extent of sporulation on 10 arbitrarily selected leaves. For ELS, sporulating lesions were observed on the adaxial (upper) leaf surface, while for LLS, they were observed on the abaxial (lower) surface. ELS sporulating lesion incidence was recorded at 61, 64, 69, 78, 83, 97, 104, 111, 113, 120, 127, 133, 141, and 148 DAP. ELS and LLS sporulation degree, along with LLS sporulating lesion incidence, was recorded at 104, 111, 113, 120, 127, 133, 141,148 DAP.

For plots that did not exhibit symptoms by the final rating (150 DAP), the maximum value of 150 days was assigned for the analysis. Destructive sampling was not conducted, and leaf sampling was limited to confirming ELS and LLS conidia during initial field monitoring only. Details of the disease rating scales are provided in Appendix Table 3, while the estimated scale for each component, illustrated with actual field images, is shown in Appendix Figure 6.

The area under the disease progress curve (AUDPC) was computed using data collected for each disease variable measured multiple time times (DAP) throughout the growing season. The AUDPC was calculated using the following formula:

$$\text{AUDPC} = \sum_{i=0}^{n} \frac{(Y_{i+1} + Y_i)}{2} (X_{i+1} - X_i)$$

where Y_i represents the disease measure for each plot at the *i*-th observation, X_i denotes the time (in days) at the *i*-th observation, and *n* is the total number of observations (Shaner and Finney 1977).

Data analysis. The calculated AUDPC values, along with the mean values recorded at single time points, were plotted to visualize the data distribution for each genotype. Residual plots, including histogram and Q-Q plots for each dataset, were used to assess the assumptions of variance and normality, and to determine the suitability of the data for linear regression analysis (See Appendix Figure 7 for plots).

Statistical analysis. Data analysis was performed using RStudio statistical software (R version 4.2.2, 2022) (RStudio Team 2020). The employed RStudio packages were “car”, “carData”, “ggplot2”, “ggpubr” “agricolae”, and “olsrr”. A linear mixed model (LMM) was applied to all field trials using randomized complete block design (RCBD) for the analysis.

The LMM for genotype and year analysis was specified as follows:

$$Y_{ij} = \beta_0 + \beta_1 \text{Genotype}_i + \beta_2 \text{Year}_j + \beta_3 \left(\text{Genotype}_i \text{ x Year}_j\right) + \epsilon_{ij}$$

where Y_{ij} represents the response variable for genotype *i* and year *j*, β_0 is the overall mean, and β_1 and β_2 are the fixed effects for genotype and year, respectively, β_3 represents the interaction effect between genotype and year, and ϵ_{ij} is the error term associated with the response. Statistical significance in all tests was decided at a *P*-value of 0.05.

The LMM for genotype and location analysis was specified as follows:

$$Y_{ij} = \beta_0 + \beta_1 \text{Genotype}_i + \beta_2 \text{Location}_j + \beta_3 \left(\text{Genotype}_i \text{ x Location}_j\right) + \epsilon_{ij}$$

where Y_{ij} represents the response variable for genotype *i* and location *j*, β_0 is the overall mean, and β_1 and β_2 are the fixed effects for genotype and location, respectively, β_3 represents the interaction effect between genotype and location, and ϵ_{ij} is the error term associated with the response. Statistical significance in all tests was decided at a p-value of 0.05.

ANOVA: Analysis of Variance (ANOVA) was to evaluate statistical differences among the fixed effects. The model effects varied depending on the variables being considered. For lesion incidence data from 2022 and 2023. Genotype and year were treated as fixed effects, and replication was considered a random effect. If interaction effects between genotype and year were not significant, these interactions were not further analyzed. However, if significant interactions were detected, analysis was conducted separately for each year, and interactions between genotype and location were also tested. In the 2023 field trials, data were analyzed with genotype and location as fixed effects and replication as a random effect. If significant interactions between genotype and location were detected, separate analyses were performed for each location.

Differences among the least square means for each genotype were assessed using Fisher's Least Significant Difference (LSD) test. The post-hoc analysis was conducted following ANOVA to determine which specific genotype means differed significantly from one another.

Correlation Analysis: Pearson's correlation coefficients s(r) was calculated to examine the relationships among different variables of each genotype, separately for the two locations in 2023.

Forward stepwise regression: The relative contribution of the variables to the Florida 1-10 scale was evaluated across locations and separate locations using forward stepwise regression. This method allows for the selection of the most significant predictors by iteratively adding variables based on their statistical significance and contribution to the overall model fit. This approach can provide a model that effectively explains the variability in disease severity scores. The selection of predictors was guided by criteria of Akaike Information Criterion (AIC) and Schwarz Bayesian Criterion (SBC).

Final model selection can be represented as:

$$Y = \beta_o + \beta_{i1}X_{i1} + \beta_{i2}X_{i2} + \cdots + \beta_{i9}X_{i9} + \epsilon$$

where Y represents the dependent variable, Florida 1-10 scale, β_0 is the intercept of the model, β_{i1}, β_{i2}, …, β_{i9} are the coefficients for each predictor, X_{i1},X_{i2}, …,X_{i9} are the independent variables or predictors, and ϵ is the error term or variability in Y not explained by the predictors.

Supplementary data. In 2022 and 2023, the genotypes varied due to seed availability for certain breeding lines. The genotypes varying during these years are listed

in Appendix Table 2.2. The lesion incidence (LI) in the 2022 and 2023 field trials was assessed by visually estimating the percentage of leaflets throughout the canopy with at least one lesion at 90, 98, 106, and 114 DAP. Data were analyzed using a linear mixed model, and Analysis of Variance (ANOVA) was conducted to evaluate the significance of the fixed effects of genotypes and their interaction with year and location when applicable. Replication was treated as random factor. Following ANOVA, Fisher's Least Significant Difference (LSD) test was used to compare the least square means. All statistical analyses were performed using RStudio statistical software (R version 4.2.2, 2022) (RStudio Team 2020).

RESULTS

Leaf spot lesion incidence in 2022 and 2023

Genotype had a significant effect on the leaf spot incidence (LI) AUDPC ($P < 0.001$), with no significant genotype by year interaction effect (G x Y) ($P > 0.05$) (Table 6.2). The suppression of lesion incidence in resistant genotypes was consistent across both years, as shown in the disease progression graphs (Figure 1), where resistance genotypes exhibited significantly lower disease levels than susceptible genotypes. Susceptible cultivars Georgia-13M, Georgia-06G, and TUFRunner'511' had significantly higher AUDPC values than genotypes with resistance segments (Table 7.1). Among the genotypes with resistance segments, IAC 322 derived from *A. cardenasii*, had the lowest AUDPC value (333.41), while RBS-95_C_9 derived from *A. stenosperma* and *A. batizocoi*, had the highest AUDPC (605.78). Other resistant genotypes derived from *A. cardenasii*, CB-1, CB-2, CB-7 and TBI-S11, had AUDPC values ranging from 397.75 to

522.56 while, RBS-158_B_10 and RBS-170_A, genotypes derived from *A. stenosperma* and *A. batizocoi* had 494.81 and 578.56.

Leaf spot field resistance components

Genotype had a significant impact on disease severity based on Florida scale (FS) AUDPC ($P < 0.001$), lesion incidence (LI) AUDPC ($P < 0.001$), levels of defoliation (LD) AUDPC ($P < 0.001$), and stem lesion severity (STL) percentage ($P < 0.01$) (Table 6.2). Significant genotype by location (G x L) interaction was detected for FS ($P < 0.01$) and LD ($P < 0.001$) (Table 6.2). Genotype significantly impacted FS at Black Shank Farm ($P<0.05$) and Lang Farm ($P < 0.001$), as well as LD at both locations ($P < 0.001$).

Cultivars, TUFRunner'511', Georgia-06G, and Georgia-13M, had the highest AUDPC values, with FS ranging from 192.41 to 204.41, LI from 1030.00 to 1172.50, and LD from 111.31 to 114.88 (Table 7.1). In contrast, genotypes with resistance segments had significantly lower values with FS ranging from 62.49 to 170.00, LI from 432.50 to 730.00, and LD from 35.38 to 78.81. The difference in STL was minimal, with susceptible cultivars ranging from 31.88 % to 51.88 % and resistant genotypes from 17.50 % to 46.88 %. Separate location analyses for FS and LD also showed that the susceptible cultivars had higher values (174.44 to 202.00 at Black Shank Farm and 193.44 to 210.38 at Lang Farm) than resistant genotypes except for RBS-95_C_9 at Lang Farm with FS of 198.69.

Genotypes derived from *A. cardenasii* (TifGP-3, IAC 322, CB-1, CB-2, CB-7, TBI-S11) showed lower values compared to susceptible cultivars. IAC 322 had the lowest FS, LI, and LD (Table 7.1). TifGP-3 also showed low FS and LI, but LD was slightly higher. CB-1 had a significantly low LI (467.50) but did not perform well in

other resistance components (Table 7.1). CB-2, CB-7 and TBI-S11 had moderate FS (93.59 to 133.78), LI (555.00 to 635.00), and LD (42.06 to 69.13) (Table 7.1). Separate location analyses also showed that IAC 322 had the lowest FS at both Black Shank Farm (and Lang Farm, and the lowest LD at Lang Farm (Table 7.4). TifGP-3 and CB-2 also showed lower FS at Lang Farm, while TifGP-3, TBI-S11 and CB-2 had lower LD compared to other *A. cardenasii* genotypes (Table 7.4).

Genotypes derived from *A. stenosperma* and *A. batizocoi* (RBS-95_C_9, RBS-158_B_10, and RBS-170_A) showed lower values for all variables than susceptible cultivars but higher than most *A. cardenasii* genotypes (Table 7.1). RBS-95_C_9 showed the highest values among the *A. stenosperma* and *A. batizocoi* genotypes (Table 10.1). Separate location analyses showed that RBS-158_B_10 and RBS-170_A had lower LD at Black Shank compared to other resistant genotypes, except TBI-S11 (Table 7.4).

Early leaf spot field resistance components

Genotype significantly affected ELS resistance components, number of days until the first noticeable sporulating lesions (ELS DSL) (P<0.01), sporulating lesion incidence (ELS SLI) AUDPC ($P < 0.01$) and sporulation degree (ELS SD) AUDPC ($P < 0.001$), with significant genotype by location (G X L) interactions for ELS SD ($P < 0.001$) (Table 9.2). Genotype significantly impacted ELS SD at Black Shank Farm ($P < 0.001$) and Lang Farm ($P < 0.001$) (Table 6.2).

Cultivars, TUFRunner’511’, Georgia-06G, and Georgia-13M, had higher ELS resistance component values, with ELS SLI ranging from 100.38 to 132.69 and ELS SD from 61.25 to 71.63. In contrast, most resistant genotypes had lower values, with ELS SLI ranging from 46.94 to 100.56 and ELS SD from 23.31 to 59.00 (Table 7.2). ELS

DSL of susceptible cultivars ranges from 77.25 to 92.38, which was shorter compared than some resistant genotypes (95.50 to 115.88), but longer to others, such as TBI-S11 and RBS-95_C_9 (Table 7.2). Separate location analyses revealed that susceptible cultivars had higher ELS SD (84.50 to 91.50 at Black Shank Farm and 38.00 to 51.88 at Lang Farm) compared to most resistant genotypes, although Georgia-13M at Lang Farm had an ELS SD equal to most resistant genotypes (Table 7.5).

Genotypes derived from *A. cardenasii* (TifGP-3, IAC 322, CB-1, CB-2, CB-7, TBI-S11) showed variable resistance to ELS. IAC 322 had the lowest ELS SLI and ELS SD, while TBI-S11 had low ELS SD compared to other genotypes (Table 7.2). However, other *A. cardenasii* genotypes showed resistance components values comparable to susceptible cultivars (Table 7.2). Separate location analyses showed that IAC 322 had the lowest ELS SD at both locations ((Table 7.5). TBI-S11 had lower ELS SD at Black Shank Farm but not at Lang Farm, while TifGP-3 had lower at Lang Farm but not at Black Shank Farm (Table 7.5).

Genotypes derived from *A. stenosperma* and *A. batizocoi* (RBS-95_C_9, RBS-158_B_10, and RBS-170_A) also showed variable resistance to ELS. These genotypes had shorter ELS DSL (69.38 days to 86.38 days) than most *A. cardenasii* genotypes (75.13 days to 115.88 days), but closer to the susceptible cultivars (77.25 days to 92.38 days) (Table 10.2). RBS-95_C_9 had significantly shorter ELS DLS compared to all resistant genotypes, though all *A. stenosperma* and *batizocoi* genotypes had higher ELS resistance component values compared to most *A. cardenasii* genotypes (Table 7.2). Separate location analyses showed that *A. stenosperma* and *A. batizocoi* genotypes had higher ELS SD (65.50 to 76.00 at Black Shank and 38.00 to 39.88 at Lang Farm)

compared to most *A. cardenasii* genotypes. Exceptions included TifGP-3 and CB-7 at Black Shank, as well as CB-1 and TBI-S11 at Lang Farm, which showed higher ELS SD (Table 7.5).

Late leaf Spot field resistance components

Genotype had a significant impact on LLS resistance components, including number of days until the first noticeable sporulating lesions (LLS DSL) ($P < 0.001$), sporulating lesion incidence (LLS SLI) AUDPC ($P < 0.001$) and sporulation degree (LLS SD) AUDPC ($P < 0.001$), with significant genotype by location (G X L) interactions observed for LLS DSL ($P <0.05$) (Table 6.2). Genotype significantly impacted LLS DSL at Black Shank Farm ($P < 0.001$) but no significant effect was observed at Lang Farm ($P >0.05$) (Table 6.2).

Cultivars, TUFRunner'511', Georgia-06G, and Georgia-13M, had higher values, with LLS SLI ranging from 96.06 to 100.69 and LLS SD from 77.50 to 89.25 (Table 7.3). In contrast, most resistant genotypes had lower values, with LLS SLI ranging from 30.25 to 63.44 and LLS SD ranging from 21.56 to 40.13 (Table 7.3). The LLS DSL of susceptible cultivars ranged from104.88 days to 108.00 days, which was shorter than the resistant genotypes, whose LLS DSL ranged from 122.38 days to 132.75 days (Table 7.3). Separate location analyses showed that at Black Shank Farm, susceptible cultivars had shorter LLS DSL (105.13 days to 108.00 days) compared to the resistant genotypes (122.38 days to 132.75 days), while at Lang Farm, there was little difference, with susceptible cultivars ranging from 104.00 days to 107.50 days and resistant genotypes from 104.00 days to 119.00 days (Table 7.5).

Genotypes derived from *A. cardenasii* (TifGP-3, IAC 322, CB-1, CB-2, CB-7, TBI-S11) showed varying levels of resistance to LLS, though the differences were not statistically significant. In comparison to ELS, LLS resistance components in resistant genotypes showed less variation. Among the *A. cardenasii* genotypes, CB-1 had the lowest LLS SLI, and both CB-1 and IAC 322 had lower LLS SD (Table 7.3). Other *A. cardenasii* genotypes had LLS SLI ranging from 48.38 to 57.69 and LLS SD ranging from 25.50 to 31.81 (Table 7.3).

Genotypes derived from *A. stenosperma* and *batizocoi* (RBS-95_C_9, RBS-158_B_10, and RBS-170_A) also showed variable resistance to LLS, though the differences were not statistically significant. These genotypes had LLS SLI ranging from 44.56 to 63.44 and LLS SD from 34.69 to 40.13 (Table 7.3). Among the resistant genotypes, RBS-170_A had the highest LLS SLI (63.44) and LLS SD (40.13) values (Table 7.3).

Relationship of leaf spot field resistance components at Black Shank Farm

The relationship among various field resistance components across different genotypes traits was analyzed for each location. At Black Shank Farm, the susceptible cultivars (TUFRunner'511', Georgia-06G, Georgia-13M) had several significant correlations ($P<0.05$). There were positive correlations between disease severity based on Florida scale (FS) and levels of defoliation (LD), lesion incidence (LI) and levels of defoliation (LD), LI and ELS sporulating lesion incidence (ELS SLI), LD and ELS SLI, and LI and ELS sporulation degree (ELS SD). On the contrary, strong negative correlations were found between LI and ELS number of days until the first noticeable sporulating lesions (ELS DSL), as well as between ELS DSL and ELS SLI. Additionally,

there was a significant (P = 0.05), moderate positive correlation between LI and stem lesion (STL), as well as between STL and LLS sporulating lesion incidence (LLS SLI) (Table 8.1).

The *A. cardenasii* derived genotypes (TifGP-3, IAC 322, CB-1, CB-2, CB-7, TBI-S11) had more significant correlations (P < 0.05) than the susceptible cultivars. There were strong positive correlations between FS and LI, FS and LD, LI and LD, FS and ELS SLI, LI and ELS SLI, LD and ELS SLI, LI and ELS SD, LD and ELS SD, and ELS SLI and ELS SD, FS and LSD, LLS SLI and LLS SD. A moderate positive correlation between LS and STL, FS and ELS SD, STL and ELS SD, FS and LLS SLI, LI and LLS SLI, LD and LLS SLI, ELS SLI and LLS SLI, LI and LLS SD, LD and LLS SD, ELS SLI and LLS SD. A strong negative correlation was found between LI and ELS DSL, ELS DSL and ELS SLI, LLS DSL and LLS SLI, LLS DSL and LLS SD. A moderate negative correlation was found between LD and ELS DSL, ELS DSL and ELS SD, FS and LLS DSL, LI and LLS DSL, LD and LLS DSL, ELS SLI and LLS DSL (, ELS DSL and LLS SD (Table 8.1).

The *A. stenosperma* and *A. batizocoi* genotypes (RBS-95_C_9, RBS-158_B_10, RBS – 170_A) had fewer significant correlations (P<0.05). A moderate positive correlation between FS and SLI, ELS SLI and LLS SLI. A strong negative correlation between ELS DSL and ELS SLI, ELS SLI and LLS DSL, LLS DSL and LLS SLI (Table 8.1).

Relationship of leaf spot field resistance components at Lang Farm

The relationship among various field resistance components at Lang Farm were analyzed similarly as for the Black Shank Farm trial. The susceptible cultivars

(TUFRunner'511', Georgia-06G, Georgia-13M) had several significant correlations ($P <$ 0.05). Strong positive correlations were found between STL and ELS SD and LI and LLS SLI. Moderate positive correlation between LD and STL, and LD and ELS SD. Strong negative correlation between ELS DSL and ELS SLI. Moderate negative correlation between LI and LLS DSL, and LLS DLS and LLS SLI (Table 8.2).

The *A. cardenasii* resistant genotypes (TifGP-3, IAC 322, CB-1, CB-2, Cb-7, TBI-S11) had several significant correlations ($P<0.05$). Strong positive correlations between FS and LD, and ELS SLI and ELS SD. Moderate positive correlation between LI and LLS SLI, LLS SLI and LLS SD. A strong negative correlation between ELS DSL and ELS SLI, ELS DSL and ELS SD, LLS DSL and LLS SLI, LLS DSL and LLS SD. A moderate negative correlation between FS and ELS SD (Table 8.2).

The *A. stenosperma* and *A. batizocoi* genotypes (RBS-95_C_9, RBS-158_B_10, RBS – 170_A) had several significant correlations ($P<0.05$). There were strong positive correlations between FS and LD, ELS SLI and ELS SD, LI and LLS SLI, LLS SLI and LLS SD. A moderate positive correlation between LI and LLS SD. A strong negative correlation was found between LLS DSL and LLS SLI, LLS DSL and LLS SD (Table 8.2).

Florida 1-10 scale associated field resistance components

Analysis of Black Shank Farm showed a moderate model fit (R2 = 0.66, adj R^2=0.64) (Table 12). The most significant predictors of the Florida scale were ELS sporulating lesion incidence (ELS SLI) ($P < 0.001$) and ELS days to sporulating lesion (ELS DSL) ($P <0.05$) (Table 9). The positive coefficients for these predictors indicate a direct relationship with Florida scale. Additionally, levels of defoliation (LD) were

included although not statistically significant (*P*=0.29), it contributed to the overall model fit and may provide valuable insights (Table 12).

Analysis of Lang Farm showed a high model fit (R=0.85, adj R^2=0.83) (Table 9). The most significant predictors included resistance component, LD (P < 0.001), ELS DSL (*P* < 0.05), LLS SLI (*P* <0.05), and LLS SD (*P* = 0.06) (Table 9). Similar to LD at Black Shank Farm, LLS SD was included in the model and contributed to the overall fit, potentially providing additional insights. ELS DSL and LLS SLI had negative coefficients, indicating an inverse relationship with the Florida scale, while LD and LLS SD had direct relationship.

The combined analysis across both locations had a high model fit (R=0.71, R^2=0.71) (Table 9). The most significant predictors were LD (P < 0.001) and ELS SLI (P < 0.001), both had direct relationship with the Florida scale (Table 9).

DISCUSSION

This study utilized various field resistance components at two locations to assess the progression of peanut leaf spot diseases across different genotypes. The genotypes included cultivars lacking resistance as well as those exhibiting resistance traits derived from wild *Arachis* species, specifically *Arachis cardenasii*, *Arachis stenosperma*, and *Arachis batizocoi*. A critical aspect of the analysis involved comparing results based on assessments of leaf spot diseases that either did not distinguish between early leaf spot (ELS) and late leaf spot (LLS) or categorized them based on sporulation. This distinction is crucial, as peanut leaf spot diseases are caused by two closely related fungal pathogens:

Passalora arachidicola (responsible for ELS) and *Nothopassalora personata* (responsible for LLS) (Shokes & Culbreath, 1997).

When evaluating peanut leaf spot without separating ELS and LLS, field resistance components included lesion incidence, disease severity measured by the Florida scale, levels of defoliation, and stem lesion severity. Cultivars without resistance traits, such as Georgia-13M, Georgia-06G, and TUFRunner'511', exhibited the highest values across most resistance components, indicating heightened susceptibility to leaf spot diseases. In contrast, genotypes with resistance segments, particularly those derived from *Arachis cardenasii*, including IAC 322, TifGP-3, TBI-S11, and CB-2, showed significantly lower values across all resistance components. Among these, IAC 322 recorded the lowest disease severity on the Florida 1-10 scale and the lowest lesion incidence. The findings align with previous research, which demonstrated that leaf spot resistance effectively reduced various field components, such as defoliation ratios and sporulation (Foster, 1980; Hassan and Beute, 1977; Johnson et al., 1986). Moreover, the limited sporulation observed in wild species like *Arachis batizocoi*, characterized by small lesion sizes and early leaf abscission, further underscores the importance of sporulation in resistance assessments (Gobina et al., 1983; Melouk & Banks, 1978).

In the context of ELS resistance components, susceptible cultivars exhibited higher values for ELS sporulating lesion incidence and ELS sporulation degree, highlighting the significance of *P. arachidicola* sporulation in ELS progression. Conversely, resistant genotypes, particularly those from *Arachis cardenasii*, demonstrated delayed sporulation and fewer sporulating lesions. This trend corroborates earlier studies that emphasized sporulation as a critical criterion for screening peanut

genotypes (Chiteka et al., 1988a; Johnson et al., 1986). For late leaf spot resistance components, susceptible cultivars again demonstrated higher values for LLS sporulating lesion incidence and sporulation degree, reinforcing previous findings that pathogen sporulation significantly influences disease progression. Resistant genotypes derived from *Arachis cardenasii*, *Arachis stenosperma,* and *Arachis batizocoi* consistently exhibited lower values for LLS resistance components, although the variability was less pronounced compared to ELS resistance components. This suggests that resistance mechanisms may not be uniformly effective across different genotypes, indicating the need for further exploration of genotype-specific responses under varying conditions.

The study revealed significant genotype-by-location interactions for some field resistance components, underscoring the critical influence of environmental factors on disease progression. For example, lesion incidence showed strong positive correlations with early leaf spot (ELS) sporulating lesions at Black Shank Farm, where ELS was dominant, while at Lang Farm, a similar correlation between lesion incidence and late leaf spot (LLS) sporulating lesions was observed, highlighting the need to evaluate disease components independently for each pathogen. The relationships among field resistance components provide valuable insights into the traits associated with resistance to *Passalora arachidicola* and *Nothopassalora personata*. Significant correlations were observed across susceptible and resistant genotypes at Black Shank Farm and Lang Farm. In susceptible cultivars, a strong negative correlation was found between early leaf spot (ELS) days to first sporulating lesions and sporulating lesion incidence, indicating that shorter time to sporulation is associated with higher numbers of sporulating lesions. In contrast, genotypes derived from wild *Arachis* species exhibited strong negative

correlations for both pathogens between days to first sporulating lesions and sporulating lesion incidence, with longer times to sporulation linked to fewer sporulating lesions.

This study builds on the foundation established by earlier research, recognizing the attributes of the Florida scale's role as a primary method for assessing leaf spot disease severity (Chiteka et al., 1988a, 1988b). While the Florida scale allows rapid evaluation of large numbers of field plots, its reliance on defoliation levels limits the exploration of critical factors such as sporulation and lesion incidence in newly developed genotypes. This study emphasizes the importance of additional resistance components and helps fill gaps in our understanding of resistance mechanisms and their impact on disease progression. Evaluation of large numbers of genotypes for detail of the components variables used in this study would likely not be feasible. However, characterization of specific components such as those addressed in this study could be done on a limited number of genotypes, to determine if they differ in which factors are responsible for differences with ratings such as the Florida 1-10 scale. This expanded assessment offers a more comprehensive understanding of resistance and insight into the factors that may affect the level and consistency of genotype performance

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Table 6.1. Analysis of the relationship between genotype and disease values based on different disease parameters in 2022 and 2023 at various locations.

Year	Disease parameter[a]	Statistical metrics[b]			
		R^2	DF	RSE	p-value
2022 and 2023	LI	0.58	165	297.30	<0.001
2023	FS	0.71	69	41.00	<0.001
	LI	0.62	69	273.80	<0.001
	LD	0.81	69	19.05	<0.001
	STL	0.65	69	17.60	<0.001
	ELS DSL	0.41	69	22.76	<0.05
	ELS SLI	0.53	69	37.73	<0.001
	ELS SD	0.80	69	13.09	<0.001
	LLS DSL	0.66	69	14.13	<0.001
	LLS SLI	0.75	69	33.40	<0.001
	LLS SD	0.75	69	21.15	<0.001

[a] Disease parameters and their abbreviations: Florida 1-10 scale (FS), lesion incidence (LI), levels of defoliation (LD), number of days until the first noticeable sporulating lesions (ELS DSL) for early leaf spot, sporulating lesion incidence (ELS SLI), sporulation degree (ELS SD) for early leaf spot; number of days until the first noticeable sporulating lesions (LLS DSL) for late leaf spot, sporulating lesion incidence (LLS SLI), sporulation degree (LLS SD) for late leaf spot.

[b] Metrics: R^2 – coefficient of determination, indicating model fit; DF – degrees of freedom, representing sample size; RSE – residual standard error, measuring model prediction error; p-value – tests statistical significance ($p < 0.05$ is significant).

The relationship between genotype and disease values was analyzed using a linear mixed model.

Table 6.2. Analyzing the effect of genotype on the disease values based on different disease parameters in 2022 and 2023 at different locations.

Year	Disease Parameter[a]	Source of variation[b]	ANOVA[ce] DF	f-value	p-value	LSD[de] DF	t-value	LSD
2022 and 2023	LI	G	11	18.34	<0.001	165	1.97	207.50
		G x Y	11	1.05	0.41	ns	ns	ns
2023	LI	G	11	7.04	<0.001	69	1.99	25.68
		G x L	11	1.34	0.22	ns	ns	ns
	FS	G	11	11.03	<0.001	69	1.99	40.90
		G x L	11	3.07	<0.01	s	s	s
		G in BSF	11	2.50	<0.05	33	2.03	70.95
		G in LF	11	26.59	<0.001	33	2.03	37.03
	LD	G	11	17.38	<0.001	69	1.99	19.00
		G x L	11	6.13	<0.001	s	s	s
		G in BSF	11	4.49	<0.001	33	2.03	29.39
		G in LF	11	27.02	<0.001	33	2.03	22.59
	STL	G	11	3.07	<0.01	69	1.99	17.55
		G x L	11	1.60	0.12	ns	ns	ns
	ELS DSL	G	11	3.06	<0.01	69	1.99	22.70
		G x L	11	0.80	0.64	ns	ns	ns
	ELS SLI	G	11	2.85	<0.01	69	1.99	37.64
		G x L	11	1.14	0.34	ns	ns	ns
	ELS SD	G	11	8.22	<0.001	69	1.99	13.05
		G x L	11	3.69	<0.001	s	s	s
		G in BSF	11	6.38	<0.001	33	2.03	23.32
		G in LF	11	4.04	<0.001	33	2.03	13.65
	LLS DSL	G	11	3.58	<0.001	69	1.99	14.10
		G x L	11	2.43	<0.05	s	s	s
		G in BSF	11	4.39	<0.001	33	2.03	22.56
		G in LF	11	0.73	0.70	ns	ns	ns

Year	Disease Parameter[a]	Source of variation[b]	ANOVA[ce] DF	f-value	p-value	LSD[de] DF	t-value	LSD
	LLS SLI	G	11	3.73	<0.001	69	1.99	33.32
		G x L	11	0.65	0.78	ns	ns	ns
	LLS SD	G	11	11.27	<0.001	69	1.99	21.10
		G x L	11	0.38	0.96	ns	ns	ns

[a] Disease parameters and their abbreviations: Florida 1-10 scale (FS), lesion incidence (LI), levels of defoliation (LD), number of days until the first noticeable sporulating lesions (ELS DSL) for early leaf spot, sporulating lesion incidence (ELS SLI), sporulation degree (ELS SD) for early leaf spot; number of days until the first noticeable sporulating lesions (LLS DSL) for late leaf spot, sporulating lesion incidence (LLS SLI), sporulation degree (LLS SD) for late leaf spot.

[b] Considers genotype (G), Genotype x Year (G x Y) interaction effect, and Genotype x Location (G x L) interaction effect

[c] Assesses whether genotypes and interaction with trial significantly affect variables. Metrics: DF, f-value, p-value.

[d] Identifies significantly different groups. Metrics: DF, q-value, LSD.

[e] Metrics: DF – degrees of freedom, representing sample size; RSE – residual standard error, measuring model prediction error; p-value – tests statistical significance ($p < 0.05$ is significant); f-value – higher values indicate more significant differences between groups; LSD value – least significant difference between group means.

Table 7.1. Least square means of disease values on different disease parameters for leaf spot in genotypes from the 2022 and 2023 trials.

Genotype	Wild *Arachis* resistance source	2022 and 2023[acd]		2023[bcd]							
		LI		FS		LI		LD		STL	
TUFRunner'511'	None	1138.25	A	192.41	AB	1030.00	C	114.63	A	51.88	A
Georgia-06G	None	1169.16	A	204.41	A	1172.50	A	111.31	A	39.38	ABC
Georgia-13M	None	1183.50	A	197.72	A	1137.50	B	114.88	A	31.88	BCD
TifGP-3	*A. cardenasii*	457.66	BCD	76.53	FG	512.50	I	60.94	BCD	20.63	D
IAC 322	*A. cardenasii*	333.41	D	62.49	G	432.50	K	35.38	E	27.50	CD
CB1	*A. cardenasii*	397.75	CD	153.81	BC	467.50	J	68.38	BC	28.13	CD
CB2	*A. cardenasii*	432.38	BCD	93.59	EFG	555.00	H	43.13	DE	33.13	BCD
CB7	*A. cardenasii*	522.56	BCD	133.78	CDE	635.00	F	69.13	BC	20.00	D
TBI-S11	*A. cardenasii*	489.06	BCD	103.66	DEF	590.00	G	42.06	DE	17.50	D
RBS-95C9	*B. stenosperma*/ *A. batizocoi*	605.78	B	165.13	ABC	730.00	D	78.81	B	46.88	AB
RBS-158B10	*A. stenosperma*/ *A. batizocoi*	494.81	BCD	170.00	ABC	625.00	F	66.13	BC	24.38	CD
RBS-170A	*A. stenosperma*/ *A. batizocoi*	578.56	BC	139.38	CD	685.00	E	58.75	CD	22.50	CD

[a] Least square means of disease values based on different disease parameters in 2022 and 2023.
[b] Least square means of disease values based on different disease parameters in 2023
[c] Disease parameters and their abbreviations: Florida 1-10 scale (FS), lesion incidence (LI), levels of defoliation (LD), stem lesion severity (STL)
[d] Genotypes are grouped based on LSD test, with letters indicating significant differences. Groups with the same letter are not significantly different.

Table 7.2. Least square means of disease values based on different disease parameters for early leaf spot in genotypes from the 2023 trials.

Genotype	Wild *Arachis* resistance source	ELS DSL[ad]		ELS SLI[bd]		ELS SD[cd]	
TUFRunner'511'	None	92.38	BCD	100.38	ABC	71.63	A
Georgia-06G	None	77.25	CDE	132.69	A	68.13	AB
Georgia-13M	None	89.63	BCDE	117.38	AB	61.25	ABC
TifGP-3	*A. cardenasii*	102.00	AB	74.56	CD	48.56	CDE
IAC 322	*A. cardenasii*	115.88	A	46.94	D	23.31	F
CB1	*A. cardenasii*	107.25	AB	72.75	CD	43.75	DE
CB2	*A. cardenasii*	95.50	ABCD	74.75	CD	51.50	CD
CB7	*A. cardenasii*	98.00	ABC	84.00	BCD	59.00	ABC
TBI-S11	*A. cardenasii*	75.13	DE	81.06	BCD	37.88	E
RBS-95C9	*A. stenosperma/ A. batizocoi*	69.38	E	100.56	ABC	51.75	CD
RBS-158B10	*A. stenosperma/ A. batizocoi*	77.88	CDE	96.38	ABC	56.75	BCD
RBS-170A	*A. stenosperma/ A. batizocoi*	86.38	BCDE	87.00	BC	57.94	BC

[a] Least square means of early leaf spot number of days until the first noticeable sporulating lesions (ELS DSL)
[b] Least square means of early leaf spot sporulating lesion incidence (ELS SLI)
[c] Least square means of early leaf spot sporulation degree (ELS SD)
[d] Genotypes are grouped based on LSD test, with letters indicating significant differences. Groups with the same letter are not significantly different.

Table 7.3. Least square means of disease values based on different disease parameters for late leaf spot in genotypes from the 2023 trials.

Genotype	Wild *Arachis* resistance source	LLS DSL[ad]		LLS SLI[bd]		LLS SD[cd]	
TUFRunner'511'	None	108.00	B	100.69	A	89.25	A
Georgia-06G	None	105.13	B	96.88	A	77.50	A
Georgia-13M	None	104.88	B	96.06	AB	83.75	A
TifGP-3	*A. cardenasii*	125.00	A	57.69	C	28.06	B
IAC 322	*A. cardenasii*	127.88	A	48.38	C	21.56	B
CB1	*A. cardenasii*	132.75	A	30.25	C	22.00	B
CB2	*A. cardenasii*	128.13	A	50.88	C	26.50	B
CB7	*A. cardenasii*	125.00	A	57.50	C	31.81	B
TBI-S11	*A. cardenasii*	124.13	A	55.44	C	25.50	B
RBS-95C9	*A. stenosperma/ A. batizocoi*	123.25	A	50.06	C	34.69	B
RBS-158B10	*A. stenosperma/ A. batizocoi*	125.00	A	44.56	C	36.88	B
RBS-170A	*A. stenosperma/ A. batizocoi*	122.38	A	63.44	BC	40.13	B

[a] Least square means of late leaf spot number of days until the first noticeable sporulating lesions (LLS DSL)
[b] Least square means of late leaf spot sporulating lesion incidence (LLS SLI)
[c] Least square means of late leaf spot sporulation degree (LLS SD)
[d] Genotypes are grouped based on LSD test, with letters indicating significant differences. Groups with the same letter are not significantly different.

Table 7.4. Least square means of disease values based on different disease parameters for leaf spot across genotypes from the 2023 trial with significant location effect.

Genotype	Wild *Arachis* resistance source	FS[ac]				LD[bc]			
		Black Shank		Lang		Black Shank		Lang	
TUFRunner'511'	None	174.44	AB	210.38	A	86.88	AB	142.38	A
Georgia-06G	None	201.50	A	207.31	A	91.63	A	131.00	A
Georgia-13M	None	202.00	A	193.44	AB	101.13	A	128.63	A
TifGP-3	*A. cardenasii*	105.69	BCD	47.38	D	76.00	ABC	45.88	DE
IAC 322	*A. cardenasii*	85.44	D	39.50	D	51.88	CD	18.88	F
CB1	*A. cardenasii*	130.25	BCD	177.38	AB	54.13	CD	82.63	B
CB2	*A. cardenasii*	135.19	ABCD	52.00	D	55.38	CD	30.88	EF
CB7	*A. cardenasii*	165.13	ABC	102.44	C	81.00	ABC	57.25	CD
TBI-S11	*A. cardenasii*	95.06	CD	112.25	C	38.75	D	45.38	DE
RBS-95C9	*A. stenosperma*/ *A. batizocoi*	131.56	ABCD	198.69	AB	59.13	BCD	98.50	B
RBS-158B10	*A. stenosperma*/ *A. batizocoi*	154.75	ABCD	185.25	AB	43.00	D	89.25	B
RBS-170A	*A. stenosperma*/ *A. batizocoi*	113.56	BCD	165.19	B	38.75	D	78.75	BC

[a] Least square means of disease values based on Florida 1-10 scale (FS).
[b] Least square means of disease values based on levels of defoliation (LD).
[c] Genotypes are grouped based on LSD test, with letters indicating significant differences. Groups with the same letter are not significantly different.

Table 7.5. Least square means of disease values based on different disease parameters for early leaf spot and late leaf spot across genotypes from the 2023 trial, with significant location effect.

Genotype	Wild *Arachis* resistance source	ELS SD[ac]				LLS DSL[bc]			
		Black Shank		Lang		Black Shank		Lang	
TUFRunner'511'	None	91.38	A	51.88	A	108.00	B	107.50	A
Georgia-06G	None	91.50	A	44.75	AB	105.13	B	104.00	A
Georgia-13M	None	84.50	AB	38.00	B	104.88	B	104.00	A
TifGP-3	*A. cardenasii*	78.63	AB	18.50	C	125.00	A	107.50	A
IAC 322	*A. cardenasii*	28.75	D	17.88	C	127.88	A	105.75	A
CB1	*A. cardenasii*	49.38	CD	38.13	B	132.75	A	115.50	A
CB2	*A. cardenasii*	66.50	BC	36.50	B	128.13	A	115.50	A
CB7	*A. cardenasii*	81.75	AB	36.25	B	125.00	A	111.50	A
TBI-S11	*A. cardenasii*	37.50	D	38.25	AB	124.13	A	105.75	A
RBS-95C9	*A. stenosperma*/ *A. batizocoi*	65.50	BC	38.00	B	123.25	A	115.50	A
RBS-158B10	*A. stenosperma*/ *A. batizocoi*	75.50	AB	38.00	B	125.00	A	119.00	A
RBS-170A	*A. stenosperma*/ *A. batizocoi*	76.00	AB	39.88	AB	122.38	A	104.00	A

[a] Least square means of disease values based on early leaf spot sporulation degree (ELS SD).
[b] Least square means of disease values based on late leaf spot number of days to first sporulating lesions (LLS DSL).
[c] Genotypes are grouped based on LSD test, with letters indicating significant differences. Groups with the same letter are not significantly different.

Table 8.1. Pearson's correlation analysis for the linear relationships between disease parameters of leaf spot in each genotype grouped by Wild *Arachis* resistance sources (none, *A. cardenasii*, *A. stenosperma* and *A. batizocoi*) evaluated in Black Shank farm, 2023.

Group			Correlation coefficient (R)[a] p-value[b]									
			LS					ELS			LLS	
			FS	LI	LD	STL	DSL	SLI	SD	DSL	SLI	SD
None	LS	FS	-									
		LI	0.32 0.31	-								
		LD	0.74 <0.01	0.60 <0.05	-							
		STL	-0.01 0.98	0.57 0.05	0.24 0.46	-						
	ELS	DSL	-0.27 0.40	-0.61 <0.05	-0.40 0.20	-0.33 0.30	-					
		SLI	0.43 0.16	0.88 <0.001	0.64 <0.05	0.43 0.16	-0.83 <0.001	-				
		SD	0.45 0.14	0.65 <0.05	0.53 0.08	0.40 0.19	-0.28 0.38	0.54 0.07	-			
	LLS	DSL	-0.23 0.47	-0.16 0.62	-0.17 0.60	0.003 0.99	0.25 0.43	-0.36 0.26	-0.27 0.39	-		
		SLI	-0.14 0.68	0.49 0.11	0.39 0.21	0.57 0.05	-0.31 0.32	0.43 0.16	0.47 0.13	-0.27 0.40	-	
		SD	0.07 0.83	0.10 0.75	0.22 0.49	0.44 0.16	0.24 0.45	0.02 0.95	0.52 0.08	-0.14 0.66	0.46 0.13	-
A. cardenasii	LS	FS	-									
		LI	0.72 <0.001	-								
		LD	0.70 <0.001	0.74 <0.001	-							

Group			Correlation coefficient (R)[a] p-value[b]									
			LS				ELS			LLS		
			FS	LI	LD	STL	DSL	SLI	SD	DSL	SLI	SD
		STL	0.24 0.27	0.21 0.33	0.47 <0.05	-						
	ELS	DSL	-0.37 0.08	-0.71 <0.001	-0.46 <0.05	-0.10 0.63	-					
		SLI	0.75 <0.001	0.89 <0.001	0.81 <0.001	0.37 0.07	-0.77 <0.001	-				
		SD	0.42 <0.05	0.66 <0.001	0.70 <0.001	0.41 <0.05	-0.46 <0.05	0.67 <0.001	-			
	LLS	DSL	-0.56 <0.01	-0.58 <0.01	-0.45 <0.05	-0.09 0.68	0.40 0.06	-0.53 <0.01	-0.29 0.16	-		
		SLI	0.56 <0.01	0.58 <0.01	0.45 <0.05	0.12 0.58	-0.40 0.05	0.53 <0.01	0.30 0.16	-0.99 <0.001	-	
		SD	0.60 <0.01	0.59 <0.01	0.54 <0.01	0.33 0.12	-0.41 <0.05	0.58 <0.01	0.38 0.07	-0.95 <0.001	0.96 <0.001	-
A. stenosperma *A. batizocoi*	CLS	FS	-									
		LI	0.28 0.38	-								
		LD	0.47 0.13	0.20 0.53	-							
		STL	0.41 0.18	-0.26 0.41	0.54 0.07	-						
	ELS	DSL	-0.52 0.08	-0.12 0.71	-0.43 0.16	-0.47 0.12	-					
		SLI	0.67 <0.05	0.41 0.18	0.49 0.11	0.18 0.58	-0.87 <0.001	-				
		SD	0.33 0.29	0.22 0.49	0.20 0.54	-0.03 0.92	-0.31 0.32	0.47 0.12	-			

Group			Correlation coefficient (R)[a] p-value[b]									
			LS				ELS			LLS		
			FS	LI	LD	STL	DSL	SLI	SD	DSL	SLI	SD
	LLS	DSL	-0.31	-0.42	-0.07	0.07	0.50	-0.66	-0.40	-		
			0.33	0.18	0.83	0.83	0.10	<0.05	0.20			
		SLI	0.33	0.45	0.10	-0.06	-0.50	0.67	0.42	-0.99	-	
			0.29	0.15	0.76	0.86	0.09	<0.05	0.17	<0.001		
		SD	0.32	0.38	0.04	-0.04	-0.52	0.66	0.45	-0.99	0.99	-
			0.31	0.22	0.90	0.91	0.09	<0.05	0.14	<0.001	<0.001	

[a] Measures the strength and direction of the relationship between disease parameters of leaf spot in each genotype. Negative values indicate an inverse relationship, while positive values indicate a direct relationship.
[b] Statistical significance of the correlation ($p < 0.05$ is significant).

Table 8.2. Pearson's correlation analysis for the linear relationships between disease parameters of leaf spot in each genotype grouped by Wild *Arachis* resistance sources (none, *A. cardenasii*, *A. stenosperma* and *A. batizocoi*) evaluated in Lang Farm, 2023.

Group			Correlation coefficient (R)[a] p-value[b]									
			LS				ELS			LLS		
			FS	LI	LD	STL	DSL	SLI	SD	DSL	SLI	SD
None	LS	FS	-									
		LI	-0.33 0.30	-								
		LD	0.48 0.11	-0.15 0.64	-							
		STL	0.44 0.16	0.36 0.24	0.59 <0.05	-						
	ELS	DSL	-0.10 0.75	0.08 0.82	-0.18 0.57	0.13 0.70	-					
		SLI	0.25 0.42	0.03 0.92	0.13 0.68	-0.10 0.76	-0.79 <0.01	-				
		SD	0.35 0.26	0.13 0.68	0.59 <0.05	0.79 <0.01	0.02 0.95	-0.14 0.66	-			
	LLS	DSL	0.21 0.52	-0.57 0.05	0.35 0.24	0.04 0.90	0.28 0.38	-0.36 0.25	0.25 0.44	-		
		SLI	-0.12 0.71	0.64 <0.05	0.20 0.54	0.50 0.09	-0.19 0.55	0.32 0.31	0.11 0.74	-0.59 <0.05	-	
		SD	0.48 0.12	0.23 0.48	0.55 0.06	0.46 0.13	0.003 0.99	0.07 0.82	0.23 0.48	-0.18 0.57	0.19 0.55	-
A. cardenasii	CLS	FS	-									
		LI	0.18 0.40	-								
		LD	0.82 <0.001	0.10 0.63	-							
		STL	0.02	0.25	-0.006	-						

Group			Correlation coefficient (R)[a] p-value[b]									
			LS				ELS			LLS		
			FS	LI	LD	STL	DSL	SLI	SD	DSL	SLI	SD
			0.93	0.24	0.98							
	ELS	DSL	-0.30	-0.38	-0.21	-0.26	-					
			0.15	0.07	0.33	0.22						
		SLI	0.37	0.36	0.31	0.25	-0.97	-				
			0.07	0.09	0.15	0.24	<0.001					
		SD	0.44	0.31	0.32	0.19	-0.85	0.85	-			
			<0.05	0.14	0.13	0.36	<0.001	<0.001				
	LLS	DSL	0.12	-0.19	0.30	0.37	-0.13	0.27	0.22	-		
			0.57	0.37	0.16	0.08	0.55	0.20	0.30			
		SLI	-0.31	0.50	-0.33	-0.19	0.02	-0.13	-0.06	-0.71	-	
			0.15	<0.05	0.11	0.37	0.92	0.54	0.78	<0.001		
		SD	0.11	0.12	-0.19	-0.25	0.20	-0.29	-0.05	-0.70	0.58	-
			0.60	0.57	0.38	0.24	0.34	0.16	0.82	<0.01	<0.01	
A. stenosperma	CLS	FS	-									
A. batizocoi		LI	0.36	-								
			0.25									
		LD	0.79	0.37	-							
			<0.01	0.23								
		STL	0.26	0.35	0.40	-						
			0.41	0.27	0.20							
	ELS	DSL	-0.55	-0.25	-0.42	-0.17	-					
			0.06	0.43	0.17	0.60						
		SLI	0.16	0.52	0.04	0.06	-0.12	-				
			0.61	0.08	0.89	0.85	0.72					
		SD	-0.11	0.37	-0.16	-0.02	0.37	0.88	-			
			0.73	0.24	0.61	0.96	0.23	<0.001				
	LLS	DSL	0.26	-0.56	-0.08	-0.07	0.19	-0.27	-0.16	-		

Group		Correlation coefficient (R)[a] p-value[b]									
		LS				ELS			LLS		
		FS	LI	LD	STL	DSL	SLI	SD	DSL	SLI	SD
		0.41	0.06	0.80	0.82	0.55	0.40	0.62			
	SLI	0.09	0.82	0.28	0.09	-0.09	0.36	0.29	-0.82	-	
		0.77	<0.01	0.39	0.77	0.78	0.26	0.35	<0.01		
	SD	-0.17	0.58	0.18	0.05	-0.14	0.25	0.17	-0.94	0.87	-
		0.59	<0.05	0.58	0.89	0.66	0.43	0.60	<0.001	<0.001	

[a] Measures the strength and direction of the relationship between disease parameters of leaf spot in each genotype. Negative values indicate an inverse relationship, while positive values indicate a direct relationship.
[b] Statistical significance of the correlation ($p < 0.05$ is significant).

Table 9. Summary of forward stepwise regression analysis for the Florida 1-10 scale (Chiteka et al. 1988) with field disease components across and separate locations.

Location	Predictor variable[a]	Statistical metrics[b]							
		Coefficient (β)	Std Error	t-value	p-value	R^2	Adjusted R^2	AIC	SBC
Black Shank Farm	ELS SLI	1.03	0.25	4.17	0.00				
	ELS DSL	0.81	0.37	2.21	0.03				
	LD	90.33	0.31	1.08	0.29				
	Overall model metrics					0.66	0.64	489.54	498.90
Lang Farm	LD	1.21	0.14	8.51	0.00				
	ELS DSL	-0.53	0.18	-2.98	0.01				
	LLS SLI	-0.37	0.13	-2.81	0.01				
	LLS SD	0.46	0.24	1.94	0.06				
	Overall model metrics					0.85	0.83	461.85	473.08
Both	LD	1.17	0.11	10.93	0.00				
	ELS SLI	0.42	0.08	4.95	0.00				
	Overall model metrics					0.71	0.71	960.43	970.69

[a] Disease parameters that predict the Florida 1-10 scale (FS): including levels of defoliation (LD), number of days until the first sporulating lesions appear (ELS DSL) for early leaf spot, sporulating lesion incidence (ELS SLI) for early leaf spot, sporulating lesion incidence (LLS SLI), and sporulation degree (LLS SD) for late leaf spot.

[b] Metrics: Coefficient (β) – effect size, representing the change in FS for a one-unit change in the predictor variable; Standard error – precision of the estimated coefficient; t-value – indicates whether the predictor variable is significant (higher suggests stronger predictor variable associated with FS); p-value – tests the statistical significance of the predictor variable (< 0.05 are statistically significant); R^2 – coefficient of determination, indicating model fits the data; Adjusted R^2 – adjusted for the number of predictors; Akaike Information Criterion (AIC) –measure of model quality; Schwarz Bayesian Criterion (SBC) – measure of model quality, with lower values indicating a better-fitting model.

Table 10.1. Analyzing the effect of genotype on the lesion incidence across non-duplicated genotypes from the 2022 and 2023 trials.

Year	Disease parameter	Linear mixed model[ae]				Source of variation[b]	ANOVA[ce]			LSD[de]		
		R^2	DF	RSE	p-value		DF	f-value	p-value	DF	t-value	LSD
2022	LI	0.79	63	262.00	<0.001	G	10	18.01	<0.001	63	2.00	261.77
						G x L	10	1.83	0.07	ns	ns	ns
2023	LI	0.67	57	252.40	<0.001	G	9	9.11	<0.001	57	2.00	252.67
						G x L	9	1.95	0.06	ns	ns	ns

[a] Analyzes genotype-variable relationships, accounting for both fixed and random effects. Metrics: R^2, DF, RSE, p-value.
[b] Considers genotype (G) and Genotype x Location (G x L) interaction effect.
[c] Assesses whether genotypes and interaction with location significantly affect variables. Metrics: DF, f-value, p-value.
[d] Identifies significantly different groups. Metrics: DF, q-value, LSD.
[e] Metrics: R^2 – coefficient of determination, indicating model fit; DF – Degrees of freedom, representing sample size; RSE – residual standard error, measuring model prediction error; p-value – tests statistical significance; $p < 0.05$ is significant); f-value – higher values indicate more significant differences between groups; t-value – larger values indicate more significant group differences; LSD –least significant difference between group means.

Table 10.2. Least square means of lesion incidence across non-duplicated genotypes from the 2022 and 2023 trials.

Selection basis	Breeding information	Year 2022[ac]			Year 2023[bc]		
		Genotype	LI		Genotype	LI	
Replicated in Both years	Susceptible cultivars	TUFRunner'511'	1246.50	A	TUFRunner'511'	1030.00	A
		Georgia-06G	1165.81	A	Georgia-06G	1172.50	A
		Georgia-13M	1229.50	A	Georgia-13M	1137.50	A
	Stable resistant lines	TifGP-3	402.81	CD	TifGP-3	512.50	B
		IAC 322	234.31	D	IAC 322	432.50	B
		CB-7	410.13	CD	CB-7	635.00	B
	Developing lines	TBI-S11	388.13	CD	TBI-S11	590.00	B
		RBS-158B10	364.63	CD	RBS-158B10	625.00	B
Not replicated in both years	Resistant lines	BBI-S25	299.44	CD	Bailey	927.50	A
		RBS-155B4	733.81	B	TifNV-HG	662.50	B
		RBS-158B6	512.63	BC			

[a] Least square means of lesion incidence (LI) in 2022 trial.
[b] Least square means of lesion incidence (LI) in 2023 trial.
[c] Genotypes are grouped based on LSD test, with letters indicating significant differences. Groups with the same letter are not significantly different.

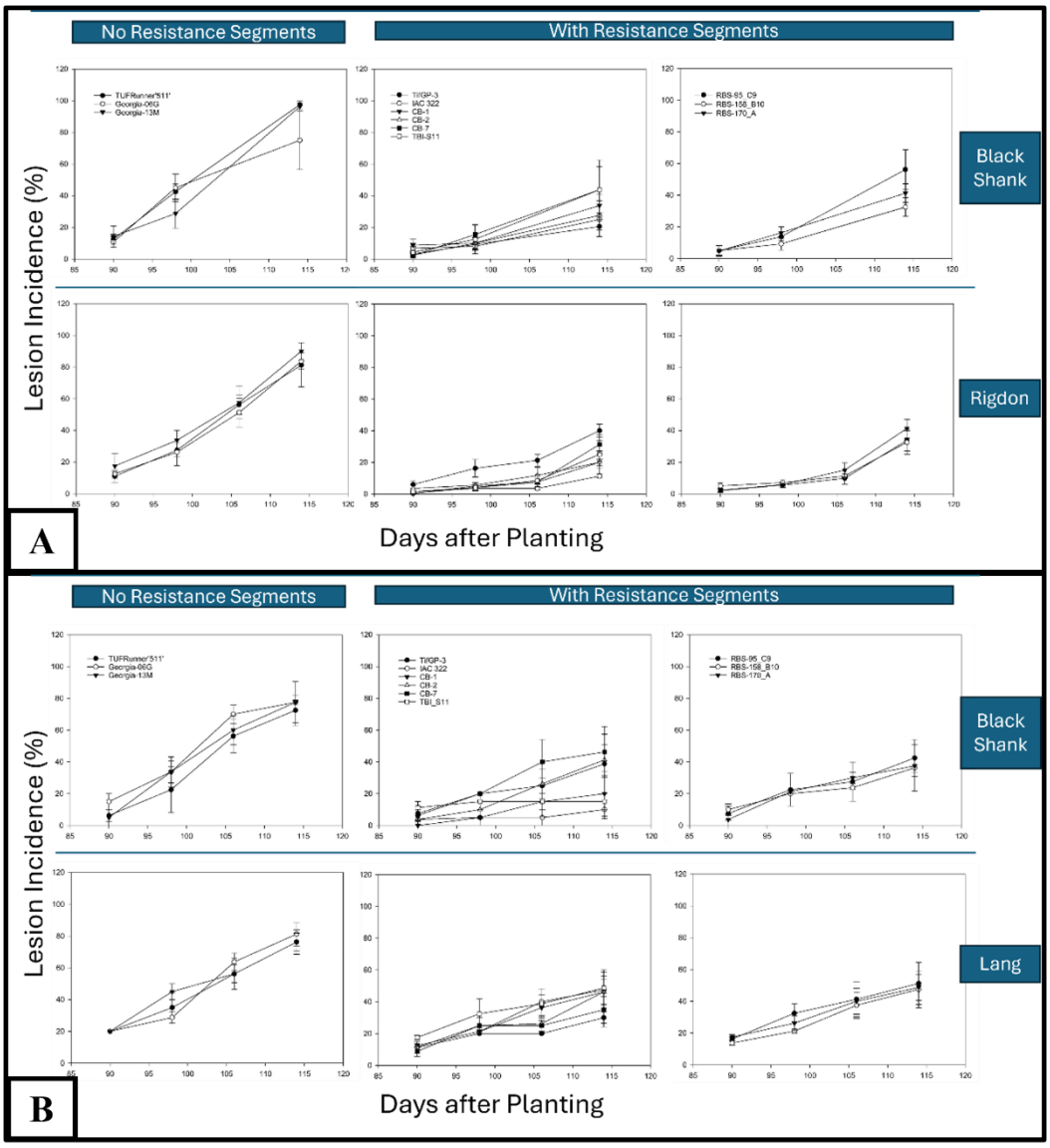


Figure 1. Leaf spot disease progression based on lesion incidence across the canopy of various peanut genotypes evaluated in 2022 and 2023 at Tifton, Georgia.
Panel A: Field evaluation in Summer 2022 at Black Shank Farm (top) and Rigdon Farm (bottom). Panel B: Field evaluation in Summer 2023 at Black Shank Farm (top) and Lang Farm (bottom). In both panels, the graphs on the left represent genotypes without resistance segments, the middle graphs show genotypes with resistance from *Arachis cardenasii*, and the right graphs display genotypes with resistance from *Arachis stenosperma* and *Arachis batizocoi*.

CHAPTER 5

CONCLUSIONS

This study offers valuable insights into the influence of peanut genotype on the early infection stages and disease progression of the leaf spot pathogens *Passalora arachidicola* and *Nothopassalora personata*. By focusing on critical early infection parameters, such as conidial adhesion and germ tube elongation, our findings indicate that resistant genotypes derived from wild *Arachis* species, particularly *Arachis cardenasii*, *Arachis stenosperma*, and *Arachis batizocoi*, exhibit significantly lower conidial counts and shorter germ tube lengths compared to the susceptible cultivar Georgia-06G. These results suggest that these resistant genotypes possess more effective defense mechanisms that reduce the infection potential of both pathogens. Notably, *A. cardenasii* is recognized for its strong resistance to both leaf spot pathogens, often demonstrating the lowest disease severity and lesion incidence. In contrast, *A. stenosperma* also displays strong resistance traits but may exhibit varying degrees of effectiveness across different genotypes. Although *A. batizocoi* serves as a valuable source of resistance, it is less extensively researched in terms of its specific traits compared to the other two species. Our findings emphasize the critical role of delaying conidial attachment and germ tube elongation in enhancing resistance, underscoring the importance of the early stages of pathogen infection in mitigating disease severity. Moreover, our comprehensive assessment of disease progression across various peanut genotypes highlights the differential effects of resistance traits on both early and late leaf

spot diseases. By comparing cultivars lacking resistance with those exhibiting traits from wild species, the study underscores the significance of specific resistance components, such as lesion incidence, disease severity, and sporulation degree. Results indicate that susceptible cultivars, including TUFRunner'511', Georgia-13M, and Georgia-06G, experience heightened disease severity and lesion incidence, while genotypes with resistance segments, particularly those derived from *A. cardenasii*, such as IAC 322, demonstrate significantly lower disease severity and lesion incidence. The strong performance of *A. stenosperma* and *A. batizocoi* in terms of disease resistance further validates their potential as genetic resources in breeding programs aimed at enhancing peanut durability against leaf spot diseases.

The identification of genotype-location interactions illustrates the influence of environmental factors on disease progression, suggesting that a broader field diversity is essential for robust applicability of the findings. This study emphasizes the necessity of examining both early and late leaf spot components, as well as the correlation between sporulation and lesion incidence, to effectively inform breeding programs and disease management strategies. While this research builds upon the established foundation of the Florida scale for assessing disease severity, it also addresses critical gaps in understanding resistance mechanisms by evaluating additional disease components. Insights gained from this study not only advance our understanding of the genetic factors influencing resistance to leaf spot pathogens in peanuts but also lay the groundwork for future research. The methodologies developed herein may facilitate the examination of additional genotypes and resistance sources, further elucidating the complexities of peanut-pathogen interactions. Despite these contributions, the study has limitations that

warrant consideration. The focus on specific peanut genotypes may restrict the generalizability of the findings to other cultivars and environmental conditions. Additionally, the research primarily concentrates on early infection stages, potentially overlooking other infection phases of disease progression. Future investigations should include a broader range of peanut genotypes and environmental conditions to validate these findings and enhance our understanding of resistance mechanisms across diverse contexts. Ultimately, this research aims to contribute to the development of more resilient peanut cultivars capable of withstanding leaf spot diseases in diverse environments. By advancing our understanding of the genetic and environmental factors that influence peanut resistance, particularly those derived from *A. cardenasii*, *A. stenosperma*, and *A. batizocoi*, this study plays a crucial role in improving disease management strategies and ensuring sustainable peanut production, which is vital for food security and economic stability in agricultural regions reliant on peanut cultivation.

APPENDIX: Supporting tables and figures

Table 1.1. Peanut genotypes tested for conidial adhesion and germ tube length of *Passalora arachidicola* and *Nothopassalora personata* in duplicated trials.

Genotype	Parentals	Breeding information	Market classification[a]	Seed source	Resistance Characteristics[b]	Reference[c]
Georgia-06G	Georgia Green x C-99R	Commercial cultivar	Runner type	Culbreath, A.K.	Susceptible to LS	Branch, W. D. 2007
IAC 322	Runner 886 x IAC 69007	Germplasm/breeding line	Runner type	Leal-Bertioli, S & Bertioli, D.	Resistance to LLS from *A. cardenasii*	Godoy, I. J. et al. 2022; Lamon S. et al. 2019
CB-7	Georgia 13M x (Georgia-13M x TifGP-3)	Cultivar	Runner type	Holbrook, C.C.	Resistance to LLS from *A. cardenasii*	Castellano, D. A. 2023; Holbrook, C. 2022 (APRES)
TBI-S11	TifNV-High O/L x (Bailey x IAC 321)	Developmental breeding line	NM	Leal-Bertioli, S & Bertioli, D.	Resistance to ELS and LLS from *A. cardenasii*	Gonzales, M. 2024; Maharjan, N. 2024 (pc)
RBS-158B10	Runner 886 x BatSten1	Developmental breeding line	NM	Leal-Bertioli, S & Bertioli, D.	Resistance to LS from *A. stenosperma*	Maharjan, N. 2024 (pc)

[a] NM – not in market (usually breeding lines)

[b] Resistance characteristics are indicated as either resistant or susceptible to LS (Leaf Spot), ELS (Early Leaf Spot), and LLS (Late Leaf Spot), based on information from the referenced sources.

[c] References are primarily published articles, except "pc," which refers to personal communication with the researchers.

Table 1.2. Peanut genotypes tested for conidial adhesion and germ tube length of *Passalora arachidicola* and *Nothopassalora personata* in the initial optimization experiment and non-replicated trial.

Genotype	Parentals	Breeding information	Market classification[a]	Seed source	Resistance Characteristics[b]	Reference[c]
Initial optimization experiment						
Georgia-06G	Georgia Green x C-99R	Commercial cultivar	Runner type	Culbreath, A.K.	Susceptible to LS	Branch, W. D. 2007
IAC 322	Runner 886 x IAC 69007	Germplasm/breeding line	Runner type	Leal-Bertioli, S & Bertioli, D.	Resistance to LLS from *A. cardenasii*	Godoy, I. J. et al. 2022; Lamon S. et al. 2019
CB-1	Georgia 13M x (Georgia-13M x TifGP-3)	Candidate cultivar	Runner type	Holbrook, C.C.	Resistance to LLS from *A. cardenasii*	Monfort, W. S. et. al 2022; Holbrook, C. 2022 (APRES)
CB-2	Georgia 13M x (Georgia-13M x TifGP-3)	Candidate cultivar	Runner type	Holbrook, C.C.	Resistance to LLS from *A. cardenasii*	Monfort, W. S. et. al 2022; Haire, B. 2022 (site); Holbrook, C. 2022 (APRES)
CB-7	Georgia 13M x (Georgia-13M x TifGP-3)	Cultivar	Runner type	Holbrook, C.C.	Resistance to LLS from *A. cardenasii*	Castellano, D. A. 2023; Holbrook, C. 2022 (APRES)

Genotype	Parentals	Breeding information	Market classification[a]	Seed source	Resistance Characteristics[b]	Reference[c]
TBI-S11	TifNV-High O/L x (Bailey x IAC 321)	Developmental breeding line	NM	Leal-Bertioli, S & Bertioli, D.	Resistance to ELS and LLS from *A. cardenasii*	Gonzales, M. 2024 (pc); Maharjan, N. 2024 (pc)
BBI-S25	Bailey x (Bailey x IAC 321)	Developmental breeding line	NM	Leal-Bertioli, S & Bertioli, D.	Resistance to ELS from *A. cardenasii*	Gonzales, M. 2024 (pc)
RBS-158B10	Runner 886 x BatSten1	Developmental breeding line	NM	Leal-Bertioli, S & Bertioli, D.	Resistance to LS from *A. stenosperma*	Maharjan, N. 2024 (pc)
Non-duplicated experiment						
TUFRunner'511'	C-99R x (88x1B-OLBC1-6-1-1-1)	Commercial cultivar	Runner type	Culbreath, A.K.	Susceptible to LS	Tillman, B. L., & Gorbet, D. W. 2017
Georgia-06G	Georgia Green x C-99R	Commercial cultivar	Runner type	Culbreath, A.K.	Susceptible to LS	Branch, W. D. 2007
IAC 322	Runner 886 x IAC 69007	Germplasm/breeding line	Runner type	Leal-Bertioli, S & Bertioli, D.	Resistance to LLS from *A. cardenasii*	Godoy, I. J. et al. 2022; Lamon S. et al. 2019
CB-7	Georgia 13M x (Georgia-13M x TifGP-3)	Cultivar	Runner type	Holbrook, C.C.	Resistance to LLS from *A. cardenasii*	Castellano, D. A. 2023; Holbrook, C. 2022 (APRES)

Genotype	Parentals	Breeding information	Market classification[a]	Seed source	Resistance Characteristics[b]	Reference[c]
TBI-S11	TifNV-High O/L x (Bailey x IAC 321)	Developmental breeding line	NM	Leal-Bertioli, S & Bertioli, D.	Resistance to ELS and LLS from *A. cardenasii*	Gonzales, M. 2024 (pc); Maharjan, N. 2024 (pc)
RBS-95C9	Runner 886 x BatSten1	Developmental breeding line	NM	Leal-Bertioli, S & Bertioli, D.	Resistance to LS from *A. stenosperma* and *A. batizocoi*	Maharjan, N. 2024 (pc)
RBS-158B10	Runner 886 x BatSten1	Developmental breeding line	NM	Leal-Bertioli, S & Bertioli, D.	Resistance to LS from *A. stenosperma* and *A. batizocoi*	Maharjan, N. 2024 (pc)
RBS-170A	Runner 886 x BatSten1	Developmental breeding line	NM	Leal-Bertioli, S & Bertioli, D.	Resistance to LS from *A. stenosperma* and *A. batizocoi*	Maharjan, N. 2024 (pc)

[a] NM – not in market (usually breeding lines)

[b] Resistance characteristics are indicated as either resistant or susceptible to LS (Leaf Spot), ELS (Early Leaf Spot), and LLS (Late Leaf Spot), based on information from the referenced sources.

[c] References are primarily published articles, except "pc," which refers to personal communication with the researchers.

The genotypes listed include those used in the initial optimization experiment (for which the methodology was optimized) and the non-duplicated experiment (where some genotypes were not included in a single trial).

Table 2.1. Peanut genotypes in the replicated field trials in different locations for leaf spot resistance in 2022 and 2023.

Genotype	Parentals	Breeding information	Market classification[a]	Seed source	Resistance Characteristics[b]	Reference[c]
TUFRunner'511'	C-99R x (88x1B-OLBC1-6-1-1-1)	Commercial cultivar	Runner type	Culbreath, A.K.	Susceptible to LS	Tillman, B. L., & Gorbet, D. W. 2017
Georgia-06G	Georgia Green x C-99R	Commercial cultivar	Runner type	Culbreath, A.K.	Susceptible to LS	Branch, W. D. 2007
Georgia-13M	Georgia-02C x Georgia-09B	Commercial cultivar	Runner type	Culbreath, A.K.	Susceptible to LS	Branch, W. D. 2014.
TifGP-3	TifNV-High O/L x IAC 322	Germplasm/breeding line	Runner type	Holbrook, C.C.	Resistance to LLS from *A. cardenasii*	Holbrook, C. C. et al. 2021
IAC 322	Runner 886 x IAC 69007	Germplasm/breeding line	Runner type	Leal-Bertioli, S & Bertioli, D.	Resistance to LLS from *A. cardenasii*	Godoy, I. J. et al. 2022; Lamon S. et al. 2019
CB-1	Georgia 13M x (Georgia-13M x TifGP-3)	Candidate cultivar	Runner type	Holbrook, C.C.	Resistance to LLS from *A. cardenasii*	Monfort, W. S. et. al 2022; Holbrook, C. 2022
CB-2	Georgia 13M x (Georgia-13M x TifGP-3)	Candidate cultivar	Runner type	Holbrook, C.C.	Resistance to LLS from *A. cardenasii*	Monfort, W. S. et. al 2022; Holbrook, C. C. 2022
CB-7	Georgia 13M x (Georgia-13M x TifGP-3)	Cultivar	Runner type	Holbrook, C.C.	Resistance to LLS from *A. cardenasii*	Castellano, D. A. 2023; Holbrook, C. C. 2022

Genotype	Parentals	Breeding information	Market classification[a]	Seed source	Resistance Characteristics[b]	Reference[c]
TBI-S11	TifNV-High O/L x (Bailey x IAC 321)	Developmental breeding line	NM	Leal-Bertioli, S & Bertioli, D.	Resistance to ELS and LLS from *A. cardenasii*	Gonzales, M. 2024 (pc); Maharjan, N. 2024 (pc)
RBS-95C9	Runner 886 x BatSten1	Developmental breeding line	NM	Leal-Bertioli, S & Bertioli, D.	Resistance to LS from *A. stenosperma* and *A. batizocoi*	Bertioli, D. J. et al., 2021 Maharjan, N. 2024 (pc)
RBS-158B10	Runner 886 x BatSten1	Developmental breeding line	NM	Leal-Bertioli, S & Bertioli, D.	Resistance to LS from *A. stenosperma* and *A. batizocoi*	Bertioli, D. J. et al., 2021 Maharjan, N. 2024 (pc)
RBS-170A	Runner 886 x BatSten1	Developmental breeding line	NM	Leal-Bertioli, S & Bertioli, D.	Resistance to LS from *A. stenosperma* and *A. batizocoi*	Bertioli, D. J. et al., 2021 Maharjan, N. 2024 (pc)

[a] NM – not in market (usually breeding lines)

[b] Resistance characteristics are indicated as either resistant or susceptible to LS (Leaf Spot), ELS (Early Leaf Spot), and LLS (Late Leaf Spot), based on information from the referenced sources.

[c] References are primarily published articles, except "pc," which refers to personal communication with the researchers.

Table 2.2. Peanut genotypes in field trials replicated across locations but not across years (2022 and 2023).

Genotype[a]	Parentals	Breeding information	Market classification[a]	Seed source	Resistance Characteristics[b]	Reference[c]
Year 2022 Trials						
BBI-S25	Bailey x (Bailey x IAC 321)	Developmental breeding line	NM	Leal-Bertioli, S & Bertioli, D.	Resistance to ELS from *A. cardenasii*	Gonzales, M. 2024 (pc); Maharjan, N. 2024 (pc)
RBS-158B4	Runner 886 x BatSten1	Developmental breeding line	NM	Leal-Bertioli, S & Bertioli, D.	Resistance to LS from *A. stenosperma* and *A. batizocoi*	Bertioli, D. J. et al., 2021 Maharjan, N. 2024 (pc)
RBS-158B6	Runner 886 x BatSten1	Developmental breeding line	NM	Leal-Bertioli, S & Bertioli, D.	Resistance to LS from *A. stenosperma* and *A. batizocoi*	Bertioli, D. J. et al., 2021 Maharjan, N. 2024 (pc)
RBS-158B10	Runner 886 x BatSten1	Developmental breeding line	NM	Leal-Bertioli, S & Bertioli, D.	Resistance to LS from *A. stenosperma*	Maharjan, N. 2024 (pc)

Genotype[a]	Parentals	Breeding information	Market classification[a]	Seed source	Resistance Characteristics[b]	Reference[c]
Year 2023 Trials						
Bailey	NC 12C x N96076L	Commercial cultivar	Virginia type	Holbrook, C.C.	Resistance to ELS from *A. cardenasii*	Isleib, T. G. et al, 2010; Bertioli, D. J. et al. 2021a;
TifNV-HG	C-99R x COAN	Commercial cultivar	Runner type		Resistance to RNK, TSWV from *A. cardenasii*	Holbrook et al. 2023

[a] NM – not in market (usually breeding lines)

[b] Resistance characteristics are indicated as either resistant or susceptible to LS (Leaf Spot), ELS (Early Leaf Spot), and LLS (Late Leaf Spot), based on information from the referenced sources.

[c] References are primarily published articles, except "pc," which refers to personal communication with the researchers.

The genotypes listed include those planted in Summer2022 and Summer 2023.

Table 3. Peanut leaf spot disease rating scale used in the field trials.

THE FLORIDA 1-10 SCALE (CHITEKA ET AL. 1988)	
SCALE	Description
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 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)
10	Plants dead

LEVELS OF DEFOLIATION 0-5 SCALE	
0	no leaves defoliated
1	>0% to 20% of leaves defoliated
2	>20% to 40% of leaves defoliated
3	>40% to 60% of leaves defoliated
4	>60% to 80% of leaves defoliated
5	>80% to 100% of leaves defoliated

ELS/LLS SPORULATING LESION INCIDENCE 0-5 SCALE	
0	no leaves with sporulating lesions
1	>0% to 20% of leaflets with sporulating lesions
2	>20% to 40% of leaflets with sporulating lesions
3	>40% to 60% of leaflets with sporulating lesions
4	>60% to 80% of leaflets with sporulating lesions
5	>80% to 100% of leaflets sporulating lesions

ELS/LLS SPORULATION DEGREE 0-3 SCALE	
0	No noticeable sporulation
1	Minimal sporulation: most lesions are slightly covered with spores (very few sporulating spots)
2	Moderate sporulation: most lesions are partially covered with spores (some noticeable sporulation)
3	High sporulation: most lesions are fully covered with spores (widespread and intense sporulation)

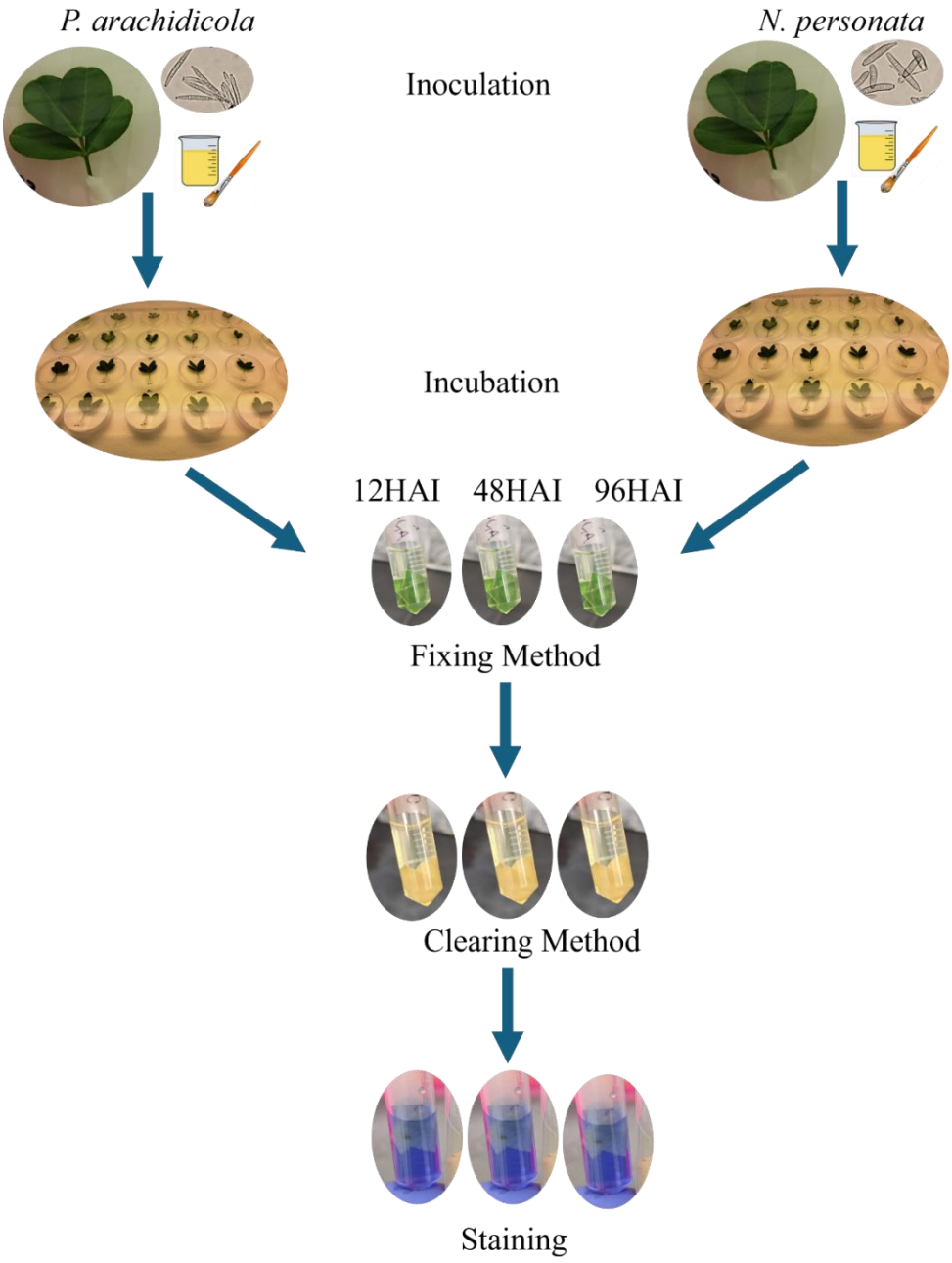


Figure 1. Flowchart depicting the overall methodology from inoculation, fixation, clearing, and staining of leaf samples for light microscopy, utilized for conidial adhesion and germ tube tests of *P. arachidicola* and *N. personata*.

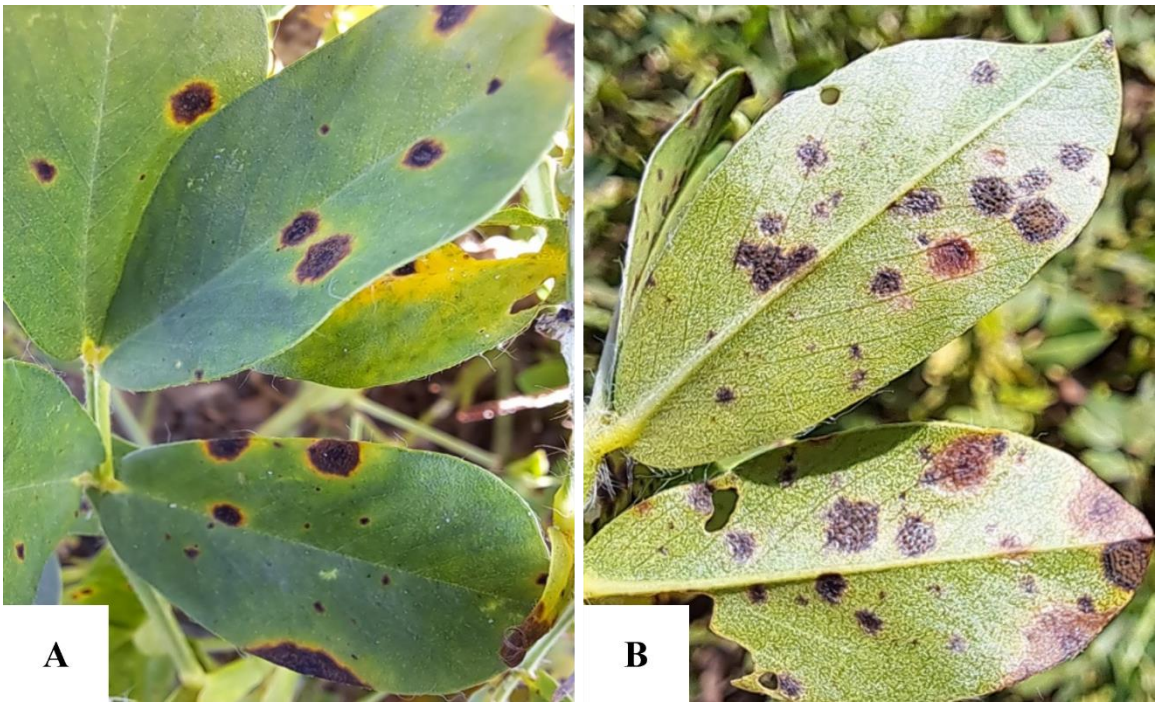


Figure 2. Field sporulation of peanut leaf spot pathogens.
A. *Passalora arachidicola* sporulates on the adaxial (upper) leaf surface.
B. *Nothopassalora personata* sporulates in the abaxial (lower) leaf surface.

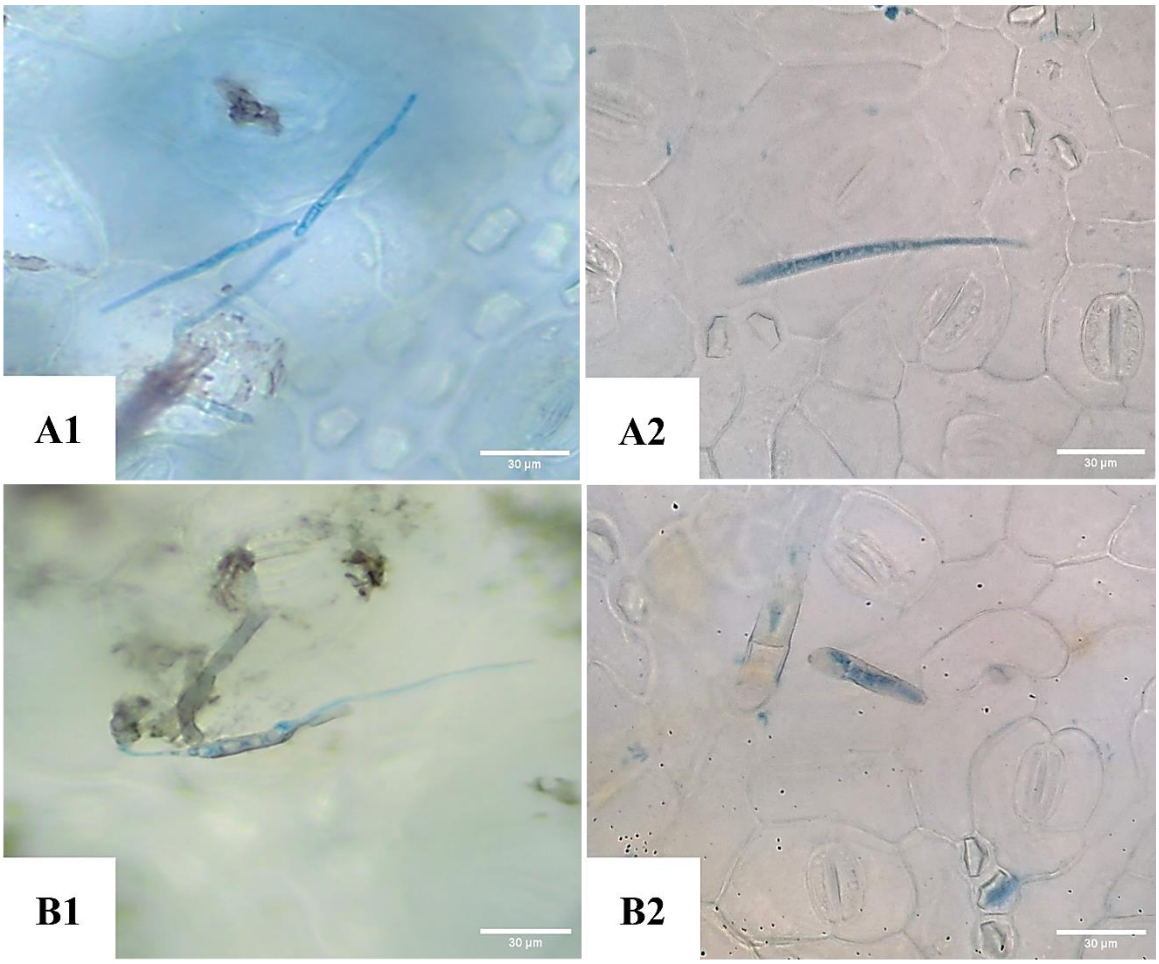


Figure 3. Differences in conidia of *P. arachidicola* (PA) and *N. personata* (NP) observed 48 hours after inoculation (HAI) under a light microscope at 400X magnification with a 30µm scale.
A and B. PA conidia are longer and thinner than NP conidia.
A. PA conidia in the susceptible genotype Georgia-06G (A1) typically show higher conidial counts compared to resistant genotype IAC 322 (A2).
B. NP conidia in the susceptible genotype Georgia-06G (B1) typically show higher conidial counts and more germinating conidia compared to resistant genotype IAC 322 (B2).

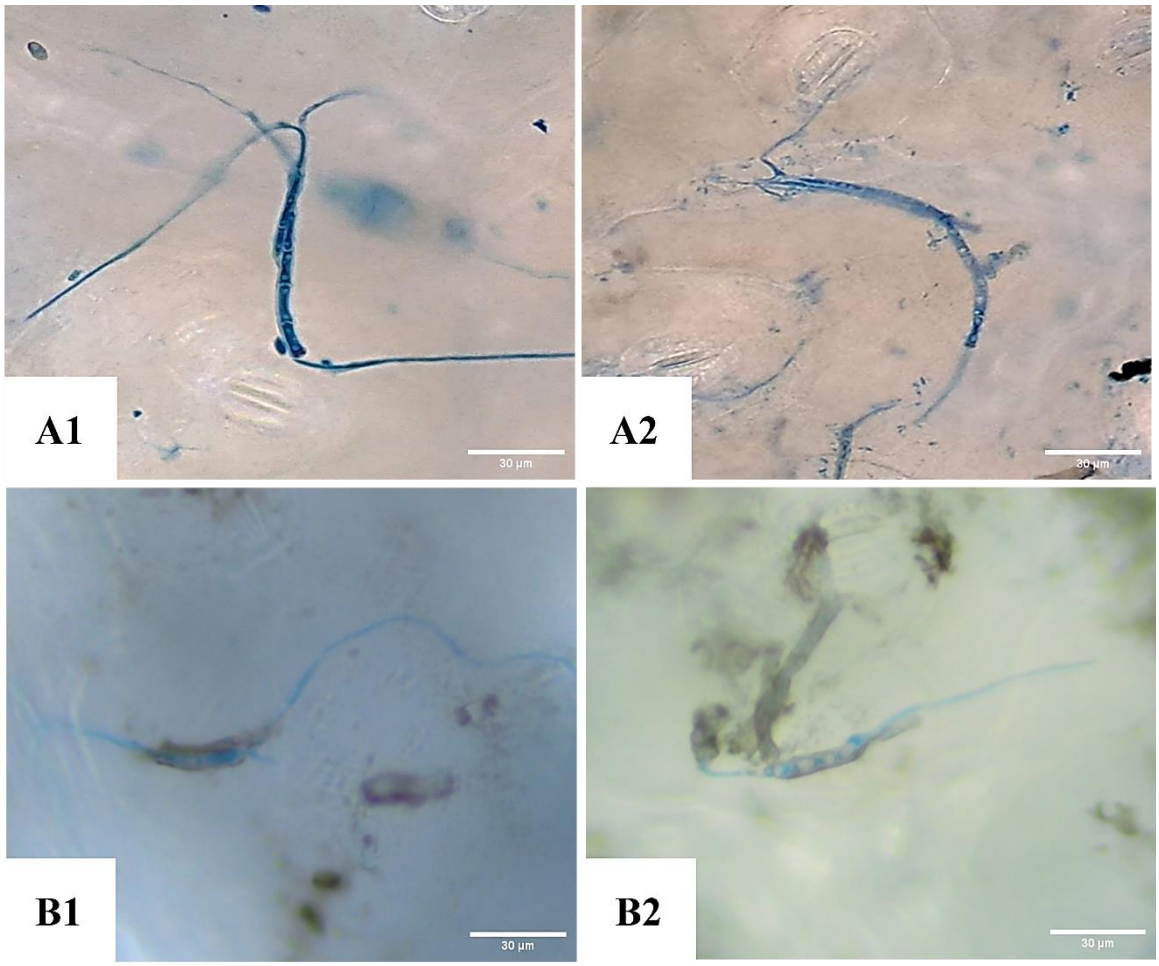


Figure 4. Differences in conidial germination of *P. arachidicola* (PA) and *N. personata* (NP) observed 48 hours after inoculation (HAI) under a light microscope at 400X magnification with a 30µm scale.
A. PA conidial germination in the susceptible genotype Georgia-06G (A1) typically show longer germ tube length compared to resistant genotype IAC 322 (A2).
B. NP conidial germination in the susceptible genotype Georgia-06G (B1) typically show longer germ tube length compared to resistant genotype IAC 322 (B2).

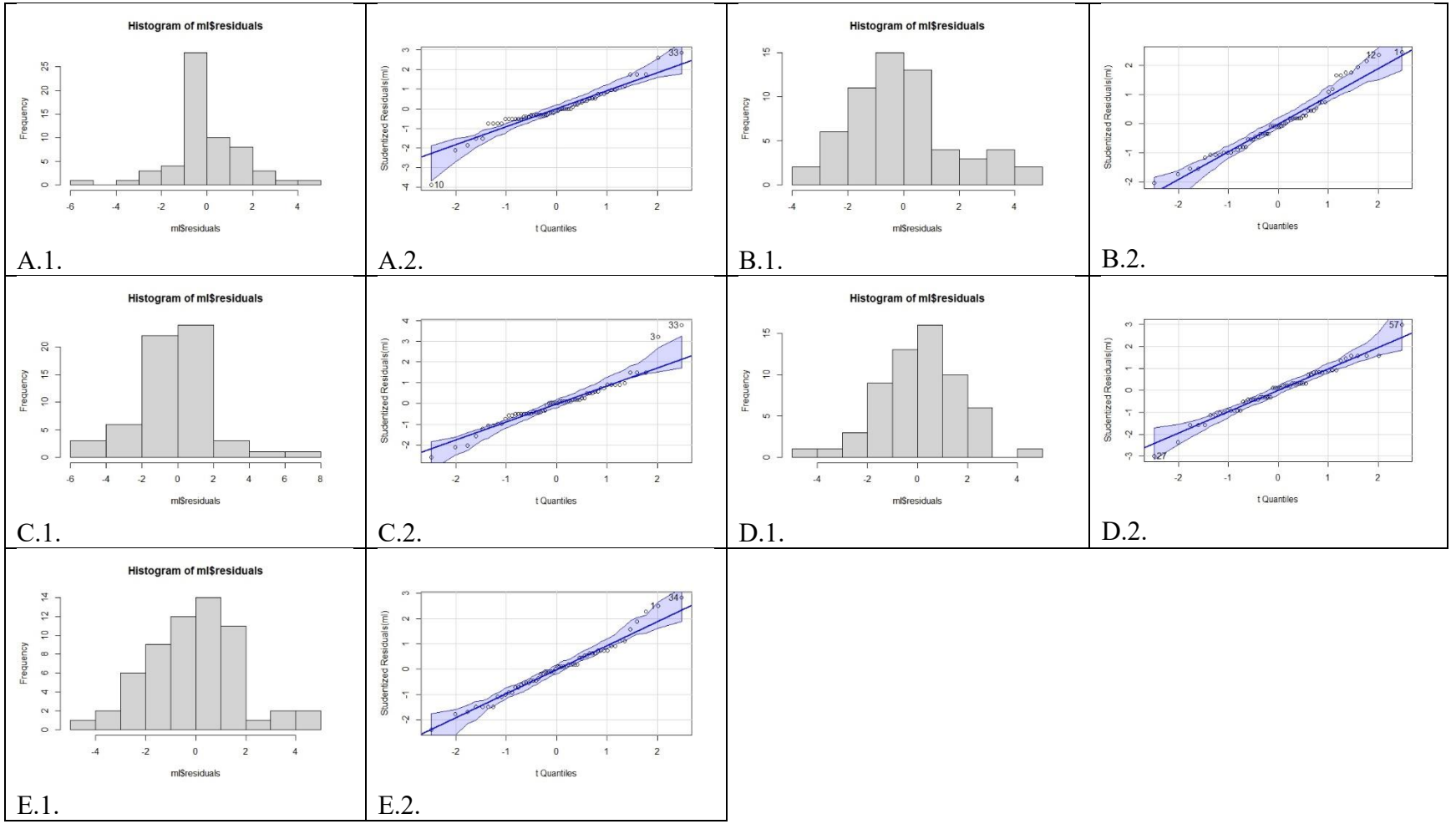


Figure 5.1. Residual plots for *P. arachidicola* and *N. personata* conidial adhesion at different observation time from duplicated trials. The plots include histogram and Q-Q plots to assess data distribution and fit for linear regression analysis.
A, B and C. Residual plots for PA conidial adhesion at 12, 48 and 96HAI.
D and E. Residual plots for NP conidial adhesion at 48 and 96HAI.

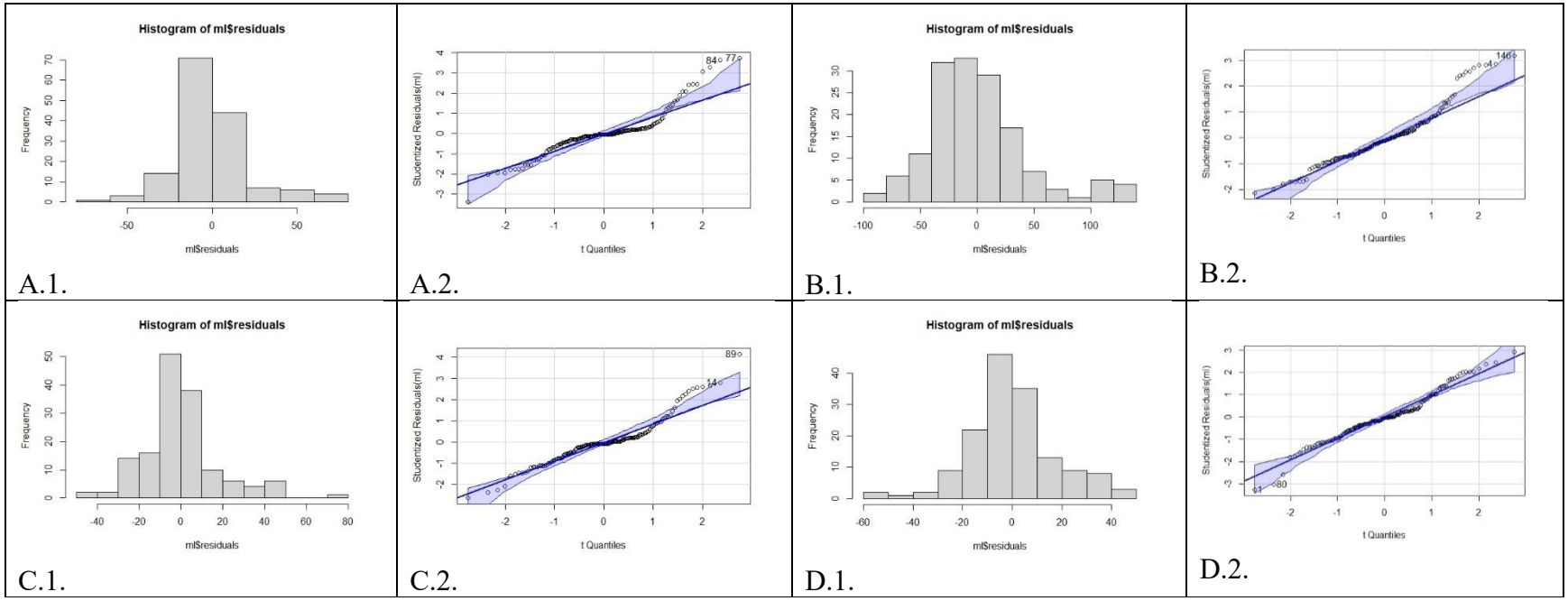


Figure 5.2. Residual plots for *P. arachidicola* and *N. personata* conidial germ tube length at different observation time from duplicated trials. The plots include histogram and Q-Q plots to assess data distribution and fit for linear regression analysis.
A and B. Residual plots for PA conidial germ tube length at 48 and 96HAI.
C and D. Residual plots for NP conidial germ tube length at 48 and 96HAI.

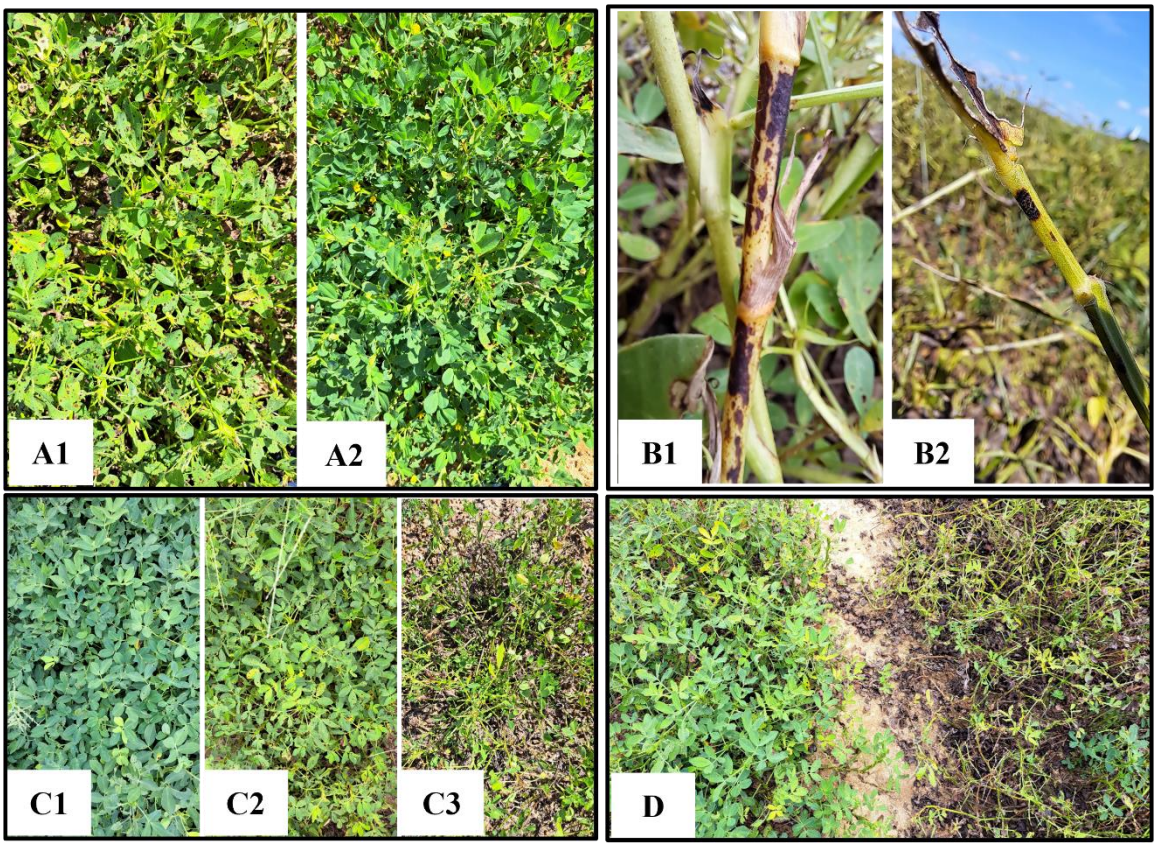


Figure 6.1. Field components evaluated for peanut leaf spot disease.
A. Lesion incidence (LI) assessed throughout the canopy, with A1 representing 90% LI and A2 representing 10% LI.
B. Stem lesion severity (STL) evaluated on 10 stems, with B1 showing 50% STL and B2 showing 15% STL.
C. Disease severity based on the Florida 1-10 scale (FS) assessed in each plot, with C1 rated as 3, C2 as 5, and C3 as 8.
D. Levels of defoliation evaluated in each plot, with the left side showing a rating of 2 and the right side showing a rating of 5.

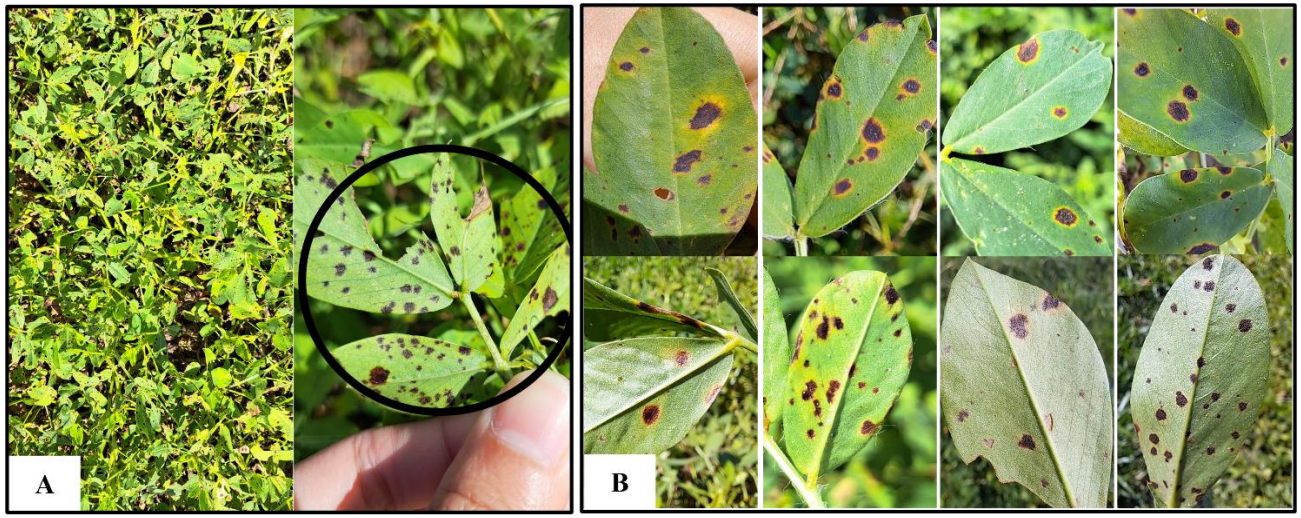
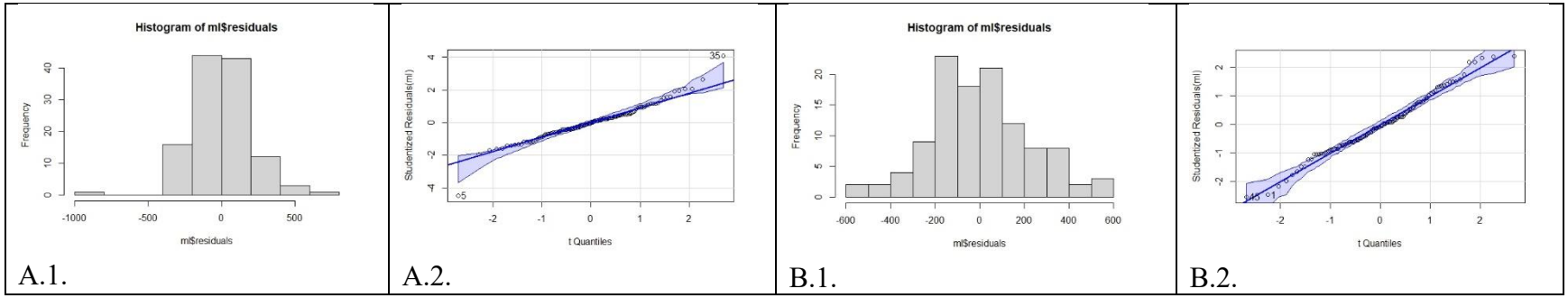


Figure 6.2. Field components evaluated for early and late leaf spot based on sporulation.
A. Sporulating lesion incidence (SLI) assessed throughout the canopy, with close inspection of sporulation. The left image represents a rating of 5.
B. Sporulating degree (SD) evaluated on 10 leaflets by closely inspecting sporulating lesions, with ratings from left to right as 0, 1, 2, and 3.



A.1. A.2. B.1. B.2.

Figure 7.1. Residual plots for lesion incidence data from field trials conducted in 2022 and 2023. The plots include histogram and Q-Q plots to assess data distribution and fit for linear regression analysis.
A: Residual plots for lesion incidence from the 2022 field trials. B: Residual plots for lesion incidence from the 2023 field trials.

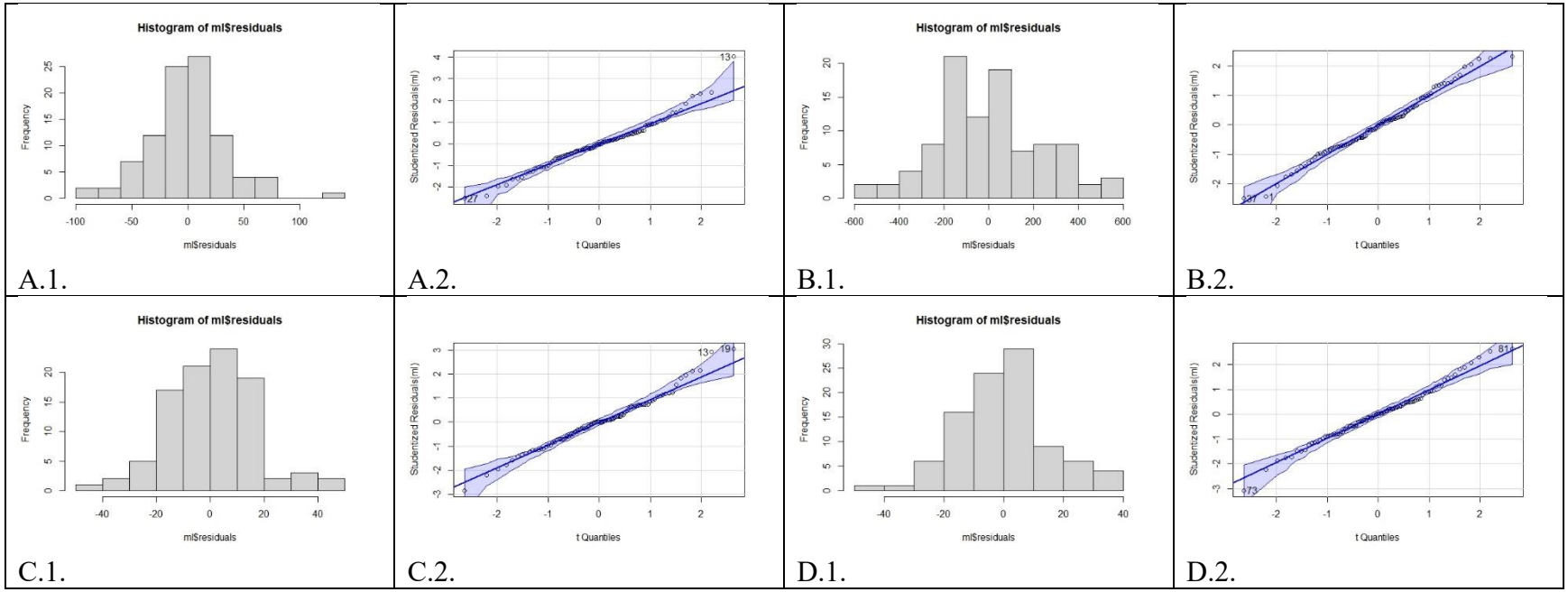


Figure 7.2. Residual plots for all ratings of leaf spot (LS) disease parameters from the field trials conducted in 2023. The plots include histogram and Q-Q plots to assess data distribution and fit for linear regression analysis.
A: Residual plots for Florida 1-10 scale. B: Residual plots for lesion incidence.
C: Residual plots for levels of defoliation. D: Residual plots for stem lesion severity.

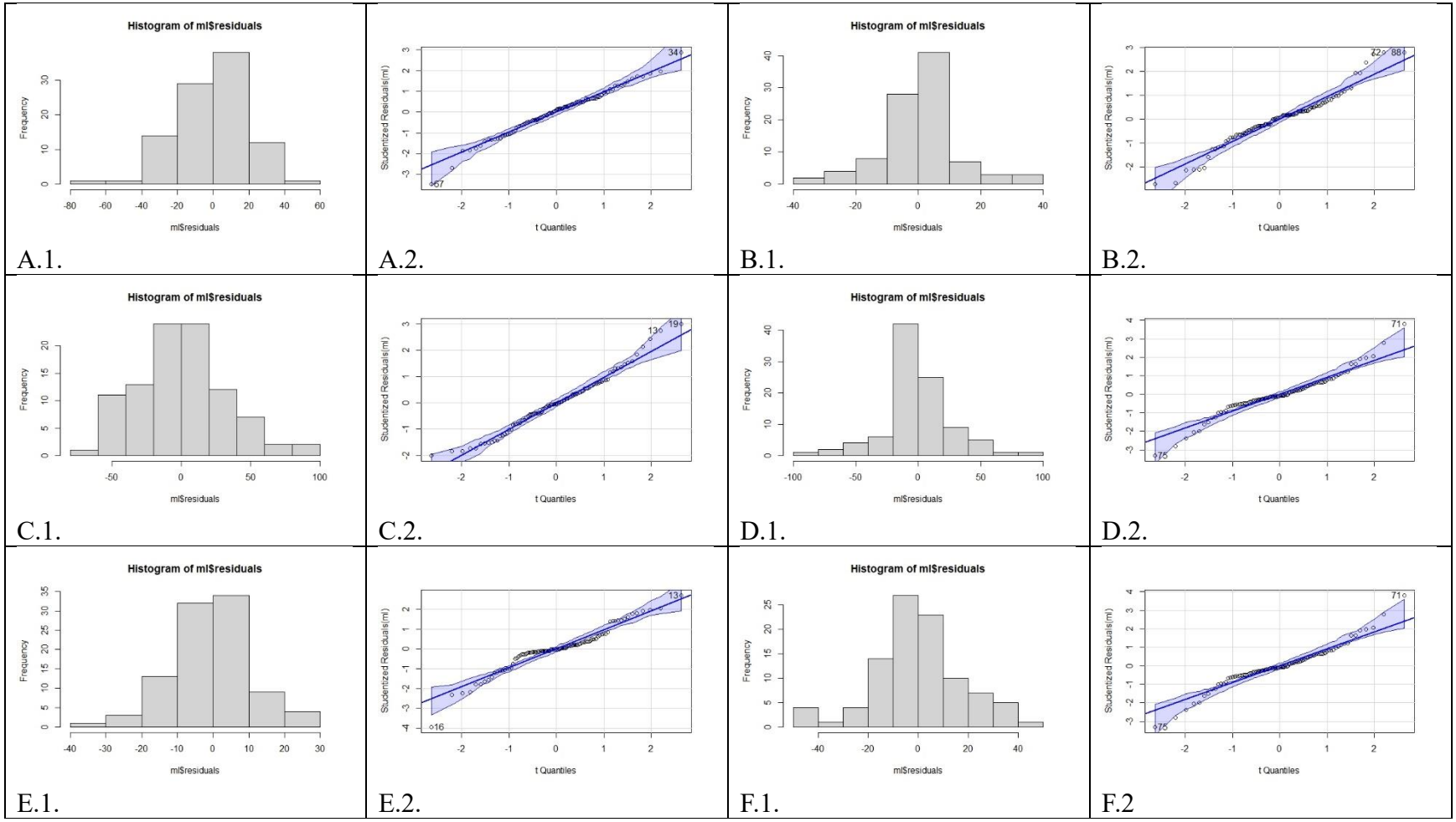


Figure 7.3. Residual plots for all ratings of early leaf spot (ELS) and late leaf spot (LLS) disease parameters from the field trials conducted in 2023. The plots include histogram and Q-Q plots to assess data distribution and fit for linear regression analysis. A and B Residual plots for ELS and LLS days to first sporulating lesions. C and D: Residual plots for ELS and LLS sporulating lesion incidence. E and F: Residual plots for ELS and LLS sporulation degree.