Comparing *Nothopassalora personata* Pre-Infection Development, Colonization, and Haustorial Characteristics Between Susceptible and Resistant Peanut Genotypes

A Thesis submitted
To the Graduate School
Valdosta State University

in partial fulfillment of requirements
for the degree of

MASTER OF SCIENCE

in Biology

in the Department of Biology
of the College of Science & Mathematics

June 2023

Damion Anthony Castellano

BS, Valdosta State University, 2019

This thesis, "Comparing Nothopassalora personata Pre-Infection Development, Colonization, And Haustorial Characteristics Between Susceptible and Resistant Peanut Genotypes" by Damion Anthony Castellano, is approved by:

Dissertation Committee Chair
Professor of Biology

Emily G. Cantonwine. Ph.D.

Committee Member

James A. Nienow, Ph.D.
Professor of Biology

Corley Holbrook, Ph.D.
Supervisory research geneticist

Associate Provost for Graduate Studies and Research

Becky K. da Cruz. Ph.D., J.D.
Professor of Criminal Justice

Defense Date

April 7th 2023

FAIR USE

DUPLICATION

I authorize the Head of Interlibrary Loan or the Head of Archives at the Odum Library at Valdosta State University to arrange for duplication of this thesis for educational or scholarly purposes when so requested by a library user. The duplication shall be at the user's expense.

Signature 

[refuse permission for this thesis to be duplicated in whole or in part.

Signature _____

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ACKNOWLEDGEMENTS

I would like to express my deep gratitude and thanks to everyone that has helped me get to this point. To my committee chair Dr. Emily Cantonwine, thank you for your endless patience and support. Thank you for helping me grow into the person I am today, and most importantly thank you for always believing in me. Thank you Dr. James Nienow for your willingness to always answer my questions and show me new techniques and equipment. Thank you Dr. Corley Holbrook for your interest in my project and our conversations about plant science.

Thanks to the wonderful faculty at VSU for your continued support and kindness. Thank you Dr. Doscher for your guidance in professional development.

I will always be grateful for the lifelong friends I've made on this journey. Thank you Joshua Brown for always being there for me, for making sure I never had to stress about finances or having a place to stay, and for putting up with me as a roommate. Thank you Andrew Harvin and Kenyanna Taylor for your support in the field and our shared experiences in the lab. Thank you Blake Lamb and Chris Le for sending job leads and help in proofreading. Thanks to Yasmin Guzman, Amber Holley, Brylie Ritchie, Daniel Rincon Diaz, Thomas Wilson, Miracle Mgbeahuruike, Darshi Patel, and Jack Mohanty.

Special thanks to my girlfriend April. Thank you for keeping me in the lab and for loving me through the hard times.

Finally, I wish to thank my family for your unconditional love and for your constant encouragement. Thank you Mom for all your support, I could not have done this without you. Thank you Billy for teaching me important life lessons, and for instilling me with the dedication to follow this path to the end. And thank you both for your efforts in reducing my stress and making sure I could focus on school. Thanks Marissa for being a great sister and friend, and thanks Grandpa for always having my back.

Chapter I

LITERATURE REVIEW

Peanuts (*Arachis hypogea* L.) are the 12th most economically impactful crop grown in the United States (Toomer 2017). In 2021, 305,538 hectares of peanuts were planted, and 4,990 kilograms per hectare were harvested for a total of $744,263,000 in revenue (Anonymous 2022). In addition to being economically important, the nutritional value of peanuts is quite high. They are about 30% protein, and hold a lot of calories in a small pod due to being over 50% oils and fats (Toomer 2017). With such high fat content, one would think the peanut quite unhealthy; however, certain types of cancer and heart disease have been shown to be reduced when peanuts are a regular part of the diet (Toomer 2017).

Late leaf spot and early leaf spot are foliar diseases that are caused by the ascomycetes *Nothopassalora personata* (Berk. & M.A. Curtis) U. Braun, C. Nakash., Videira and Crous and *Passalora arachidicola* (Hori) U. Braun respectively. Early leaf spot disease produces a reddish-brown lesion, typically surrounded by a yellow ring. Late leaf spot disease produces a chocolate brown lesion, and usually does not have a yellow ring, but when present, the yellow ring is typically less pronounced ring of early leaf spot disease. The first infections come from ascospores or conidia originating in field soils on infected leaf litter of previous crops. Primary inoculations typically occur when it rains, where the spores are splashed from the leaf litter to the surface of leaves. Under continued

moist conditions at 85% humidity or above, and temperatures between 15 and 25°C (Alderman and Nutter 1994), spores germinate to produce one or more germ tubes that can penetrate stomata 3-5 days after inoculation (Johnson and Cantonwine 2013). For late leaf spot, incubation periods, the time before symptoms first appear, can be as short as 9-10 days after inoculation (Dwivedi et al 2002; Lamon et al 2021), or as late as 15 days (Pande et al 2002). Latent periods, or the time between infection and sporulation, are 13-39 days after inoculation depending on host and environment (Wadia and Butler 1994). Similar environmental conditions, incubation periods and latent periods have been reported for early leaf spot (Cantonwine et al 2008). In the field, once sporulation occurs, so does secondary infection, as the asexual conidia are easily disseminated by splashing water, winds, or insects, initiating new infections if environmental conditions are optimal. Both early and late leaf spot cause defoliation, which reduces the plant's ability to make the energy it needs for growth, survival, and reproduction. When left untreated, leaf spot diseases can decrease total yield by 65-70% (Anco et al 2020). In 2019, foliar leaf spot diseases caused 9.95 million dollars in damages (Kemerait and Little 2019)

In 2019, costs of leaf spot control reached 34.1 million dollars (Kemerait and Little 2019). Fungicide regimens to control for leaf spot disease incur the highest costs of peanut management, because six to eight sprays are needed throughout the growing season (Woodward et al. 2014). In addition to the economic expense of fungicides, there are other concerns of fungicide use, including non-target toxicity concerns within the environment and fungicide

resistance of target and non-target fungi. Because most systemic fungicides use a single mode of action, it is recommended to use multiple types of fungicides in conjunction with one another (Culbreath et al 2002), to reduce the likelihood of pathogen populations developing resistance to these modes of action (Ktiller and Scheinpflug 1987).

Planting cultivars of peanut with genetic resistance to one or more of the leaf spot pathogens can allow similar disease management levels with 2 to 3 fewer fungicide applications (Cantonwine et al. 2006). Genetic resistance in peanut is partial, meaning instead of immunity to the pathogen, there is reduced severity. One reason for this is the fact that many traits such as resistance, environmental stress tolerance, production, and yield are controlled by polygenes, also known as quantitative traits (Sehgal et al. 2016). Quantitative trait loci (QTL) are regions of the genome that contain polygenes and contribute to the magnitude of a quantitative trait, also referred to as continuous, trait that is often influenced by a number of different genes. These genes are difficult to identify because the phenotype is not a complete reflection of a gene, only a partial one. (Seghal et al. 2016).

QTL mapping uses molecular markers to link phenotypes of interest to a physical location on the genome (Lamon et al 2021) based on variations within experimentally crossed plant populations (Clevenger et al. 2018). Two common molecular markers are Simple Sequence Repeats, also known as SSRs or microsatellites, and Single Nucleotide Polymorphisms, or SNPs. Using the FAIR guiding principles of making data “findable, accessible, interoperable, and

reusable", finding markers is easier than ever (Wilkinson et al 2016). Even so, peanut studies have far less available markers than other crops (Chu et al. 2016). The importance of QTL mapping in peanut was demonstrated by Chu et al. (2019) when QTLs for ELS disease resistance on chromosome 3, and QTLs for LLS resistance on chromosome 5, consistent in genotypes from *A. cardenasii,* were identified. Mapped QTLs allowed for the identification of candidate genes related to leaf spot disease regulation in two other peanut genomes, *A. duranensis* and *A. ipaensis*. (Chu et al. 2019).

Although early and late leaf spot symptoms are similar, the infection strategies of *P. arachidicola* and *N. personata* within host tissues differ. While both pathogens produce cercosporin-like molecules, which are toxins that break down lipids and cause cell death in plant tissues (Jenkins 1938; You et al 2008), only *N. personata* produces haustoria. Haustoria are specialized hyphae that penetrate plant cell walls to extract amino acids and sugars from plants through extra-haustorial membranes (Szabo and Bushnell 2001). Haustoria occur in many obligate biotrophs of the phyla ascomycota, basidiomycota, and oomycota. This wide range of phyla suggests that haustoria may have evolved more than once, and that they may act differently in different hosts (Voegele and Mendgen 2003). As a hemibiotroph, *N. personata* is thought to utilize haustoria to feed off living tissue during its biotrophic phase, ending its life as a necrotroph, feeding off dead tissues. Though there is little evidence, it has been suggested that haustoria can "induce susceptibility" and allow for easier secondary infections (Voegele and Mendgen 2003). This could give *N. personata* a competitive edge

against *P. arachidicola* when competing for space to grow, or contribute to the explosive epidemic rate of progression that is common for late leaf spot (Fulmer et al. 2023). After major hyphal colonization, it is suggested that *N. personata* begins to release cercosporin-like phytotoxins creating lesions on the leaf and feeding off the dead tissue. This is in contrast with *P. arachidicola*, which is presumed to release phytotoxins during hyphal colonization (Mims et al 1989).

The haustoria of *N. personata* are highly branched tree-like structures that allow for a large amount of surface area for nutrient absorption (Mims et al 1989). To my knowledge, no other cercosporoid fungi have been confirmed to produce true haustoria. In 1934, *Cercospora cruenta*, a pathogen that infects cowpea, was reported to produce small, unbranched haustoria (Latham 1934), but the pathogen was later described without the presence of haustoria (Miles et al 2009). *Cercosporella virgaureae* has recently been documented to have an intracellular structure of strongly lobed hyphae resembling an appressorium (Kirschner 2009). It is unknown whether or not these “appresoria" are utilized in nutrient uptake, but their highly branched structure is reminiscent of the haustoria of *N. personata* (Kirschner 2009). The appressoria do not create an invagination in the cell membrane of the plant, and simply rest on the cell membrane with no discernable extra-haustorial matrix. Another key difference is that they do not create a branched tree-like structure, but rather a lobed cup-like structure (Kirschner 2009).

Phenotyping resistance to the leaf spot pathogens has focused almost entirely on factors that occur after infection. Few studies have tested the ability of

peanut to resist the pathogen prior to, or during infection. In 1974, Abdou et al. demonstrated that resistance to late leaf spot could be displayed before infection by monitoring germination of conidia and seeing whether or not germ tubes were attracted to stomata. Since then, not many similar studies have been performed. To our knowledge, there are no published studies that evaluate resistance to the leaf spot pathogens during the infection process. Examples of this would include disruption of hyphal colonizing habits and other factors that prevent tissue colonization of the fungus.

Chapter II

CHARACTERIZING NOTHOPASSALORA PERSONATA COLONIZATION PATTERNS ACROSS SUSCEPTIBLE AND RESISTANT PEANUT GENOTYPES

Introduction

Even before the discovery of DNA's structure in 1953, plant breeders backcrossed disease resistant plants with plants having desirable agricultural traits to decrease yield loss due to disease (Schumann and D'arcy 2010). Disease resistant plant varieties are one of the most environmentally safe and convenient ways to fight plant disease (Bent 1996). Components of resistance that are most often analyzed are disease incidence, disease severity, and yield impact (Chiteka et al. 1988). While these resistance components are generalized and highly important information for growers, a better understanding of the mechanisms of resistance can help breeders develop cultivars with enhanced resistance.

Nothopassalora personata (Berk. & M.A. Curtis) U. Braun, C. Nakash., Videira and Crous is the fungal causative agent of late leaf spot disease, which causes small necrotic leaf spots and premature defoliation in peanut (*Arachis hypogea* L). *N. personata* is a hemibiotroph, which means that during the early stage of infection, the fungus acts as a biotroph, acquiring nutrients from healthy plant tissue, and is capable of feeding on dead tissue as a necrotroph during

later stages of infection. *N. personata* is able to feed on healthy cells with specialized hyphae called haustoria.

The haustoria of *N. personata* are not as extensively studied as the haustoria of other plant pathogens such as *Puccinia graminis*. As a biotroph, haustoria play a key role in the infection cycle of *P. graminis* by suppressing hypersensitive responses in the plant (Leonard and Szabo 2005). The haustoria of *P. graminis* are also responsible for most of the amino acid uptake of the pathogen, as the pathogen synthesizes very little (Leonard and Szabo 2005). Most of the first cells the *P. graminis* infects with haustoria are epidermal cells (Leonard and Szabo 2005).

Plants resistant to rust (*Puccinia graminis*) have been shown to possess multiple defense mechanisms to prevent haustorial development, or quickly kill the cell in response to haustorial infection. In resistant plants, rust species have been shown to elicit a hypersensitive response and cause rapid cell death with the introduction of a pathogen. This is not the case in susceptible plants (Heath 1997). While peanut leaves infected with *N. personata* do not elicit a hypersensitive response, even in resistant cultivars, perhaps there are other similarities between the interaction between haustoria and plant cells. Since haustoria in rust have been shown to suppress the hypersensitive responses in the plant (Leonard and Szabo 2005), perhaps the same is true for *N. personata*.

Most of what is known about how *N. personata* colonizes peanut tissues is based on work by Jenkins and Woodruff in the 1930s (Jenkins 1938; Woodroof 1933). Jenkins used thin microtome sections of leaves to diagram highly

branched haustoria in mesophyll cells of leaves. In 1989, Mims viewed haustoria extending through cell walls in scanning electron micrographs and determined that haustoria were present in all leaf cells except xylem and phloem cells, with highest frequencies in epidermal and spongy mesophyll cells. It is unclear what role haustoria play in the infection process of *N. personata,* or how long the pathogen utilizes haustoria, though it is suggested that haustoria may continue to absorb nutrients from dead plant tissues (Mims et al. 1989). The purpose of this study is to help elucidate how the pathogenic mycelium spreads throughout the leaf tissue, and when and where haustoria form.

Materials and Methods

Experimental design

The genotypes evaluated in this experiment were GA-13M, a variety susceptible to *N. personata*, and three resistant genotypes, CB18, CB7, and CS195. CS195 was derived from the cross of GA-13M and TifGP-3 (Holbrook et al. 2022). CB7 and CB18 were derived from the same cross and were back-crossed with GA13M an additional time. TifGP-3 was derived from the cross of TifNV-High O/L and IAC 322. TifNV-High O/L contains an introgression from *A. cardenasii* on chromosome A09 that confers nematode resistance. TifNV-High O/L also has resistance to tomato spotted wilt virus (Holbrook et al. 2017). IAC 322 is a breeding line which contains three introgressions from *A. cardenasii* on the A02 and A03 chromosomes shown to contribute to leaf spot resistance (Lamon et al. 2021).

Sampling took place at three different field sites on October 05, 2021. Field site one was the multistate peanut test at the UGA Coastal Plains Experimental Station Blackshank farm. Site one was planted on May 20, and plants received a fungicide regimen of Chlorothalonil (720 g/L) applied at 1.5 pt/A on July 22, August 07, August 19 and September 03, 2021. Field sites two and three were located at the UGA Coastal Plains Experimental Station Gibbs farm. Site two was planted on June 03 and sprayed minimally, receiving one application of Convoy at 60 DAP. Site three was planted on June 02 and was and was not sprayed with fungicide. All three fields were planted into randomized blocks with three replications. In each sampled plot, several leaves, selected arbitrarily from the upper canopies, were cut at the petiole and wrapped in damp cotton before being placed in an iced cooler for transit. Leaves were stored at 20ºC for 1 to 3 days before sectioning.

Sectioning, clearing and staining

For each sampled plot, a 3 mm wide and a 1 mm wide lesion were excised from leaves using a razor blade, making sure to include at least 3 mm of healthy tissue surrounding each lesion. The smaller 1 mm lesions were not taken from leaves at field site 1 due to a large amount of early leaf spot (*Passalora arachidicola* (Hori) U. Braun), which is difficult to distinguish from late leaf spot at that size. All 3 mm lesions presented a similarly narrow chlorotic ring around the necrotic tissue and were sporulating. The 1 mm lesions lacked chlorotic rings.

Leaf sections were immediately submerged in 10% KOH for one hour to remove the waxy coating on the cuticle, following the Visikol website

suggestions. Tissues were then completely submerged in Visikol® for Plant Biology™ (Visikol, Inc. 53 Frontage Road, Suite 120, Shelbourne Building, Hampton, NJ 08827) overnight at 37°C for optical clearing. If clearing was insufficient, the tissue was submerged in new Visikol for another night at 37°C. The cleared tissue was then submerged in Lactophenol Cotton Blue stain for 30 minutes in a hot bath set at 37°C. The tissue underwent two 1-minute water washes before being stored at room temperature in water. Staining and destaining was repeated on the 3 mm lesions just prior to observation. Wet mount slides were prepared in water with the abaxial side of the lesions facing up.

Data Collection

Under a light microscope (Olympus model CH30RF) the true necrotic diameter of the lesion was measured, and sporulation was assessed using a 0-5 scale where 0 is no sporulation, 1 is one to three groups of sporodochia, 2 is five to ten groups of sporodochia, 3 is a large number of sporodochia with large spaces in between, 4 is a large number of sporodochia with very little spacing in between, and 5 is the entire lesion covered with sporodochia. The width of the chlorotic ring, appearing as a band of discoloration along the necrotic margins, was measured for the 3 mm lesions (Figure 2.1). Necrotic tissues were the areas where sporulation occurred and where hyphae were densely packed. Assessments were made at four evenly distributed subsampled locations around each lesion on the abaxial side. Observations were limited to the epidermal tissue layer that included the stomatal guard cells, as well as the spongy

mesophyll layer directly beneath the epidermal tissue layer. The distance of the farthest haustoria and the distance of the farthest colonizing hyphae were measured from the necrotic edge. The density of haustoria was also measured by counting the number of haustoria visible in a 400X field of view, $1.6x10^5$ μm^2 area, arbitrarily selected beyond the necrotic edge, and for the 3 mm lesions, within a discolored region presumed to be the chlorotic ring. The diameters of the largest haustoria and the cell it occupied were measured with an ocular micrometer to calculate the linear proportion of the cell occupied by haustoria. Similar proportion calculations were done for hyphal distance by haustorial distance, and for the 3 mm lesions, hyphal distance by chlorotic ring width, and haustorial distance by chlorotic ring width.

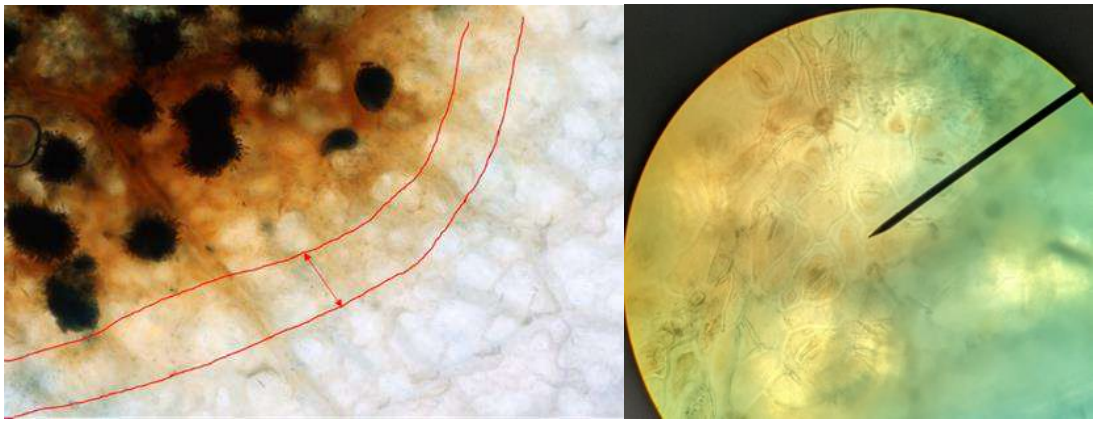

Figure 2.1: Chlorotic ring width at 100X, between the red lines (a) and at 400X (b).

Statistical analysis

Descriptive statistics for lesion size, spore rank and chlorotic ring widths, when applicable, were conducted by genotype, lesion size and field site. The effects of genotype, lesion size, and field site on dependent variables were tested using GLM Univariate analyses of variance (UNIANOVA). Factors were analyzed separately when factor interactions were significant ($P>0.05$). Tukey's Honest Significant Difference tests were used to assess which factor levels were significantly different. Correlations among variables were tested using Pearson Correlation analysis (Tables 2.6 and 2.7). Sporulation rank was included as a covariate for variables with significant correlation ($P>0.05$). All Statistical analyses were performed with SPSS (version 26).

Results

Characterization of diseased lesions.

Lesions with an estimated size of 1 mm were 1.00 mm to 1.10 mm in actual size (Table 2.1), while lesions with an estimated size of 3 mm were 2.88 mm to 2.99 mm in actual size (Table 2.3). 1 mm lesions had a mean spore rank of 1-2, or one to ten groups of sporodochia (Table 2.2). 3 mm lesions had a mean spore rank of 2-5, or five to ten groups of sporodochia to the entire lesion being covered in sporodochia (Table 2.4). The mean width of chlorotic rings were between 190 µm and 238 µm (Table 2.5).

Table 2.1: True lesion size for the 1 mm lesions by genotype.

	Field site 2[a]		Field site 3[b]	
	Mean (mm)	SD	Mean (mm)	SD
GA13M	1.06	0.036	1.05	0.067
CS195	1.10	0.025	1.00	0.050
CB7	1.03	0.025	1.08	0.040
CB18	1.06	0.017	1.06	0.074

[a] Field 2, located at Gibbs farm, received one application of Convoy at 60 DAP.
[b] Field 3, located at Gibbs farm, received no fungicide treatment.

Table 2.2: Mean spore rank for the 1 mm lesions by genotype.

	Field site 2[a]	Field site 3[b]
	Mean	Mean
GA13M	1.0	1.7
CS195	1.0	1.7
CB7	1.0	1.0
CB18	2.0	1.0
P value	0.692	0.488

[a] Field 2, located at Gibbs farm, received one application of Convoy at 60 DAP
[b] Field 3, located at Gibbs farm, received no fungicide treatment

Table 2.3: True lesion size for the 3 mm lesions by genotype.

	Field Site 1[a]		Field site 2[b]		Field site 3[c]	
	Mean (mm)	SD	Mean (mm)	SD	Mean (mm)	SD
GA13M	2.93	0.108	2.96	0.096	2.99	0.577
CS195	2.96	0.066	2.89	0.090	2.96	0.076
CB7	2.98	0.100	2.93	0.064	2.96	0.139
CB18	2.97	0.076	2.88	0.068	2.93	0.110

[a] Field 1, planted at Blackshank farm, received a fungicide regimen of Chlorothalonil (720 g/L) applied at 1.5 pt/A on July 22, August 07, August 19 and September 03
[b] Field 2, located at Gibbs farm, received one application of Convoy at 60 DAP
[c] Field 3, located at Gibbs farm, received no fungicide treatment

Table 2.4: Mean spore rankings of the 3 mm lesions by genotype.

	Field Site 1[a]	Field site 2[b]	Field site 3[c]
	Mean	Mean	Mean
GA13M	4.3	4.7	4.3
CS195	2.0	3.0	5.0
CB7	3.0	5.0	2.7
CB18	3.7	3.3	3.7
P value	0.068	0.185	0.164

[a] Field 1, planted at Blackshank farm, received a fungicide regimen of Chlorothalonil (720 g/L) applied at 1.5 pt/A on July 22, August 07, August 19 and September 03
[b] Field 2, located at Gibbs farm, received one application of Convoy at 60 DAP
[c] Field 3, located at Gibbs farm, received no fungicide treatment

Table 2.5: Mean widths of the chlorotic ring by genotype.

	Field Site 1[a]		Field site 2[b]		Field site 3[c]	
	Mean (µm)	SD	Mean (µm)	SD	Mean (µm)	SD
GA13M	235	41.16	223	12.50	238	1.443
CS195	226	34.67	190	27.04	230	4.330
CB7	229	5.774	230	5.000	222	6.292
CB18	226	2.887	230	9.014	217	13.77

[a] Field 1, planted at Blackshank farm, received a fungicide regimen of Chlorothalonil (720 g/L) applied at 1.5 pt/A on July 22, August 07, August 19 and September 03
[b] Field 2, located at Gibbs farm, received one application of Convoy at 60 DAP
[c] Field 3, located at Gibbs farm, received no fungicide treatment

Table 2.6: Pearson correlation test for 1mm lesions.

		Spore Rank	Mycelium Distance	Haustoria Distance	Haustoria Density	Cell Diameter	Haustoria Diameter
Spore rank [a]	r	---	0.243	0.171	0.304	**-0.468**	0.202
	P-Value	---	0.253	0.424	0.149	0.021	0.344
Mycelium Distance [b]	r	0.243	---	**0.762**	-0.144	-0.177	0.309
	P-Value	0.253	---	>0.005	0.502	0.407	0.142
Haustoria Distance [c]	r	0.171	**0.762**	---	-.0359	-0.291	0.201
	P-Value	0.424	>0.005	---	0.085	0.167	0.345
Haustoria Density [d]	r	0.304	-0.144	-.0359	---	0.171	-0.038
	P-Value	0.149	0.502	0.085	---	0.424	0.861
Cell Diameter [e]	r	**-0.468**	-0.177	-0.291	0.171	---	-0.084
	P-Value	0.021	0.407	0.167	0.424	---	0.698
Haustoria Diameter [f]	r	0.202	0.309	0.201	-0.038	-0.084	---
	P-Value	0.344	0.142	0.345	0.861	0.698	---

Bold text indicates significant correlation.

[a] Spore rank is a 0-5 scale where 0 is no sporulation, 1 is one to three groups of sporodochia, 2 is five to ten groups of sporodochia, 3 is a large number of sporodochia with large spaces in between, 4 is a large number of sporodochia with very little spacing in between, and 5 is the entire lesion covered with sporodochia.

[b] Mycelium distance is the measure of the space between the necrotic edge and the tip of farthest hypha found.

[c] Haustoria distance is the measure of the space between the necrotic edge and the farthest haustoria found.

[d] Haustoria density is the number of haustoria per $1.6x10^5$ μm^2 area.

[e] Cell diameter was taken from epidermal cells containing haustoria. In cells with shapes other than circular, the longest diameter was taken.

[f] Haustoria Diameter was taken from the approximate largest haustoria in a $1.6x10^5$ μm^2 area.

Table 2.7: Pearson correlation test for 3mm lesions.

		Spore Rank	Mycelium Distance	Haustoria Distance	Chlorotic Width	Haustoria Density	Cell Diameter	Haustoria Diameter
Spore Rank [a]	r	---	**0.344**	0.303	0.128	-0.053	-0.327	-0.56
	P-Value	---	0.040	0.072	0.456	0.757	0.052	0.746
Mycelium Distance [b]	r	**0.344**	v	**0.625**	**0.652**	-0.226	-0.266	**-0.375**
	P-Value	0.040	NA	>0.005	>0.005	0.185	0.117	0.024
Haustoria Distance [c]	r	0.303	**0.625**	---	**0.623**	-0.041	-0.010	0.078
	P-Value	0.072	>0.005	---	>0.005	0.810	0.953	0.652
Chlorotic Width [d]	r	0.128	**0.652**	**0.623**	---	-0.154	-0.070	-0.263
	P-Value	0.456	>0.005	>0.005	---	0.369	0.685	0.121
Haustoria Density [e]	r	-0.053	-0.226	-0.041	-0.154	---	0.020	0.014
	P-Value	0.757	0.185	0.810	0.369	---	0.910	0.936
Cell Diameter [f]	r	-0.327	-0.266	-0.010	-0.070	0.020	---	**0.511**
	P-Value	0.052	0.117	0.953	0.685	0.910	---	0.001
Haustoria Diameter [g]	r	-0.56	**-0.375**	0.078	-0.263	0.014	**0.511**	---
	P-Value	0.746	0.024	0.652	0.121	0.936	0.001	---

[a] Spore rank is a 0-5 scale where 0 is no sporulation, 1 is one to three groups of sporodochia, 2 is five to ten groups of sporodochia, 3 is a large number of sporodochia with large spaces in between, 4 is a large number of sporodochia with very little spacing in between, and 5 is the entire lesion covered with sporodochia.
[b] Mycelium distance is the measure of the space between the necrotic edge and the tip of farthest hypha found.
[c] Haustoria distance is the measure of the space between the necrotic edge and the farthest haustoria found.
[d] Chlorotic Distance was the diameter of the band of discoloration along the necrotic margins.
[e] Haustoria density is the number of haustoria per $1.6x10^5$ μm^2 area.
[f] Cell diameter was taken from epidermal cells containing haustoria. In cells with shapes other than circular, the longest diameter was taken.
[g] Haustoria Diameter was taken from the approximate largest haustoria in a $1.6x10^5$ μm^2 area

Characterization of fungal colonization.

Haustoria were deeply lobed and always within the confines of a single cell (Figure 2.2). Haustoria were observed in both the epidermal cells and in spongy mesophyll cells immediately below the epidermis. For the 1 mm lesions, the mean distance of the farthest haustoria was 102 µm (min = 65 µm, max = 120 µm) from the necrotic edge. Although some genotype differences in haustoria distance were observed for the 1 mm lesions, with distances being among the farthest for GA13M at both field sites 2 and 3, genotype differences were not consistent across field sites (Table 2.8). A similar pattern was observed for the linear proportion of the plant cell occupied by haustoria, with haustoria of GA13M taking up numerically or statistically more of the plant cells diameter than one or more of the resistant genotypes, but with inconsistent genotype differences across field sites. (Table 2.8).

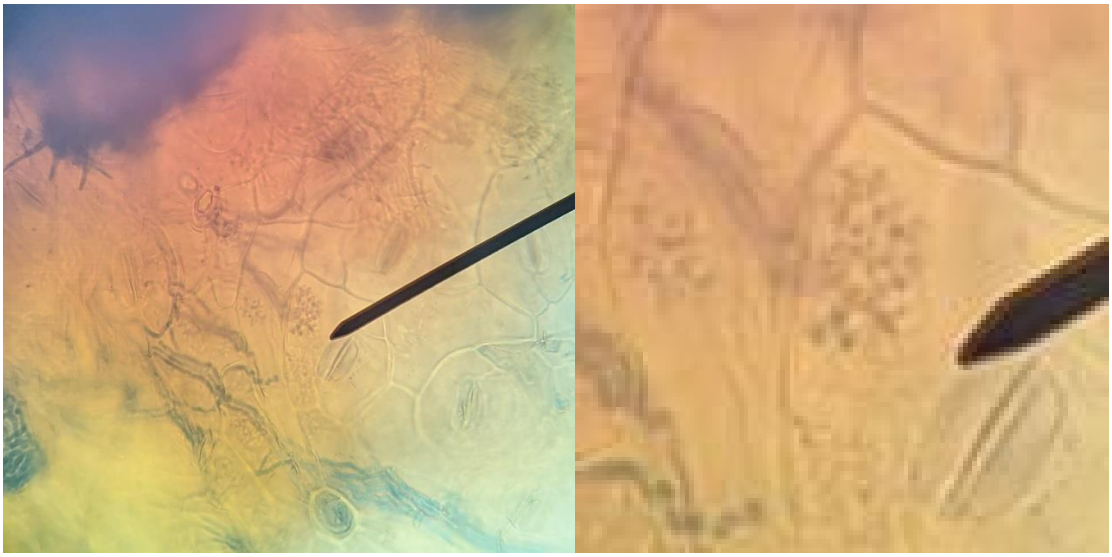

Figure 2.2: Haustoria found just below the epidermal cells.

For the 3 mm lesions, the mean distance of the farthest haustoria was 133 µm (min = 75 µm, max = 164 µm) away from the necrotic edge. Haustoria distance did not differ by genotype (Table 2.9). Mean haustoria distances were 0.58 to 0.61 times the distance of the chlorotic ring for all genotypes (P = 0.947). No haustoria were found beyond the chlorotic ring in the 3 mm lesions. GA13M had significantly fewer haustoria per unit area in the 3 mm lesions than the other genotypes (P=0.026), but no differences were observed for the 1 mm lesions (Tables 2.8 and 2.9). There were significantly higher densities of haustoria in the smaller lesions than the large for all genotypes across all field sites (Figure 2.3; P = 0.011).

Table 2.8: Haustoria characteristics associated with 1 mm diameter lesions.

Genotype	Density[a]	Distance (µm)[b]		Proportion of cell occupied by haustoria[c]	
		Field 2[x]	Field 3[y]	Field 2	Field 3
GA13M	7.6 A	108.9 A	114.2 A	0.48 A	0.44 A
CB18	7.9 A	101.0 AB	111.0 A	0.39 B	0.38 AB
CB7	8.2 A	80.0 B	105.8 AB	0.38 B	0.39 AB
CS195	7.7 A	101.0 AB	96.9 B	0.42 AB	0.35 B
P-Value	0.713	0.028	0.007	0.025	0.046
Error*	0.47	7.70	3.63	0.030	0.027

[a]: Density is defined as number of haustoria per $1.6x10^5$ µm^2 area. The data for haustorial density are for fields 2 and 3 combined. Fields 2 and 3 are minimally sprayed with fungicide and unsprayed respectively.
[b]: Distance is the measure of the space between the necrotic edge and the farthest haustoria found.
[c]: Proportion of cell occupied by haustoria is calculated by dividing the diameter of the haustoria by the diameter of the cell
[x]: Field 2, located at Gibbs farm, received one application of Convoy at 60 DAP
[y]: Field 3, located at Gibbs farm, received no fungicide treatment
*: Standard error of the mean

Table 2.9: Haustoria characteristics associated with 3 mm diameter lesions.

Genotype	Density[a]	Distance (µm)[b]	Proportion of Cell Occupied by Haustoria[c]		
			Field 1[x]	Field 2[y]	Field 3[z]
GA13M	5.3 A	139.9 A	0.40 A	0.40 B	0.41 A
CB18	6.3 B	130.9 A	0.39 A	0.36 A	0.44 A
CB7	6.3 B	132.7 A	0.41 A	0.43 C	0.39 A
CS195	6.4 B	127.8 A	0.37 A	0.44 C	0.46 A
P value	0.026	0.569	0.261	<0.005	0.078
Error*	0.291	8.01	0.023	0.008	0.023

[a]: Density is defined as number of haustoria per $1.6x10^5$ μm^2 area. The data for haustorial density are for all fields combined. Field 1 is heavily sprayed Fields 2 and 3 are minimally sprayed with fungicide and unsprayed respectively.
[b]: Distance is the measure of the space between the necrotic edge and the farthest haustoria found.
[c]: Proportion of cell occupied by haustoria is calculated by dividing the diameter of the haustoria by the diameter of the cell
[x]: Field 1, planted at Blackshank farm, received a fungicide regimen of Chlorothalonil (720 g/L) applied at 1.5 pt/A on July 22, August 07, August 19 and September 03
[y]: Field 2, located at Gibbs farm, received one application of Convoy at 60 DAP
[z]: Field 3, located at Gibbs farm, received no fungicide treatment
*: Standard error of the mean

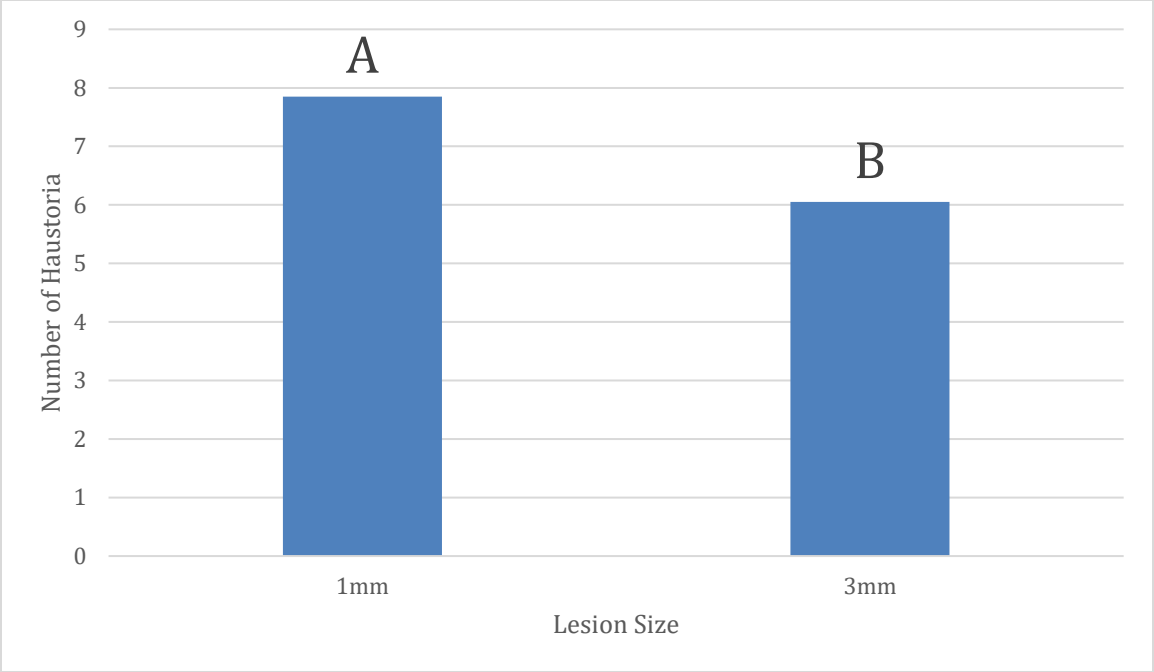

Figure 2.3: Haustoria density per $1.6x10^5$ μm^2 by lesion size across genotypes.

Colonizing hyphae appeared as tightly packed parallel groups radiating away from the center of necrosis (Figure 2.4). This pattern was present in both lesion sizes and all genotypes. Colonizing hyphae were observed between the epidermal tissue layer and the spongy mesophyll cells immediately below the epidermis, appearing to be attached to the bottom of the epidermal layer.

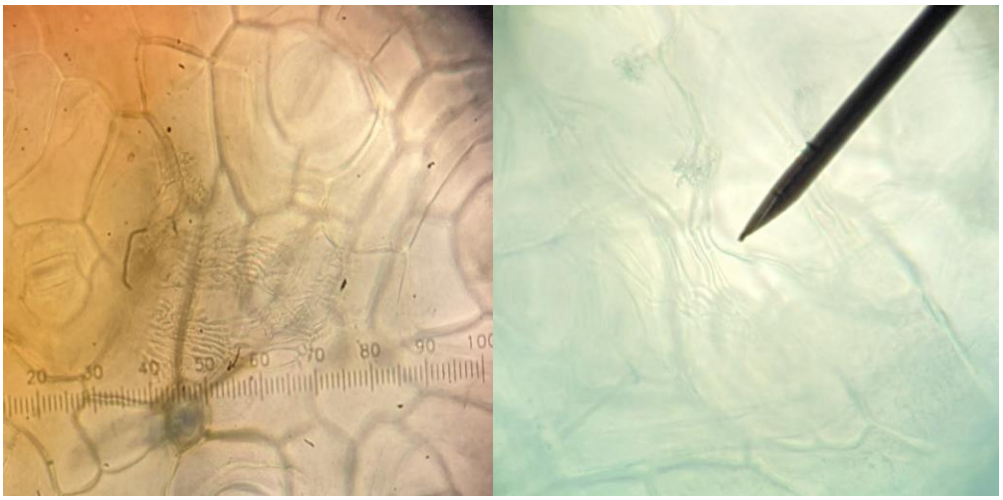

Figure 2.4: Parallel hyphae structure.

Regardless of lesion size, genotype, or field site; colonizing hyphae extended 1.53 to 1.87 times farther from the necrotic edge than haustoria ($P > 0.05$). In 1 mm lesions, hyphae of CB7 did not travel as far as the other genotypes (Figure 2.5; $P < 0.05$). For the 3 mm lesions, the colonizing hyphae of GA13M extended farther than the other genotypes, and extended beyond the chlorotic ring (Figures 2.6 and 2.7; $P = 0.012$). GA13M was the only genotype to extend beyond the chlorotic ring.

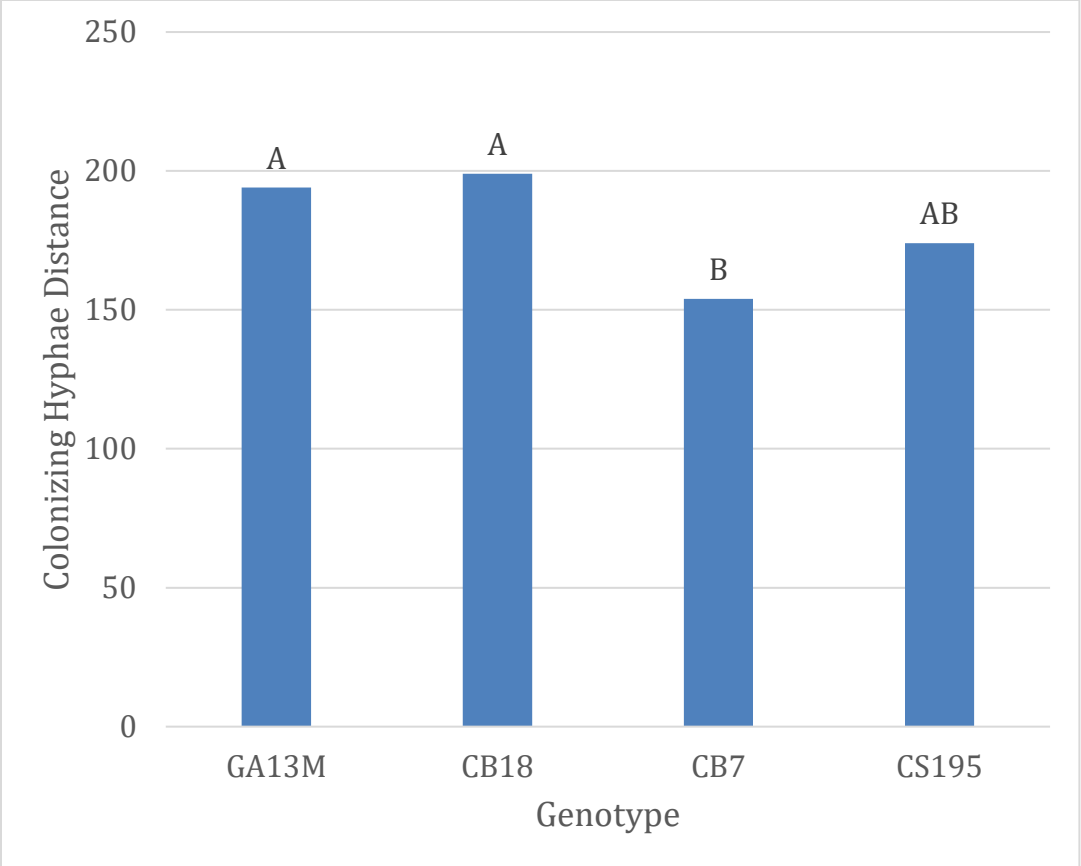

Figure 2.5: Distance of mycelium extending beyond the necrotic edge by genotypes across all field sites in 1 mm lesions.

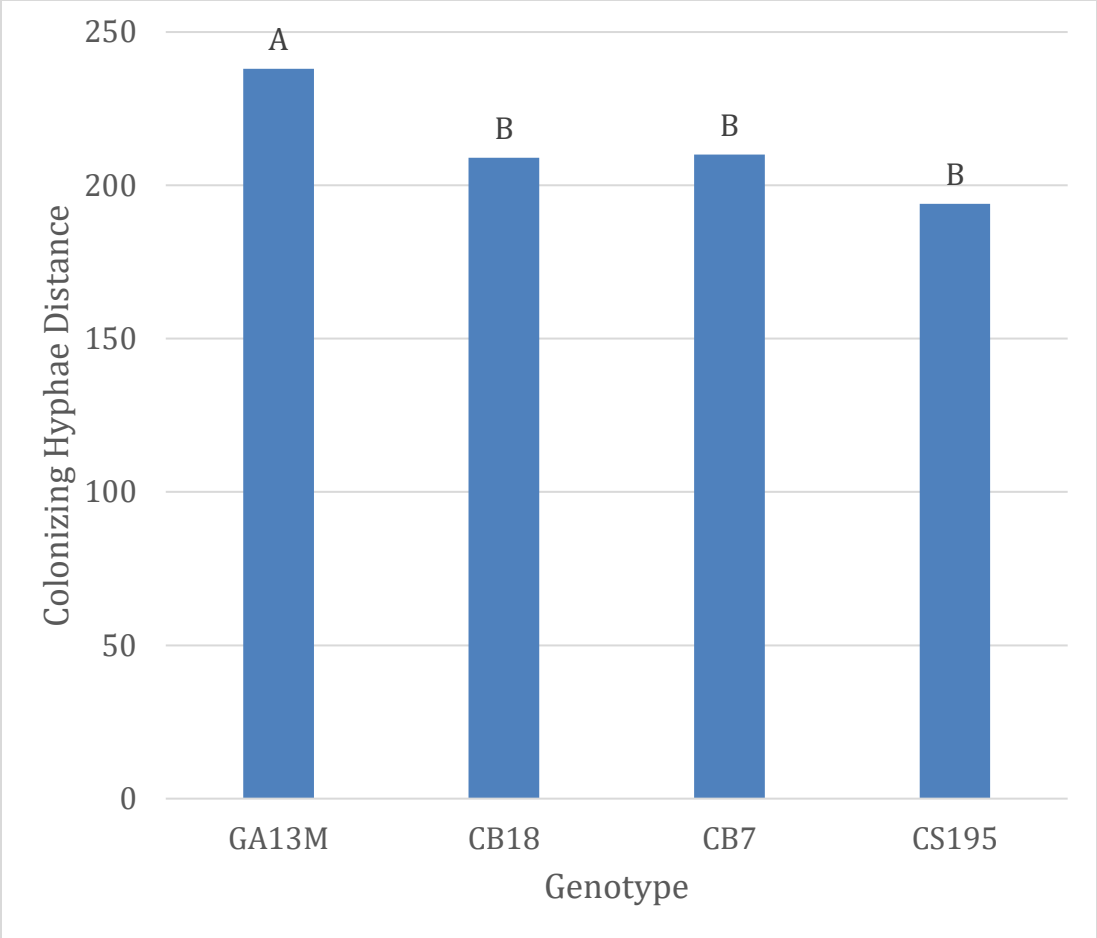

Figure 2.6: Distance of mycelium extending beyond the necrotic edge by genotypes across all field sites in 3 mm lesions.

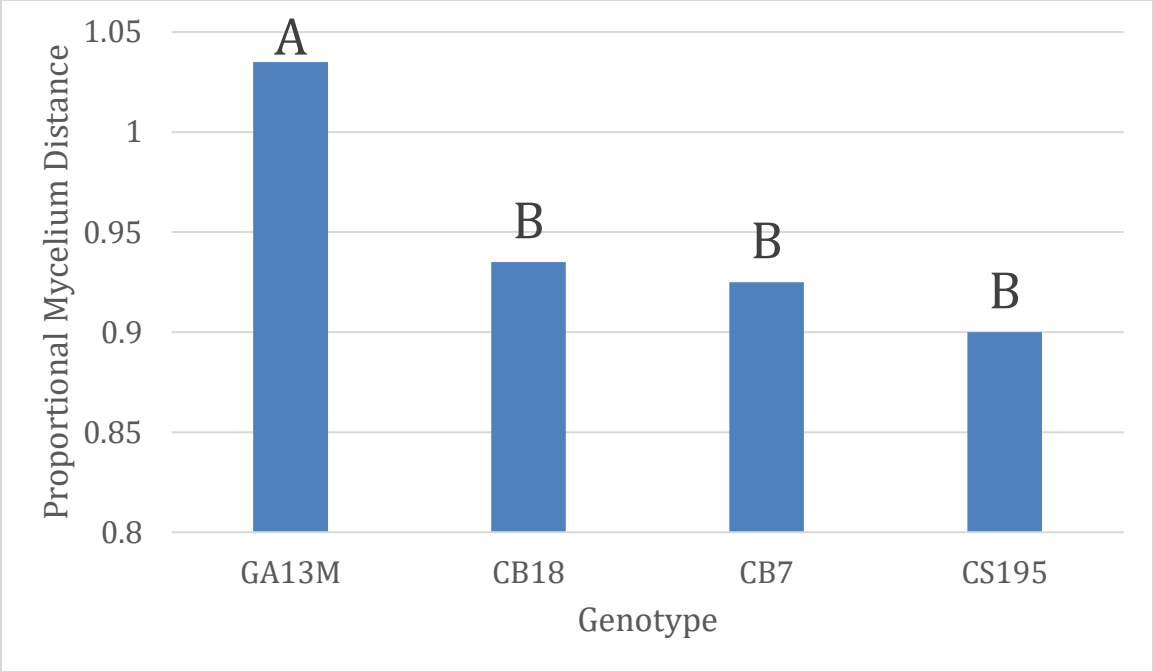

Figure 2.7: Proportional distances of mycelial relative to the chlorotic ring width for the 3 mm lesion size across field site.

Discussion

The methods in this study worked well to observe *N. personata* colonization of epidermal cells and the spongy mesophyll layer immediately below the epidermis, but was not sufficient for haustoria in deeper tissues, where they also occur (Jenkins, 1938; Mims et al., 1989). Although samples were taken from field infected leaves, and lesion age was not standardized, attempts to standardize by lesion size and presence of a yellow ring around the 3 mm lesion appear to have been an effective standardization method. Microscopic measures of lesion diameters were remarkably close to the estimated size categories and there was no indication that variances among the dependent variables were due to inadequate standardization protocols as variances of samples were low

(Tables 2.1 to 2.4). Sporulation ratings of the lesions evaluated in this study ranged from 0 to 5, with the variances being similar for all genotypes. This variance was taken into account by including sporulation as a covariate in analyses where sporulation was correlated or nearly correlated (mycelium distance; P=0.040 and haustoria distance; P=0.072). The chlorotic ring widths were similar between genotypes (Table 2.5), further suggesting similar aged lesions and successful standardization. Jenkins (1938) reported that chlorotic rings were found around late leaf spots only on mature lesions, which is consistent with what was observed for all genotypes in this study.

This study provides some insights into the characteristics of *N. personata's* haustoria, colonizing hyphae, and how QTLs conferring late leaf spot resistance may affect their development. The trend of higher haustorial densities with the 1 mm lesions than the 3 mm lesions suggests that haustoria may be utilized more frequently in the earlier stages of infection, although it is also possible that larger lesions have more area for haustoria to spread out. There were a couple genotype differences related to haustoria with the 1 mm lesions, but these were not consistent across field sites 2 and 3. However, for the 3 mm lesions, haustoria were less dense for GA13M than the resistant genotypes across all three field sites. If the reduced density of haustoria is indicative of fewer total haustoria for GA13M, this observation is opposite of what might be expected for a susceptible genotype. However, the observation that GA13M had longer colonizing hyphae than the resistant genotypes in 3 mm lesions, and that they were always 1.53-1.87 times farther than haustoria, suggests that the

colonizing hyphae could function during the biotrophic phase of the fungus. This would be consistent with other cercosporoid hemibiotrophs, including *P. arachidicola*, and biotrophs, including *Cladosporium fulvum*, both of which use intracellular hyphae for absorption during biotrophy (Rivas and Thomas 2005). Considering the differences in intracellular hyphal lengths between the resistant genotypes and GA13M in 3 mm lesions, as well as CB7 in 1 mm lesions, it is likely that limitations to hyphal expansion within leaf tissues is a component of resistance. Reduced colonization would be expected to result in reduced lesion size, which has been reported to be a component of late leaf spot resistance (Vasavirama and Kirti 2012).

Mims (1989) reported that haustoria remained in dead tissue. This was consistent with our findings, and could indicate that the haustoria are able to both feed on living tissue, and dead tissue (Mims et al. 1989).

Hyphal colonization consistently occurred as a cluster of three or more hyphae that were minimally branched and growing in a parallel pattern, most likely firmly affixed to the lower surface of epidermal cells. Woodruff (1933) and Jenkins (1938) also reported subepidermal hyphae, but their observations, based on cross-section, did not describe the parallel pattern observed in this study (Figure 2.4). Some of these hyphae were observed to branch off, entering cells as haustoria, or terminated as haustoria, but this was not always the case. If the colonizing hyphae of *N. personata* are biotrophic, their dense clumping could support maximization of nutrient uptake.

An important limitation to this study is that all of our observations were restricted to the epidermal layer or adjacent mesophyll cells of the leaf. Since haustoria are known to occur throughout the mesophyll (Woodruff, 1933; Jenkins 1938; Mims et al. 1989), it would be a mistake to overstate these results. Therefore, although this study did not detect resistance components related to the frequency, location, or size of haustoria, it is possible that these differences may have occurred within the mesophyll tissues. Likewise, it is possible that the differences detected for colonizing hyphae between the susceptible and resistance genotypes may not hold up when mesophyll tissues are examined. Microtome sectioning and freeze fracturing of tissues would help to elucidate how consistent fungal colonization is throughout the leaf tissues.

Chapter III

MEASURING DIFFERENCE IN RESISTANCE TO LATE LEAF SPOT DISEASE BEFORE AND DURING INFECTION

Introduction

Nothopassalora personata (Berk. & M.A. Curtis) U. Braun, C. Nakash., Videira and Crous is the fungal causative agent of late leaf spot in peanut (*Arachis hypogaea* L.). This disease can cause premature defoliation and yield loss if not controlled. Plant breeders have created several peanut cultivars with high levels of pathogen resistance using introgressive hybridization of cultivated peanut and wild peanut species. The genotypes tested in this study are crosses between TifNV-High O/L and IAC322, each with different introgressions from *A. cardenasii* which have shown resistance in the field and lab (Lamon et al 2021).

Most components of resistance evaluated occur after the plant has been infected with the pathogen. Incubation period, latent period, number of lesions per leaf, percent necrosis present on leaf, amount of sporulation, and area under disease progress curve are all examples of this. A 1974 study by Abdou et al., demonstrated resistance can be displayed before infection has occurred by observing differential responses to specific genotypes. One of these responses included reduced ability of the germ tubes to locate open stomata for penetration. The purpose of this study was to evaluate the components of resistance that occur before infection. In this study percent germination of conidia, germ tube

length, and percent penetration were all evaluated, as well as the final number of lesions per leaf.

Materials and Methods

Experimental design

Genetic material came from F2 and F3 plants derived from a cross between TifNV-High O/L and IAC 322, with three introgressed regions from *A. cardenasii* on chromosomes A02 and A03 (Lamon et al. 2021). The genotypes, S85, S159, S91, and S273 had various presences of these introgressions. The Genotype S85 had an introgression on the bottom of chromosome A02, S159 on the top of A02, S91 on the bottom of A03, and S273 with A02 top + A03 bottom. Runner 886 was the susceptible genotype, and the parents, IAC 322 and TifNV-High O/L were also included. Seeds were planted in the NESPAL greenhouse at UGA Tifton campus on February 8, 2020. The seeds were given anti-fungal seed treatments. Three seeds were planted in each pot and there were three pots per genotype.

Detached leaf assay

Inoculum was collected from dried, sporulating lesions that had been sectioned from leaf tissues infected with *N. personata*. Tissues were placed in 0.005% Tween-20 solutions and agitated so the conidia would dislodge. The solution was filtered using cheesecloth. The inoculum was then diluted to a concentration of 6×10^4 spores per mL. Percent germination of the inoculum was calculated by spraying the inoculum on water agar, and an average of 76% of conidia germinated 3 days after inoculation.

Leaves from 8-week-old plants grown from seed in ten-inch pots in a greenhouse were used in this study. Three leaves were selected from the first or second fully expanded leaves from the top were cut at the petiole. The petioles were wrapped in damp cotton immediately after excision, and the leaves were placed onto water agar plates. Inoculum was dispersed via pressurized spray can, and was standardized as an arm's length of distance (approximately 30cm) and one second of spraying. After inoculation the plates were sealed with parafilm to retain humidity and were placed under a grow light for a 12-hour photoperiod at 23°C. Every two days the top of the lids was sprayed with water and the plates were re-parafilmed to ensure high humidity. At 9 days after inoculation, sections of leaf tissue were fixed and cleared in Visikol solution.

Data collection and statistical analysis

Percent germination was determined by counting 30 spores and marking how many germ tubes were present. Percent penetration was counted using 30 germinated spores and were classified as penetrated if the germ tubes terminated in the middle of a stomate. Germ tube length was recorded using the same 30 germinated spores for percent penetration. Disease severity was estimated at 28 days after inoculation as the number of leaf spots per leaflet. Data were analyzed in SPSS using univariate ANOVA.

Results

There was no significant difference between genotypes in percent penetration, percent germination, or germ tube length (Table 3.1). This suggests that resistance related to these introgressions occurs after penetration.

Table 3.1 – Components of resistance pre-infection compared between genotypes.

	Proportion of Penetration	Proportion of Germination	Germ tube length (µm)
IAC	0.07	1	35.26
TIF	0.06	0.86	31.93
A02Bot	0	0.80	28.10
A02Top	0.07	0.93	31.13
A03Bot	0.03	0.93	33.70
A02Top A03Bot	0.04	0.80	30.20
R886	0.06	0.82	27.07
P value	0.443	0.972	0.592

The entry with the A02 top + A03 bottom introgression showed numerically fewer number of final lesions while TifNV-High O/L showed the highest, but the difference was not significant (Figure 3.1).

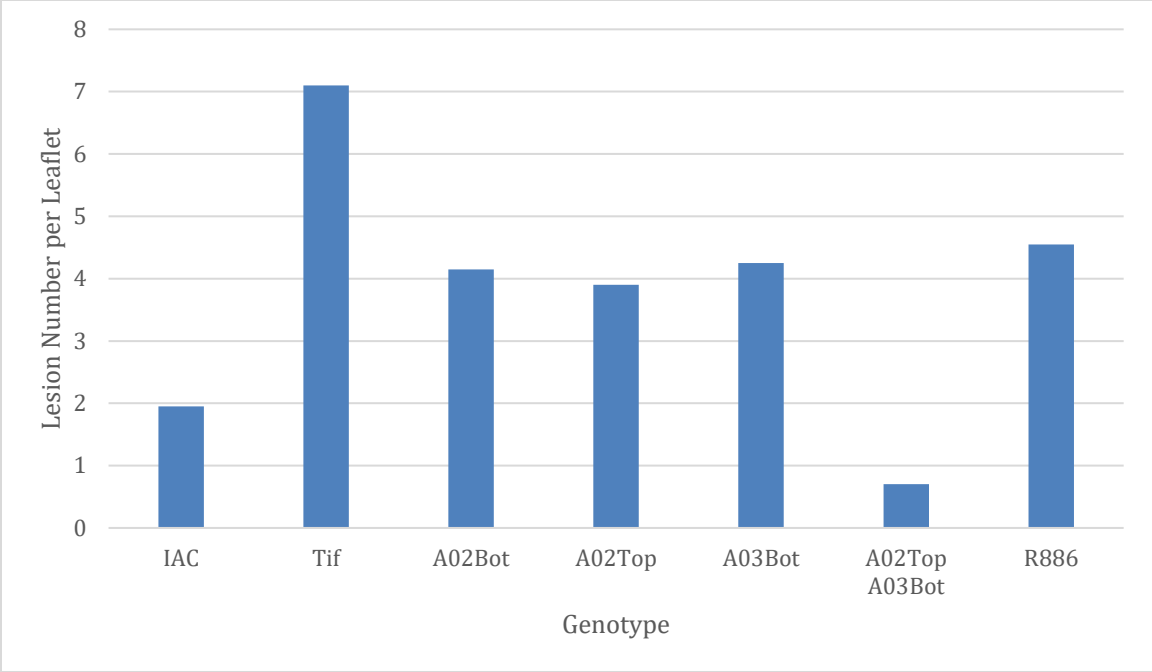

Figure 3.1: Final number of lesions per leaflet at 28 days after inoculation (P=0.260).

Discussion

Penetration and disease were low in this study, and although genotype IAC and the germplasm with introgressions A02 Top and A03 Bottom had numerically lower lesion numbers than Tif, the variation was too high to find statistical significance. Lesion number differences among these genotypes were found in a prior inoculation assay (Lamon et al., 2021). The low level of disease may have been due to inconsistent environmental conditions during the incubation period. The experiment was conducted in April of 2020 at the beginning of a lockdown caused by the SARS coronavirus 2 outbreak. Typically, a detached leaf assay would be conducted in a growth chamber, where photoperiod and temperature can be easily controlled. This experiment, however,

was conducted on a table in an uncontrolled environment. While photoperiod was still easily controlled for, temperature fluctuations could have been possible. Running the dryer in the laundry room could have caused the heat to rise in that particular room, or the air conditioner running could have made parts of the room colder than others. Another explanation for poor disease development could have been a lower inoculum concentration than a similar study (Lamon et al., 2021) or poor deposition of conidia on the leaf surfaces. While it was easy to find 30 conidia to assess on most leaflets, spores were often in clumps, and some leaf tissues lacked spores.

There was no evidence in this study to suggest that the introgressed genes function before pathogen penetration. Penetration frequencies, germination rates, and germ tube sizes did not differ by genotype. The experiment was not repeated, and therefore this report should be considered as preliminary findings.

Chapter IV: Conclusions

Genetic resistance in plants is important, but much is still unknown about how infection is slowed or stopped. Understanding differences in pathogen behavior between different levels of resistance is an important step in elucidating the physiology behind these resistance genes. This research suggests that resistant peanut genotypes may have some way of slowing down intercellular fungal growth. Our results showed that while the number of haustoria present was not lowered by higher levels of resistance, haustoria were found further away from the lesion in the susceptible genotype than two of the resistant genotypes. Perhaps resistant genotypes also have some way of delaying the production of haustoria to newly infected cells. In the larger 3mm lesions, haustoria were actually more abundant in resistant genotypes. Perhaps a component of resistance slows down the fungal lifecycle, keeping the fungus in the biotrophic stage for extended times.

This research also helped characterize the colonization behaviors of *N. passalora*. Mycelium were always found 1.53-1.87 times farther than the farthest haustoria, showing how far the mycelium colonize before producing haustoria. If a future study could discover the rate of mycelium colonization, predictions could be made on the rate of haustorial development as well. We also found a higher number of haustoria in the smaller 1mm lesions than in the larger 3mm lesions.

This supports our hypothesis that the fungi utilize more haustoria early in the fungal lifecycle.

References Cited

Abdou, Y. A. M., Gregory, W. C., & Cooper, W. E. (1974). Sources and nature of resistance to Cercospora arachidicola Hori and Cercospora personata (Beck & Curtis) Deighton in Arachis Species. Peanut Science 6-11

Alderman, S. C., Nutter, F. W., Jr. (1994). Effect of temperature and relative humidity on development of Cercosporidium personata in peanut on Georgia. Plant Disease. 78, 690–694. doi: 10.1094/PD-78-0690

Anco, D. J., Thomas, J. S., Jordan, D. L., Shew, B. B., Monfort, W. S., Mehl, H. L., Small, I. M., Wright, D. L., Tillman, B. L., Dufault, N. S., Hagan, A. K., & Campbell, H. L. (2020). Peanut yield loss in the presence of defoliation caused by late or early leaf spot. Plant Disease. 104:5, 1390-1399

Anonymous. (2022). National Agricultural Statistical Service. USDA, Published online at https://quickstats.nass.usda.gov/

Bent, A. F. (1996). Plant disease resistance genes: function meets structure. The Plant Cell 8:10, 1757.

Cantonwine, E. G., Culbreath, A. K., Stevenson, K. L., Kemerait Jr, R. C., Brenneman, T. B., Smith, N. B., & Mullinix Jr, B. G. (2006). Integrated disease management of leaf spot and spotted wilt of peanut. Plant disease, 90:4, 493-500.

Cantonwine, E. G., Culbreath, A. K., Holbrook, C. C., & Gorbet, D. W. (2008). Disease progress of early leaf spot and components of resistance to Cercospora arachidicola and Cercosporidium personata in runner-type peanut cultivars. Peanut Science, 1 January 2008; 35:1, 1–10.

Chiteka, Z. A., Gorbet, D. W., Shokes, F. M., Kucharek, T. A., & Knauft, D. A. (1988). Components of resistance to late leafspot in peanut. I. Levels and variability-implications for selection. Peanut Science, 15:1, 25-30.

Chu, Y., Gill, R., Clevenger, J., Timper, P., Holbrook, C. C., & Ozias-Akins, P. (2016). Identification of rare recombinants leads to tightly linked markers for nematode resistance in peanut. Peanut Science, 43, 88–93. doi: 10.3146/ps16-12.1

Chu, Y., Chee, P., Culbreath, A. K., Isleib, T. G., Holbrook, C. C., & Ozias-Akins, P. (2019). Major QTLs for resistance to early and late leaf spot diseases are identified on chromosomes 3 and 5 in peanut (Arachis hypogaea). BioRxiv

Clevenger, J., Chu, Y., Chavarro, C., Botton, S., Culbreath, A., Isleib, & T. G., et al. (2018). Mapping late leaf spot resistance in peanut (Arachis hypogaea) using QTL-seq reveals markers for marker-assisted selection. Front. Plant Science. 9:83. doi: 10.3389/fpls.2018.00083

Culbreath, A. K., Stevenson, K. L., & Brenneman, T. B. (2002). Management of late leaf spot of peanut with benomyl and chlorothalonil: A study in preserving fungicide utility. Plant Disease 86:4, 349-355

Dwivedi, S. L., Pande, S., Rao, J. N., & Nigam, S. N. (2002). Components of resistance to late leaf spot and rust among interspecific derivatives and their significance in a foliar disease resistance breeding in groundnut (Arachis hypogaea L.). Euphytica, 125, 81–88.

Fulmer, A. M., Kemerait, R. C., Brenneman, T., Culbreath, A. K., & Cantonwine, E. G. (2023). Susceptibility of historically dominant runner-type peanut cultivars of the southeastern United States to early and late leaf spot. *Plant Health Progress*, (*In Press*).

Heath, M. (1997). Signalling between Pathogenic Rust Fungi and Resistant or Susceptible Host Plants. Annals of Botany, 80:6, 713–720, https://doi.org/10.1006/anbo.1997.0507

Holbrook, C. C., Ozias-Akins, P., Chu, Y., Culbreath, A. K., Kvien, C. K., & Brenneman, T. B. (2017). Registration of “TifNV-High O/L” Peanut. Journal of Plant Registrations, 11:3, 228. doi: 10.3198/jpr2016.10.0059crc

Holbrook, C., Ozias-Akins, P., Chu, Y., Lamon, S., Bertioli, D. J., Leal-Bertioli, S. C. M., Culbreath, A. K., & Godoy, I. (2022). Registration of TifGP-3 and TifGP-4 peanut germplasm lines. Journal of Plant Registrations. 2022:16, 120– 123. https://doi.org/10.1002/plr2.20179

Jenkins, W. A. (1938). Two fungi causing leaf spot of peanut. Journal of Agricultural Research 56:5, 317-332.

Johnson, R. C. and Cantonwine, E. G. (2014). Post-infection activities of fungicides against Cercospora arachidicola of peanut (Arachis hypogaea). Pest Management Science, 70, 1202-1206. https://doi.org/10.1002/ps.3671

Kemerait, R., & Little, E. (2019). 2019 Georgia plant disease loss estimates. University of Georgia Extension, Published online at https://extension.uga.edu/publications/detail.html?number=ap102-12

Kirschner, R. (2009). Cercosporella and Ramularia, Mycologia, 101:1, 110-119, DOI: 10.3852/07-038

Ktiller, W., & Scheinpflug, H. (1987). Fungal resistance to sterol biosynthesis inhibitors: a new challenge. *Plant Dis*, *71*, 1066-1074.

Lamon, S., Chu, Y., & Guimaraes, L. A., et al. (2021). Characterization of peanut lines with interspecific introgressions conferring late leaf spot resistance. Crop Science. 61, 1724– 1738.

Latham, D. H. (1934). Life history of a Cercospora leaf spot fungus of cowpea. Mycologia, 26:6, 516-527.

Leonard, K. J., & Szabo, L. J. (2005). Stem rust of small grains and grasses caused by Puccinia graminis. Molecular plant pathology 6:2, 99-111.

Miles, A. K., et al. (2009). Infection, colonisation and sporulation by Pseudocercospora macadamiae on macadamia fruit. Australasian Plant Pathology 38:1, 36-43.

Mims, C. W., Luttrell, E. S., & Alderman, S. C. (1989). Ultrastructure of the haustorium of the peanut late leaf spot fungus Cercosporidium personata. Canadian Journal of Botany, 1989, 67:4, 1198-1202

Pande, S., Rao, J. N., & Dwivedi, S. L. (2002). Components of resistance to late leaf spot caused by Phaeoisariopsis personata in inter-specific derivatives of groundnut. Indian Phytopathology, 55:4, 444-450.

Rivas, S., & Thomas, C. M. (2005). Molecular interactions between tomato and the leaf mold pathogen Cladosporium fulvum. Annual Review of Phytopathology, 43:1, 395–436.

Schumann, G. L., & D'Arcy, C. J. (2010). Essential plant pathology (No. PA 581.2 S38 2013.). St. Paul, MN: APS Press.

Sehgal, D., Singh, R., & Rajpal, V. R. (2016). Quantitative trait loci mapping in plants: Concepts and approaches. Sustainable Development and Biodiversity, 31–59. doi:10.1007/978-3-319-27090-6_2

Szabo, L. J., & Bushnell, W. R. (2001). Hidden robbers: The role of fungal haustoria in parasitism of plants. Proceedings of the National Academy of Sciences, 98:14, 7654–7655. doi:10.1073/pnas.151262398

Toomer, O. T. (2017). Nutritional chemistry of the peanut (Arachis hypogaea). Critical Reviews in Food Science and Nutrition, 29, 1–12.

Vasavirama, K., & Kirti, P. B. (2012). Increased resistance to late leaf spot disease in transgenic peanut using a combination of PR genes. Funct Integr Genomics, 12, 625–634. https://doi.org/10.1007/s10142-012-0298-8

Voegele, R. T. & Mendgen, K. (2003). Rust haustoria: Nutrient uptake and beyond. New Phytologist, 159, 93-100. https://doi.org/10.1046/j.1469-8137.2003.00761.x

Wadia, K. D. R. & Butler, D. R. (1994). Relationships between temperature and latent periods of rust and leaf-spot diseases of groundnut. Plant Pathology, 43, 121-129. https://doi.org/10.1111/j.1365-3059.1994.tb00561.x

Wilkinson, M. D., Dumontier, M., Aalbersberg, I. J. J., Appleton, G., Axton, M., Baak, A., Blomberg, N., & Boiten, J. W., et al. (2016). The FAIR guiding

principles for scientific data management and stewardship. Scientific data. 3:160018.

Woodroof, N. C. (1933). Two leaf spots of the peanut (Arachis hypogaea L). Phytopathology, 23:8, 627-640.

Woodward, J. E., Brenneman, T. B., Kemerait, R. C., Culbreath, A. K., & Smith, N. B. (2014). On-farm evaluations of reduced input fungicide programs in peanut fields with low, moderate, or high levels of disease risk. Peanut Science. 41, 50–57. doi: 10.3146/PS11-23R2.1

You, B. J., Lee, M. H., & Chung, K. R. (2008). Production of cercosporin toxin by the phytopathogenic Cercospora fungi is affected by diverse environmental signals. Canadian Journal of Microbiology, 54:4, 259–269. doi:10.1139/w08-002