SURVEY OF FUNGAL PATHOGENS AFFECTING WATERMELON PRODUCTION THROUGHOUT TEXAS GROWING REGIONS

A Thesis

by

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ABSTRACT

Texas ranks 3rd in US watermelon production, with 40% of Texas counties generating $83.2 million in 2019. Fungal pathogens such as *Stagonosporsis* spp. and *Fusarium* spp. can cause significant revenue loss annually. Fungal watermelon pathogens are not well documented in Texas, and the impact of varying environmental conditions on those fungi are not well understood. A survey was conducted to document fungal pathogens in Texas during the 2020 growing season. Thirty symptomatic stem, leaf, and/or root samples were collected from 5 fields in 5 Texas counties and assayed on 25% PDA+ antibiotics. Isolates were identified morphologically and confirmed using PCR. *Stagonosporopsis citrulli, Fusarium proliferatum, Fusarium brachygibbosum, Fusarium incarnatum, Bipolaris* spp*., Alternaria alternata,* and *Rhizopus oryzae* were isolated. Pathogenicity tests were conducted using representative isolates of *S. citrulli, F. proliferatum, F, brachygibbosum, F. incarnatum, Bipolaris* spp., and *A. alternata.* All isolates caused disease symptoms on watermelon, though *S. citrulli* and the 3 *Fusarium* spp. inoculated plants consistently had the highest disease severity ratings. Environmental data (humidity, temperature, dew point, air pressure, wind speed, wind gust, and precipitation) were collected from weather stations near each location from 6 weeks prior to until time of collection. These data (components) were processed using Principal Components Analysis to determine the most influential factors of the environments and which environments were the most unique. PCA showed humidity and dew point were the most influential components for all counties. Three of the 5 counties had no significant differences among their environmental components, while Maverick

and Glasscock counties were significantly different from the other 3. Glasscock county had the most significant differences among the environmental components from all other counties sampled. Glasscock had the lowest total recovery of fungi, and it can be inferred based on the data that environmental components had a role in this outcome. The fungi isolated and documented as pathogenic in this work are more widespread in Texas than previously known and may be associated with reduced plant vigor and reduced yield quality and quantity in the field. More research is needed to determine each pathogen's production and economic impacts on Texas watermelon.

DEDICATION

She is made of depths even the ocean could not fathom. – Jessica Katoff

To those who have inspired my spark of curiosity, added stones to build my character, and have made me who I am today.

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Contributors

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NOMENCLATURE

GSB	Gummy Stem Blight
FON	Fusarium Wilt/ *Fusarium oxysporum* f.sp. *niveum*
FO	Fusarium wilts / *Fusarium oxysporum*
IPM	Integrated Pest Management
BURL	Burleson County
GLASS	Glasscock County
HID	Hidalgo County
M	Maverick County
RH	Relative Humidity
WS	Wind Speed
WG	Wind Gust
PCA	Principal Components Analysis
DPI	Days Post Inoculation

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CHAPTER I

INTRODUCTION

1.1 Watermelon Production

Watermelon (*Citrullus lanatus* (Thumb.) Matsum. et Nakai) is a part of the Cucurbitaceae family, which also includes cucumber (*Cucumis sativus* L.), and squash (*Cucurbita pepo* L.). This family is comprised of two sub-families and eight tribes consisting of roughly 115 genera and 960 species (Jeffery, 1990, Kocyan, 2007). Watermelon originated in Asia and belongs to the subfamily Cucurbitoideae, the Benincaseae tribe, and the subtribe of Benincasinae (Maynard, 2001, Schaefer, 2009). In 2019, the annual global production of watermelon was 100,414,933 metric tons with 1,680,514 metric tons produced within the United States (FAO 2020).

Texas ranks 3rd in watermelon production in the U.S., where watermelon is the largest acreage and revenue generating annual horticultural crop grown throughout the state valued at $83,202,000 in 2019 (USDA 2019). Texas has four major watermelon production regions that span the state: the Lower Rio Grande Valley, the Winter Garden, the Rolling Plains, and the High Plains. East Texas also produces watermelon, though not at the scale of the other regions. Over 40% of Texas counties grow watermelon annually with the top five being Hidalgo, Brooks, Knox, Gaines, and Wood counties. Partly due to the extensive area watermelon production is spread across in Texas, plant disease challenges and environmental conditions can greatly influence melon production across the state. Commonly grown seedless varieties grown are Fascination,

Captivation, and Tri-X 313. Fascination and Captivation are both resistant to Fusarium Wilt race 1 and Anthracnose race 1, while Tri-X 313 has no disease resistance. Black Diamond, Jubilee, Crimson Sweet are commonly grown seeded varieties. Black Diamond has no observed disease resistance, Jubilee has shown resistance to Fusarium wilt race 1 and Anthracnose race 1, while Crimson Sweet is resistant to Fusarium Wilt race 0 (IPM Center). There are no current watermelon varieties that are resistant to all 4 races of Fusarium wilt (Dutta, 2018). Beyond general susceptibility, as in the case of Florida and Texas Giant, little information is available regarding resistance to other *Fusarium* spp. induced diseases.

1.2 Major Fungal Pathogens that Affect Watermelon Production in Texas

There are over 8,000 fungi or fungal-like organisms (FLOs) that cause disease in plants. These eukaryotic organisms lack chlorophyll and therefore cannot make their own food. Fungi and FLOs absorb water and nutrients with the use of their hyphae, which in the case of plant pathogenic microbes can cause disease and potentially death of the whole plant. Fungi, including plant pathogens, can be categorized into either saprotrophs, biotrophs, necrotrophs, or hemibiotrophs. Among plant pathogenic fungi, saprotrophs live on decaying plant material, biotrophs gain nutrients from living plants, and necrotrophs kill the host plant's cells and tissues as they are colonized, while fungi considered to be hemibiotrophs begin their life cycles as biotrophs then become necrotrophic towards the end of their life cycles (Carris, 2012).

Two major fungal pathogens that affect watermelon production in Texas are Gummy Stem Blight (GSB, *Stagonosporopsis cucurbitacearum* (Fr.) Aveskamp,

Gruyter & Verkley*, S. citrulli,* and *S. caricae*) (Stewart et al., 2015) and Fusarium wilt (FON, *Fusarium oxysporum* f.sp. *niveum* (E.F. Sm.) W.C. Snyder & H.N. Hans). While these are thought to be the most significant fungal diseases in Texas watermelon production, other pathogens that can pose challenges to production include: anthracnose (*Colletotrichum orbiculare* (Berk. & Mont.) Arx), Alternaria leaf blight (*Alternaria cucumerina* (Ellis & Everh.) J.A. Elliott), Charcoal rot (*Macrophomina phaseolina* (Tassi) Goidanich), and Rhizopus rot (*Rhizopus stolonifer* Ehrenb) (Keinath, Wintermantel, Zitter, 2017).

Gummy stem blight is a major cucurbit disease of concern in all cucurbits producing areas globally (Rennberger and Keinath, 2018). GSB was first reported in 1891 on cucumber crop in France and was reported on watermelon in Florida in 1917 (Sherf, 1986, Sherbakoff, 1917). The causal agent of GSB has three morphologically similar but genetically distinct species, *S. cucurbitacearum, S. citrulli*, and *S. caricae*, with *S. citrulli* being the most commonly found in the southeastern United States (Brewer et al. 2015). *Stagonosporopsis* spp. discussed in this work are soilborne necrotrophic ascomycete pathogens. *Stagonosporopsis* spp. may be seedborne and often enters watermelon production fields in infested transplants and as a polycyclic pathogen, new infections occur rapidly in fields (Santos, Café-Filho 2006). Symptoms of GSB include tan to dark brown spots in circular to triangular shape along the leaf margins. Water-soaked lesions appear on leaves, petioles, hypocotyls, and the stems of watermelon. These lesions appear due to the cell wall-degrading enzymes produced by the pathogen. The most recognizable symptoms of GSB are the red/brown gummy

exudate commonly produced on the surface of stem cankers and pycnidia found on foliar lesions, without these signs it can be difficult to identify in the field without expertise. Symptoms are often reported to appear during the mid to late growing season (Keinath, 2017). *Stagonosporopsis* sp. produce conidia that are cylindrical with rounded ends, 1-2 celled, and averaged sized of 11.6 µm long. Ascospores are produced in groups of 8 in asci within perithecia. Ascospores are hyaline, 2 celled and have an average size of 13.7 µm. Perithecia typically overwinter on crop debris, such as short plant stems left in the field after crops are mowed and are spread due to wind and splashing water (Zitter 1992, Santos, Café-Filho 2006, Keinath, 2017). The lignified and thickened crown area of a watermelon stem is particularly durable debris and will not decay rapidly and therefore can provide inoculum a place to overwinter into the next production season and new crop (Keinath, 2008). In fact, previous studies have reported *S. citrulli* can survive on buried infected watermelon crown for up to 30 weeks (Keinath 2002).

Fusarium (Schlechtendahl Emend. Snyder and Hansen) is a genus of soilborne necrotrophic plant pathogenic fungi (Gordon 2017). *Fusarium* spp. may produce macroconidia, microconidia, and chlamydospores depending on the species. These fungi can live in the soil without a host as mycelium and hardy chlamydospores until a suitable host becomes available (Larone 1995), with optimal growth temperatures ranging from 25 to 30°C with 95-100% humidity. Despite being one of the most important genera of toxigenic fungi, *Fusarium* has had a dynamic taxonomic history (Geiser et al., 2004). The recent advent of multilocus phylogenetic methods allows for more nuanced identification of species boundaries of fungi than morphological identification alone,

which under-estimated species diversity (Aoki & O'Donnell, 1999; Geiser, Juba, Wang, & Jeffers, 2001; O'Donnell, 2000; Taylor et al., 2000). Subsequently, the complexities of relationships among *Fusarium* species have been revealed in greater detail than available in the past. To improve our understanding Fusarium spp. species and f. sp. diversity with multilocus sequencing, proper primer selection in PCR identification is essential. Currently, ITS 1 and 4 (White et al., 1990) is no longer deemed sufficient due to the high genetic similarity in species of *Fusarium*. Primers such as elongation factor (EF) are essential to obtain adequate resolution due to high genetic similarity within not only *f. sp* and species, but the genus as a whole (Geiser, 2004). Amplification of the translation elongation factor gene (EF1-α or TEF1 gene) provides species-level detection in addition with the FUSARIUM-ID database to obtain accurate identification (Karlsson, 2016).

Fusarium oxysporum (FO), is a saprophyte that can survive in soils for many years, infect host plants during their entire growing season, and is considered as the most damaging species of Fusarium (Smith, 2007). This species of fungi produces the typical macroconidia, microconidia, and chlamydospores but does not have a documented sexual stage (Nieuwenhuis 2016). FO has more than 120 *formae specialis* (f. sp.) sub-classifications within the species, with a yet more narrow classification of pathogen races with varying virulence on a given plant host within a *formae specialis*. FO can cause varying plant disease symptoms, including vascular wilt, root rot, seed rot, and stem rots, some of which produce mycotoxins in cereal crops that can be problematic with respect to food safety and human and animal health.

Fusarium oxysporum f. sp. *niveum* (FON) causes Fusarium wilt on watermelon and is one of the major limiting factors of production in the world (Chang, 2008). This disease was first detected in the U.S. in 1894 in South Carolina and Georgia (Smith, 1894). Symptoms include vascular discoloration, particularly around the crown and upper taproot, withering/ wilting leaves followed by either death of a vine or whole plant (Amaradasa et al., 2018). The ability of the pathogen to infect and colonize the host successfully depends on temperature, light, nutrients, type and concentration of inoculum, and infection method (Martyn, 1989). Humid and wet conditions are ideal for *F. oxysporum f. sp. niveum* to infect host plants. The four races of FON described are Race 0, 1, 2, and 3, with Race 2 currently being the of the most economic importance. Races 0, 1 and 2 are currently found in Texas watermelon fields (Zhou, 2010). Other *formae specialis* of *F. oxysporum* may cross infect watermelons but severe symptoms rarely appear (Keinath 2017).

Alternaria Leaf Blight (*Alternaria cucumerina*) is ubiquitous throughout watermelon production fields. This disease develops after extended wet periods with high relative humidity and commonly produces yellow necrotic leaf spots, particularly in the middle of older leaves on the plant (Umamaheswari et al., 2007). Anthracnose (*Colletotrichum orbiculare*) can also be found in most cucurbit growing regions and affects watermelon at all growth stages. While there are 7 races of *C. orbiculare*, only 3 cause disease on watermelon: Races 1, 2, and 3 (Boyhan et al., 1994). Like Alternaria leaf blight, anthracnose favors warm, wet, and humid conditions for infection and disease development. Anthracnose symptoms include brown to black angular leaf spots

near leaf veins, seedling damage, and fruit rot (Keinath, 2017). Cercospora Leaf spot (*Cercospora citrullinia*), a prevalent foliar disease causes small leaf spots that can lead to defoliation of vines when severe. Leaf spots appear on older leaves and are irregular in shape with a yellow halo and dark brown center. Cercospora leaf spot can restrict fruit development or scalding due to defoliation and is found in many growing regions in Texas (Keinath, 2017).

Charcoal Root Rot (*Macrophomina phaseolina*) is a soilborne pathogen that can infect a wide range of plants, including watermelon (Cohen et al., 2016). Unlike the previous pathogens discussed, optimal environmental conditions for *M. phaseolina* disease development are hot and dry weather. Plants may be infected at various stages of development, from seedling stage to older plants. Infection may occur early on and symptoms become notable only later in the season when conditions are favorable for the pathogen, well after initial infection. Symptomatic plants may be stunted or wilted and will eventually develop black discoloration in the roots and lower stem area during the latter stages of disease development (Keinath, 2017).

Rhizopus Rot (*Rhizopus stolonifer*) is a common postharvest disease and is associated with poor handling from field to store, and ripe to overripe melons injured in the field are especially vulnerable to infection. Inoculum is ubiquitous, but it is thought that exposure in the field during harvest handling is a significant source of inoculum and creating opportunity for infections (Kwon, 2010). Infection and disease development of Rhizopus rot favors warm, humid conditions during storage and transportation of the fruits (Baggio et al., 2016). Though this fungus favors these conditions, Rhizopus Rot

can still occur at cooler temperatures as low as 13°C at a reduced rate (Scruggs, et al., 2016).

1.3 Optimal Environmental Conditions for Disease Development and Environmental

Texas' growing seasons and environmental conditions differ greatly over the large geographic area of the state (Table 2), which affects planting and harvesting times, and other production operations of the Texas watermelon industry. This variation in environmental conditions may also affect pathogen population occurrence and frequency, depending on the pathogens' ideal environment for optimal growth (Table 1).

Table 1: Optimal conditions for disease development of watermelon pathogens in Texas

Disease	Peak Temperature Range	Moisture Levels/Relative Humidity	Climate
FON	25°-30° C	High	Warm temperate climate with dry winter or wet all year round
GSB	20°-25°C	High/ continuous leaf wetness	Warm, wet
Anthracnose	26°-32°C	Medium/High	Warm, wet climate
Charcoal Root Rot	>30°C	Low	Warm, dry climate
Alternaria Leaf Blight	21°-32°C	High	Warm, wet
Rhizopus rot	20°-30°C	High	Warm, wet climate

Keinath, Wintermantel, and Zitter, 2017

Table 2: Average temperatures and average precipitation per location surveyed.

Growing Region	Planting Month Temperature (°C)	Harvest Month Temperature (°C)	Planting Month Precipitation (cm)	Harvest Month Precipitation (cm)	Average for Region Yearly (°C) (cm)
Rio Grande Valley	January High: 21° Low: 8°	May High:31 ° Low: 17°	Rain: 0.33-0.39	Rain: 0.45	High: 30° Low: 16° Rain: 8.92
Winter Garden	January High: 17° Low: 4-7°	June High: 34-36° Low: 21-22°	Rain: 0.46-0.48	Rain:0.56-1.29	High: 29° Low: 15° Rain: 9.75
Rolling Plains	February High:15-20° Low: 0-4°	July High: 35° Low: 20°	Rain:0.57-0.618	Rain: 1.02	High: 25° Low: 10° Rain: 10.42
High Plains	February High: 23-28° Low: 2-8°	August High: 28° Low: 11°	Rain: 0.40-0.41 Snow: 0-0.40	Rain: 0.94 Snow: 0	High: 22° Low: 4° Rain:7.24 Snow:3.15

https://www.usclimatedata.com/ U.S. Climate Data

1.4 Current approaches to management

Many producers use a combination of management practices to control disease symptoms in the field. Fungicide applications are heavily relied on for disease control. However, not all pathogens can be effectively controlled with fungicide applications due to less-than-ideal efficacy, particularly when disease pressure is high. Additionally, development of fungicide resistance is a concern for many vegetable pathogens and can result in unchecked disease progression. Customer preference can also drive changes in disease management practices. For example, the post-harvest disease Rhizopus rot had been controlled with dicloran dips in the past, but market demand changes have led to limited use of these dips in recent years (Scruggs and Quesada-Ocampo, 2016).

Resistant varieties, such as Fascination and Captivation, are often used by Texas growers, but these are only resistant to FON race 1 and Anthracnose race 1. FON race 2 has been found in Texas fields. Currently, there are only non-harvestable pollinizer seeded varieties available that are resistant to race 2 and these are not profitable to the grower (UGA, 2018). With the emergence of FON race 3, the use of resistant varieties as a management strategy is not ideal in all areas since there is no resistance to race 2 or race 3 currently on the market.

Other management efforts that make up part of an integrated pest management program include manipulating the growing environment, the exact methods of which depend on the biology of the disease being managed. For example, given the development of many foliar diseases requires excess moisture for a period of time, the likelihood of an epidemic can be reduced by limiting the frequency of sprinkler

irrigation when possible. Management efforts for soilborne pathogens such as Fusarium wilt and charcoal root rot include the use of crop rotation, the use of resistant varieties, and chemical application when appropriate, though options for chemical management are limited. Developing a better understanding of favorable environmental conditions associated with the occurrence of fungal plant pathogens will lead to more accurate recommendations for disease control practices, including judicious use of fungicides and cultural techniques as part of an IPM program. Currently, plant pathogen distribution across Texas watermelon production areas is not well understood or documented. Furthermore, the exact environmental conditions required for major pathogens to thrive and cause disease is not well documented in Texas.

My research interest is to better understand the distribution and frequency of watermelon fungal pathogens across Texas, and to better understand environmental parameters associated with greater occurrences of pathogens. This will provide additional insight to which areas and/or conditions have greater potential for disease epidemics. Ultimately, a better understanding of these facets of plant pathology will help producers make more well-informed choices about best disease management strategies, potentially saving time and resources in the future. My research objectives are as follows:

1. To determine the occurrence and frequency of fungal watermelon pathogens across surveyed watermelon production sites in Texas, with special focus on *Stagonosporopsis* spp.

2. To determine relationships between environmental components and the recovery of fungal isolates recovered across Texas.
3. To determine pathogenicity of the fungi found in Texas watermelon production fields.

CHAPTER II

SURVEY OF FUNGI ISOLATED FROM WATERMELON ACROSS TEXAS

Objective - To determine the occurrence and frequency of fungal watermelon pathogens across surveyed watermelon production sites in Texas, with special focus on Stagonosporopsis spp.

2.1 Methods

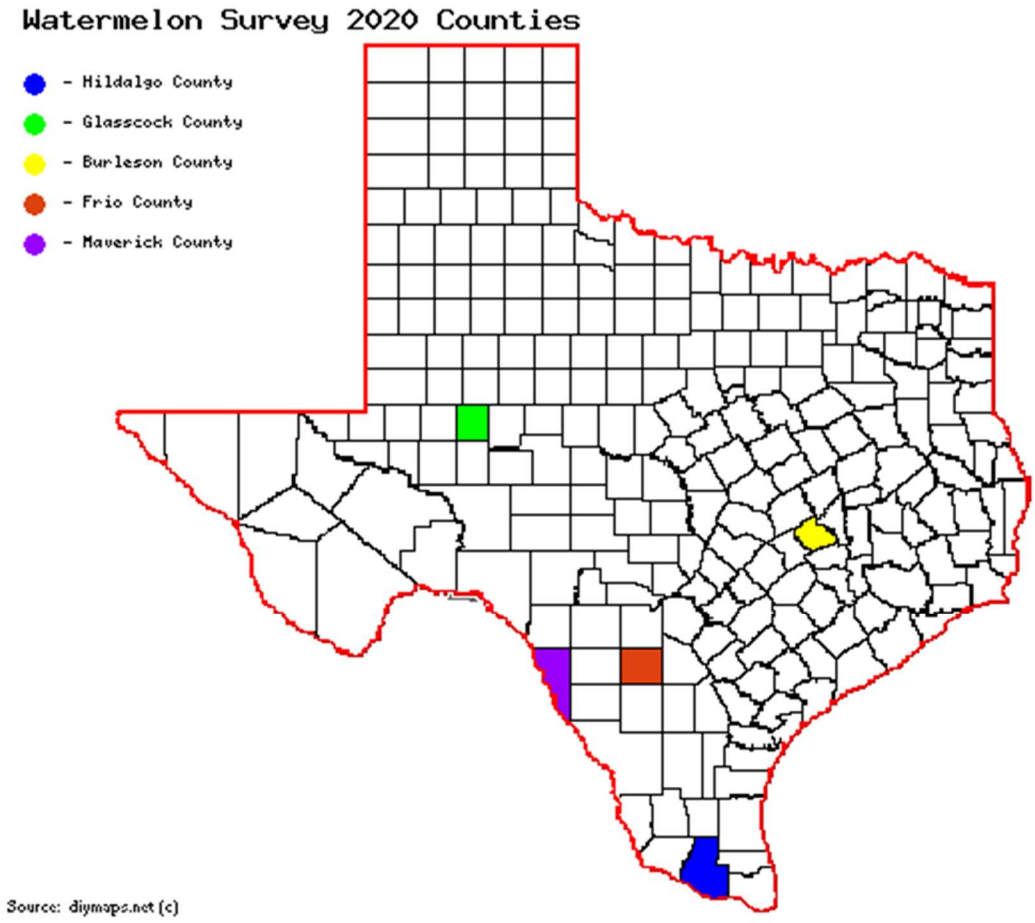
2.1.1 Location Selection & Sample Collection

Survey locations were selected in the 2020 season by contacting producers from the Texas Watermelon Association member list and speaking with committee members for contacts. Five field locations were chosen in the following counties: Frio, Hidalgo, Glasscock, Maverick, and Burleson.

Sampling areas within the field were chosen after obtaining input from the growers about areas of concern within their fields. Thirty symptomatic tissue samples were arbitrarily chosen and collected at each location 1-2 weeks before harvest (Figure 1). Samples consisted of leaves, stems, and/or roots showing symptomatic tissue such as chlorotic leaves, leaf curling, brown lesions, wilting, leaf spots, and necrosis (Index Figure 1). If a root sample was taken, at least one vine and melon attached to that vine was also taken for stem and tissue sampling to determine if the pathogen was present in roots, the vine, and fruit. GPS coordinates were taken at each collection site within a field to track pathogen distribution throughout each field (Supplementary Table 1). Samples were placed in coolers with ice, taken back to the lab within 2 days of

collection, and stored at 4°C until processed, which was within one week after the date of collection.

Figure 1: Map of surveyed counties in 2020.



2.1.2 Processing Samples & Pathogen Identification

Two ~7 cm stem sections with lesions and one~ 3 cm –leaf section with lesion were taken from each sample. Sections were rinsed in a 250 mL beaker covered with tea infuser under running water for 1 minute, surface disinfested by placing in 1% NaClO solution for 30 seconds for stem and root tissue and 15 seconds for leaf or delicate stem/root tissue and rinsed with reverse osmosis water for 15 seconds to remove the bleach solution. The sections were then cut into four to five~ 2 cm segments and plated on 25% PDA + antibiotics (streptomycin and chlortetracycline at 100ppm). Plates were placed in an incubator at 27°C for up to one month and checked for growth twice per week until ~15mm diameter of growth occurred. Pure isolates were obtained using hyphal tip technique, and sub-cultured to maintain the isolates.

Fungal isolates were morphologically identified to at least genus level using microscopy and taxonomic reference materials (Keinath 2017, Invasive.org, Barnett, 1998). Isolates with high relative frequency were selected for PCR identification to species level. Representative isolates were selected, and 7-day old cultures were used for DNA extraction following the protocol from the Zymo Quick – DNA Fungal/Bacterial Kit (Zymo Research). PCR was conducted using Thermo Scientific Phusion Flash High-Fidelity PCR Master Mix (Fisher Scientific) kit per manufacturer instructions using EF 1 and 2 primers for Fusaria isolates (Karlsson, 2016), and LSU and β-tubulin for all remaining isolates (Brown, 2014 & Stielow, 2015). PCR products were visualized on agarose gels and purified for sequencing with ExoSAP-IT™ *Express* PCR Product Cleanup Reagent ThermoFisher# 75001.20 ULclean up kit and sent to the Eton

Bioscience, Inc. in San Diego, California sequencing facility for Sanger sequencing. Resulting sequences were subjected to the NCBI BLAST database and sample identification was determined on the highest percent matches (Altschul et al., 1990).

2.2 Results

In total, 9 unique fungi from 6 genera were isolated from surveyed plants (Figures 2 & 3). *Stagonosporopsis* spp. were isolated from approximately 3-6% (n=11) of stem and root tissue samples from four of the five locations: Burleson, Glasscock, Hidalgo, and Frio counties. Frio County having the highest rate of recovery, and Maverick with no isolations (Figure 2). All isolates recovered were identified as S. citrulli (Table 3). *Fusarium* spp. was also prevalent at all sites and recovered from 6-76% of samples from each location. Three unique types of Fusaria were observed morphologically and identified as *F. brachygibbosum*, *F. proliferatum*, and *F. incarnatum* using PCR (Table 3, Figure 4). *Fusarium incarnatum* was the most frequently recovered across locations, as 62% of 94 total *Fusarium* spp. isolates. *Fusarium brachygibbosum* was the second most commonly recovered as 25% of *Fusarium* spp. isolates. *Alternaria* spp. was found across all sites and was recovered from 23-100% of stem and leaf tissue samples from each location. *Bipolaris* spp. was isolated from 7-23% of stem and leaf samples and *Rhizopus* spp. was isolated from 3-13% of stem and leaf samples collected. *C. orbiculare,* was also isolated from two sites, Hidalgo (3% of samples) and Frio (10% of samples) counties.

Figure 2: Pathogen incidence per county by genus. Thirty samples were taken at each location.

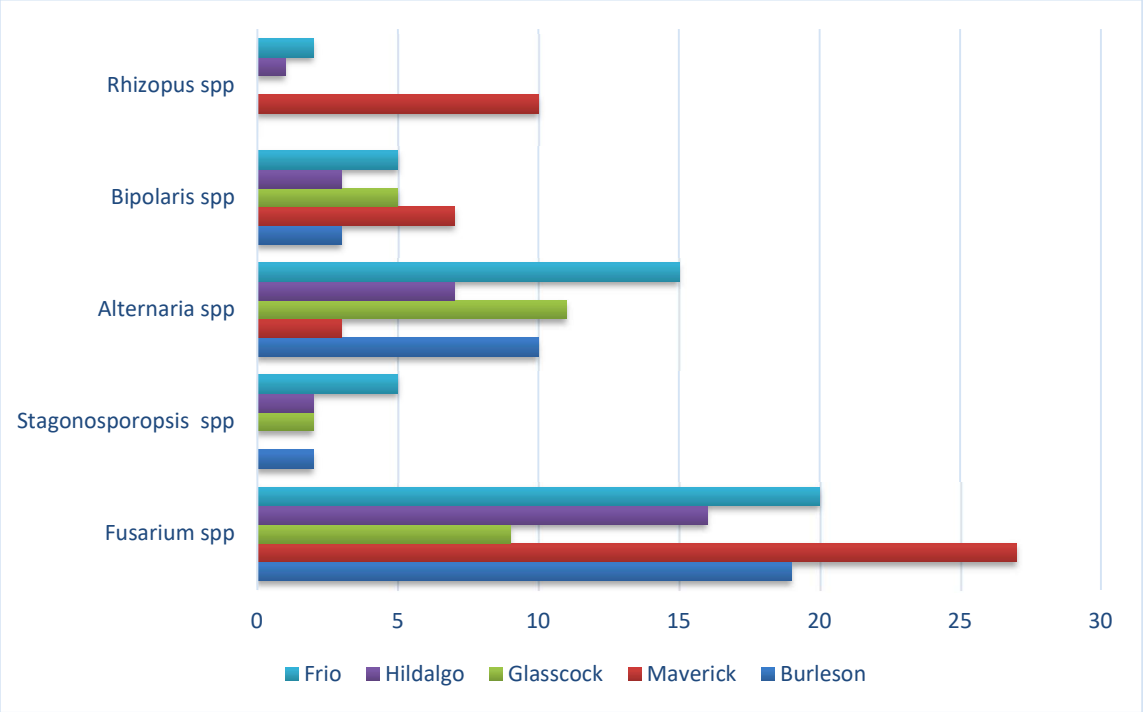


Figure 3: Pathogen recovery by sample tissue type. Pathogens were recovered from either root, stem, or leaf tissue. Data shows not all fungi were recovered from all types of tissue samples.

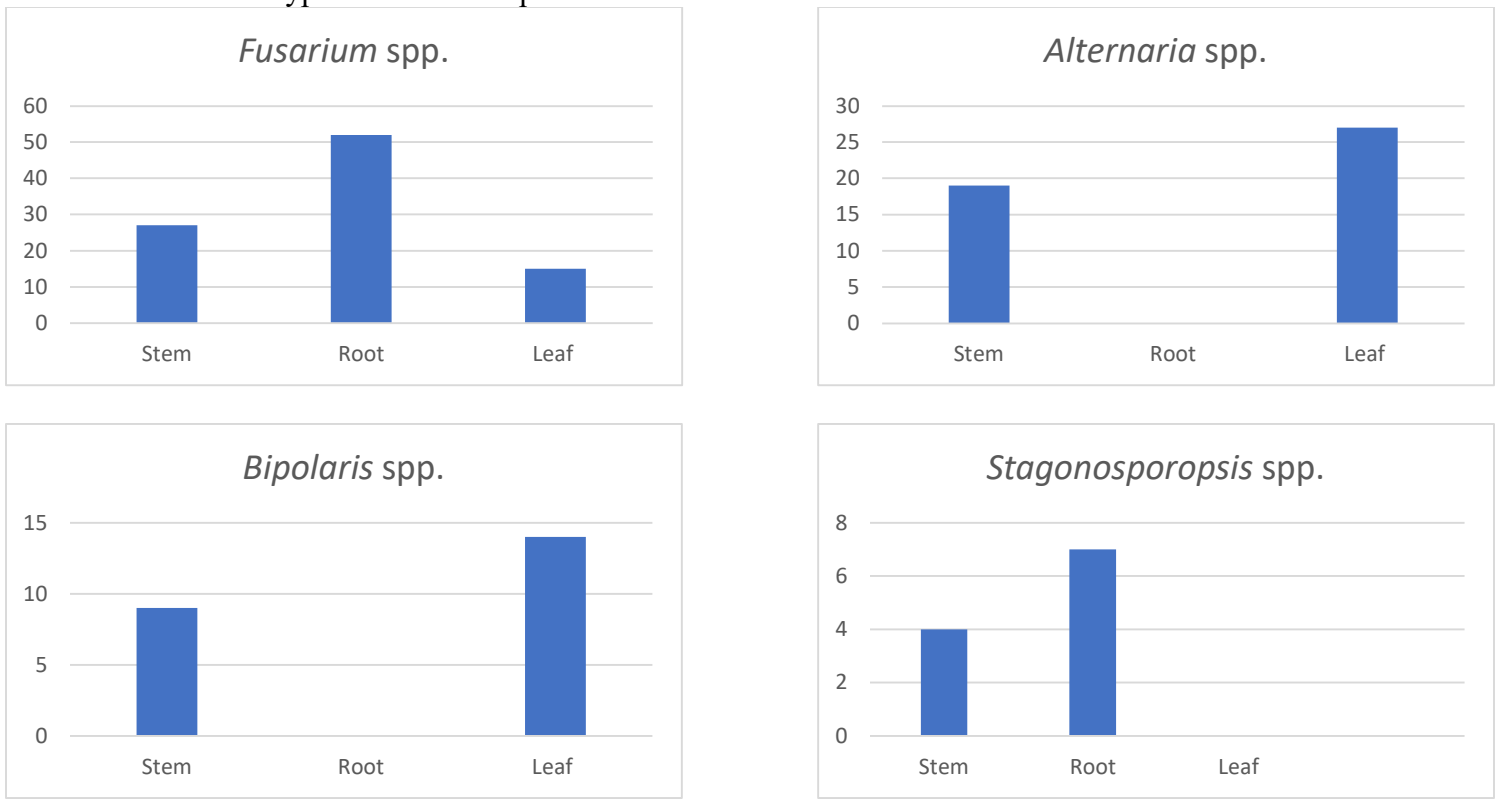


Figure 3: Continued

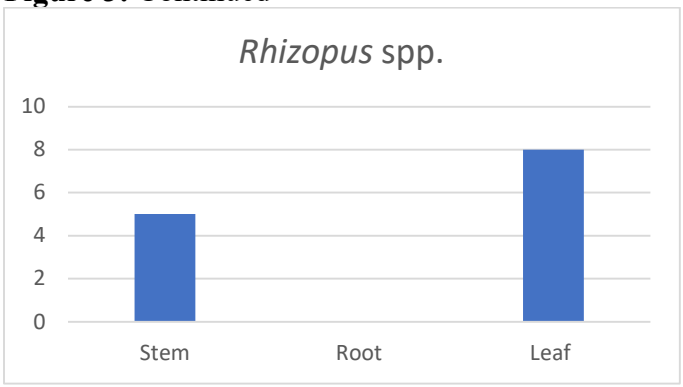
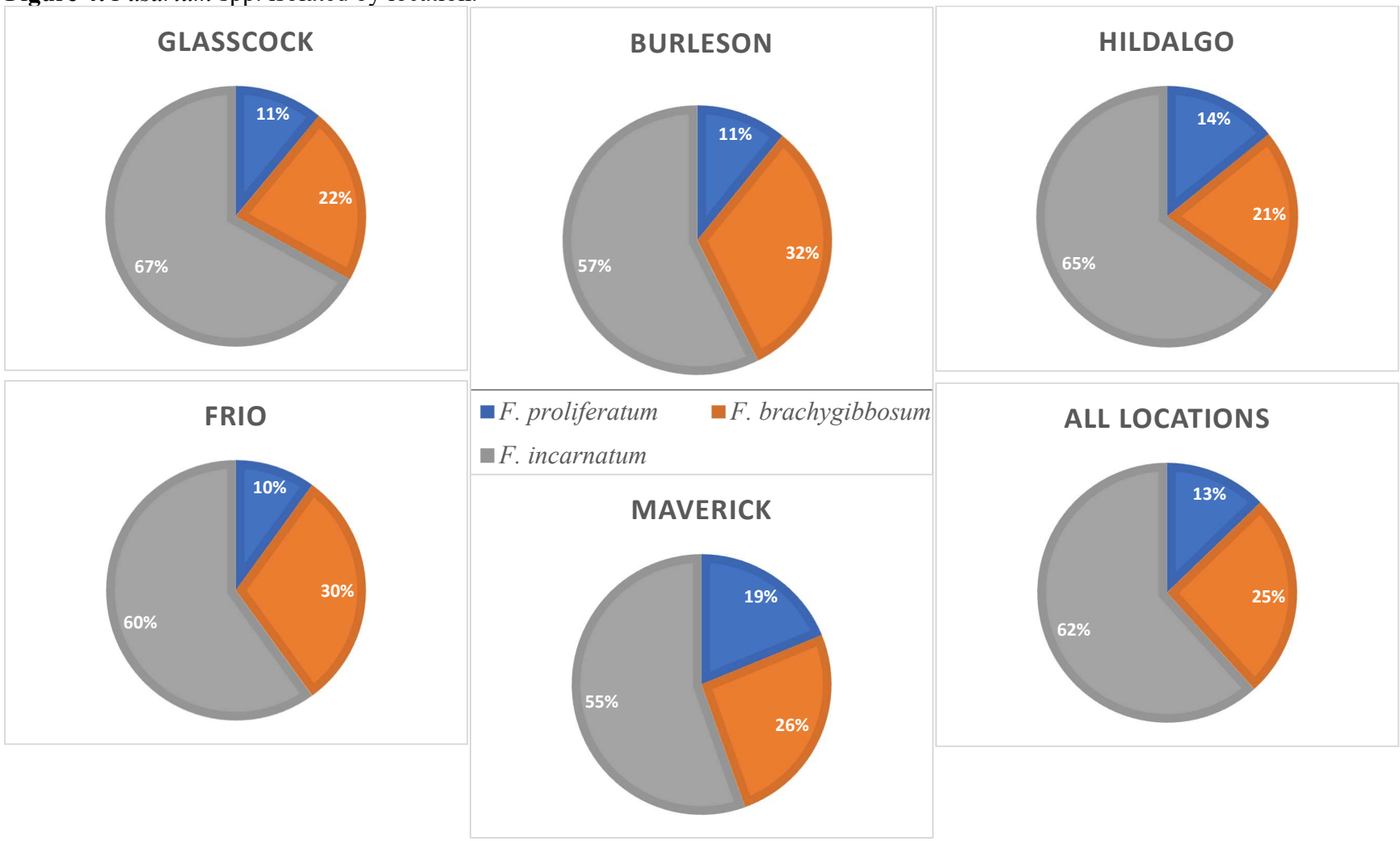


Table 3: Representative isolate identification PCR identification results.

Fungal ID	Primer	Blast Accession Number Match Example	% Match	% Coverage
Alternaria alternata	ITS/LSU	MN615420.1	100	100
Bipolaris drechsleri	LSU	NG_070031.1	100	100
Bipolaris sorokiniana	ITS	MT635282.1	100	100
Fusarium brachygibbosum	EF	MK648153.1	100	100
Fusarium incarnatum	EF	MT163656.1	99.82	100
Fusarium proliferatum	EF	MT095058.1	100	100
Rhizopus oryzae	ITS/LSU	MH877020.1	100	100
Stagonosporopsis citrulli	ITS/LSU	KJ855546	100	100

Figure 4: *Fusarium* spp. isolated by location.



2.2.1 Fungal Characteristics

Stagonosporopsis citrulli colonies were dark grey to black in PDA culture and produced pycnidia and pseudothecia, asexual and sexual fruiting bodies, respectively. Pycnidia were 120-180 µm in size while pseudothecia were typically slightly larger, 125-210 µm. Asexual conidia were formed in pycnidia, were 1 or 2 celled, cylindrical with rounded ends, and averaged in size from 6-10 µm x 3-5 µm Ascospores were 2 - celled, produced in asci within the pseudothecia, occurred in groups of 8 within each ascus, and were 13-16 µm x 3-6 µm. (Figure 5.H).

In PDA culture, *Fusarium brachygibbosum* had light to dark pink/red pigmentation with deep red sporodochia. Macroconidia were slightly curved in shape, with 4-6 septa. Microconidia are rarely observed in culture and were not observed in these isolates. The average size of the macroconidia is 17.6 µm x 2.7 µm, which was consistent with observations in pure cultures. Chlamydospores were globose and observed to occur intercalarily and terminally (Figure 5.A).

Fusarium incarnatum (syn. *F. semitectum, F. pallidoroseum*) is a part of the *F. incarnatum-equiseti* complex. Colonies were typically white or cream in culture with cream color sporodochia on PDA. This fungus produced two types of macroconidia: sickle-shaped and spindle-shaped, with a size range of 20-30 µm x 3-5 µm. Microconidia are rarely produced in culture but range in size from 1-3 µm x 4-10 µm. (Figure 5.B)

Fusarium proliferatum produced a light to deep purple pigmentation, similar to FON when grown on PDA. Macroconidia were fusiform with 2-3 septa, and were either

tapered, curved, or both in shape. Spore size ranged from 26-39 µm x 3.5-6 µm. Chains of microconidia produced on polyphialides were single celled and greatly differed in shape, the most common were oval, kidney, or spindle shaped, and were typically 5.9 µm x 15.1 µm in size (Figure 5.C).

Alternaria alternata (Fr.) Keissl. isolates grown on PDA were dark to olive green with conidia 16 - 70 µm. Conidia were multicellular, ovoid, or ellipsoidal in shape and short-beaked, and in chains emerging from conidiophores. Conidia had transverse septa ranging from 3-8 and 0-2 longitudinal septa. (Figure 5.G)

Rhizopus oryzae colonies were gray with blackish dots throughout and produced sporangia and sporangiophores. Sporangia were globose in shape and ranged from 30 µm to 225 µm, while sporangiospores were oval with spore sizes of 4- 10 µm in size (Figure 5.E).

Bipolaris drechsleri and *Bipolaris sorokiniana* produced elliptical, straight, or curved shaped conidia and conidiophores. Conidiophores had 5-9 septa with an average size of 60-120 µm x 12-20 µm. (Figure 5.D&F) Fungal colonies were white to grey pigmentation in culture.

Figure 5: Fungal Microscopy Photos. Fungal spores and cultures: micro- and macroconidia of A) Fusarium brachygibbosum B) Fusarium incarnatum C) Fusarium proliferatum D) Alternaria alternata conidia.

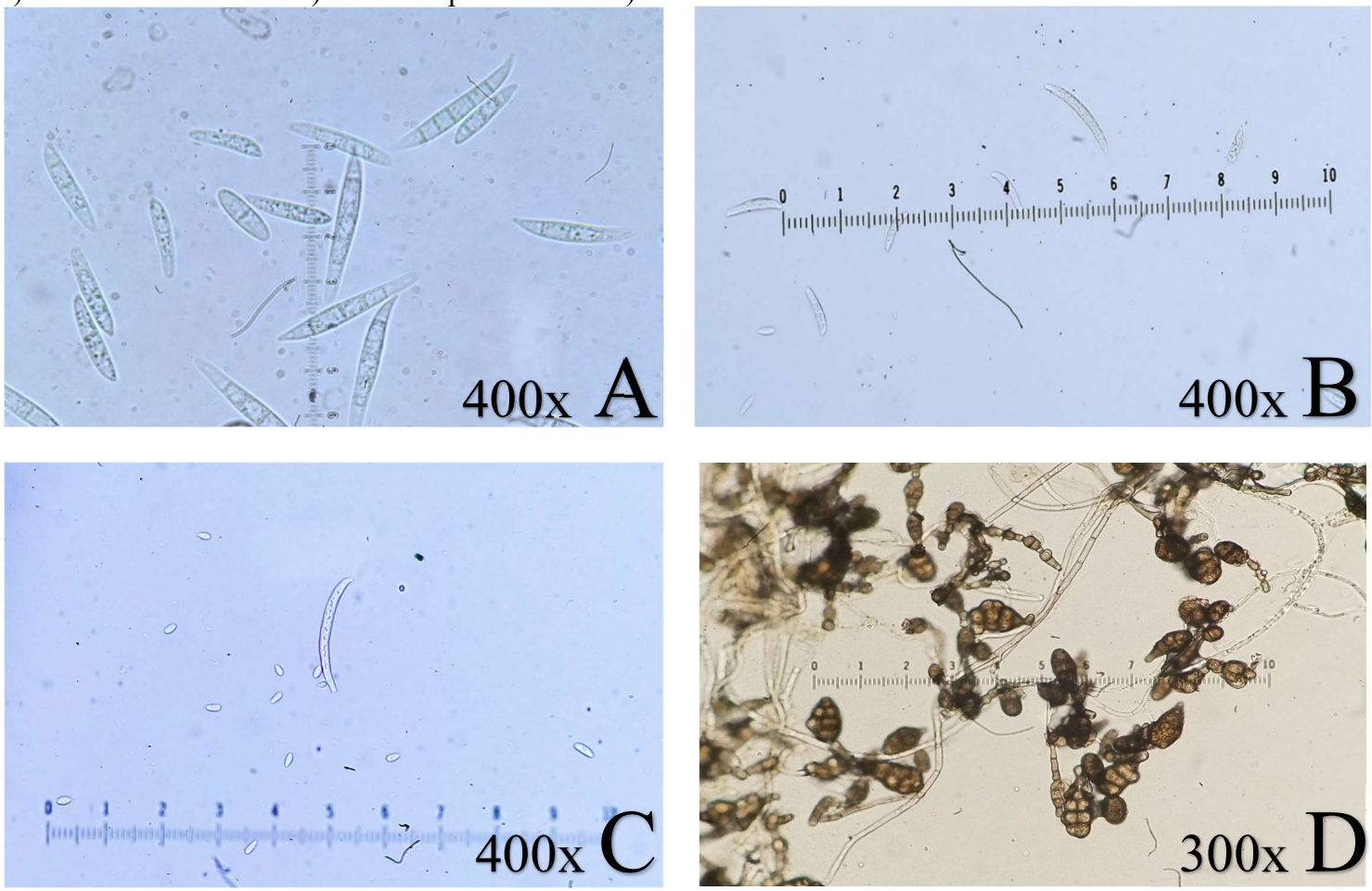
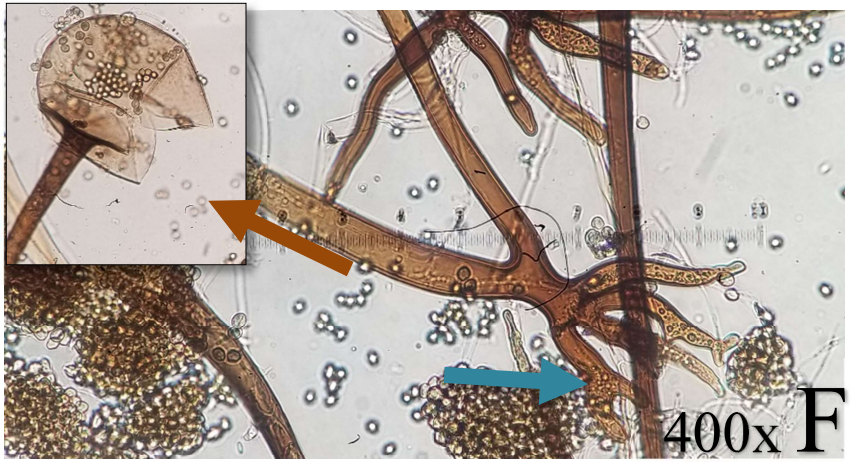
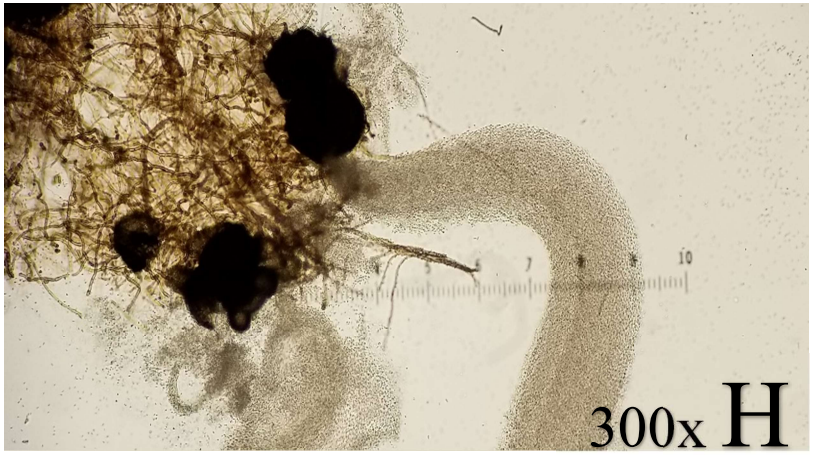


Figure 5: (Continued) E) *Bipolaris drechsleri* conidia; *F*) *Rhizopus oryzae* sporangium (orange arrow) rhizoid (blue arrow); G) *Bipolaris sorokiniana* conidia; H) *Stagonosporopsis citrulli* pycnidia and conidial ooze.





2.3 Discussion

This study has shown *S. citrulli* is geographically widespread throughout Texas watermelon production fields, occurring in 4 of the 5 major watermelon growing regions in the state. While *S. citrulli* has been documented in the southeastern United States, its distribution had not been well documented in Texas (Rennberger, Keinath, 2018). The findings of this work contribute to the understanding and management of GSB and can serve as a springboard for future studies.

Two of the Fusaria species recovered have been shown to cause disease on other members of the Cucurbitaceae, specifically *F. incarnatum* causes fruit rot on cantaloupe (Wonglom, 2020) and *F. proliferatum* causes Fusarium wilt on oriental melons (Seo, 2017). The three Fusaria species found in this study are previously unreported to cause disease on watermelon in Texas. However, in recent studies in Sonora, Mexico in 2015 *F. brachygibbosum* has been reported to cause disease on watermelon (Renteria-Martinez, 2015), while *F. incarnatum* to cause postharvest fruit rot in cantaloupes in Thailand (Wonglom, 2020), and *F. proliferatum* causes disease on oriental melon in Korea (Seo, 2017). Symptoms associated with field samples these fungi were isolated from included leaf tip scorching, browning of vascular tissue, and wilting, and in severe cases lesions and stem rot, which is consistent with other reports of these pathogens on cucurbits.

FON has been reported to be one of the more common pathogenic Fusaria observed on Texas watermelon (Martyn and Bruton, 1989), and was expected to be recovered in this study. The absence of FON in this survey could be due to growers

generally avoiding fields known to have FON, or the time of sample collection with respect to environmental conditions. FON favors temperatures between 25-30°C and the four weeks before samples were taken average daily temperatures in all locations ranged from 34-37°C. The warmer temperatures that occurred later in the growing season may have caused FON to become less active and allow other fungi to colonize plant tissues. Additionally, varieties grown by producers in this study were resistant to FON race 1, which could be a contributing factor to why FON was not recovered in this survey. Alternatively, plants infected with FON may have already succumbed to the disease by the time of collection. While there are differences in conidiophore structures, both FON and *F. proliferatum* have similar purple pigmentation in culture and similar shaped macro and microconidia and soilborne plant disease symptoms often overlap. This high degree of similarity may have contributed to past misidentification of *F. proliferatum* as FON by personnel without expertise and microscopy skills to observe the differences between the two species.

A. alternata causes leaf spot that affects most cucurbits, which was first reported in Greece in 1988 on cucumbers then on melons in 1990 (Keinath et, al., 2017). Alternaria leaf spot was first reported in the United States in 2006 in Wicomico County, Maryland when dark brown, circular lesions appeared on melons (Zhou & Everts, 2008). Considering the prevalence of *A. alternata* found in this study across all locations surveyed, this pathogen has likely been under reported within watermelon fields in Texas. Given the regular use of foliar fungicides for controlling foliar diseases in Texas watermelon production, the prevalence of this fungus warrants additional

analysis with respect to pathogenicity and fungicide efficacy. *R. oryzae* has been documented to cause soft rot on melons post-harvest in Korea. This pathogen was found on wounded melons that developed cracks during harvest causing them to rot quickly (Kwon, 2010). *R. oryzae* has also been shown to cause postharvest rot on other crops, including in apple in south Korea (Kwon, 2011). Additional work is warranted to determine the impact of *R. oryzae* on post-harvest melons.

While both *B. sorokiniana* ((Sacc.) Shoemaker) and *B. drechsleri* ((Sacc.) Shoemaker) were recovered from stem tissue. *B. sorokiniana* is an ascomycete that causes root rot disease of wheat and barley (Bockus, 2010 & Mathre, 1997). This fungus is the sexual reproductive stage of *Cochliobolus sativus* ((S. Ito & Kurib.) Drechsler ex Dastur), which is rarely seen in nature. Brown lesions are common symptoms on seedlings, crown, and roots. This seedling disease favors warm and humid conditions similar to other major watermelon pathogens. In 2017, another *Bipolaris* species (*Bipolaris spicifera)* was reported to causes leaf spot on watermelon in Egypt (Farag, 2017) and in 2009 this species was reported to cause disease symptoms on seedlings, crown, and roots of watermelon in Morocco (Mhadri, 2009).

Recovering and identifying previously undocumented fungi in Texas watermelon plants provides not only producer, but extension specialists and researchers with a better understanding of the current pathogens posing challenges to watermelon production. These fungi may have been undocumented due to past incorrect identification or lack of surveys and reporting, or simply due to producers or scouts not recognizing mild to moderate symptoms among the vines in the fields. Incorrectly attributing disease

symptoms in the field to expected pathogens, such as FON, may lead to ineffective management resulting in wasted resources and loss of yield. With respect to future work, pathogenicity tests will be done with the isolates found in this survey to determine if these fungi cause disease symptoms on watermelons. Lastly, additional studies need to be conducted on the fungi found in this survey to better understand the potential economic impact on Texas watermelon production.

CHAPTER III

ENVIROMENTAL COMPONENTS

Objective: To determine relationships between environmental components and the recovery of fungal isolates recovered across Texas.

3.1 - Methods

3.1.1 Environmental components Data

Weather underground was used to obtain environmental data for the five sample collection locations in chapter 2: Burleson (Burl), Frio, Glasscock (Glass), Hidalgo (Hid), and Maverick (Mav) counties. Data were harvested from weather stations within 5 miles, or as close as possible to each field location. Data collected included temperature, relative humidity (RH), dew point, wind speed (WS), wind gust (WG), air pressure, and precipitation (Supplementary Table 2). Data included the 45 days before sample collection for each location.

3.1.2 Analysis of relationships between environmental components and fungi isolated from watermelon across Texas.

Daily averages were calculated for each location to prepare data for analysis for all components of the environmental data used for comparative analysis: temperature, RH, dew point, WS, WG, air pressure, and precipitation. Principle components analysis was performed in R studio to reduce the dimensionality of these large datasets while increasing interpretability and minimizing information losses (Jolliffee and Cadima, 2016). PCA standardizes the raw data so all variables will contribute equally and on the same scale within the analysis due to the sensitivity of PCA to the variances in raw data.

All locations were included in the analysis. Tukey tests were conducted for each environmental condition to examine differences in environmental components among locations.

Pathogen incidence data from chapter 2 was used in this objective. Analysis of the potential inferences of relationships between environmental components and incidence of fungal microbes recovered in chapter 2 were examined.

3.2 Results

The environmental components that had the strongest positive influence across all locations were humidity and dew point. Of these two components, dew point had the strongest impact (Figure 6). Wind speed, wind gust, air pressure, and precipitation were very similar to each other in how impactful their influences were on the environment (Figure 6).

A Biplot for all 45 days prior to the sample dates Burleson, Frio, and Hidalgo showed no significant differences among their environmental components, meaning their environments were overall similar at these locations (Figure 7). Maverick county was significantly different from Burleson, Frio, Hidalgo, and Glasscock (Figure 7), while Glasscock was the most different from all other environments. Two biplots were created to examine the 2 months within the 45 days and a trend can been seen among the counties (Figures 8&9). The biplot for May showed results similar to the combined 45 days, with Frio, Hidalgo, and Burleson counties having similar environmental components. Maverick Co. environmental components was significantly different from the previous three counties, while Glasscock had the greatest statistically significant

difference from all other counties. The June biplot shows Maverick County was statistically similar to Frio, Burleson, and Hidalgo, while these 3 locations had slight differences in the environmental components compared to the May biplot. Environmental components of Glasscock County had the greatest statistically significant difference from all other counties surveyed in June.

Figure 6: Principal Components Analysis correlation circle of environmental components across all locations.

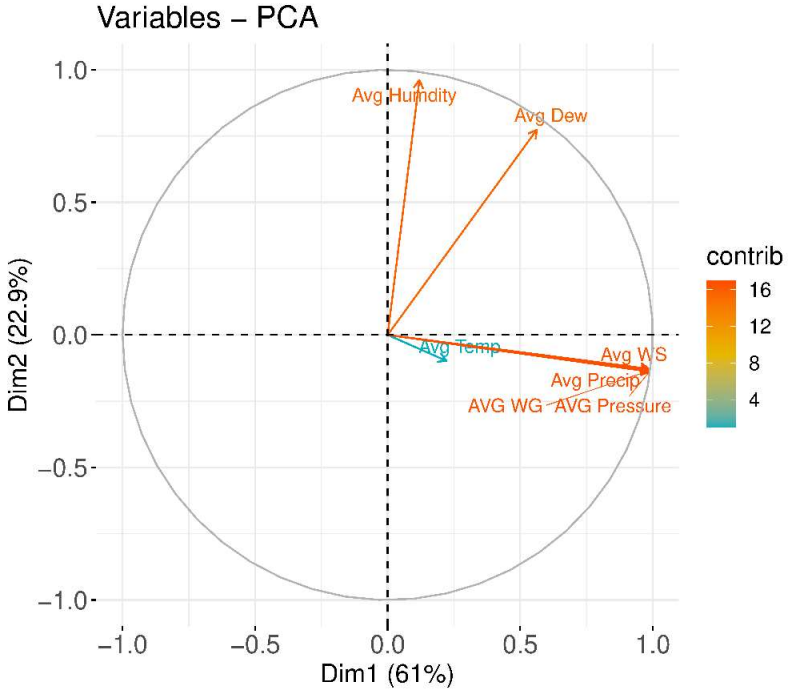


Figure 7: Principal Components Analysis biplot of daily environmental components per location 45 days before sample date.

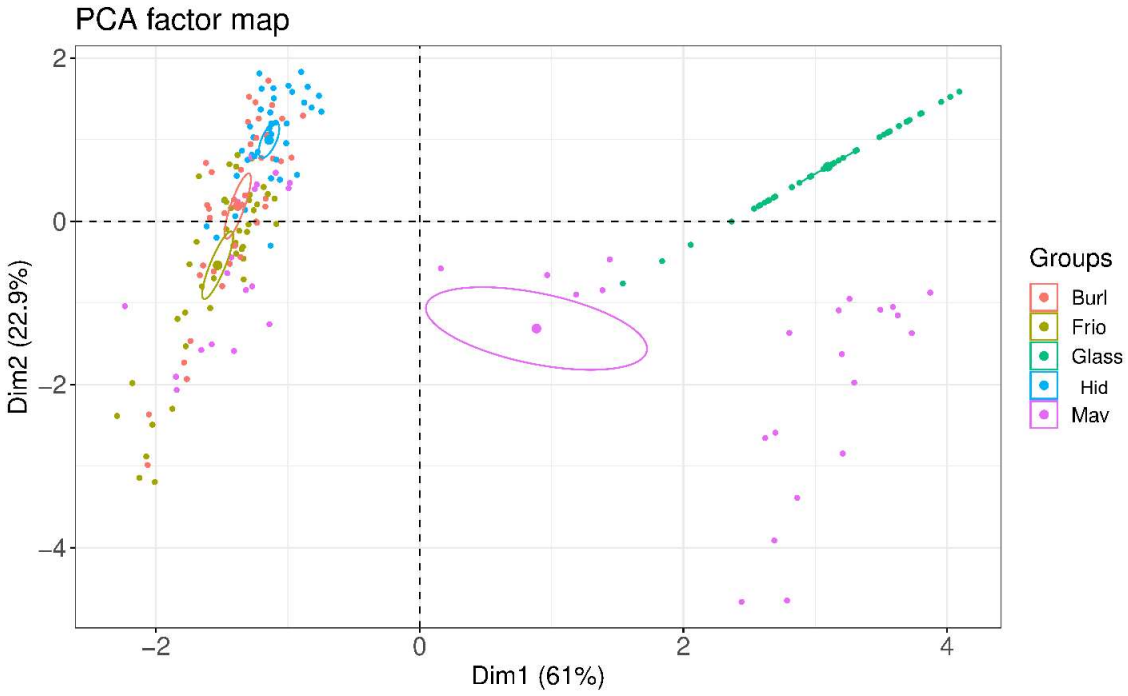


Figure 8: Principal Components Analysis biplot of daily environmental components per location June 2020.

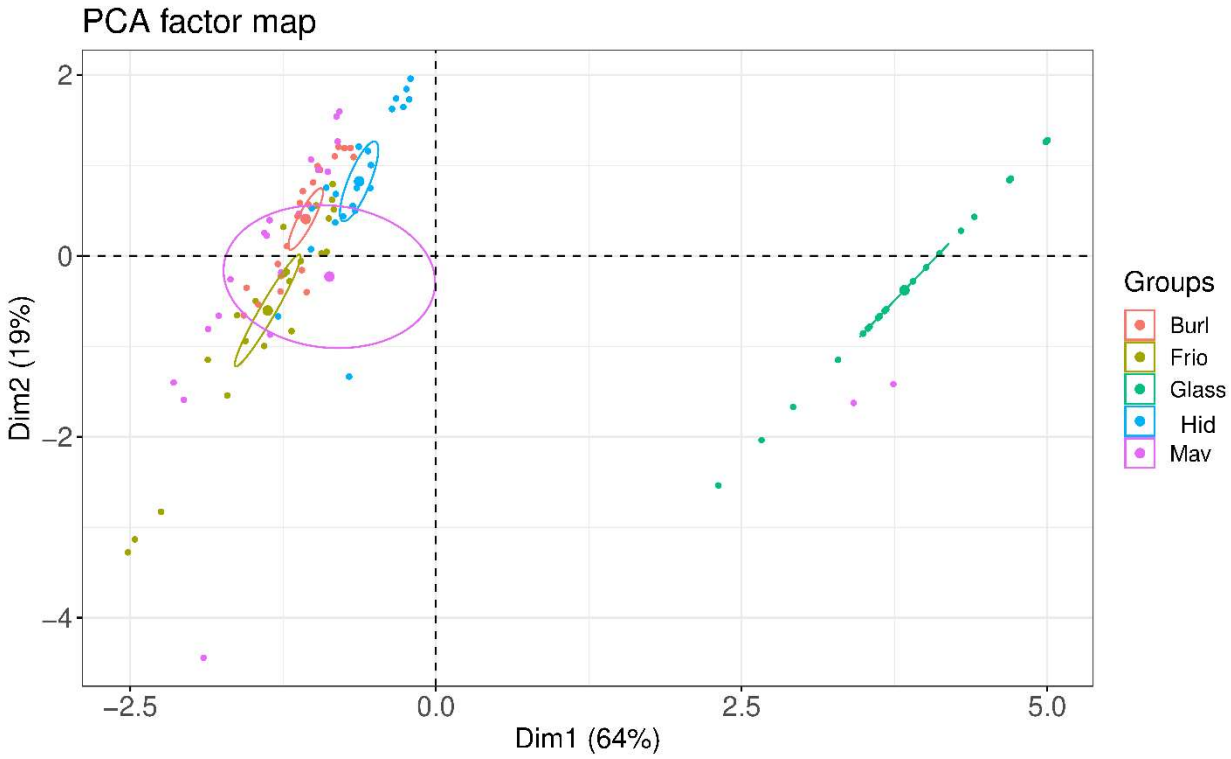


Figure 9: Principal Components Analysis biplot of daily environmental components per location May 2020.

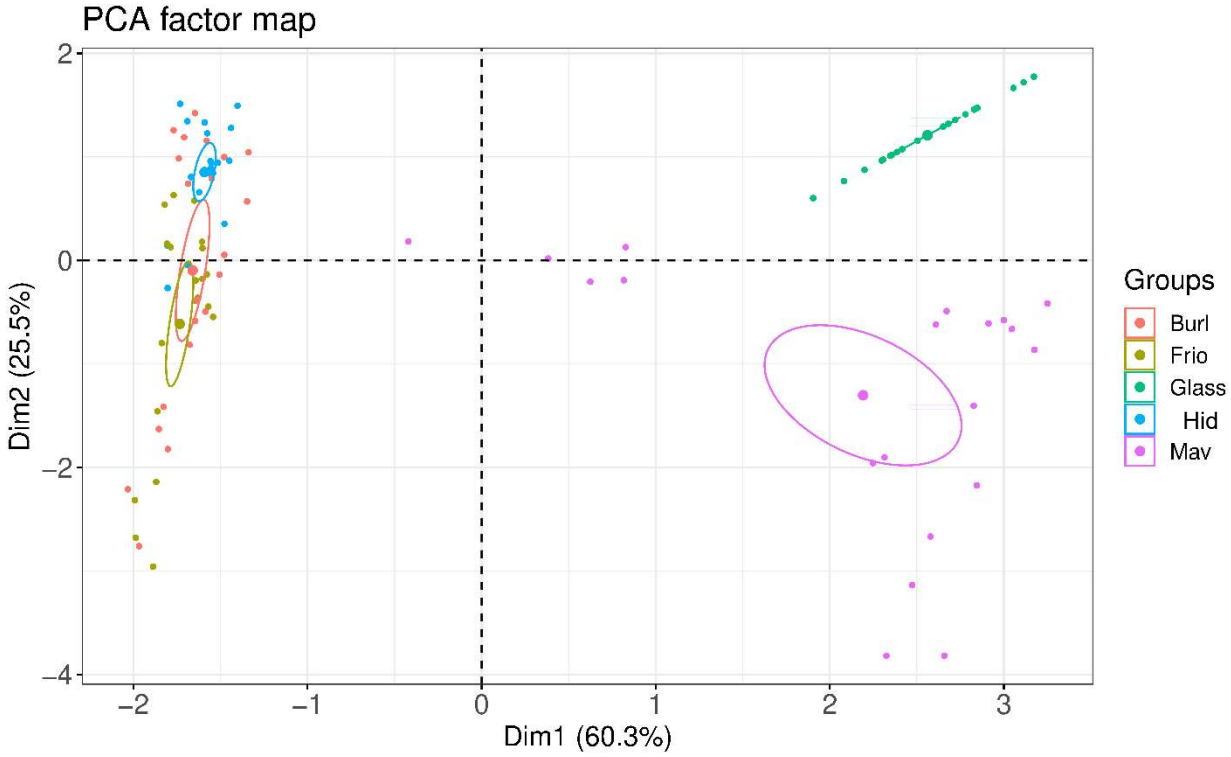
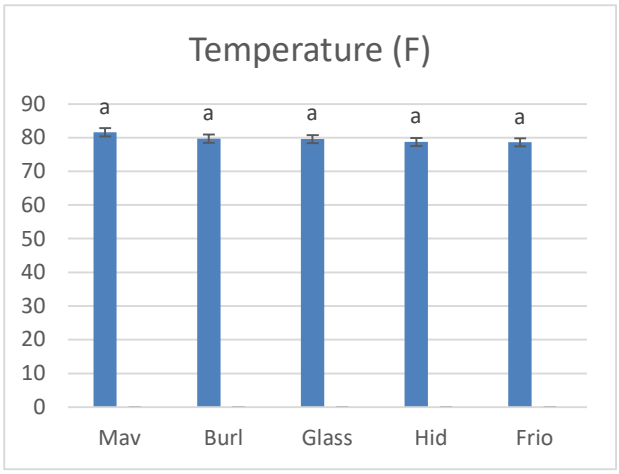
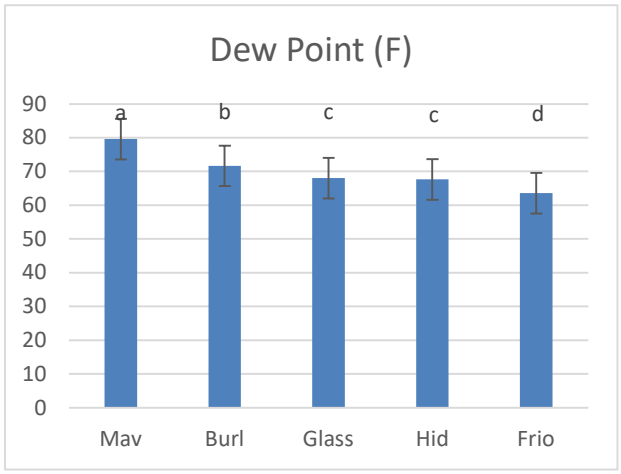
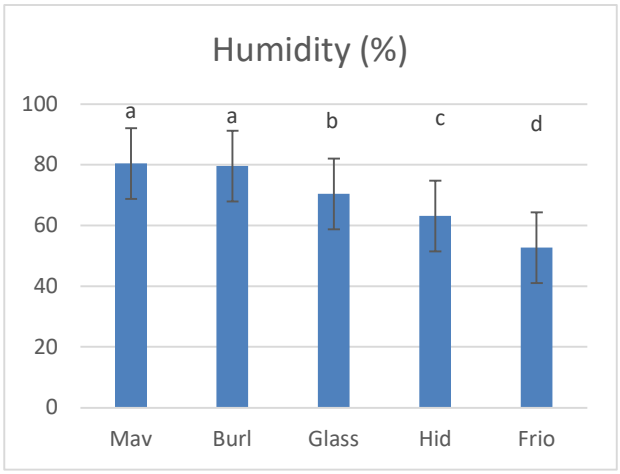


Figure 10: Tukey Tests for each environmental component with standard error. Different letters indicate significant differences.







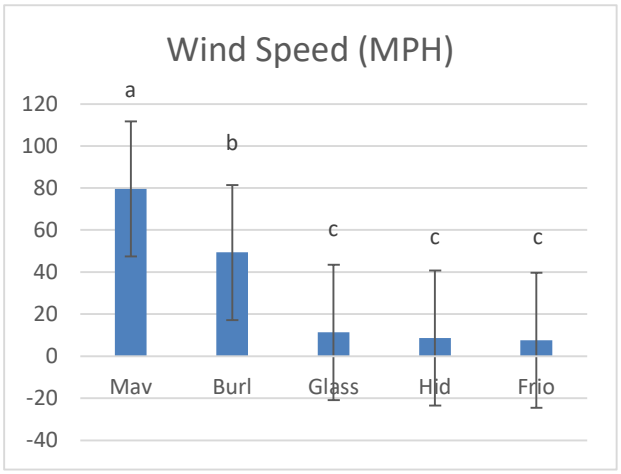
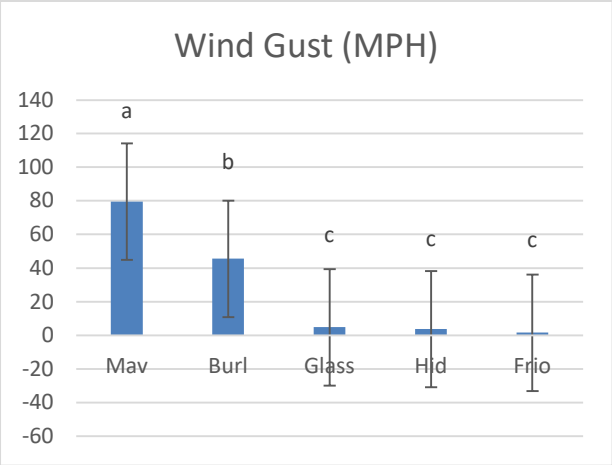
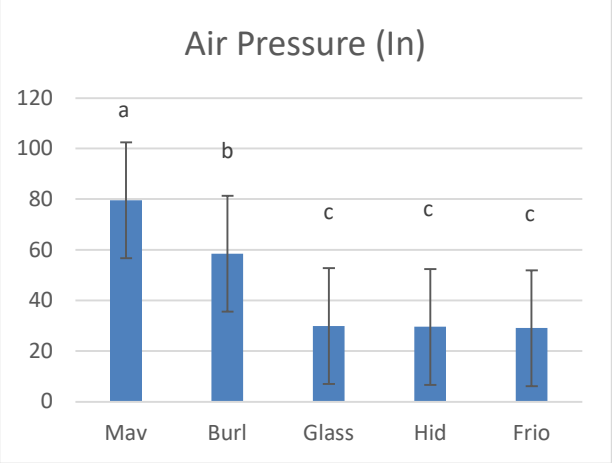
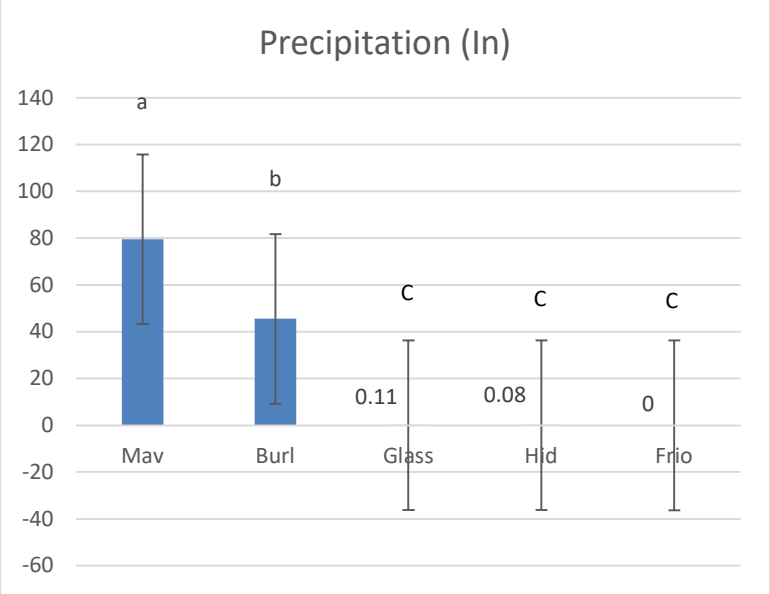


Figure 10: Continued







Tukey tests (Figure 10) showed temperature had no statistically significant differences across all locations. Maverick and Burleson had the highest values in all environmental components and were statistically different from each other and the remaining locations in all components with the exception of humidity, where they were statistically similar to each other.

Wind speed, wind gust, pressure, and precipitation were statistically similar across Hidalgo, Frio, and Glasscock counties, while these locations had significant differences in humidity and dew point. Specifically, Glasscock and Hidalgo were statistically similar in average dew point, though statistically different in humidity, while Frio was statistically different from the other locations for both dew point and humidity.

3.3 Discussion

Of the five locations studied, Glasscock had the most unique environment in the preceding 45 days before sampling. Humidity and dewpoint were the greatest influences in the differences among locations. An environmental component that was previously considered to be significant to pathogen incidence, temperature, did not highly affect each location as previously assumed (Figure 10) and components WS, WG, and air pressure affected the locations more significantly (Figure 6).

Environmental components not only affect plant growth, but the ability of plant pathogens to grow and thrive as well. Favorable components for fungal plant pathogens not only allow for overwintering, but generation of inoculum, which when combined

with a susceptible host plant or substrate, can lead to increased disease pressure and incidence of symptomatic plants in the field.

In light of the favorable components for each fungus described in chapter 2, the results from the PCA analysis can be used to make inferences about differences in pathogen incidence per location. Glasscock county had the most unique environment, and also had least amount of fungi recovered from each species overall (Figure 2). Considering environmental and pathogen recovery data as a whole, it could be inferred that the ideal environmental components for these fungi recovered in chapter 2 were not met. This may have been due to the lower precipitation and wind conditions observed in Glasscock County not providing adequate means for these fungi to spread within the field (Figure 10). Soilborne pathogens such as the Fusaria spread through water splashing onto the soil releasing spores, without adequate rainfall or overhead irrigation to achieve this requirement, infection does not spread quickly throughout the field. The same can be inferred for foliar pathogens such as *Alternaria alternata*, without significant wind, spores will not spread throughout a field.

Maverick county had the highest average for humidity, dew point, and precipitation making this county the ideal environment for the three *Fusarium* species such as high humidity for excess water and precipitation to allow spread of spores (Figure 2 & 10) (Larone 1995). When sampling occurred in June, Frio and Burleson counties had similar environmental components which could possibly explain why these 3 locations showed the highest incidence for *Fusarium* species (Figure 8). A possible reason why *S. citrulli* was not recovered from Maverick, even with abundant moisture as

high humidity, dew point, and precipitation, could be due to wind speed and wind gust. *Stagonosporopsis citrulli* requires continual leaf wetness to cause disease (Figure 1), wind quicky dries the leaves not allowing the optimal duration and frequency for infection to occur.

CHAPTER IV

PATHOGENICITY TESTING

Objective: To determine pathogenicity of the fungi found in Texas watermelon production fields.

4.1 Methods

4.1.1 Seedling Establishment

The varieties Black Diamond, Florida Giant, and Sugar Baby were used in this experiment, as they are susceptible to a variety of pathogens, including several found in this study. Twenty-one seeds of each of the three varieties were grown separately in 63-10cm pots filled with Pro-Mix LP15 multi-purpose soil (Pro-Mix) for 4 weeks in the growth chamber replicating typical spring/summer growing conditions: 14-hour light at 24°C and 10-hour dark at 22°C with 65-70% humidity. Plants were watered as needed to maintain growth. Plants were fertilized with Liquid Miracle Grow once according to manufacturer recommendation. Pots were grouped into 3 pots per replication, with 7 replications in a randomized complete block.

4.1.2 Inoculum Preparation & Inoculation

One representative isolate of each of the most common fungi isolated from watermelon samples in chapter 2 were used to determine pathogenicity. These were: *S. citrulli, F. brachygibbosum*, *F. incarnatum*, *F. proliferatum*, *B. sorokiniana,* and *A. alternata*. Inoculum was prepared using methods previously described: *S. citrulli* by

Keinath (1995), *Fusarium* spp. by Xue (2004), *Bipolaris* spp. by Sun (2020), and *Alternaria alternata* by Tymon (2016). Each isolate was grown for one week on petri plates of 25% PDA+ antibiotics (streptomycin and chlortetracycline at 100ppm, 25PDA+a). Inoculum was prepared for each isolate per the methods stated above. Sterile water was added to the spore solution until a concentration of 10^5 conidia/µL was achieved. Inoculum was stored at 4°C and used within 3 days after preparation.

Three seedlings from each variety were used for controls, and three additional seedlings from each variety were used for each of the 6 isolate inoculations (Figure 11). At 4 weeks old, control seedlings were punctured with a sterile 16-gauge needle on 3 leaves and sprayed until runoff with sterile water. After the control plants are sprayed, they were placed in the growth chamber immediately to reduce the possibility of contamination. Seedlings to be inoculated were punctured with a new sterile small gauge needle and sprayed until runoff with the prepared inoculum solution of a single isolate. Seedlings were then placed in a growth chamber with a photoperiod of 14hr light at 30°C, 10hr dark at 24°C and 95% humidity for 3 weeks. To track temperature and humidity, a Govee Smart Hygrometer monitor was used placed in the growth chamber to ensure the targeted temperature and humidity was achieved and maintained.

4.1.3 Disease Symptom Assessment

At 3 days post inoculation (dpi), seedlings were checked for symptoms (Figure 12) and rated on a scale of 1 (0% leaf area symptomatic), 2 (< 1% leaf area symptomatic), 3 (1-10% leaf area symptomatic), 4 (11-25% leaf area symptomatic) and 5 (>75-100% leaf area symptomatic) (Table 4), and symptomatic area was defined as

chlorosis, leaf necrosis, wilting, and brown lesion development. Plants were rated individually within the 7 inoculation treatments, and ratings were averaged to create the final score for the treatment overall. Seedlings were rated every 5 days after the initial rating. At 21 dpi, seedlings were rated (Table 4), checked for symptoms (Figure 13), and surface disinfested, as previously described in chapter 2. Symptomatic areas of the seedlings were cut into 3cm sections of root, stem, and leaf tissue, and plated on 25PDA+a, and incubated for 1 week. Resulting cultures were confirmed to be the target fungi using morphological identification.

4.2 Results

Stagonosporosis citrulli and the Fusaria species consistantly had high disease severity ratings throughout the 21 days. Specifically, plants inoculated with *S. citrulli* (GSB) isolates had the greatest foliar disease symptoms, while the Fusaria inoculated plants showed the greatest wilt symptoms (Figure 13). After 21 dpi, 90% of plants inoculated with GSB had a disease rating of 5. *Fusarium incarnatum* and *F. brachygibbosum* produced the most severe disease symptoms out of the three *Fusarium* spp. At 7 dpi, plants inoculated with the three Fusaria had wilt symptoms appear when 3 days passed between watering, though initially plants would recover after being watered. The plants that were not inoculated with *Fusarium* spp. did not show wilt symptoms with water stress as severely and always recovered after watering. Noticing these symptoms, I continued waiting 3 days between watering plants inoculated *Fusarium* spp. isolates. After the third occurrence of induced water stress, approximately 15 dpi, plants no longer fully recovered after watering (Figure 11, 12, & 13).

Table 4: Average rating of symptomatic tissue determined by ratings for all plants within the treatment and taking the average.

Isolate	Average 3 dpi Disease Rating	Average Final Disease Rating 21 dpi
Stagonosporopsis citrulli	2	5[z]
Fusarium brachygibbosum	2	4
Fusarium incarnatum	2	4
Fusarium proliferatum	2	4
Alternaria alternata	1	3
Bipolaris spp.	1	2

[z] = Disease ratings were: 1=no symptoms, 2= <1% leaf area symptomatic, 3=1-10% leaf area symptomatic, 4=11-25% leaf area symptomatic, 5= >50% leaf area symptomatic.

Figure 11: Control and inoculated plants before inoculation, 3 dpi, and 21 dpi.

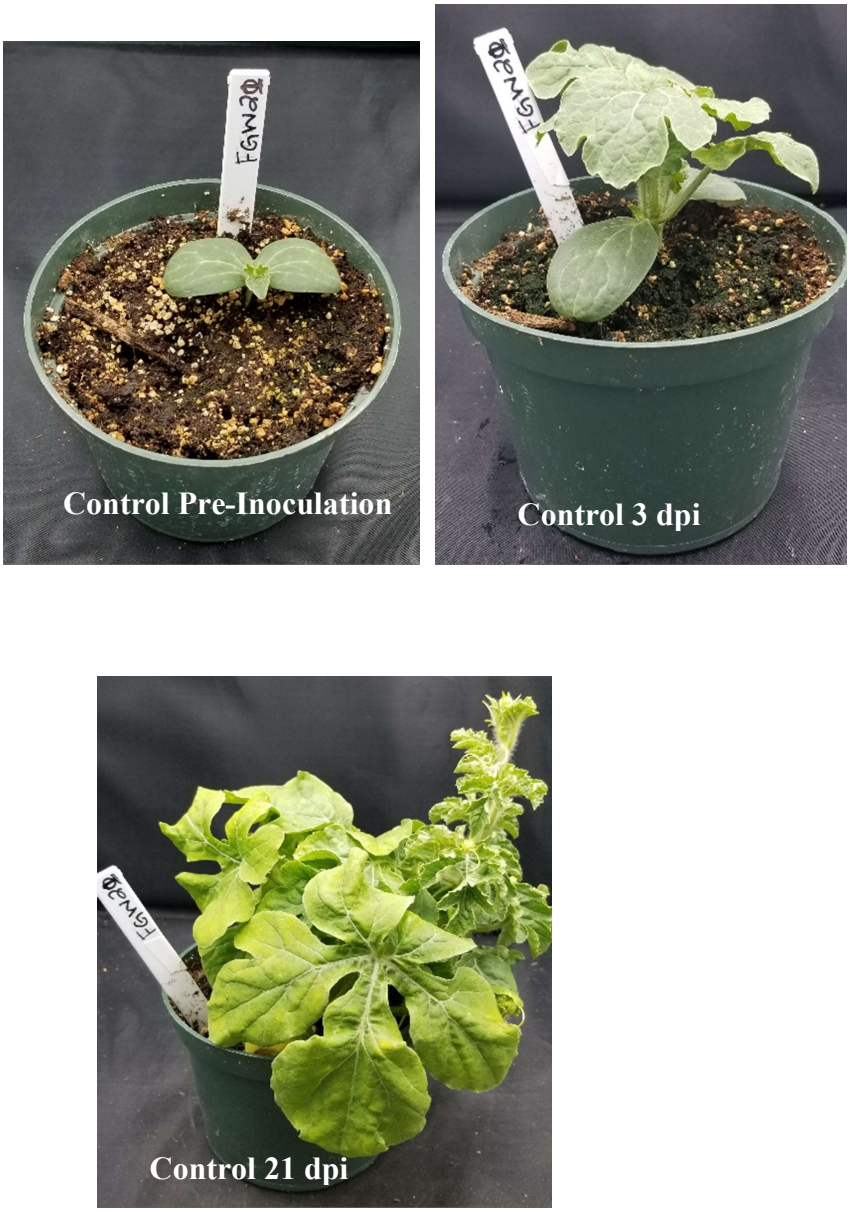
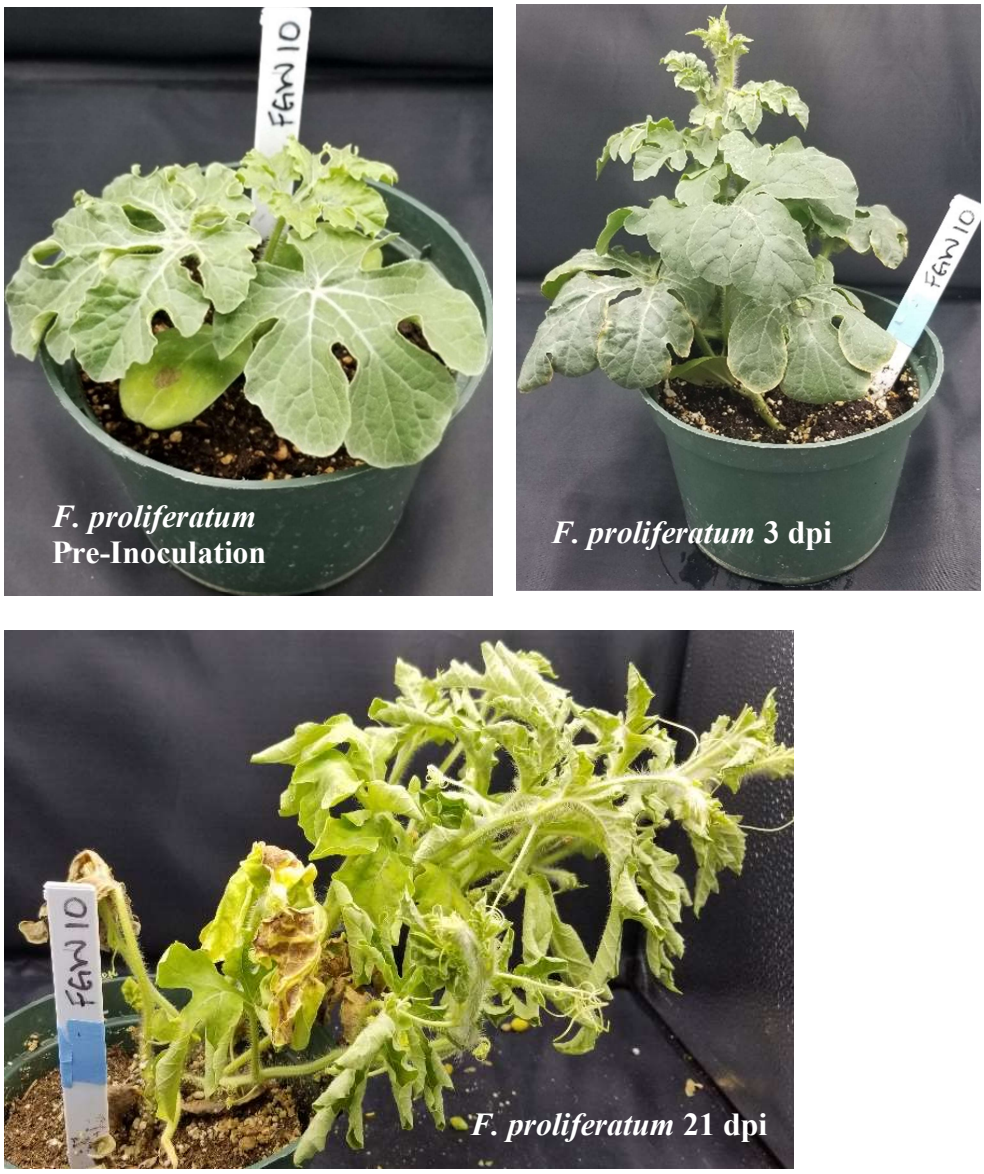


Figure 11: Continued



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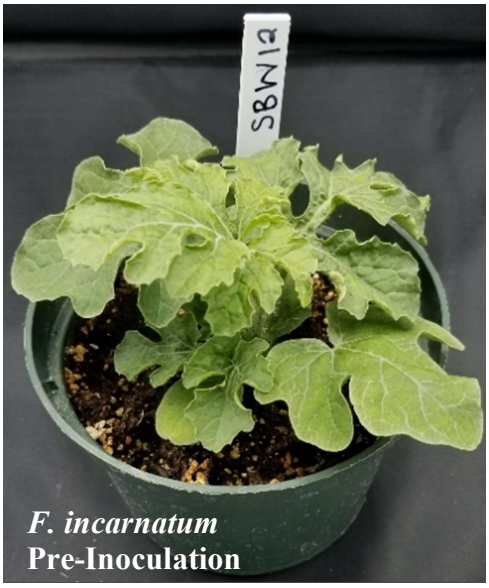

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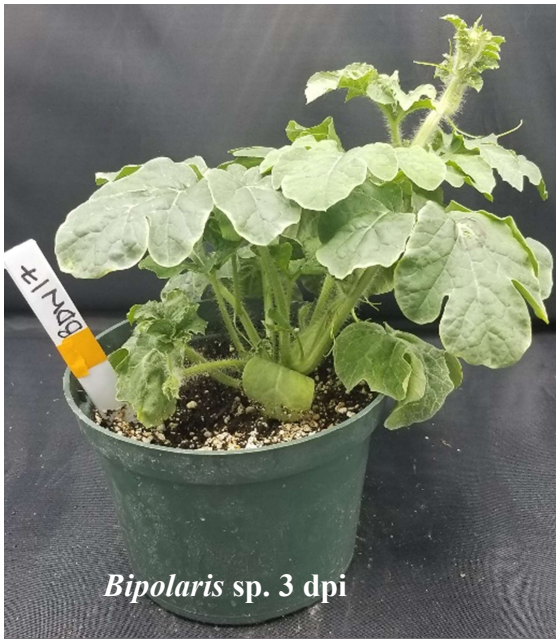

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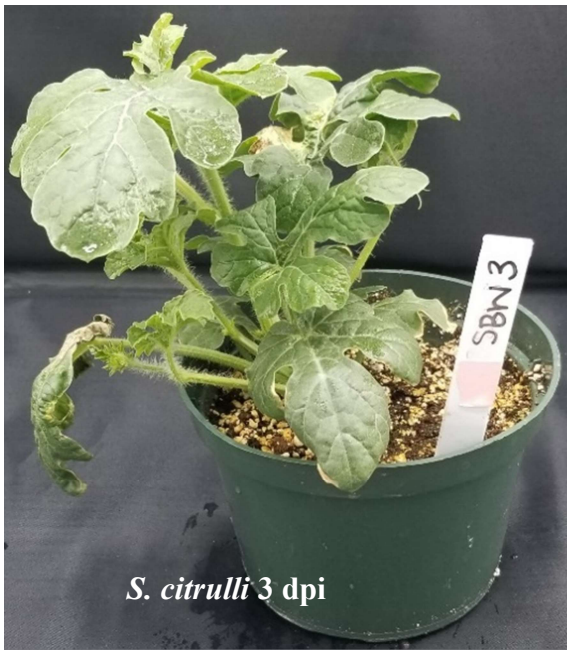


Figure 11: Continued

Figure 11: Continued

Figure 12: Close up of symptoms of (1) Fusarium proliferatum, (2) Fusarium brachygibbosum, and (3) Stagonosporopsis citrulli at 3 dpi.

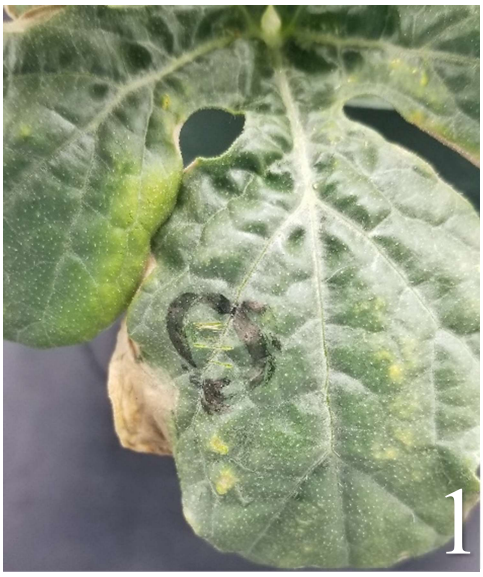
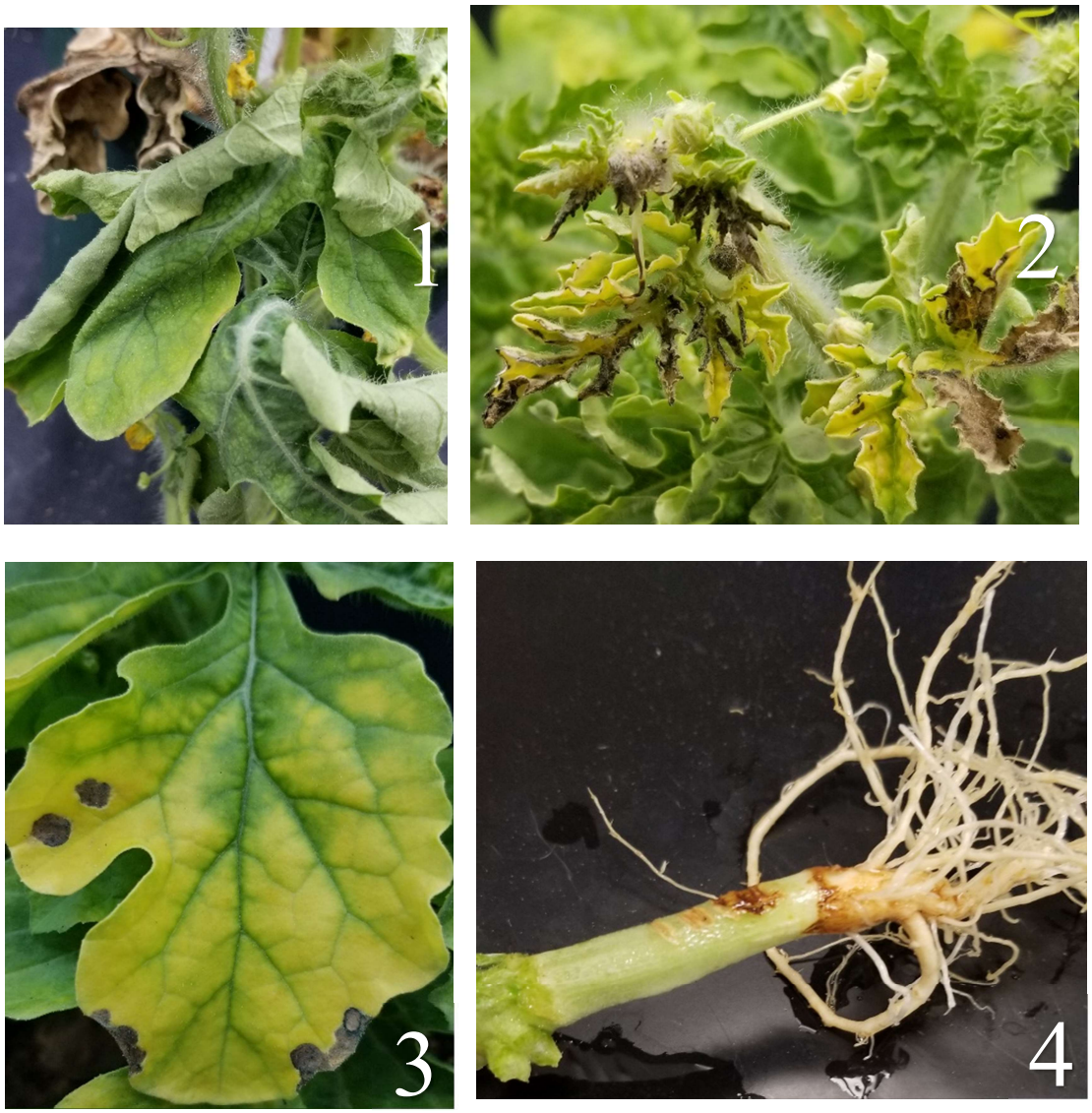


Figure 13: Close up of symptoms of (1) Fusarium brachygibbosum, (2) Alternaria alternata, (3) Bipolaris sp. and (4) Stagonosporopsis citrulli, 21 dpi.



After culturing, isolates of all inoculated fungi were recovered from tissue samples collected from inoculated plants, which fulfilled Koch's postulates. The *Fusarium* spp. and *S. citrulli* isolates were recovered from root and stem tissue, while *Bipolaris* sp. and *A. alternata* were recovered from stem and leaf tissue. The control group had slight contamination (n=2) from the other two of the Fusaria isolates used due to crowded vines touching in the growth chamber (*F. brachygibbosum* and *F. incarnatum)*.

4.3 Discussion

Stagonosporopsis citrulli inoculation resulted typical GSB symptoms on each watermelon variety. This pathogenicity test confirmed *S. citrulli* caused symptoms such as root lesions, chlorosis, and necrosis of leaves, and produced minor lesions on stem tissue, which are all typical of symptoms seen in the field. This pathogen has been previously documented throughout the southern United States (Keinath, 2011), but not well documented in Texas.

While *Fusarium* species such as FON are known to infect cucurbits and cause wilt symptoms (Keinath, 2017), pathogenicity and resulting symptoms of other species of *Fusarium* on watermelon were unknown. Plants inoculated with *F. brachygibbosum, F. incarnatum, F. proliferatum* in this study all exhibited wilting symptoms, and especially so when under water stress. Plants inoculated with *F. brachygibbosum* exhibited the most striking symptoms across all varieties in the conditions of the growth chamber. The wilting symptoms observed in plants inoculated with the 3 Fusaria

species were first detected when plants were under water stress. Wilt symptoms were observed when plants were not under water stress several days later, indicating the symptoms are more apparent when plants are stressed. Watermelon fields within Texas are not always irrigated well and rely at least partially on rain to water crops, which is unpredictable at best. Due to unpredictable water supply, wilting symptoms may not be seen as an indication of disease resulting in early symptoms not being noticed in the field and may go unnoticed altogether under low disease pressure.

In this study, symptoms from both *A. alternata* and *Bipolaris* were consistent with a leaf blight. *Alternaria alternata* and *Bipolaris* spp. symptoms could affect fruit growth due to the chlorotic leaves throughout the plant reducing sugar production and other nutrients required for fruit production. These symptoms could lead to general plant health decline and cause eventual death if not identified and managed correctly (Zhao, 2016, Farag, 2017, Mhadri, 2009). While *A. alternata* is often described as a weak pathogen, symptoms in this study indicated it may be more significant than previously reported due to the recovery rate from tissue samples in this survey. Additionally, *A. alternata* is also a postharvest disease, Alternaria Rot, on other cucurbits such as cantaloupe and cucumber (Keinath, 2017), the impacts of which require additional research to fully understand. *Bipolaris* spp. is a more widespread pathogen on watermelon than previously documented from Mhadri (2009), Zhao (2016) and Farag (2017), and needs to be monitored by producers and extension personnel not only in Texas but in US watermelon production. Future work could include surveys for

Alternaria spp. and *Bipolaris* spp. in Texas watermelons followed by molecular identification work and additional pathogenicity tests with other species and isolates.

The likelihood of nutrient deficiency causing chlorosis symptoms in this study is low due to all seedlings being fertilized with Liquid Miracle Grow 14 days after planting. The experiment was concluded 5 weeks after fertilization, while the label recommended to repeat application every 7 weeks in containers. Other factors that could cause chlorosis, such as mites, were not observed in this study. While 95% of pathogenicity confirmation cultures did not have any contamination, two control plants that were touching inoculated plants in the growth chamber did have *F. brachygibbosum* and *F. incarnatum* isolated at the conclusion of the study. Though this limited cross contamination occurred, the inoculated target isolates were still recovered from each plant.

These results have confirmed the pathogenicity of *S. citrulli* and have shown that 5 fungi previously undocumented on watermelon caused disease symptoms on three different varieties of watermelon. The symptoms *Fusarium* spp. isolates produced in this study may be mistaken as water stress within fields, as early symptoms may resolve after irrigation. Additional work is needed to better understand the impacts of these newly described pathogens on fruit production and economic profitability of watermelon and other cucurbits in Texas.

CHAPTER V

CONCLUSIONS

In total, seven fungal species were isolated from root, stem, and leaf samples collected from Texas watermelon fields. Only one of the three causal species of GSB, *Stagonosporopsis citrulli*, was present in Texas. Isolates were recovered from 4 of the 5 locations, indicating this fungus is more widespread than previously documented in the state. Six of the 7 fungi were not previously documented on watermelon in Texas and may play a greater role than previously thought, particularly the Fusaria. Prior to this work *Fusarium incarnatum* and *Fusarium proliferatum* had not been previously reported to cause disease on watermelon but had been reported to cause disease on other cucurbits. Additionally, *Fusarium brachygibbosum* has not been documented to cause disease on watermelon in the United States. The three Fusaria species found in this survey were associated with varying levels of necrosis, chlorosis, and minor water stress induced wilting in the field. These symptoms differ from typical FON infection and were more consistent with stem rot and general vine decline.

The environmental components examined in this work, particularly humidity and dew point, appear to have a role in the occurrence of pathogens at different locations across Texas. Specifically, the composition of isolate recovery varied across the state with Glasscock Co. having the least amount of total fungi recovered and the most unique environment per the PCA analysis. If adequate environmental conditions for disease development such as excess moisture and/or adequate means for spread through wind and precipitation are not met, significant symptoms and therefore pathogens may not be noticed initially. To confirm this, additional analysis over several growing seasons is needed to better understand the

relationship between environmental components within the field, these pathogens, and the diseases they cause.

The fungi used in pathogenicity tests in this study were associated with reduced plant health. Specifically, plants inoculated with *F. brachygibbosum, F. incarnatum*, and *F. proliferatum* showed symptoms including chlorosis, necrosis, and wilt, particularly during water stress. Interestingly, plants inoculated with *Fusarium* spp. initially recovered after watering until symptoms progressed in severity at the end of the study. These results show the importance of detailed observations and record keeping in the field regarding the relationship between disease symptom recognition and water stress. Plants inoculated with *S. citrulli* showed typical GSB symptoms, indicating that Texas isolates induce typical GSB symptoms. *Bipolaris* sp. and *A. alternata* inoculations resulted in leaf spot symptoms. The disease symptoms observed in the pathogenicity tests in this work can lead to reduced quality and yield of fruits in the field and warrant additional investigation, particularly under varying environmental conditions.

Additional field studies are needed to understand the effects of these plant pathogens and environmental conditions on plant health. Examination of interactions among these recovered isolates and other plant pathogens is needed, particularly under challenging growing conditions during production. The results from this study can facilitate improvements in field scouting and better management practices, which would ultimately assist producers and extension personnel in optimizing future watermelon production in Texas.

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APPENDIX A

SUPPLEMENTARY DATA

Supplementary Table 1: Date, location, and GPS coordinates of 2020 Samples taken (DOC-Date of Collection).

DOC	County	Weather DOC	Sample ID	Latitude	Longitude
6/16/2020	Frio	94°/74°	DL1-F1	28.75001	99.23496
6/16/2020	Frio	94°/74°	DL2	28.75011	99.23494
6/16/2020	Frio	94°/74°	DL3	28.75005	99.23479
6/16/2020	Frio	94°/74°	DL4	28.75014	99.23479
6/16/2020	Frio	94°/74°	DL5	28.75027	99.23470
6/16/2020	Frio	94°/74°	DL6	28.75018	99.23486
6/16/2020	Frio	94°/74°	DL7	28.75027	99.23495
6/16/2020	Frio	94°/74°	DL8	28.75032	99.23492
6/16/2020	Frio	94°/74°	DL9	28.75041	99.23471
6/16/2020	Frio	94°/74°	DL10	28.75053	99.23466
6/16/2020	Frio	94°/74°	DL11	28.75207	99.23438
6/16/2020	Frio	94°/74°	DL12	28.75208	99.23434
6/16/2020	Frio	94°/74°	DL13	28.75363	99.23434
6/16/2020	Frio	94°/74°	DL14	28.75240	99.23484
6/16/2020	Frio	94°/74°	DL15	28.75036	99.23544
6/16/2020	Frio	94°/74°	DL16	28.74997	99.23554
6/16/2020	Frio	94°/74°	DL17-F2	28.74684	99.32889

6/16/2020	Frio	94°/74°	DL18	28.74625	99.32684
6/16/2020	Frio	94°/74°	DL19	28.74587	99.32522
6/16/2020	Frio	94°/74°	DL20	28.74579	99.32376
6/16/2020	Frio	94°/74°	DL21	28.74585	99.32396
6/16/2020	Frio	94°/74°	DL22	28.74685	99.32630
6/16/2020	Frio	94°/74°	DL23	28.74654	99.32525
6/16/2020	Frio	94°/74°	DL24-F3	28.74849	99.32674
6/16/2020	Frio	94°/74°	DL25	28.74939	99.32642
6/16/2020	Frio	94°/74°	DL26	28.74960	99.32636
6/16/2020	Frio	94°/74°	DL27	28.74876	99.32724
6/16/2020	Frio	94°/74°	DL28	28.75030	99.32673
6/16/2020	Frio	94°/74°	DL29	28.75111	99.32648
6/16/2020	Frio	94°/74°	DL30	28.75351	99.32568
6/17/2020	Hidalgo	92°/74°	JP1	27.47979	98.13405
6/17/2020	Hidalgo	92°/74°	JP2	27.47985	98.13421
6/17/2020	Hidalgo	92°/74°	JP3	27.47978	98.13435
6/17/2020	Hidalgo	92°/74°	JP4	27.47978	98.13497
6/17/2020	Hidalgo	92°/74°	JP5	27.47977	98.13567
6/17/2020	Hidalgo	92°/74°	JP6	27.47985	98.13578
6/17/2020	Hidalgo	92°/74°	JP7	27.47984	98.13695
6/17/2020	Hidalgo	92°/74°	JP8	27.47977	98.13834

6/17/2020	Hidalgo	92°/74°	JP9	27.47977	98.13900
6/17/2020	Hidalgo	92°/74°	JP10	27.47979	98.14165
6/17/2020	Hidalgo	92°/74°	JP11	27.47922	98.14522
6/17/2020	Hidalgo	92°/74°	JP12	27.47916	98.14497
6/17/2020	Hidalgo	92°/74°	JP13	27.47924	98.14494
6/17/2020	Hidalgo	92°/74°	JP14	27.47923	98.14310
6/17/2020	Hidalgo	92°/74°	JP15	27.47924	98.14253
6/17/2020	Hidalgo	92°/74°	JP16	27.47898	98.14077
6/17/2020	Hidalgo	92°/74°	JP17	27.47865	98.13334
6/17/2020	Hidalgo	92°/74°	JP18	27.47855	98.13283
6/17/2020	Hidalgo	92°/74°	JP19	27.47861	98.13283
6/17/2020	Hidalgo	92°/74°	JP20	27.47864	98.13210
6/17/2020	Hidalgo	92°/74°	JP21	27.47865	98.13172
6/17/2020	Hidalgo	92°/74°	JP22	27.47842	98.13063
6/17/2020	Hidalgo	92°/74°	JP23	27.47847	98.13062
6/17/2020	Hidalgo	92°/74°	JP24	27.47839	98.13103
6/17/2020	Hidalgo	92°/74°	JP25	27.47842	98.13152
6/17/2020	Hidalgo	92°/74°	JP26	27.47849	98.13148
6/17/2020	Hidalgo	92°/74°	JP27	27.47819	98.13375
6/17/2020	Hidalgo	92°/74°	JP28	27.47810	98.13313
6/17/2020	Hidalgo	92°/74°	JP29	27.47783	98.13083
6/17/2020	Hidalgo	92°/74°	JP30	27.47783	98.13090

6/23/2020	Glasscock	89°/67°	GCF1	31.74101	101.57805
6/23/2020	Glasscock	89°/67°	GCF2	31.74121	101.57770
6/23/2020	Glasscock	89°/67°	GCF3	31.74123	101.57758
6/23/2020	Glasscock	89°/67°	GCF4	31.74148	101.57726
6/23/2020	Glasscock	89°/67°	GCF5	31.74193	101.57647
6/23/2020	Glasscock	89°/67°	GCF6	31.74223	101.57598
6/23/2020	Glasscock	89°/67°	GCF7	31.74234	101.57568
6/23/2020	Glasscock	89°/67°	GCF8	31.74271	101.57520
6/23/2020	Glasscock	89°/67°	GCF9	31.74298	101.57518
6/23/2020	Glasscock	89°/67°	GCF10	31.74286	101.57549
6/23/2020	Glasscock	89°/67°	GCF11	31.74275	101.57565
6/23/2020	Glasscock	89°/67°	GCF12	31.74264	101.57586
6/23/2020	Glasscock	89°/67°	GCF13	31.74139	101.57841
6/23/2020	Glasscock	89°/67°	GCF14	31.74189	101.57759
6/23/2020	Glasscock	89°/67°	GCF15	31.74202	101.57738
6/23/2020	Glasscock	89°/67°	GCF16	31.74281	101.57603
6/23/2020	Glasscock	89°/67°	GCF17	31.74286	101.57594
6/23/2020	Glasscock	89°/67°	GCF18	31.74414	101.57491
6/23/2020	Glasscock	89°/67°	GCF19	31.74496	101.57534
6/23/2020	Glasscock	89°/67°	GCF20	31.74463	101.57584
6/23/2020	Glasscock	89°/67°	GCF21	31.74420	101.57663

6/23/2020	Glasscock	89°/67°	GCF22	31.74376	101.57732
6/23/2020	Glasscock	89°/67°	GCF23	31.74267	101.57862
6/23/2020	Glasscock	89°/67°	GCF24	31.74304	101.57803
6/23/2020	Glasscock	89°/67°	GCF25	31.74351	101.57724
6/23/2020	Glasscock	89°/67°	GCF26	31.74466	101.57536
6/23/2020	Glasscock	89°/67°	GCF27	31.74444	101.57524
6/23/2020	Glasscock	89°/67°	GCF28	31.74397	101.57600
6/23/2020	Glasscock	89°/67°	GCF29	31.74398	101.57683
6/23/2020	Glasscock	89°/67°	GCF30	31.74310	101.57746
6/25/2020	Maverick	97°/75°	RH1	28.88361	100.58519
6/25/2020	Maverick	97°/75°	RH2	28.88372	100.58520
6/25/2020	Maverick	97°/75°	RH3	28.88376	100.58537
6/25/2020	Maverick	97°/75°	RH4	28.88390	100.58541
6/25/2020	Maverick	97°/75°	RH5	28.88394	100.58559
6/25/2020	Maverick	97°/75°	RH6	28.88396	100.58566
6/25/2020	Maverick	97°/75°	RH7	28.88440	100.58613
6/25/2020	Maverick	97°/75°	RH8	28.88456	100.58614
6/25/2020	Maverick	97°/75°	RH9	28.88457	100.58633
6/25/2020	Maverick	97°/75°	RH10	28.88467	100.58643
6/25/2020	Maverick	97°/75°	RH11	28.88561	100.58752
6/25/2020	Maverick	97°/75°	RH12	28.88690	100.58807

6/25/2020	Maverick	97°/75°	RH13	28.88686	100.58802
6/25/2020	Maverick	97°/75°	RH14	28.88676	100.58800
6/25/2020	Maverick	97°/75°	RH15	28.88660	100.58771
6/25/2020	Maverick	97°/75°	RH16	28.88818	100.58725
6/25/2020	Maverick	97°/75°	RH17	28.88786	100.58700
6/25/2020	Maverick	97°/75°	RH18	28.88751	100.58645
6/25/2020	Maverick	97°/75°	RH19	28.88674	100.58566
6/25/2020	Maverick	97°/75°	RH20	28.88581	100.58468
6/25/2020	Maverick	97°/75°	RH21	28.88508	100.58375
6/25/2020	Maverick	97°/75°	RH22	28.88525	100.58343
6/25/2020	Maverick	97°/75°	RH23	28.88557	100.58372
6/25/2020	Maverick	97°/75°	RH24	28.88614	100.58450
6/25/2020	Maverick	97°/75°	RH25	28.88665	100.58500
6/25/2020	Maverick	97°/75°	RH26	28.88703	100.58544
6/25/2020	Maverick	97°/75°	RH27	28.88773	100.58610
6/25/2020	Maverick	97°/75°	RH28	28.88776	100.58620
6/25/2020	Maverick	97°/75°	RH29	28.88798	100.58652
6/25/2020	Maverick	97°/75°	RH30	28.88840	100.58704
8/15/2020	Burleson	106°/80°	WG1	30.58721	96.50446
8/15/2020	Burleson	106°/80°	WG2	30.58714	96.50437
8/15/2020	Burleson	106°/80°	WG3	30.58714	96.50447

8/15/2020	Burleson	106°/80°	WG4	30.58716	96.50455
8/15/2020	Burleson	106°/80°	WG5	30.58716	96.50455
8/15/2020	Burleson	106°/80°	WG6	30.58665	96.50495
8/15/2020	Burleson	106°/80°	WG7	30.58640	96.50536
8/15/2020	Burleson	106°/80°	WG8	30.58611	96.50553
8/15/2020	Burleson	106°/80°	WG9	30.58591	96.50592
8/15/2020	Burleson	106°/80°	WG10	30.58555	96.50616
8/15/2020	Burleson	106°/80°	WG11	30.58798	96.50442
8/15/2020	Burleson	106°/80°	WG12	30.58780	96.50466
8/15/2020	Burleson	106°/80°	WG13	30.58762	96.50494
8/15/2020	Burleson	106°/80°	WG14	30.58744	96.50526
8/15/2020	Burleson	106°/80°	WG15	30.58713	96.50549
8/15/2020	Burleson	106°/80°	WG16	30.58682	96.50586
8/15/2020	Burleson	106°/80°	WG17	30.58675	96.50592
8/15/2020	Burleson	106°/80°	WG18	30.58674	96.50607
8/15/2020	Burleson	106°/80°	WG19	30.58583	96.50695
8/15/2020	Burleson	106°/80°	WG20	30.58558	96.50740
8/15/2020	Burleson	106°/80°	WG21	30.58813	96.50515
8/15/2020	Burleson	106°/80°	WG22	30.58811	96.50518
8/15/2020	Burleson	106°/80°	WG23	30.58827	96.50492
8/15/2020	Burleson	106°/80°	WG24	30.58827	96.50498
8/15/2020	Burleson	106°/80°	WG25	30.58815	96.50542

8/15/2020	Burleson	106°/80°	WG26	30.58840	96.50584
8/15/2020	Burleson	106°/80°	WG27	30.58857	96.50562
8/15/2020	Burleson	106°/80°	WG28	30.58881	96.50536
8/15/2020	Burleson	106°/80°	WG29	30.58933	96.50475
8/15/2020	Burleson	106°/80°	WG30	30.58934	96.50411

Supplementary Table 2: Weather data sources utilized.

Website	URL	Information	Date Accessed
US Climate Data	www.usclimatedata.com	Monthly Averages: High/Low Temperatures, Precipitation	June 2020 to present
Weather Underground	www.wunderground.com	Daily Averages: High/Low Temperatures, RH, Precipitation, Wind Speed/Direction	August 2020 to present
National Oceanic and Atmospheric Administration	www.noaa.gov	Monthly Averages: High/Low Temperatures, Precipitation	June 2020 to present

APPENDIX B

FIELD DISEASE SYMPTOMS

Figure 14: Field Disease Symptoms.

Figure 14: Continued

Figure 14: Continued

Figure 14: Continued

Figure 14: Continued