Integrated Approach to Understanding Tomato Sour Rot and Improving Disease Management on the Eastern Shore of Virginia

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ABSTRACT

Sour rot of tomatoes, caused by *Geotrichum candidum*, occurs in the field and postharvest settings regularly, although postharvest losses are severe only in some years on the Eastern Shore of Virginia (ESV) and other tomato production regions. Fungicide products and cultural control methods are tested for efficacy utilizing a traditional wounding technique that does not properly reflect natural sour rot infections. A new inoculation technique was optimized for *G. candidum* using negative pressure to infiltrate the tomato stem scar with pathogenic spores. This new method creates consistently high rates of infection and more successfully creates infections in mature green and breaker fruit. The population of *G. candidum* on the Eastern Shore of VA (ESV) was characterized using multilocus sequencing technique. The resulting phylogenetic tree defines four distinct groups, including two with uncommon loci that distinguish them from the majority of the population. Thirty-seven *G. candidum* isolates were inoculated to media amended with ten fungicides and antimicrobial compounds commonly used in tomato production and postharvest treatments. Propiconazole and tebuconazole completely inhibited growth of all colonies. Cultivar trials were conducted to determine if resistance or tolerance to *G. candidum* occurs. Ten commonly grown round and Roma cultivars on the ESV were similarly susceptible to *G. candidum*, even at low inoculum levels. Field and postharvest surveys of sour rot on tomato fruit attempted to correlate disease incidence with weather conditions in order to better understand the cause of sporadic infection. Few patterns were seen consistently throughout harvest periods and years. Rainfall was positively correlated with disease 2-3 days before surveys and

temperature was negatively correlated with disease 5-7 days before surveys. No in-field weather conditions were correlated with postharvest disease incidence. Greenhouse trials were conducted to assess the influence of water congested tomato fruit on susceptibility to sour rot. Tomato plants were exposed to water inundation to mimic rainfall and varying levels of irrigation, both in order to congest tomato fruit. Though water congestion was achieved, tomato fruit were equally susceptible to sour rot infections.

Dedication

This dissertation is dedicated to Lenore Paul who introduced me to the world of agriculture and encouraged my pursuit of science.

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CHAPTER 1: INTRODUCTION AND REVIEW

Tomato Production:

The Eastern Shore of Virginia (ESV) produces a variety of vegetable crops annually, with fresh market tomato fruit (*Solamum lycopersicum* L.) as the most valuable. In 2010, roughly $51.5 million worth of fresh market tomato fruit were produced in Virginia on 4500 acres (USDA, 2010). Virginia ranks annually third to fifth in the United States for tomato production value. Approximately 80% of Virginia's tomato crop is grown on the ESV, which includes Accomack and Northampton counties.

Tomato production in this region follows standard tomato plasticulture methods based on industry practices, including plastic mulch, drip irrigation, transplants, and plants strung on stakes. Mature green fruit are hand harvested and ripened with ethylene in postharvest coolers. Cultivars grown on the ESV are similar to those grown in the southeast coast throughout the year, with BHN602 and BHN669 (BHN Seed, Immokalee, FL) dominating summer harvests. Increasingly, commercial tomato companies are developing their own proprietary cultivars. Although disease control inputs are high in this system, pathogens still can significantly reduce harvestable yields. Production and pest management costs of fresh tomato fruit on the ESV can range from $8,000-$12,000 per acre.

Once mature green fruit are harvested they are collected in large gondolas transported to the packinghouse. Ideally, fruit are immediately washed on arrival, but it is common practice to hold fruit overnight to remove the field heat before rinsing off organic material. Holding fruit is not advised because fruit experience a high amount of pressure weighing down for extended periods of time, which leads to cracking and internal bruising (Bartz et al., 2007; Bartz et al., 2009; Sommer, 1982). Once removed from the gondolas, fruit are cleaned with chlorinated water warmer than the internal temperature of the tomato fruit in large dump tanks. High temperature water is needed because water lower than pulp temperature causes locule material within the fruit to constrict, creating a vacuum and

drawing dump tank water, and all infectious agents suspended in it, into the tomato fruit through the stem scar (Bartz, 1981; Bartz, 1982; Smith 2006). Another route that wash water and infectious agents can enter tomato fruit is when they are submerged 122cm below the water line. At that depth, water has enough pressure to infiltrate tomato fruit, bringing in pathogens (Bartz, 1982). Modern packinghouses are moving away from dump tanks and towards the use of wash water sprays on the packing line. Eliminating recirculating water and pressure at depth reduces risk of contamination by plant and human pathogens. Also, wash water pH must remain between 4-6 pH in order for free chlorine to stay reactive. Incorrect pH and improper chlorine levels can lead to decreased shelf life, phytotoxicity, and presence of human pathogens within tomato fruit (Bartz, 2001; Bartz, 2007; Boyette, 1993; Suslow, 2000).

After fruit are washed, they are covered with a food grade wax and boxed. Mature green fruit are placed in storage rooms that expose fruit to ethylene to continue ripening. Rooms can be cooled to regulate the rate of ripening so fruit are at the correct stage at their final destination.

In most tomato growing seasons there are two distinct harvest periods. Typically the first crop is planted in April with summer harvest beginning during the first weeks of July. The second crop is planted in late July and fall harvest begins the first weeks of September. Though seasonal variation occurs, summer growing periods tend to be hot and dry, while fall growing periods are cooler and wet.

Sour rot and other postharvest diseases:

Geotrichum candidum is the causal agent of tomato sour rot and the pathogen is present at all steps of tomato production, though cause the most serious losses in the postharvest setting. Symptomatic fruit are culled out during the early stages of postharvest handling, though infected fruit may present themselves later in the ethylene ripening room and during distribution. This leads us to assume that *G. candidum* can infest fruit and remain latent until the fruit and storage environment is more hospitable for

the pathogen. In the postharvest setting there are other diseases present. These diseases are bacterial soft rot (*Pectobacterium carotovorum*), black mold rot (*Alternaria arborescens*), Rhizopus rot (*Rhizopus stolonifer*), blue mold (*Penicillium spp.*), and Fusarium fruit rot (*Fusarium oxysporum*). *Pectobacterium carotovorum* is a soft rotting pathogen that displays similar symptomology as *G. candidum* and is easily confused without proper confirmation. The distinguishing characteristic of sour rot is the presence of velvety white sporulation and mycelial growth (Bartz, 2009; Moline, 1984). Bacterial soft rot is watery and smells of vinegar, whereas sour rot has distinct spore growth and a sweet/sour smell.

Geotrichum candidum infections occur both in the field and during the postharvest setting. The pathogen can only infect tomato fruit, thus vegetative tissue is not damaged. In the field, sour rot causes a soft, watery rot with a velvety white coating on infected tissue. Fruit infections are found on damaged and overly vine-ripe fruit, acting as a saprophyte. At the beginning of the season, the most common sour rot infections occur on tomato fruit with blossom end rot and fruit with damage to the cuticle (personal observation). Infected fruit are a major source of inoculum and can spread spores quickly within the plant canopy to other fruit. In the postharvest setting infected fruit within boxes can spread to other fruit with shared contact surfaces and fruit in contact with inoculum from infected fruit that have lost integrity (Bartz et al., 2007).

Geographic distribution and host range:

Geotrichum candidum is a cosmopolitan pathogen and can be found in many locations on various hosts. It is considered natural flora, a spoilage pathogen of foods, and a human pathogen. It is naturally present in raw milk, as well as used in the cheese industry. It can be found in all agricultural environments, including soil, water, and dust. It can cause epidermal and lung infections in immune-compromised patients. As a plant pathogen, *G. candidum* causes disease in tomato (*Solanum*

lycopersicum), carrot (*Daucus carota* subsp. *sativus*), potato (*Solanum tuberosum*), stone fruits (*Prunus* spp.), cucumber (*Cucumis sativus*), pumpkin (*Cucurbita maxima*), and other fruits and vegetables. *Geotrichum candidum* is found in all tomato producing regions of the United States, as well as the majority of tomato-producing countries throughout the world.

Human health aspects:

Study of the *Geotrichum candidum* and tomato pathosystem is important due to in-field and postharvest losses, but also because of human health implications. *Geotrichum candidum* is pathogenic in respiratory and gastrointestinal tracts of humans and other mammals (Carmichael, 1957). Strains of *G. candidum* show proteolytic and alkalinizing activity which can increase the pH of tomato pulp. The pH can increase 2.7 units, creating an environment conducive for the growth of *Salmonella enterica* and other human pathogens (Wade, 2003, Wade and Bouchat, 2003). A survey revealed that 12.5% of asymptomatic tomato fruit in grocery stores were infected by *Geotrichum* species (Tournas, 2005) and another study found 1 out of 6 fruit infected with *G. candidum* also contained *Salmonella typhimurium* (Wells and Butterfield, 1999).

Epidemiology:

Several epidemiological observations of *G. candidum* in tomato have been made, though no clear conclusions have developed. The pathogen is shown to infect round tomatoes in all growing regions, particularly after periods of heavy rainfall during fall harvest (Bartz, 1981; Bartz 2012; Rideout, personal comm.). Roma cultivars develop in isolated incidences but are thought to be more tolerant to infection (Jay Taylor, personal comm.). Larger epidemics have been associated with bruised fruit, fruit harvested from canopies with free moisture, and fruit wounded by insects or mechanical injury. Minor

and internalized infections show few to no symptoms, but the pathogen reproduces quickly and decayed fruit can be observed in the ripening room or packing lines if left overnight.

Etiology and Predisposing factors for disease:

Predisposing factors to sour rot infection are still unclear, but it is believed that water plays a significant role in infections of tomato fruit. A citrus sour rot (*Geotrichum citri-aurantii*) study revealed factors that increased susceptibility, including developmental stage, duration of storage, and ethylene treatments (Baudoin, 1982).

With citrus sour rot, water uptake also increases susceptibility. It was observed that lemons harvested after rainfall were more susceptible than those harvested during dry, sunny periods (Baudoin, 1982). Water content was slightly higher in post-rainfall fruit. Fruit can be water congested, which develops from excess soil moisture and reduced transpiration, making tissue more prone to damage and infection. Water congestion can also cause rapid increases in fruit size which produces micro-cracks in the cuticle and epidermis, exposing tomato fruit tissue to *G. candidum* spores (Bartz et al., 2007). Tomatoes in dump tanks filled with water at a lower temperature than fruit pulp have been shown to experience increased water content, leading to congested fruit, making them more susceptible to infection. Water congestion can be measured by fruit weight gained after submersion. Fruit congested with at least 0.1 grams of a bacterial water suspension reached higher rates of infection than fruit that were submerged in bacterial water suspension without becoming water congested. Water congested fruit expressed symptoms faster than non-congested fruit (Bartz, 1982). More investigation is required to determine the exact impact of excess water on fruit susceptibility.

Field observations show increased sour rot infections after rain events. Extensive sour rot infections on the ESV have occurred mainly late in the season and after large rainstorms (Bartz, 2006;

Bartz, 2007; Bartz et al., 2007; Rideout, 2009). Fall harvested tomato fruit on the ESV had significant sour rot infections after large rainfalls in 2006-2009, all in September. Infections increase to "epidemic" status during unusually wet fall harvests, especially when tropical storm systems occur. The apparent ideal weather conditions for sour rot development include rainfall, heavy dew, fog, and abrupt temperature changes (Bartz, 2007; Bartz et al., 2012).

Fruit developmental stage can impact sour rot incidence and severity; such as harvesting fruit earlier in development to reduce losses. Fewer sour rot infections occur in mature green and breaker fruit than in red fruit (Brady, 1982). Tomato sour rot infections were inhibited in green fruit when compared to red fruit, and some of the lesions in green fruit appeared to become arrested (Moline, 1984). It was also found in citrus sour rot that fruit later in development, specifically in reference to color, increased susceptibility (Baudoin, 1982).

Characterization:

Morphology

Geotrichum candidum was first observed infecting tomato fruit in 1923 by Prichard and Porte, though classification and nomenclature was not agreed upon until decades later. This species of is genetically diverse and can be organized into sub-groups based on the host, region, and environment (Gente et al., 2002). *Geotrichum candidum* in culture is hyaline, septate, and grows in a flat, radiating pattern from the source. *Geotrichum* is closely related to yeasts, but is only considered "yeast like" because it does not reproduce by budding. Vegetative hyphae branch and the lateral branches become sporulating chains; when broken apart the infectious bodies are arthrospores (Carmichael, 1957; Cole and Kendrick, 1969; Duran, 1972). The pathogen in culture and on susceptible fruit produces milky white colonies with velvety texture composed of the spore chains. Optimal growth is at 25°C in fruit and

30°C on media. Minimal growth occurs at 5°C, which is well below the recommended storage temperature of tomato fruit (12.8°C) (Moline, 1984; Plaza et al., 2003).

The genus *Geotrichum* cannot be identified by microscopic morphology alone because many related and unrelated fungi form arthrospores and have similar characteristics. Within *G. candidum* there is variable phenotypical characteristics and very marked polymorphisms, making molecular characterization more effective (Prillinger et al. 1999). Also, standard identification micro-methods, which were developed for the yeasts of clinical importance, are not suitable for yeasts encountered in agro-food industries (API 32C Biomerieux, France; Auxacolor 2, Biorad, RapID Yeast System, etc.) (Gente et al., 2006).

Molecular Identification

As stated previously, *G. candidum* is a difficult species to identify using strictly morphological characteristics. The extensive host range, diverse ecological niches, and exchange of genetic material within this pathogen have led to high number of polymorphisms, making molecular methods ideal for identification and sub-group classification of *G. candidum* isolates (Prillinger et al., 1999). A common method for microorganisms identification is comparison of the internal transcribed spacers 1 and 2 (ITS1 and ITS2), but it is not effective with *Geotrichum* species due to many divergences that are not reflected in the ITS region. Research groups have devised unique methods and tools to identify isolate diversity, which includes RAM-PCR and MLST.

RAM-PCR (random amplified microsatellites technique) has been used to successfully identify *G. candidum* from other *Geotrichum* species, as well as sub-groups that have been associated with ecological niches. Primer GATA4 is able to discriminate within species and could also be used to classify the strains in terms of their original substrate (Gente et al, 2001; Gente et al, 2006).

Unfortunately, this method results in a gel with unique banding patterns for each isolate. In order to view clear banding patterns, PCR cycle time and temperature need to be optimized and results are not reproducible in different locations.

Multi-locus sequencing technique (MLST) is the most recent method developed to identify *G. candidum* sub-group diversity. The method analyzes six housekeeping genes (Table 1.1) and sequences the PCR products to compare the alleles (Alper et al., 2013). It was found that MLST differentiation is more efficient than RAM-PCR and also offers non-ambiguous data that can be exchanged and compared, as well as used for evolutionary studies.

Management strategies:

Cultural

Improper harvest and post-harvest handling of tomato fruit can increase infection due to injury. Load vibration and load compression during transportation from field to packinghouse adds additional stress. Increased bruising, made worse if fruit are puffy due to poor seed-set and air in locular cavities, can increase fruit susceptibility to sour rot infections (Bartz, personal, comm.). It is also recommended that plant canopies and fruit are completely dry during harvest, though recommended methods may not actually be practiced.

Once harvested, preventative steps can be employed to reduce disease pressure if *G. candidum* spores are present on the fruit surface. Proper dump tank water temperature must be monitored and maintained at 2.8-5.6°C higher than tomato pulp temperature so the temperature differential does not cause a vacuum to form, pulling potentially contaminated water into fruit (Bartz, 1988). It is also advised that dump tanks be less than 122 centimeters because water pressure at greater depths causes rapid infiltration of contaminated water into fruit (Bartz, 1982).

Chemical

Efficacy of sour rot chemical control is variable and limited because *G. candidum* does not respond to standard fungicides. At the beginning of postharvest handling, fruit are cleaned with 125 ppm free chlorine. Dump tanks with 125 ppm free chlorine (initial concentration) can significantly reduce the spore load on fruit surfaces (Bartz, 2001; Boyette et al., 1993). Chlorine levels in tanks must be monitored routinely because excessive levels can encourage the uptake of dump tank water into the fruit and greater than 500 ppm free chlorine causes phytotoxicity (Bartz, 1988).

Some fungicides actually enhance sour rot infections on fruit, including benomyl, imazalil, thiophanate methyl, vinclozolin, cyprodinil, and carbamate (Moline, 1984). Sodium bicarbonate and potassium sorbate have slight inhibitory properties, while propiconazole (Mentor 45WP, Syngenta Crop Protection, Greensboro, NC) is the only chemical with significant inhibitory effects (McKay et al, 2012a; McKay et al., 2012b; Moline, 1984, Rideout, personal comm.). It is effective at 128-256 ppm when used as a postharvest drench/dip for tomato (*Solanum lycopersicum*), citrus (*Citrus* spp.), nectarine and peach (*Prunus persica*) (McKay, 2011a; McKay, 2011b; Rideout, personal comm.).

Development of a non-invasive infection technique:

Currently, the methodology utilized for screening materials on harvested tomato fruit is invasive and involves severe wounding prior to inoculation with *G. candidum*. Wounded fruit that have been inoculated in the field or during harvest would be visually detected and removed from the line during culling, leaving internalized infections that are difficult to visually detect. Additionally, responses to post-harvest treatments may differ during wounding methods. Fruit develop defense mechanisms as a result of wounding, in order to prevent pathogen invasion. It is has been hypothesized that plants have

evolved mechanisms that integrate both pathogen-specific and general wounding responses (Castro-Mercado et al., 2009). An alternative method that more closely resembles natural internal infections would allow us to study the tomato fruit's response to infection more effectively than with wounding.

Dump tank studies have shown water and bacteria are able to infiltrate tomato fruit, which is a possible mechanism for a new inoculation method. Three main situations can result in significant fruit infection by infiltration with a pathogen suspension; 1) excessive depth of fruit in dump tank, 2) water temperature less than fruit pulp temperature, and 3) poor epidermis integrity. Minor contributors that influence infection rates include chlorine level and time submerged in the dump tank (Bartz, 1981; Bartz, 1988).

1) Hydrostatic pressure can force water and pathogens into tomato fruit when they are immersed more than 122cm under the water surface (Bartz, 1982). Based on this concept, it was discovered that negative and positive pressure can be applied to fruit in order to infiltrate fruit (Hadjok, 2007; Wade, 2003).

2) Temperature differentials of more than 9.4°C can also force water into fruit (Bartz, 1982). This phenomenon is caused by a tomato fruit coming into contact with water cooler than the pulp temperature, making the inner pulp contract, causing a vacuum to form and allowing infiltration of the surrounding liquid.

3) When fruit contain high levels of water, tissue expands which can cause microcracks in the cuticle and epidermis of the fruit. Microcracks, small cracks in the cuticle due to rapid expansion, and other small surface wounds have been shown to increase the chance of infiltration of pathogens into tomato fruit (Bartz, 1982). Surface wounds may result from insect, environmental, or mechanical damage, and also from water congestion. Once in the dump tank, wounded tomato fruit quickly come in direct contact with *G. candidum* spores, thus allowing entry by the pathogen.

Water and microorganism infiltration ultimately depends on the integrity of the stem scar. Fruit with fresh stem scars are more vulnerable to infiltration than fruit with ones more than 24 hours old (Bartz, 1981). Once stem scars dry there is extreme surface tension prohibiting water and inoculum entrance into the fruit. The addition of surfactant increases the amount of infiltration of liquid in fruit and decreases the time between immersion and onset of infiltration into tomato fruit (Bartz, 1982). Once liquid suspensions are infiltrated into fruit, tissue immediately below the stem scar is more likely to contain bacteria than other tissues (Bartz, 1981).

Using these proven concepts, two inoculation methods have been developed to infect fruit for studies investigating infiltration by other pathogens. Young (1974) immersed bean plants (*Phaseolus vulgaris* L. cv. 'Masterpiece') in *Pseudomonas phaseolicola* suspensions then placed them into an autoclave. The autoclave was turned on for 2-3 minute cycles to create a high pressure environment, then released to equilibrate with ambient air pressure. The pressurization cycles were conducted until leaves were fully infiltrated. This procedure was used to inoculate leaves and quantify the *P. phaseolicola* colonies after a period of time. Another procedure developed by Hadjok et al (2007) used a vacuum chamber to infiltrate human pathogens and spoilage bacteria into fresh produce. They successfully infiltrated tomato fruit with *Salmonella enterica*, *Escherichia coli*, *Pectobacterium carotovorum*, and *Pseudomonas fluorescens*. Whole tomato fruits were submerged in these bacterial suspensions for 20 minutes then transferred to a vacuum chamber. A vacuum was created and released for 3 cycles of 2 minutes each.

Through infiltration the pathogen is forced into the interior of tomato fruit, which is an ideal environment for growth (Bartz, 1982). When fruit are infiltrated, water's microbial concentration does not need to be as great as surface contamination to achieve the same level of infection. As a result of infiltration with the bacterium *Pectobacterium carotovorum* in tomato fruit, symptoms and signs present

themselves as soon as 24 hours, which is 2-5 days earlier than wound inoculations with the same bacterium (Bartz, 1982).

Objectives:

This research aims to better understand the tomato sour rot pathosystem so recommendations can be made to growers that will reduce the impact of sour rot on tomato production. Studies are clustered into two categories: approaches to better understand the biology of tomato sour rot and practical experiments for immediate disease prevention. The development of new methods to tests fungicides allows us to more realistically evaluate products for control of sour rot. Fungicides currently used within postharvest or tomato production systems were evaluated to determine their potential for preventing disease. In addition, fungicide sensitivity was evaluated *in vitro* across a range of *G. candidum* isolates. Gaining a better understanding of the influence of rain and other weather conditions on disease occurrence allows growers to be more effective at utilizing cultural practices and practicing sound harvest and postharvest methods to reduce disease occurrence. Characterizing isolates of *G. candidum* from the ESV allows us to compare the population to other regions. All of this was accomplished by completing five main objectives.

I. Development of a non-wounding inoculation technique for tomato fruit.
    1. Using a vacuum chamber to infiltrate tomato fruit with *G. candidum* spore suspension.
    2. Comparing susceptibility of ripeness stages using vacuum inoculation.
II. Fruit susceptibility of commercial cultivars to *G. candidum*
    1. Compare fruit susceptibility to *G. candidum* of 10 commonly grown cultivars.

2. Study to determine required infectious dose to cause disease.

III. Influence of irrigation and rain events on susceptibility of tomato fruit to postharvest *G.* infection.

1. Influence of rain events at varying times directly before harvest.
2. Differing irrigation regimes and their influence on tomato fruit susceptibility.

IV. Occurrence of sour rot infections in tomato fruit.

1. In-field surveys.
2. Packaged fruit in the postharvest setting.

V. Characterization of *G. candidum* in tomato growing regions.

1. Isolation of pathogen from soil and infected fruit.
2. *In vitro* sensitivity of pathogen to various fungicides.
3. MLST analysis of isolates.

Table 1.1: Six loci used for *G. candidum* MLST and the corresponding proteins.

Primer	Protein
ala1	alanyl-tRNA synthetase
cdc19	pyruvate kinase
erg10	acetyl-coA acetyltransferase
gln4	Glutaminyl-tRNA synthetase
pgi1	phosphoglucoisomerase
pgm2	phosphoglucomutase

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CHAPTER 2: DEVELOPING AN ARTIFICIAL INOCULATION METHOD FOR TOMATO FRUIT WITH *GEOTRICHUM CANDIDUM*

Abstract:

Sour rot of tomatoes occurs in the field and postharvest settings regularly, though in select years significant postharvest losses occur on the Eastern Shore of Virginia (ESV) and other tomato production regions. Chemical and cultural efficacy trials are conducted in order to assess the ability to control postharvest sour rot of tomatoes. The current postharvest tomato fruit inoculation protocol for sour rot studies alters its physiology and induces a wound response, which is not reflected in commercial tomato production sour rot infections. A non-invasive inoculation method was developed utilizing negative pressure to infiltrate tomato stem scars. Wound-inoculation of fruit results in 75% infection; while vacuum-inoculation of fruit resulted in 100% infection. Wound inoculation is primarily successful in red fruit, though fresh market tomato fruit on the ESV includes handling of mature green, breaker, and red fruit. Vacuum inoculation resulted in successful infection of 63% mature green, 87% breaker, and 100% red fruit, unlike wound inoculation which resulted in significantly less infection at each developmental stage. Standard fungicide screenings utilizing both wound and vacuum inoculation resulted in different levels of control. Mentor 45WP (propiconazole, Syngenta Crop Protection, Greensboro, NC) successfully controlled 97-100% of sour rot infections while utilizing the wound inoculation method, but Mentor controlled only 37-57% infection when fruit were vacuum inoculated. Vacuum inoculation is effective at producing consistently high rates of sour rot in tomato fruit.

Introduction:

Geotrichum candidum is the causal agent of tomato sour rot (*Solanum lycopersicum*) and other fresh produce. It is also present in dairy products and acts as a spoilage pathogen in produce and processed foods. *Geotrichum candidum* is a "yeast like" fungi that grows in a mycelial form and under ideal conditions lateral branching occurs, forming arthrospores that break apart and act as infectious material (Cole and Kendrick, 1969; Duran, 1972). Healthy tomato fruit are infected by *G. candidum* through microcracks, wounds, infiltration, and contact with decaying fruit (Bartz, 2009). This disease is a limiting factor for tomato production on the Eastern Shore of Virginia (ESV) and other tomato producing regions. The pathogen causes losses in the field, but the primary concern is during post-harvest handling. Postharvest *G. candidum* infections occur more frequently after moist harvest conditions, abrupt drops in temperature due to rainfall, and improper post-harvest handling procedures (Bartz et al., 2012).

There are no in-field fungicides targeted to prevent sour rot infections and only one postharvest fungicide is available to prevent further losses. Propiconazole (Mentor 45WP, Syngenta Crop Protection, Greensboro, NC) was labeled for use in Virginia in 2013 and is the only postharvest treatment that is effective at preventing sour rot infections (unpublished data). Beyond chemical intervention, tomato sour rot incidence can be decreased by storage at proper temperatures, sanitation of fruit and packaging systems, and harvesting mature green fruit rather than ones with color (Bartz, 2009).

The current postharvest tomato fruit inoculation protocol for sour rot for studies examining treatment effects includes wounding fruit, altering its physiology and inducing a wound response. Fruit have developed defense strategies as a result of wounding, in order to prevent further pathogen invasion. It is has been hypothesized that plants have evolved mechanisms that integrate both pathogen-specific and general wounding responses (Castro-Mercado et al., 2009; Vilanova et al., 2013). We propose a new

procedure that allows fruit to remain intact while the spore suspension is inserted, thus more resembling internalized field infections, and effectively evaluating potential compounds, cultivars, and storage conditions for control. Negative and positive pressure has been used to infiltrate fruit with pathogens. Hydrostatic pressure forces water and bacterial pathogens into tomato fruit when they are immersed more than 122cm under the water surface in dump tanks (Bartz, 1982; Bartz & Showalter, 1981). Also, tomato fruit and other fresh produce such as iceberg lettuce (*Lactuca sativa*), broccoli florets (*Brassica oleracea* var. *italica*), and sliced Spanish onions (*Allium cepa*) have been successfully inoculated by applying vacuum cycles to whole produce in order to internalize *Escherichia coli* and *Salmonella enterica* into the inner tissue (Hadjok, 2007).

This research focuses on the development of a non-wounding inoculation technique for tomato fruit, then comparing its utility to the established wounding protocol. This was accomplished by fulfilling the following objectives:

1. Develop a non-invasive inoculation protocol of tomato fruit.
2. Compare efficacy of vacuum method with current wounding method.
3. Examine vacuum method's efficacy of causing infection of tomato fruit at different developmental stages.
4. Use both inoculation methods in a standard fungicide screening to determine usefulness.

Materials and Methods:

Tomato fruit (Compari TOV, Village Farms, Heathrow, FL) were at the breaker stage of development and the surface was wiped clean with 70% ethanol before treatment. Breaker stage tomato fruit are fruit that have a definite change in color from green to tannish-yellow, pink, or red on no more

than 10% of the surface (USDA, 1997). Silwet L-77 treatments were accomplished by applying 5-10 µL of the compound to the stem scar (with the amount depending on stem scar size) and allowing the liquid to remain for 5 minutes. Treatments that included water or fungal suspension were applied directly on the stem scar with a pipette in the amount of 15 µL (PR 1000 Rainin classic, Mettler-Toledo Inc., Columbus, OH). Tomato fruit were placed into a vacuum desiccator (Fisher Scientific, Waltham, MA) for 2 minutes of vacuum (-0.1 MPa pressure) and 1 minute of normal atmospheric pressure. This was repeated twice more as described in Hadjok et al (2008) and Ocampa-Garcia (2010). The spore suspension treatments included in this study were: 1) water, 2) Silwet L-77 (Momentive Performance Materials, Columbus, OH), 3) water + *G. candidum*, and 4) water + Silwet L-77 + *G. candidum* and 5) standard wound inoculation. Spore suspensions of *G. candidum* were prepared at a concentration of 10^6 spores per milliliter and quantified using a hemocytometer (Hausser Scientific, Horsham, PA).

Standard wound inoculations were accomplished by inserting a sterile nail (2 mm diameter) 2mm deep into the tomato fruit at 4 locations around the stem scar on the shoulder. A 15 µL spore suspension (10^6 spores/mL) was then pipetted into each wound. All treatments included 15 fruit and were replicated twice.

Once all fruit were treated, they were placed into produce trays (Monte Package Company, Riverside, MI) within covered plastic containers (Rubbermaid, Atlanta, GA) containing moist paper towels to maintain at least 90% relative humidity and stored at 20°C. Incidence of sour rot symptoms around inoculation sites was recorded after seven days of incubation. Disease incidence data were subjected to an analysis of variance (ANOVA) and comparison of means using Tukey's HSD (α=0.05), which was performed using JMP 10 (SAS Corporation, Cary, NC).

To visually confirm spore location after inoculation, aniline blue was substituted in place of *G. candidum* and applied to the stem scar. Wound and vacuum inoculation methods were carried out as normal, then tomato fruit were cut in half for visual evidence of liquid movement.

Tomato fruit (BHN602) were inoculated at three developmental stages for comparison of susceptibility: mature green, breaker, and red. Mature green fruit are entirely green but are able to continue ripening if exposed to ethylene (USDA, 1997). Prior to inoculation, the fruit surface was cleaned with 70% ethanol. A spore suspension of *G. candidum* was introduced into the fruit utilizing the vacuum inoculation technique described previously. Silwet-L77 was applied to the stem scar and allowed to remain for 5 minutes, then 15 μL of inoculum (10^6 spores/mL) was pipetted onto the stem scar and fruit placed into the vacuum desiccation chamber. The vacuum produced -0.01 MPa of pressure for 2 minutes, was allowed to equilibrate at ambient pressure for 1 minute, and the procedure was repeated twice more. Infected fruit were incubated at 90% relative humidity and stored at 20°C as previously described. Each treatment included 15 fruit and was replicated twice. Fruit were considered positive for sour rot infection when characteristic symptoms (greasy, water-soaked appearance) and white spore masses were present around inoculation sites. Incidence of sour rot symptoms around inoculation sites was recorded after seven days of incubation. Data were analyzed using logistic regression and Likelihood Ratio (L-R) tests (α=0.05) in JMP.

Trials to assess a fungicide's ability to prevent tomato sour rot infections in the postharvest setting were conducted utilizing both inoculation methods. Propiconazole (Mentor 45WP, Syngenta Crop Protection, Greensboro, NC) is labeled for tomato sour rot control in the postharvest setting. Previous trials by members of this lab have shown decreased sour rot incidence when propiconazole is

applied. Propiconazole efficacy trials were conducted utilizing both wound and vacuum inoculation methods to determine if the chemical is able to prevent disease under both conditions.

The surface of breaker stage fruit was cleaned with 70% ethanol before treatment. Seven treatments were examined: 1) non-inoculated control; 2) wound inoculation, no fungicide; 3) vacuum inoculation, no fungicide; 4) wound inoculation followed by propiconazole application; 5) vacuum inoculation followed by propiconazole application; 6) propiconazole application followed by wound inoculation; and 7) propiconazole application followed by vacuum inoculation. Propiconazole applications were accomplished by submerging fruit in 43ºC (2.8ºC above pulp temperature) 0.60 g/liter solution (labeled rate, Mentor 45WP, Syngenta Crop Protection, Greensboro, NC) for 2 minutes to simulate dump tank treatment. Fruit inoculated with *G. candidum* spores first were allowed to incubate for 4 hours before propiconazole treatment, and fruit initially treated with propiconazole were air dried for 4 hours before inoculation with spores. After inoculation and treatment, infected fruit were incubated at 90% relative humidity and stored at 20°C as previously described. Incidence of sour rot symptoms around inoculation sites was recorded after seven days of incubation. Data were analyzed using logistic regression and Likelihood Ratio (L-R) tests (α=0.05) in JMP.

Results and Discussion

Tomato fruit treatment with Silwet-L77 before inoculation with *G. candidum* resulted in significantly more infected fruit than without the surfactant (Fig. 2.1). Surfactant was able to decrease surface tension of corky tissue within the stem scar, allowing a greater number of spores to enter the fruit and resulting in greater disease incidence (Bartz, 1982). More dependable infection rates are ideal for fungicide efficacy and other disease management studies. Concerns arose when full-strength Silwet-L77 was applied directly to fruit and produced slight phytotoxicity; local epidermal cells around stems

scars became sunken and necrotic. The result of treatment is injured fruit, similar to the wounding process, and defeating the purpose of developing a non-wounding inoculation method. In the future, a diluted rate of surfactant should be used in order to reduce phytotoxicity and still aid in stem scar infiltration. This observation also raises the possibility that wound inoculation could have produced higher infection rates if they had been accompanied by Silwet treatment.

Tomato fruit inoculated by vacuum method with Silwet-L77 resulted in a significantly greater rate of infection than wound inoculations (Fig. 2.2). Visualization with aniline blue in place of *G. candidum* spore suspension (Image 2.1) showed that spores added during wound inoculation remain close to the surface of the wounded tissue and, thus, have to potentially overcome desiccation and tomato fruit wound defense responses. Spores inoculated into fruit via vacuum remain in a moist environment, are not exposed to UV light, and infiltrated tissue probably does not experience wound responses.

When fruit at differing developmental stages were inoculated by either vacuum or wound methods, the interaction between inoculation method*developmental stage was not significant (Table 2.1). The main factors in the model (developmental stage and inoculation method) were both significant. Red fruit sustained the greatest infection rate, followed by breaker, then mature green tomato fruit. This has been observed in field infections and previous studies done by this lab. This is supported by the known increase in polygalacturonase during tomato fruit ripening. This pectin degrading enzyme accumulates at high levels during ripening and leads to fruit softening at the end stages of ripening allowing greater susceptibility to *G. candidum* (Brady, 1982). In laboratory tests, transgenic fruit not producing polygalacturonase had decreased sensitivity to *G. candidum* inoculum and sustained over 50% less infection (Kramer, 1992). Vacuum inoculated fruit at each developmental stage resulted in a significantly greater infection rate than the corresponding stage fruit inoculated by wounding.

When treated with propiconazole, tomato fruit resulted in significantly less infection than untreated fruit. Wound inoculated fruit that were then treated with propiconazole resulted in significantly less infection than treat fruit that were vacuum inoculated (Table 2.2). The interaction between inoculation method and propiconazole treatment was not significantly different. Vacuum inoculation resulted in greater disease incidence than wound inoculation, and propiconazole treatment significantly reduced disease incidence. Though propiconazole use still resulted in infected fruit, the severity was not as great as control fruit. Visual observations (data not shown) of propiconazole-treated fruit inoculated via vacuum showed restricted growth and limited sporulation. Visual estimates of lesion size were not effective at expressing differences in infection progress so a more appropriate method should be developed in order to properly show differences.

Investigation of the dose response of tomato fruit to *G. candidum* spore suspensions revealed that rates as low as 10^2 spores/mL were effective at causing successful sour rot infection (see Chapter 5). Inoculation with the industry standard rate of 10^6 spores/mL for fungicide efficacy trials resulted in roughly 100% infection and this may mask differences between cultivars, fungicides, and inoculation methods. Differences found in this study comparing inoculation methods must consider that infection rates were close to the maximum possible, and future studies should include lower inoculum concentrations in order to determine if there are differences.

The vacuum inoculation method provides more consistent infections and can provide reliable results for future screening of fungicides, cultivars, and cultural practices when large declines in infection are expected. Though the inoculation methods lead to different levels of disease control by propiconazole, there is still a use for both methods. Neither method is an exact replica of naturally-occurring latent postharvest infections, but both mimic types of sour rot infections that need to be controlled. In the future it is recommended that both wound and vacuum inoculation methods are utilized to screen for new chemical and cultural controls.

Figure 2.1: Infection rate of tomato fruit treated with and without Silwet-L77 before inoculation with *G. candidum* spores (10^6/mL). Silwet-L77 was applied to the stem scar surface at variable amounts that corresponded to size of stem scar. Means were compared by Tukey's HSD, and bars with different letter show significant differences ($\alpha = 0.05$).

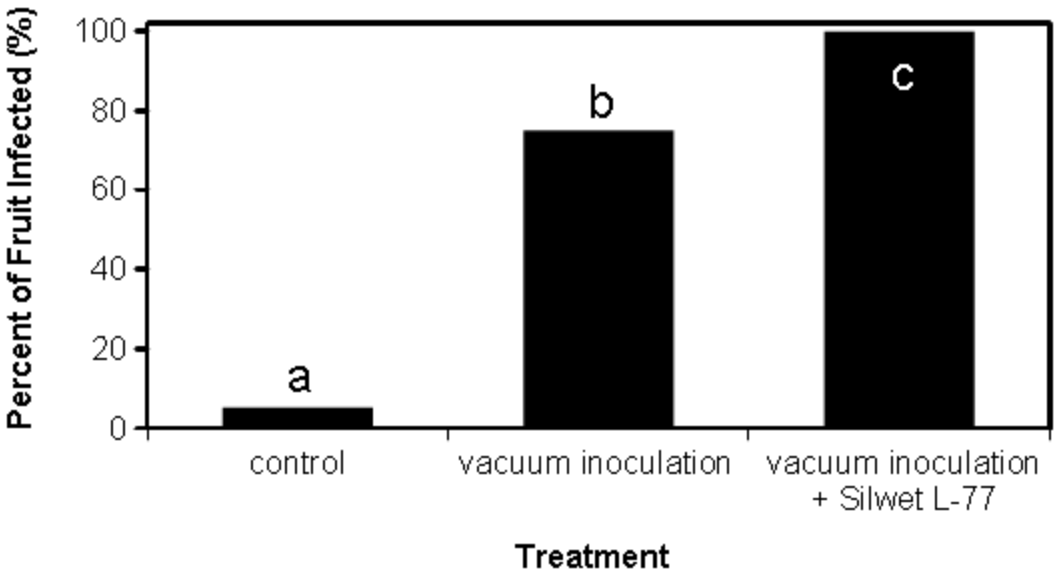

Figure 2.2: Infection rate of tomato fruit inoculated with *G. candidum* by wounding versus vacuum methods. Means were compared by Tukey's HSD, and bars with different letter show significant differences ($\alpha = 0.05$).

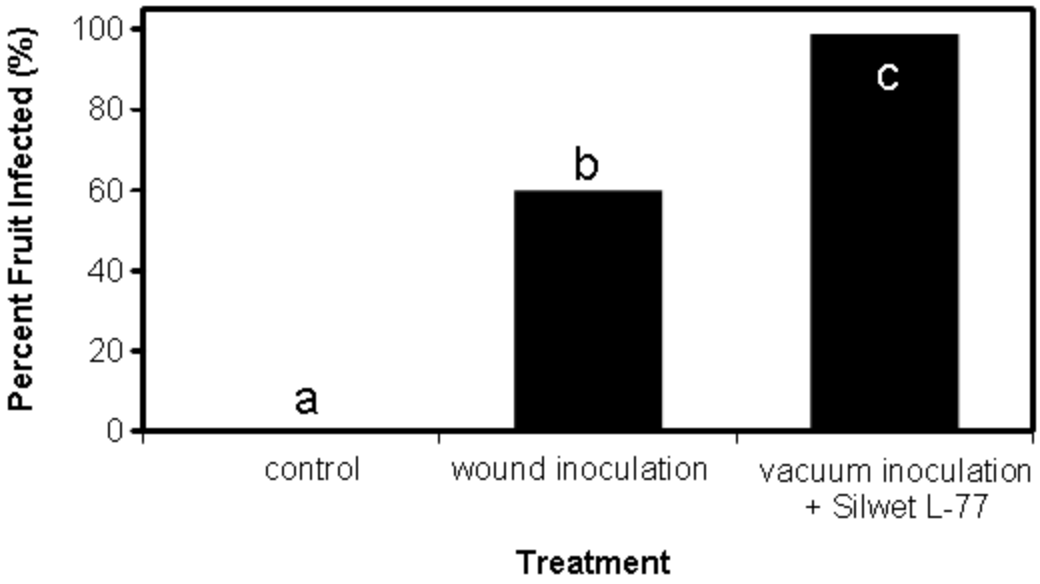

Figure 2.3: Demonstration of inoculum movement utilizing the traditional wounding method (A) and vacuum infiltration method (B). Suspensions were amended with aniline blue to show movement of solution into a tomato fruit.

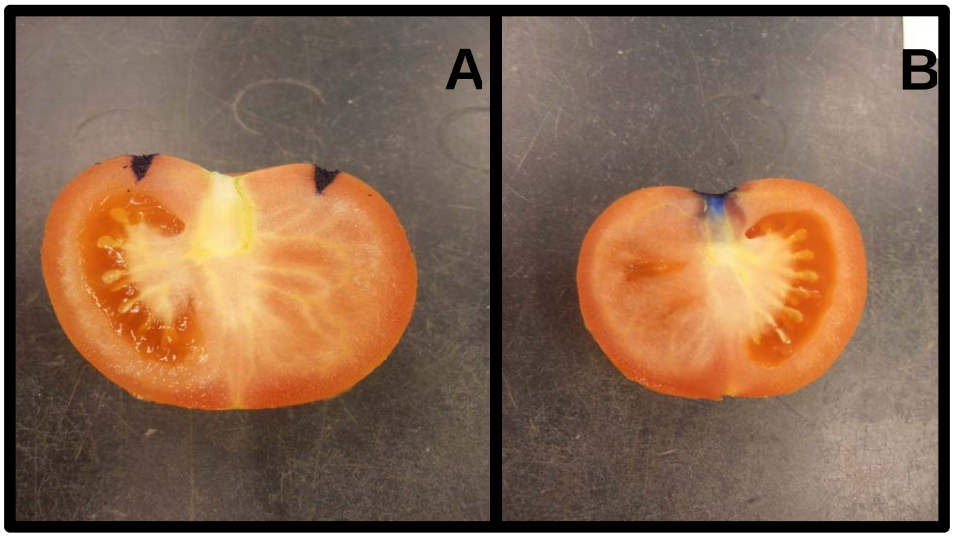

Table 2.1: Infection rate (%) of tomato fruit at green, breaker, and red stages of development utilizing two inoculation methods (wounding and vacuum) with *G. candidum* spore suspension. Data were analyzed using logistic regression and Likelihood Ratio (L-R) tests (α=0.05) in JMP.

	Fruit Developmental Stage			
Inoculation Method	**Green**	**Breaker**	**Red**	**Mean**
Wound	23.3	66.7	93.3	61.1
Vacuum	63.3	86.7	100	83.3
Mean	43.3	76.7	96.7	

Source of Variation	**Chi Square**	**P > Chi Square**
Color	52.66	< 0.0001*
Inoculation Method	9.49	0.0021*
Color * Inoculation Method	1.23	0.5397

Table 2.2: Infection rate (%) of wound or vacuum inoculated tomato fruit by *G. candidum* and treated with propiconazole after inoculation. Fruit were submerged in 43ºC (2.8ºC above pulp temperature) 0.60 g/liter solution (labeled rate, Mentor 45WP, Syngenta Crop Protection, Greensboro, NC) for 2 minutes to simulate dump tank treatment. Logistic regression and Likelihood Ratio Tests were used to assess significance ($\alpha = 0.05$).

	Propiconazole Application		
Inoculation Method	**After Inoculation**	**None**	**Mean**
Wound	3.3	88.3	45.8
Vacuum	26.7	98.3	62.5
Mean	15	93.3	

Source of Variation	**Chi Square**	**P > Chi Square**
Propiconazole Treatment	91.9	<0.0001*
Inoculation Method	129.2	<0.0001*
Propiconazole * Inoculation Method	2.9	0.2337

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CHAPTER 3: CHARACTERIZATION AND COMPARISON OF *G. CANDIDUM* FROM TOMATO GROWING REGIONS.

Abstract

A newly developed multilocus sequencing technique (MLST) and *in vitro* fungicide sensitivity trials were utilized to characterize local populations of *Geotrichum candidum* causing sour rot on tomato fruit grown on the Eastern Shore of Virginia. Thirty-seven pathogen isolates were collected from infected fruit at commercial tomato fields, a packing house, a cull pile, and boxed fruit. Six primer pairs were used for amplification and sequencing; ala1, cdc19, erg10, gln4, pgi1, and pgm2. Sequences were aligned using Clustal W, and neighbor-joining analysis was done to produce a phylogenetic tree. Field isolates showed genetic similarity and there was a general separation between those and post-harvest related isolates (packinghouse, cull pile, boxed fruit). One subgroup of isolates exhibited similarity to *Galactomyces reessii* at the pg1 locus. *In vitro* fungicide sensitivity trials were conducted on potato dextrose agar amended with 8 fungicides and antimicrobial products. Active colonies were placed on amended plates at three concentrations of product and the diameters of colonies were compared to control plates. Propiconazole and tebuconazole inhibited 100% growth at all labeled rates, while other demethylation inhibitors partially reduced *G. candidum* growth. Characterization of *G. candidum* on the Eastern Shore of Virginia revealed a genetically diverse population for such an isolated and small location, and responded relatively uniformly to products used in tomato production and postharvest handling.

Introduction

Geotrichum candidum is the causal agent of sour rot of tomato fruit (*Solanum lycopersicum*) and other fresh produce. It is also present in dairy products and acts as a spoilage pathogen in produce and processed foods. *Geotrichum candidum* is a "yeast-like" fungus that grows in a mycelial form and under ideal conditions laterally branches and segments to form arthrospores, which act as the infectious material (Cole and Kendrick, 1969; Duran, 1972). Healthy tomato fruit are infected by *G. candidum* through microcracks, wounds, infiltration, and contact with decaying fruit (Bartz, 2009). This disease is a limiting factor for tomato production on the Eastern Shore of Virginia (ESV) and other tomato production regions. It causes losses in the field, but the primary concern is during postharvest handling. *Geotrichum candidum* infections are more common after wet harvest conditions, abrupt drops in temperature due to rainfall, and improper post-harvest handling procedures.

Fungi and yeasts have been traditionally identified by their morphological and phenotypic characteristics. Morphological identification of *G. candidum* is not realistic or accurate and identification of *Geotrichum* species based on phenotypic characteristics is not possible because phenotypic identification generally does not agree with genotypic data (Prillinger, 1999). Many isolates can only be morphologically identified to genus, leaving a need for molecular tools for species identification. Molecular techniques can be utilized in order to identify the species of *Geotrichum* isolates. Studies related to cheese and dairy research have not only found methods to clearly identify species, but are specific enough to identify multiple subspecies of *G. candidum* in association with different environmental niches and hosts (Alper, 2013; Gente et al., 2001; Gente et al., 2006; Prillinger, 1999). As of yet, *G. candidum* isolates from tomato and populations on the ESV have not been characterized. Effective tomato sour rot disease management is difficult to establish without a full

understanding of the populations present. The goal of this study is to develop a *Geotrichum* collection of isolates from ESV and characterize them based on molecular and *in vitro* techniques.

Fungicides are an important component of disease management in tomato production, but few fungicides have been found effective against sour rot. Four triazoles were tested because previous trials have shown potential for *G. candidum* control by members of this group and McKay et al. (2012a, 2012b). Propiconazole (Mentor 45WP, Syngenta Crop Protection, Greensboro, NC) is labeled for tomato sour rot control in the postharvest setting. Other triazoles have not been as thoroughly tested so three additional compounds were studied to determine their effect on *G. candidum* growth. Many other fungicides are used in tomato production to control diseases so some were tested to see if their application resulted in *G. candidum* growth restriction. Azoxystrobin (Quadris 2.08SC, Syngenta Crop Protection) is used to control tomato early blight (*Alternaria solani*), black mold (*Alternaria alternata*), anthracnose (*Colletotrichum coccodes*), and late blight (*Phytophthora infestans*). Myclobutanil is also used on tomato when powdery mildew (*Leveillula taurica*) is a concern. Dicloran (Botran 5F, Gowan, Yuma, AZ) is not labeled for tomato production but is a common fungicide in vegetable production so tomato fields on the ESV may be exposed to the product during crop rotations.

Postharvest fungicides for other fruits and vegetables may have the potential for controlling *G. candidum* infections as well. For this reason fludioxonil (Scholar SC, Syngenta Crop Protection) was used to determine pathogen sensitivity. It is currently labeled for tree and pome fruits, sweet potato, and tomato fruit in the postharvest setting (Förster, et al., 2007). It can control Rhizopus rot (*Rhizopus stolonifer*), black mold (*Alternaria alternata*), and gray mold (*Botrytis cinerea*).

Understanding a pathogen's *in vitro* sensitivity to fungicides is a first step for identifying products for the development of long-term disease and fungicide resistance management strategies. Azoxystrobin-resistant pathogen populations have been found in tomato production regions because

azoxystrobin is commonly used to manage many diseases. Azoxystrobin-resistant isolates have been detected in the United States, including *Alternaria alternata* (pistachio), *A. solani* (potato), *Didymella bryoniae* (cucurbits), *Pyricularia grisea* (turf), *Pythium aphanidermatum* (turf), and *Uncinula necator* (grapes) (Syngenta, 2014). Though *G. candidum* is not a target pathogen, it may have been exposed to many applications of the chemical and decreased sensitivity may have resulted. Fludioxonil resistance is not as common, though it is found in *Botrytis cinerea* on strawberry (Fernández-Ortuño, 2013), *Penicillium expansum* on apple (Xiao, 2009), and *Fusarium* spp. on potato (Peters, 2008).

Materials and Methods

Pathogen Isolation

Geotrichum candidum was isolated from infected tomato fruit using a sterile loop and streaked on acidified potato dextrose agar (PDA) (39.0g/L) (Cole-Palmer, Vernon Hills, IL) amended with novobiocin (2.5 mg/L) (Becton, Dickinson and Co., Sparks, MD) to prevent growth of opportunistic bacteria (Brown, 1979; Sneh, 1996). Isolates were collected in twelve commercial tomato fields, one commercial packinghouse, and one cull pile located on the ESV (Table 3.1). Isolates were incubated at 30°C for 3 days, then colonies were re-streaked from individual colonies five times to produce clean isolates. Once a pure culture was obtained, samples were stored on sealed PDA plates at 8°C until further testing.

DNA purification

Agar plugs taken from *G. candidum* isolates were placed on PDA and incubated at 24°C for three days. Colony surfaces were scraped with a sterile loop, fungal material was collected in a 2ml tube with phosphate buffer, then frozen at -80°C. DNA was extracted with FastDNA SPIN Kits and a

FastPrep-24 instrument (MP Biomedical, Santa Ana, CA). DNA extraction followed the manufacturer's protocol for yeast and fungi. Extracted DNA was stored at -20°C until analyzed.

Molecular identification and analysis

Isolates were characterized using the multilocus sequencing technique (MLST) recently published by Alper et al (2013). Six primer pairs were used for amplification and sequencing, namely *ala1*, *cdc19*, *erg10*, *gln4*, *pgi1*, and *pgm2*. ExoSAP-IT enzyme digest was used for PCR product cleanup (Affymetrix, Santa Clara, CA). PCR products were sent to the University of Chicago Comprehensive Cancer Center for sequencing, utilizing Sanger sequencing (3730XL, Life Technologies, Carlsbad, CA). Contig assemblies and multiple sequence alignments were performed using Lasergene 11 Core Suite (DNASTAR, Madison, WI). Sequences were aligned using the Clustal W Method and neighbor-joining analysis was done to produce a phylogenetic tree. Allele profiles of each locus were determined based on nucleotide polymorphisms. A unique number was assigned to each distinct allele sequence for each locus. The unique combination of six loci numbers was used to determine the sequence type (ST) of each strain, using isolate number 23 as the basis of comparison (Alper et al., 2013). Isolate 23 is a genetic match to a *G. candidum* isolate collected and maintained at Virginia Tech's Eastern Shore Agricultural Research and Education Center (ESAREC) in Painter, VA and has been used extensively in past postharvest fungicide efficacy trials. Sequence types and group designations are unique to this study and do not correspond to Alper et al.'s ST values.

Fungicide sensitivity screening- in vitro

Isolates of *G. candidum* were screened for their sensitivity to the following commercial fungicides: azoxystrobin (Quadris 2.08SC, Syngenta Crop Protection, Greensboro, NC), dicloran

(Botran 5F, Gowan, Yuma, AZ), difenoconazole (Inspire, Syngenta Crop Protection, Greensboro, NC), fludioxonil (Scholar SC, Syngenta Crop Protection, Greensboro, NC), myclobutanil (Rally 40WSP, Dow AgroSciences, Indianapolis, IN), propiconazole (Mentor 45WP, Syngenta Crop Protection, Greensboro, NC), prothioconazole (Proline SC, Bayer Crop Science, Research Triangle PK, NC), and tebuconazole (Tebuzol 45DF, United Phosphorus, King of Prussia, PA). PDA was prepared and amended with three concentrations of each fungicide, representing the recommended lowest, highest and mid-range label rates (Table 3.2) after autoclaving.

G. candidum isolates were grown on PDA for 2 days at 30°C. Agar plugs (4 mm diameter) cut from the outer edge of the growing culture were placed pathogen-side down in the center of fungicide-amended and non-amended plates. Plates were incubated for 72 hours at 30°C, the optimal temperature for the pathogen. The colony diameter was measured in two perpendicular directions on each culture plate, the diameter of the mycelial plug subtracted, and the measurements averaged. The percentage of growth reduction was calculated using:

$$(100 - [\text{growth on amended plate} / \text{growth on non-amended}]) \times 100$$

This test was replicated three times for each active ingredient concentration and isolate. The percentage of growth reduction was subjected to an analysis of variance (ANOVA) and comparison of means using Tukey's HSD (α=0.05), using JMP (SAS Corporation, Cary, NC).

Results

Sequence analysis

Diversity of *G. candidum* isolates was evaluated by the allelic variation at six loci. This revealed 23 STs, with 15 STs represented by a single strain (Table 3.3). There were few patterns within STs when

geographic location was considered. ST5 included isolates 22 and 25, both from packinghouse samples, as well as isolate 24 from the cull pile located within close proximity to the packinghouse. ST8 contained the two isolates from Seaford field and only one of the isolates from grape tomato samples. ST10 included two isolates taken from boxed tomato fruit but the other three boxed-fruit isolates from the same sub-field harvest were not of the same ST. Lastly, both Melfa field isolates were included within ST11.

The phylogenetic relatedness of isolates was analyzed with a neighbor-joining tree that was based on the sequences of the six loci. Allelic diversity that was previously described is reflected in the resulting tree (Figure 3.1), with STs clustered together within the tree. Isolates 19 and 38 from Bundick field and 26 from AREC are separate from the other isolates because of their unique gln4 allele type that was different from all others, with 16 polymorphic sites (Table 3.4). Isolate 5 and 17 from Newman and Bundick fields respectively, are strongly removed from the rest of the isolates on the tree because of their unique allele type at the pgi1 locus, which was different from all other isolates with 54 polymorphic sites (Figure 3.1).

In vitro Fungicide testing:

Demethylation inhibitors

All isolates were completely inhibited by all propiconazole and tebuconazole concentrations. The three concentrations of prothioconazole were statistically different, though the medium concentration resulted in the highest growth reduction at 66.9% (Figure 3.2). The interaction of isolate*concentration was significant, which is reflected in the highly variable sensitivity results. Field isolate 13 (Bobtown, Fall 2012) was statistically the most sensitive isolate to prothioconazole, resulting in 73-83% growth reduction.

On average, all levels of difenoconazole greatly restricted *G. candidum* growth (Figure 3.3). Low and medium concentrations resulted in significantly different growth restrictions (84.4% and 85.9% respectively), though the high concentration (85.1%) was statistically similar to both concentrations. Isolates and the interaction of isolates*concentration were also significant, but isolates exhibited clearly different levels of sensitivity to difenoconazole.

The low concentration of myclobutanil resulted in a large reduction of *G. candidum* growth, while medium and high concentration completely prevented growth (Figure 3.4). There were no significant differences between the isolates and their sensitivity to myclobutanil.

Fungicides used in vegetable field production

Most *G. candidum* isolates were only partially sensitive to azoxystrobin at the labeled rates (Figure 3.5). The three concentrations of fungicide resulted in 30-40% growth reduction across most isolates. Differences among isolates and the interaction of treatment level and concentration*isolates were significant as well. Field isolate 14, collected summer 2012, displayed increased growth when grown on all concentrations of azoxystrobin, and field isolate 17 (Fall 2013), had increased growth on the highest concentration of azoxystrobin. These two isolates show evidence for azoxystrobin resistant populations on the ESV.

The low concentration of dicloran resulted in about 50% reduction in growth across all isolates, with each increasing concentration resulting in a significantly higher reduction in growth (Figure 3.6). Differences between isolates were significant at each treatment level, as well as the interaction between concentration and isolate. At the highest concentration, isolates 6, 13, and 14 (Painter cull pile, Summer 2012; Bobtown, Summer 2012; Painter, Fall 2012) were completely inhibited.

Postharvest Production

Sensitivity to fludioxonil was variable at all concentrations (Figure 3.7). Low and medium concentrations reduced growth significantly less than the high concentration. Low and medium levels resulted in 0-44% growth reduction, while high levels of the chemical resulted in 10-84% reduction in growth. The interaction of isolates and concentration was also significant. Growth reduction of isolates 5, 7, and 36 was either minimal and growth was not reduced at all, which could be evidence of fludioxonil's diminished activity or resistant populations developing.

Discussion

Multilocus sequence typing was successful at characterizing tomato populations of *G. candidum* and revealing unexpected diversity from such an isolated population. Results from Alper *et al*. (2013) show more diversity among their isolates, having a greater number of alleles except for *ala1*. Though our *pgi1* locus had less allelic diversity than *pgi1* of Alper *et al*., the number two allele had 54 polymorphic sites. A BLAST search revealed that the *pgi1* sequence of isolate 5 and 17 is only 90% identical to other *G. candidum* sequences (Accessions JQ668857, JQ668860, JQ668745, JQ668749, JQ668743, JQ668742, JQ668741) and is 96% identical to a strain of *Galactomyces reessii* (Accession KF042635.1). *Galactomyces reessii* is not a plant pathogen, but a free living yeast-like relative of *G. candidum* that is used to transform 3-methylbutyric acid (MBA) to 3-hydroxy-3-mtheylbutyric acid (HMBA), which is a dietary supplement used to improve animal growth and health (Dhar, 2002). All other loci for isolates 5 and 7 (Group D) are consistent with *G. candidum* isolates from a BLAST search so we still assume that those isolates are within the same species but different subspecies.

The resulting phylogenetic tree of the 38 isolates analyzed revealed four major groups. Two large groups, A and B, do not have clear geographic differences. Nine out of twelve isolates associated

with postharvest handling of tomato fruit (packinghouse, boxed fruit, or cull pile) were located in Group B. 47% of Group A isolates were collected during the summer production seasons and 73% of Group B isolates were collected during fall production seasons.

This tree scheme allowed us to identify two phylogenetically distant groups among *G. candidum* isolates, suggesting the possibility of subspecies for group C and D (Figure 3.1).

In vitro fungicide sensitivity analysis revealed antimicrobial products that successfully restrict the growth of the pathogen, though it was hard to compare the rates of different compounds since the ratios of low versus medium versus high were different. Though the majority of products tested were ineffective, propiconazole and tebuconazole completely restricted growth at all labeled rates. Propiconazole is currently marketed as a postharvest treatment on tomato fruit (Mentor, Syngenta Crop Protection, Greensboro, NC) and has been shown to successfully prevent infection progress *in vivo* (unpublished data). Tebuconazole is currently only labeled for in-field application on tree fruits so it cannot be applied legally to tomatoes at this time. Interestingly, *G. candidum* is not equally sensitive to all triazole products. Propiconazole and tebuconazole successfully prevented growth, but difenoconazole in growth media resulted in 80% growth reduction and prothioconazole only resulted in 42-80% growth reduction.

Myclobutanil also completely restricted *G. candidum* growth at medium and high concentrations. It is a broad-spectrum fungicide that is used in tree fruit, vegetable, and ornamental production for the control of a wide range of pathogens. It is currently labeled for the in-field control of tomato powdery mildew. There is no pre-harvest interval with tomatoes so it can be applied the day of harvest. If there are concerns of sour rot appearing in harvested tomato fruit due to optimal weather conditions, an in-field application of myclobutanil may have potential to protect again postharvest infection.

Repeated use of fungicides possessing the same mode of action may lead to the development of insensitive pathogen populations (www.frac.info). Populations frequently develop insensitivity to azoxystrobin when it is not applied properly or used frequently and evidence from these *in vitro* studies shows that more than one *G. candidum* population on the ESV may have developed insensitivity. Both locations where insensitive isolates were collected have a long history of tomato production and azoxystrobin use. These results indicate that more responsible use of azoxystrobin may be needed to restrict the development of reduced-sensitivity populations.

Propiconazole was only recently labeled for postharvest use on tomatoes and is still not commonly used, making tomato producers rely on disinfesting fruit and wash water with chlorinated water. Tomato fruit producers on the ESV assume proper levels of bleach successfully kill all microorganisms in wash water, but in reality it is only effectively eliminate bacterial organisms (Bartz, 2001; Boyette, 1993). Previous studies in our lab show evidence that postharvest sanitation may play a more important role in postharvest sour rot loss than in-field conditions (unpublished data). If this is true, relying on chlorine or peroxide products for disinfesting tomato fruit and wash water may not be effective at reducing postharvest losses.

These studies are only one component to identifying compounds for the control of tomato sour rot. *In situ* studies must be conducted before further recommendations can be made. Also, further *G. candidum* isolate collection and analysis should be completed to include more production fields and variety in tomato growth stage and cultivar to determine if genetic diversity increases with more diverse samples.

The MLST method successfully characterized *G. candidum* isolates within the ESV tomato production region. This is a diverse population for a relatively small sampling area. Alper's study (2013) was completed on samples from the dairy and cheese industry, including samples from smear cheeses,

grass, corn silage, milk, and bioreactor contaminant, and resulted in slightly more diversity than our study. Soil samples and *G. candidum* from other fresh produce on the ESV would be of interest to characterize, as well as isolates from other regions.

Table 3.1: Description of *G. candidum* isolates tested including field locations, date, source, and other descriptive information.

Description			Isolate Number
Field	Date	Source	
AREC	09/07/12	field	10
AREC	10/26/11	field	23
AREC	09/07/12	field	26
AREC	09/07/12	Roma, field	32
Bobtown	09/05/12	field	13
Bowen	10/08/12	field	37
Bundick	09/05/12	field	17
Bundick	09/05/12	field	19
Bundick	09/05/12	field	29
Bundick	09/05/12	field	38
Custis	09/05/12	field	2
Grapeland	07/09/12	field	34
Jones	08/16/12	field	4
Jones	10/08/12	field	15
Lipman's boxed fruit	08/22/12	green, field	27
Melfa	10/07/12	field	31
Melfa	10/07/12	field	33
Newman	08/16/12	field	3
Newman	09/15/12	field	5
Newman	08/16/12	field	36
Pacific grape tomato	07/16/12	from ground	7
Pacific grape tomato	07/16/12	from ground	11
Painter	07/17/12	field	14
Seaford	08/16/12	field	9
Seaford	08/16/12	field	16
Yeardley	10/08/12	field	20
	10/26/11	packinghouse	12
	10/26/11	packinghouse	22
	10/26/11	packinghouse	25
	10/26/11	packinghouse	30
Painter	07/17/12	cull pile	6
Painter	10/26/11	cull pile	21
Painter	10/26/11	cull pile	24
Painter	10/26/11	cull pile	28
	08/25/12	postharvest, box, green	8
	08/22/12	postharvest, box, green	1
	10/22/12	postharvest, box	18
	10/22/12	postharvest, boxed	35

Table 3.2: Active ingredients used in amended PDA to test *G. candidum* isolate sensitivity. Rates tested reflect the spectrum of products' labeled formulated rates.

Active Ingredient	Product	FRAC Code	Manufacturer	Rate		
				Low	Medium	High
azoxystrobin	Quadris 2.08SC	11	Syngenta Crop Protection, Greensboro, NC	0.469 ml/L	0.820	1.210
dicloran	Botran 5F	14	Gowan, Yuma, AZ	1.5 ml/L	4.750	8.000
difenoconazole	Inspire	3	Syngenta Crop Protection, Greensboro, NC	0.312 ml/L	0.430	0.547
fludioxonil	Scholar SC	12	Syngenta Crop Protection, Greensboro, NC	0.781 ml/L	1.640	2.500
myclobutanil	Rally 40WSP	3	Dow AgroSciences, Indianapolis, IN	0.098 ml/L	0.439	0.781
propiconazole	Mentor 45WP	3	Syngenta Crop Protection, Greensboro, NC	0.1498 g/L	0.599	1.198
prothioconazole	Proline 480SC	3	Bayer CropScience, Research Triangle Park, NC	0.195 ml/L	0.320	0.445
tebuconazole	Tebuzol 45DF	3	UPI, King of Prussia, PA	0.460 ml/L	0.814	1.16

Table 3.3: Genotypes of 38 *G. candidum* isolates. Group designations are those indicated within the phylogenetic tree (Figure 1). Each unique combination of six locus numbers was used to determine the ST of each strain. The same ST was used for isolates sharing the same allelic profiles.

Isolate #	Group	Strain Type (ST)	Housekeeping genes allele number					
			ala1	cdc19	erg10	gln4	pgi1	pgm2
6, 23, 36	A	1	1	1	1	1	1	1
14, 15, 20	A	2	1	1	1	1	1	2
7, 9, 16	A	8	1	2	2	1	1	1
13, 37	A	9	1	2	2	1	1	2
10	A	12	1	2	2	1	1	5
30	A	17	3	1	1	1	1	1
21	A	18	4	1	1	1	1	1
11	A	21	5	2	2	1	1	2
3	A	22	6	1	1	1	1	1
8	A	23	7	2	2	3	1	1
29	B	3	1	1	1	1	1	3
4, 32	B	4	1	1	2	1	1	4
22, 24, 25	B	5	1	1	2	1	1	3
12	B	7	1	1	3	1	1	3
2, 18, 35	B	10	1	2	2	1	1	3
27, 31, 33, 34	B	11	1	2	2	1	1	4
1	B	14	1	2	3	1	1	4
28	B	16	2	1	1	1	1	3
38	C	13	1	2	2	2	1	2
26	C	15	1	2	4	2	1	3
19	C	20	4	2	2	2	1	2
5	D	6	1	1	2	1	2	1
17	D	19	4	2	2	1	2	3

Table 3.4: Positions of the polymorphic sites for alleles identified for each locus. Allele 1 is presented as reference for each locus. For other alleles, only sites that differ from allele 1 are shown and positions with identical nucleotides are represented by dots. Double dashes represent insertions.

	position										
ala1	27	30	44	45	46	48	50	51	52	69	273
allele 1	--	--	C	G	A	A	--	G	A	T	T
allele 2	•	•	•	•	•	•	•	•	•	C	•
allele 3	•	•	•	•	•	•	•	•	•	C	A
allele 4	•	•	•	•	•	•	A	•	•	•	•
allele 5	•	T	•	•	•	•	•	•	•	•	•
allele 6	•	•	G	A	•	G	•	--	--	•	•
allele 7	G	G	G	•	G	•	•	•	•	•	•

	position			
erg10	26	447	645	651
allele 1	A	C	C	C
allele 2	•	T	•	•
allele 3	•	T	T	T
allele 4	--	T	•	•

	position
cdc19	300
allele 1	G
allele 2	A

	position																			
gln4	36	37	42	52	61	73	82	91	175	310	331	336	343	376	394	415	427	446	478	550
allele 1	A	--	--	C	G	C	T	T	T	T	A	A	C	C	A	C	C	C	C	C
allele 2	•	•	A	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
allele 3	--	•	A	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
allele 4	•	A	A	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
allele 5	•	•	A	T	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
allele 6	•	•	A	T	A	T	C	C	C	C	C	G	T	T	G	T	T	T	A	T

	position																			
pgi1	4	6	12	22	36	42	54	63	66	69	78	81	89	90	99	114	153	156	186	195
allele 1	C	G	T	T	T	G	T	C	C	T	G	T	G	C	G	T	T	C	T	C
allele 2	T	A	C	C	C	A	C	T	T	C	T	C	A	A	C	C	C	T	C	T

	219	225	234	237	249	276	294	297	318	321	332	333	336	342	343	351	357	360	362	381
	C	C	G	T	T	T	C	T	C	C	C	T	G	T	C	A	T	T	T	C
	T	T	T	C	C	A	T	C	T	T	T	C	A	C	T	G	C	C	C	T

	382	396	399	405	411	417	418	441	444	459	462
	A	C	C	T	A	C	C	C	C	T	T
	G	T	T	C	G	T	T	T	T	A	C

	position			
pgm2	3	18	93	159
allele 1	T	T	C	C
allele 2	C	•	•	•
allele 3	C	C	T	T
allele 4	C	C	T	•
allele 5	C	•	T	•

Figure 3.1: Phylogenetic tree (neighbor-joining) of 18 *G. candidum* strains based on ALA1, CDC19, ERG10, GLN4, PGI1, and PGM2 complete gene contigs. The phylogenetic trees were constructed using the neighbor- joining method with 1,000 bootstraps Phylogenetic analysis were conducted in Lasergene 11 Core Suite.

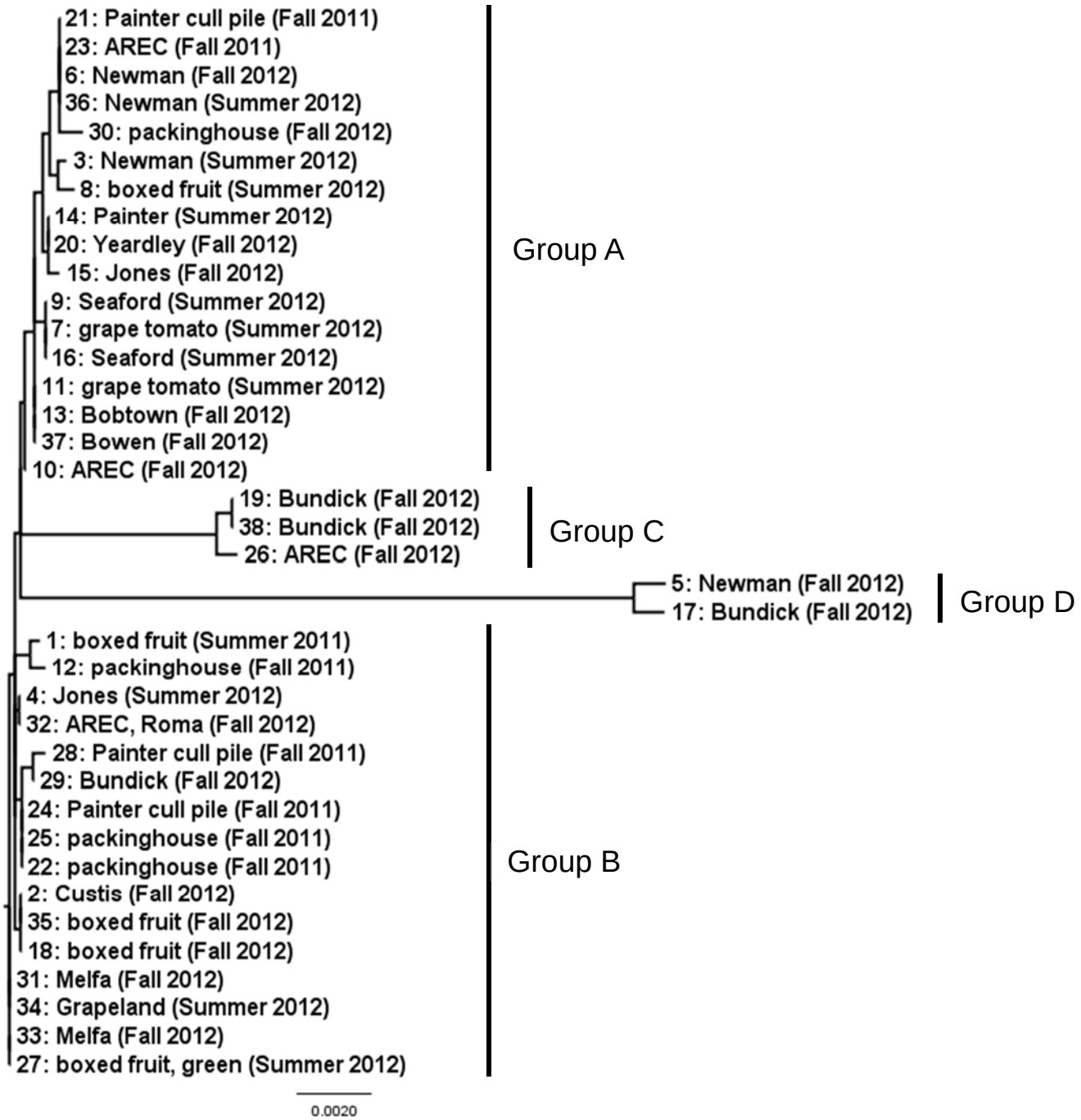


Figure 3.2: Low (A), medium (B), and high (C) concentrations of prothioconazole acid in amended PDA and the resulting inhibition of *G. candidum*. Tukey HSD compared means and bars with distinct letter show significant differences ($\alpha = 0.05$). Letters shown represent the first and last of the letter sequence showing significance.

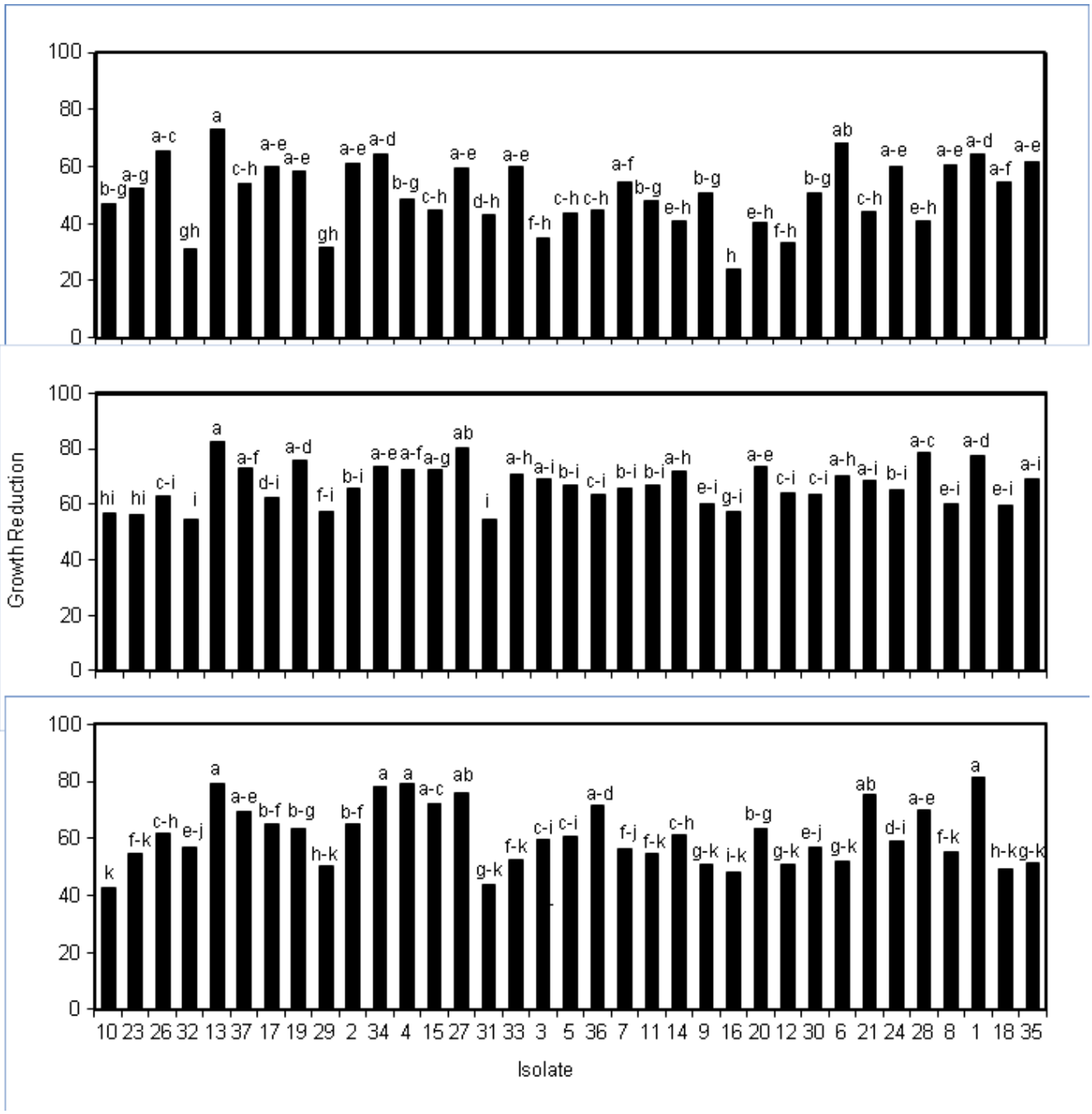


Figure 3.3: Low (top), medium (middle), and high (bottom) concentrations of difenoconazole in amended PDA and the resulting reduction in growth of *G. candidum*. Tukey HSD compared means and bars with distinct letter show significant differences ($\alpha = 0.05$). Letters shown represent the first and last of the letter sequence showing significance.

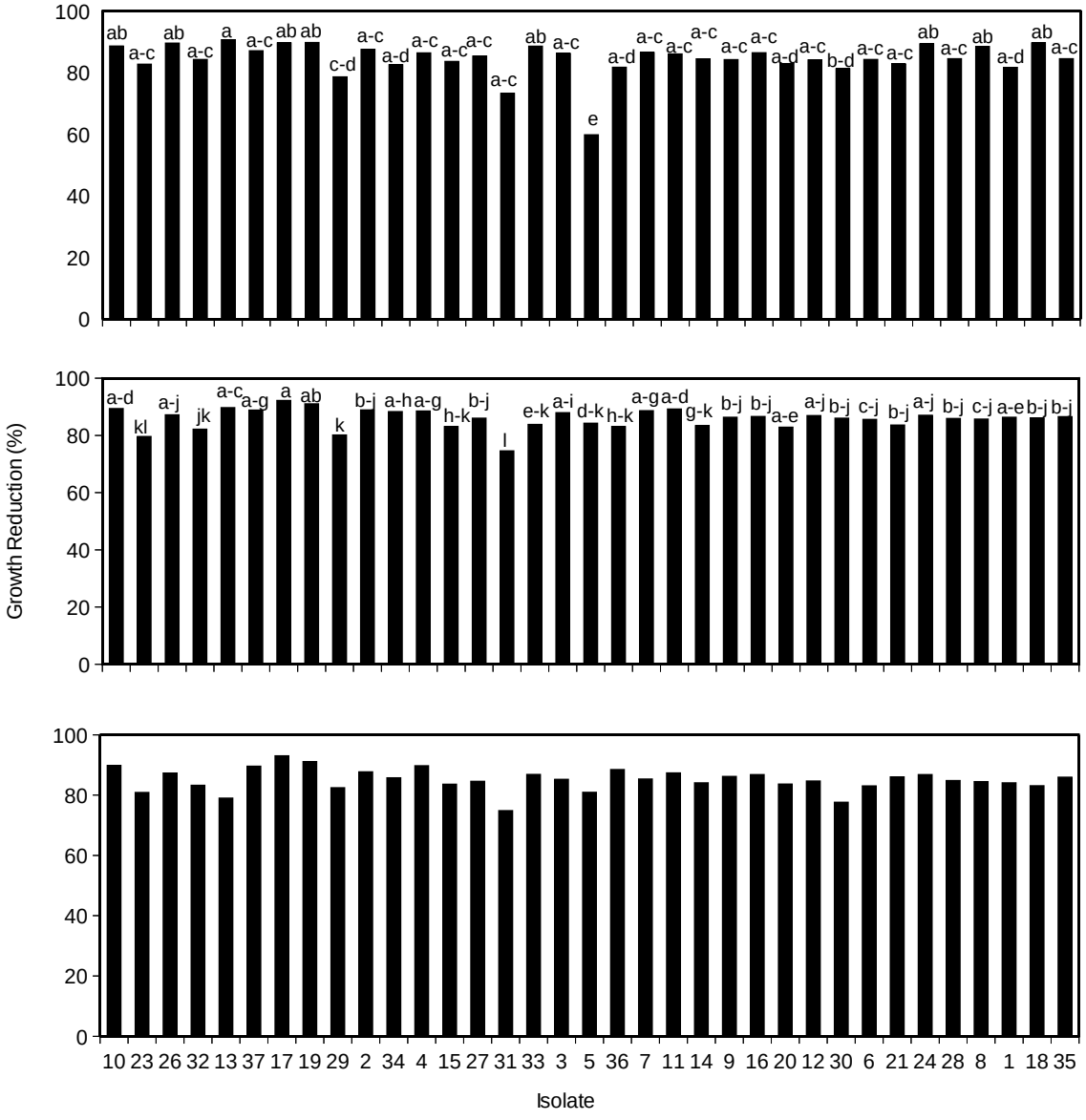


Figure 3.4: Low, medium, and high concentrations of myclobutanil in amended PDA and the resulting reduction in growth of *G. candidum*. Differences between isolates were not significant. Tukey HSD compared means and bars with distinct letter show significant differences ($\alpha = 0.05$). Letters shown represent the first and last of the letter sequence showing significance.

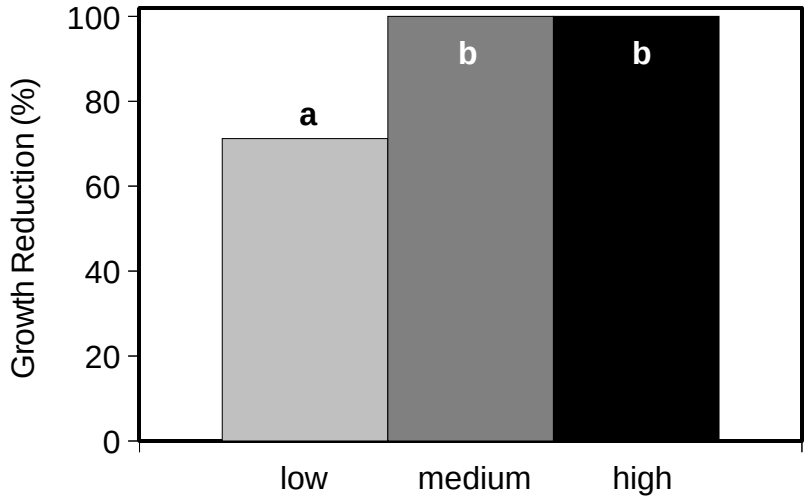


Figure 3.5: Low (top), medium (middle), and high (bottom) concentrations of azoxystrobin in amended PDA and the resulting reduction in growth of *G. candidum*. Tukey HSD compared means and bars with distinct letter show significant differences ($\alpha = 0.05$). Letters shown represent the first and last of the letter sequence showing significance.

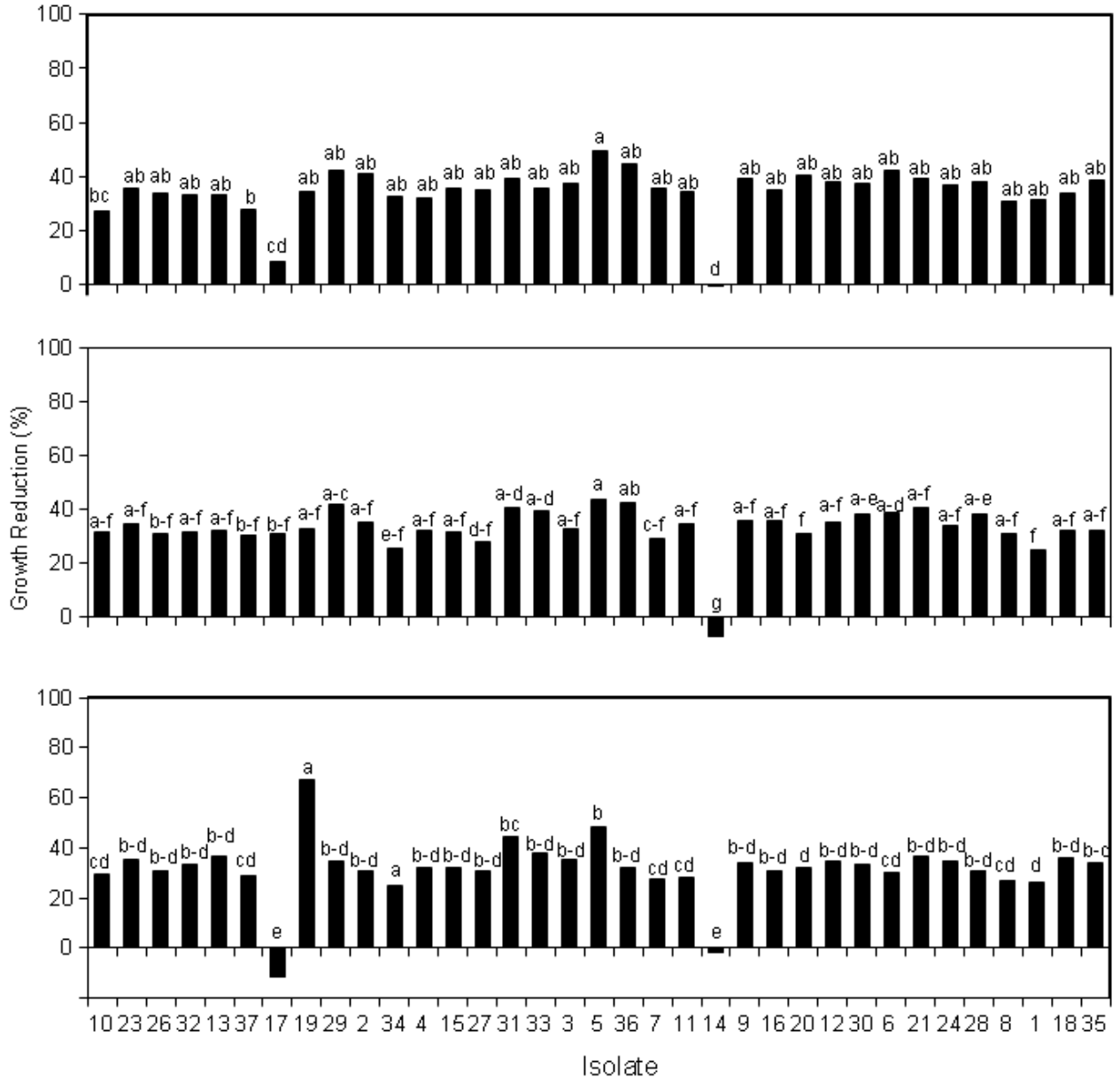


Figure 3.6: Low (top), medium (middle), and high (bottom) concentrations of dicloran in amended PDA and the resulting reduction in growth of *G. candidum*. Tukey HSD compared means and bars with distinct letter show significant differences ($\alpha = 0.05$). Letters shown represent the first and last of the letter sequence showing significance.

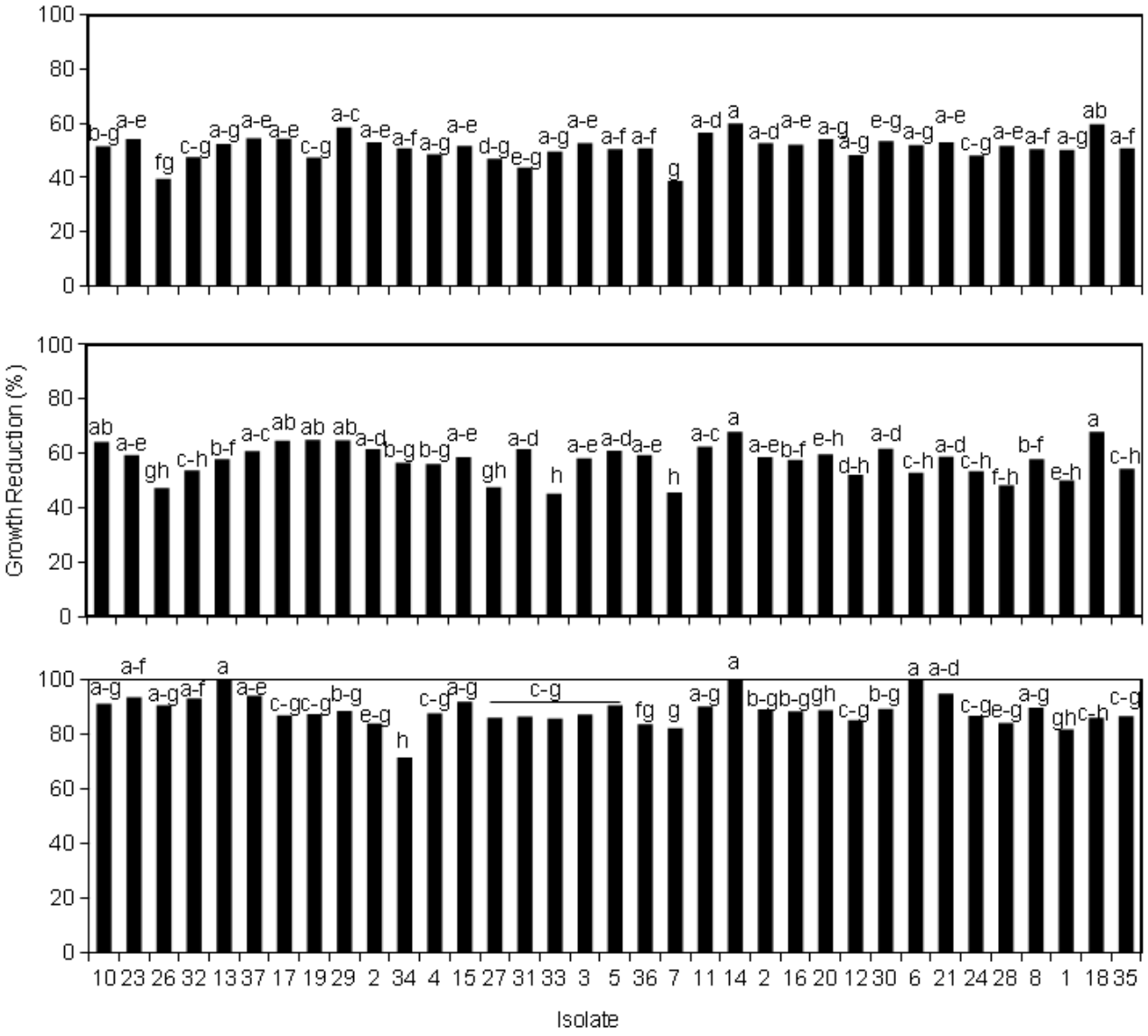
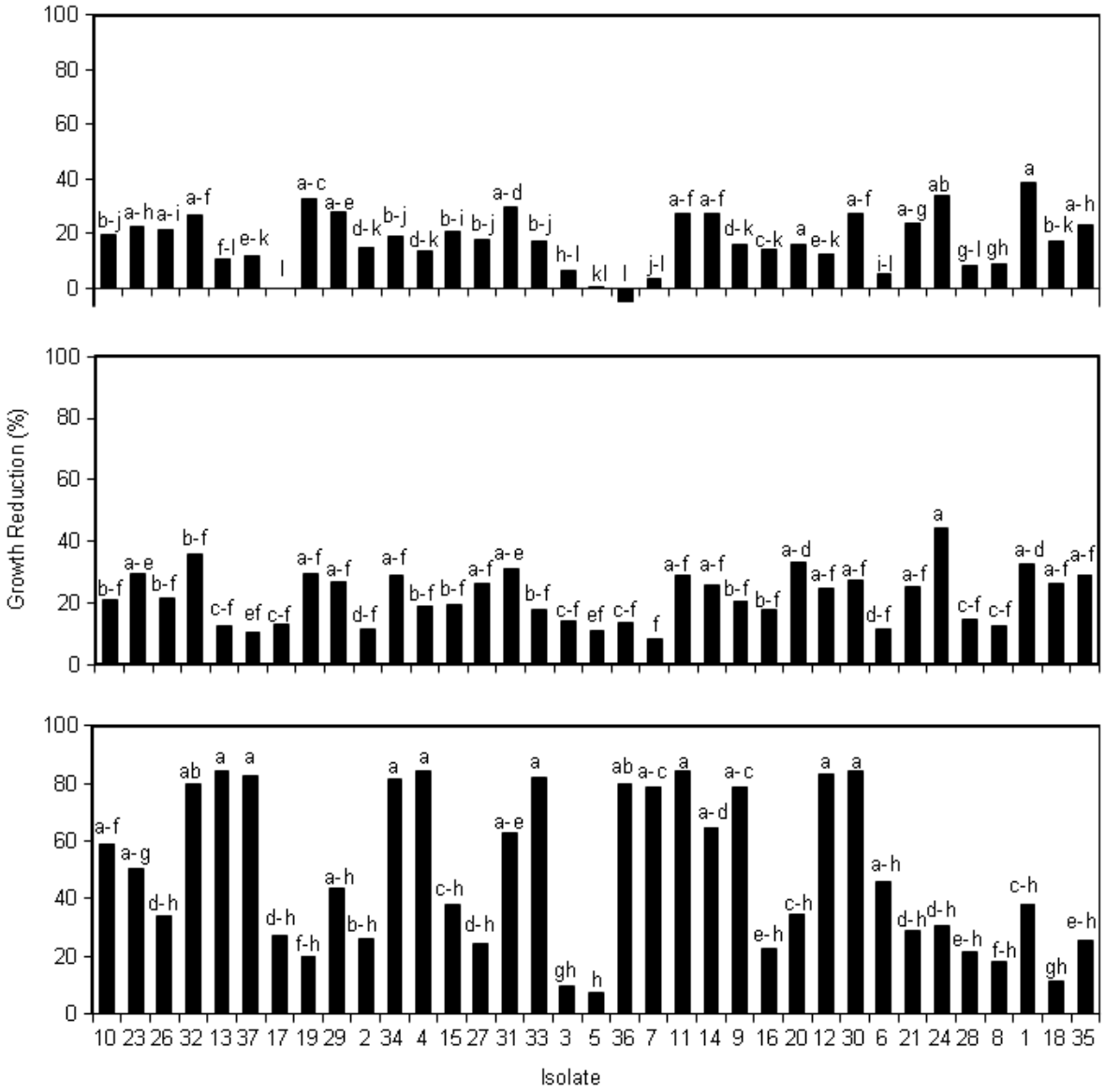


Figure 3.7: Low (A), medium (B), and high (C) concentrations of fludioxonil in amended PDA and the resulting reduction in growth of *G. candidum*. Tukey HSD compared means and bars with distinct letter show significant differences ($\alpha = 0.05$). Letters shown represent the first and last of the letter sequence showing significance.



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CHAPTER 4: SOUR ROT EPIDEMIOLOGY

Abstract

In-field and postharvest surveys were conducted to summarize losses of tomatoes on the Eastern Shore of Virginia due to *Geotrichum candidum*, causal agent of sour rot. Plants containing market-ready tomato fruit were randomly selected within four fields during summer harvest and four fields during fall harvest over a 2-year period. Incidence of sour rot per plant was scaled-up to estimate total loss per field. Daily temperature, relative humidity, and precipitation were recorded and summed for periods of 1 through 7 days prior to each sampling date. Values were compared to in-field sour rot losses. Sanitized and boxed fruit were also assessed for postharvest sour rot infections and the values compared to weather data. Sour rot incidence was greater during summer harvests than fall harvests. Few correlations were found with weather and in-field sour rot incidence across both seasons and years. Rainfall was positively correlated with disease 2-3 days before surveys and temperature was negatively correlated with disease 5-7 days before surveys. No in-field weather conditions were correlated with postharvest disease incidence. Weather parameters and scale of study need to be reevaluated in order to determine conditions that influence sour rot incidence. Postharvest handling and sanitation practices are suspected to play a larger role in postharvest sour rot loss than initially assumed.

Introduction

Geotrichum candidum is the causal agent of sour rot of tomato fruit (*Solanum lycopersicum*) and other fresh produce. This disease is a major limiting factor of tomato production in all tomato growing regions, including the Eastern Shore of Virginia (ESV). It can cause losses in the field but the primary concern is infections that develop during postharvest handling. There are no in-field fungicides targeted to prevent sour rot infections and only one newly labeled postharvest product, propiconazole (Mentor 45WP, Syngenta Crop Protection, Greensboro, NC), is available to prevent further losses. Cultural controls are relied on heavily to prevent postharvest sour rot infections.

Observations indicate *G. candidum* infections are most severe after wet harvest conditions, abrupt drops in temperature due to rainfall, and improper post-harvest handling procedures (Bartz, 2007; Bartz et al., 2012). It is also believed that sour rot infections are more significant during the fall growing season, thus growers have altered their cultivar selection, planting date, and harvest time based on this (personal observation). In certain years, *G. candidum* can reach "epidemic" status and result in an estimated 25% loss of packaged fruit (Richard Davis, Lipman Produce, personal comm.). Infected fruit may remain asymptomatic for a number of days during processing and de-greening, presenting symptoms during storage and throughout distribution (Bartz, 2007; Coates and Johnson, 1997).

Sour rot occurs in a variety of geographic locations and environments, making it difficult to identify the exact in-field and postharvest conditions that influence disease incidence. Recently, high incidence of sour rot of peach and nectarine has increased in California, which are produced in semi-arid conditions and environmental moisture was not found to play a role in infection rate (Yaghmour et al., 2012). Florida citrus is raised in mild and humid conditions and experiences sour rot caused by related pathogen (*Geotrichum citri-aurantii*). Disease incidence there is influenced by the microclimate of the ground and lower canopy (Brown, 1979). Citrus fruit closer to the ground and fruit with wind damage

displayed more disease incidence. Other studies that investigate environmental conditions associated with sour rot incidence focus on sanitation and packinghouse conditions and rarely include in-field environmental factors.

The purpose of this study was to determine disease incidence of tomato sour rot in field and postharvest settings, and to highlight correlations between disease occurrence and weather. These objectives were fulfilled by completing in-field disease incidence and weather monitoring, as well as disease incidence observed in postharvest boxed tomato fruit.

Materials and Methods

In-field Assessments

Sour rot disease incidence was determined by extensive in-field scouting according to the Florida Tomato Scouting Guide published by University of Florida Extension (Pernezny, 2008) and methods used by local scouts on the ESV. In both 2012 and 2013 four commercial fields on the ESV were chosen for scouting during the summer harvest season (July-August) and four different fields for fall harvest season (September-October) (Table 4.1). Within each field, ten random sites were selected where seven consecutive plants were selected and the number of infected mature green fruit was counted. Locations within fields were selected randomly at each visit. This was done twice a week starting 2 weeks before the first harvest and continued to the end of the third harvest, usually totaling 6-8 weeks of surveys for each harvest period.

Rainfall and relative humidity (RH) were recorded at a central location (Melfa, VA weather station) and degree days were calculated from a base of 18.3ºC and daily average temperature made up of hourly temperature readings. All weather data and disease incidence values were summed for 7, as well as 6, 5, 4, 3, 2, and 1 day prior to each sampling date. Percentage of lesion incidence on fruit for

each survey was compared to cumulative rainfall, degree days, and relative humidity to determine if weather components influence sour rot incidence in the field. Multivariate analysis was completed using JMP (SAS Corporation, Cary, NC) with all previously mentioned variables, then non-parametric Spearman's rank correlation coefficients (Spearman's ρ) were analyzed for significance (α=0.05) (Madden & Hughes, 1999).

Postharvest Epidemiology

Twenty boxes (25-40 fruit/box) of cleaned, waxed, and packaged ethylene-treated tomato fruit at breaker stage or later were incubated at 29°C for two weeks at 90% relative humidity. Disease incidence was recorded for sour rot and other postharvest diseases. Like previous in-field surveys, cumulative rainfall, degree days, and relative humidity were summed for the 7 days prior to boxed fruit's harvest, then decreasing by one less day down to a single day's worth of weather data. Statistical analysis was completed as previously described for in-field disease levels. Multivariate analysis was completed using JMP (SAS Corporation, Cary, NC) with all previously mentioned variables, then non-parametric Spearman's rank correlation coefficient (Spearman's ρ) was completed to find significance (α=0.05).

Results and Discussion

In-field

The hypothesized seasonality of sour rot incidence was not supported by our data. Instead of greater disease incidence during fall harvests, results show greater disease incidence during summer harvest during both 2012 and 2013 (Figure 4.1). Generally, summer harvest periods had more rain and higher temperatures (Table 4.2), which is average for the region (SERCC, 2014). Disease develops

much quicker during summer heat, leading to faster disease progress once it is established within a field (Plaza et al., 2003). Early Fall 2012, 17.07cm rainfall accumulated over a 24-hour period during Hurricane Sandy. Tomato fruit on plants during the storm were not yet at the mature green stage and the majority dropped from plants due to wind, so the excessive rainfall is not believed to play a role in sour rot incidence later on during harvest. In Fall 2013 the Bobtown field had higher disease incidence than other fields, which may be due to standing water that was regularly observed within proximity to plants with high levels of infection. Standing water was due to excess irrigation and rain water draining into low points within the field. Other fields during the sampling period were drier and less disease was observed.

Relative humidity was significantly correlated with disease incidence at many time points throughout all seasons and years surveyed (Table 4.2). During the Summer 2012 harvest period, disease incidence was positively correlated with RH 1, 2, 6, and 7 cumulative days from disease surveys, and only 3 cumulative days out from disease surveys in Summer 2013. Fall 2012 and 2013 disease surveys were very different, showing RH significantly negatively correlated with disease incidence on all cumulative days (1-7) before disease surveys.

Rainfall was positively correlated with 2 and 3 cumulative days before disease surveys in Summer 2012 and positively correlated with 2-7 cumulative days from disease surveys in Summer 2013 (Table 4.2). Similar to RH values, rainfall during all cumulative days (1-7) was negatively correlated with disease incidence during Fall 2012 harvest. Rainfall was also negatively correlated with disease incidence but only for 3-7 cumulative days before disease surveys.

Degree days were only found to be significant during the 2012 harvest seasons (Table 4.2). During the summer harvest period disease incidence was negatively correlated with degree days for the

cumulative 5-7 days before disease surveys. Degree days during 1-7 cumulative days before surveys were negatively correlated with disease incidence.

Relative humidity and rainfall negatively correlated to disease incidence during fall harvest periods, which goes against common phytopathological assumptions. General trends show that disease incidence follows a pattern, though the precise weather conditions monitored did not significantly influence disease. Future sour rot epidemiology studies should investigate other possible weather and microbial factors that may have a greater influence, such as more closely describing the abrupt weather changes before storm conditions that could internalize spores on the surface of fruit, and the spore levels on fruit and exposed soil.

Postharvest

Overall sour rot incidence in 2012 was relatively low, as were incidences of other common postharvest diseases (Figure 4.2). Postharvest tomato disease surveys included four pathogens; sour rot and bacterial soft rot (*Pectobacterium carotovorum*) were the dominant diseases present, black mold (*Alternaria alternata*), and Fusarium fruit rot (*Fusarium oxysporum* f. sp. *lycopersici*) were also present. There were no significant correlations between postharvest sour rot incidence and weather parameters when multivariate analysis was conducted (Table 4.4). These data do not reveal any trends that show specific and consistent correlation with sour rot incidence and large-scale weather conditions.

Lack of significance between postharvest disease incidence and in-field weather before harvest leads to the assumption that postharvest handling plays a larger role in postharvest disease development than originally thought. The wrong weather parameters may have been tested and alternate parameters are needed for future studies. High levels of bacterial soft rot in the postharvest setting (Fig. 4.2) are

indicative of poor postharvest handling, specifically dump tank management, which is a major component of proper postharvest handling.

Packinghouse sanitation and its relationship to stone fruit, citrus, and tomato sour rot incidence has been closely studied, showing many areas within the packinghouse that can serve as sources of inoculum. In several California peach and citrus packinghouses, *Geotrichum* spp. was found at four major locations within the house; fruit unloading, de-stemming brushes, cleaning brushes, and final packing table (Yaghmour, 2012). *Geotrichum candidum* is regularly found on the surface of harvested fruit which leads to inoculum on packinghouse machinery and in dump tank water (Brown, 1979). Many packinghouse studies have resulted in recommendations for proper dump tank maintenance and controlled temperature and humidity (Bartz, 2001; Bartz, 2007; Smilanick, 2007, Suslow, 2000), but cleaning and sanitation of the entire packinghouse facility is not mentioned in many publications and is not commonly practiced. If *G. candidum* has been found in various locations throughout packinghouses then sanitation suggestions similar to those in the food safety industry may be beneficial for preventing postharvest plant diseases, such as sour rot.

Table 4.1: Eastern Shore, VA fields surveyed for disease incidence. Summer 2012 and 2013 sampling included July 7- August 8; Fall 2012 sampling included August 28-October 8; and Fall 2013 sampling included September 3-October 4.

	2012	2013
Summer	Grapeland	Bundick
	Marshall	Custis
	Melfa	Grapeland
	Painter	Yeardley
Fall	Bowen	Bobtown
	Jones	Bowen
	Melfa	Jones
	Yeardley	Yeardley

Table 4.2: Average temperature and total rainfall for summer and fall harvest periods in 2012 and 2013. At the beginning of the fall 2013 harvest period 17.07cm of rain accumulated over a 24 hour period during hurricane Sandy. The value in parenthesis is total rainfall during the Fall 2012 harvest period without the hurricane influence.

	Average Temperature (ºC)	Total Rainfall (cm)
Summer 2012	30.6	13
Summer 2013	25.9	13.8
Fall 2012	24	28.5 (11.43)
Fall 2013	21	4.7

Figure 4.1: Cumulative in-field percentage of estimated sour rot in each field. Summer 2012 and 2013 sampling included July 7- August 8; Fall 2012 sampling included August 28-October 8; and Fall 2013 sampling included September 3-October 4.

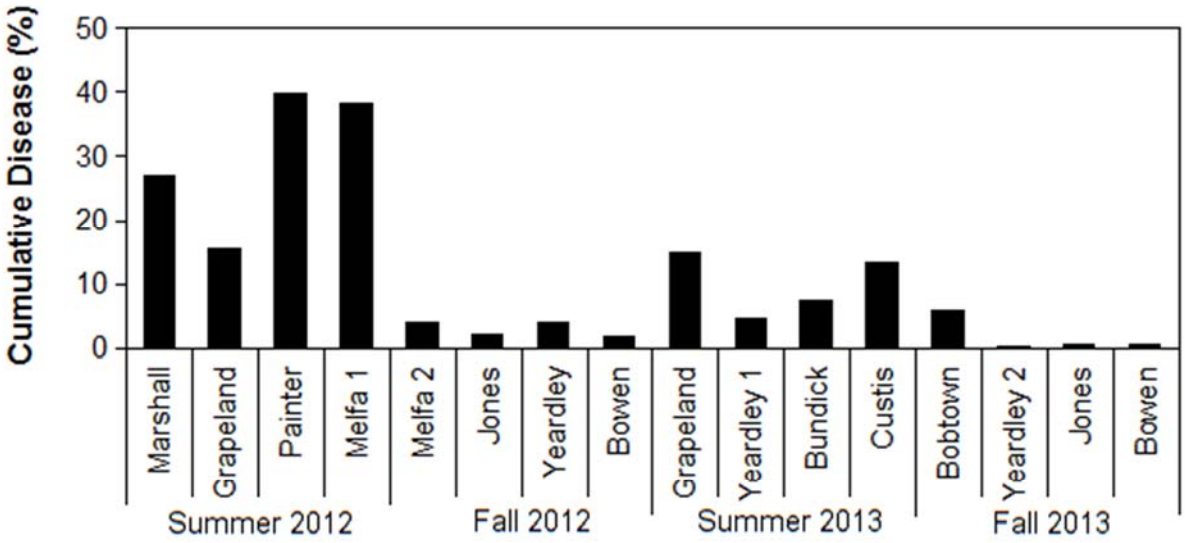

Table 4.3: Multivariate analysis of disease incidence to humidity, rainfall, and degree days during the summer and fall harvest periods in 2012 and 2013. Spearman's rank correlation coefficient (Spearman's ρ) used to compare means.

	July 7-August 8, 2012			**July 8-August 8, 2013**		
Cumulative Days Before Sampling	Relative Humidity	Rainfall	Degree Days	Relative Humidity	Rainfall	Degree Days
1	0.38*	0.33	-0.30	0.01	-0.19	-0.11
2	0.45*	0.63*	-0.33	0.11	0.44*	-0.10
3	0.30	0.49*	-0.28	0.39*	0.60*	-0.06
4	0.30	0.18	-0.28	0.18	0.52*	-0.14
5	0.25	0.17	-0.37*	0.16	0.40*	-0.11
6	0.39*	0.16	-0.40*	0.14	0.44*	-0.15
7	0.40*	0.16	-0.42*	0.04	0.35*	-0.18
	August 28-October 8, 2012			**September 3-October 4, 2013**		
Cumulative Days Before Sampling	Relative Humidity	Rainfall	Degree Days	Relative Humidity	Rainfall	Degree Days
1	-0.55*	-0.55*	-0.35*	-0.58*	0.00	-0.03
2	-0.57*	-0.56*	-0.47*	-0.64*	-0.19	0.07
3	-0.50*	-0.49*	-0.66*	-0.56*	-0.37*	-0.04
4	-0.47*	-0.47*	-0.73*	-0.50*	-0.37*	-0.03
5	-0.49*	-0.49*	-0.70*	-0.48*	-0.53*	-0.10
6	-0.44*	-0.44*	-0.69*	-0.48*	-0.53*	0.01
7	-0.46*	-0.46*	-0.72*	-0.46*	-0.53*	-0.13

*$\alpha = 0.05$

Figure 4.2: Postharvest disease incidence in boxed tomato fruit, namely sour rot (*Geotrichum candidum*), bacterial soft rot (*Pectobacterium carotovorum*), black mold (*Alternaria alternata*), and fusarium rot (*Fusarium oxysporum*).

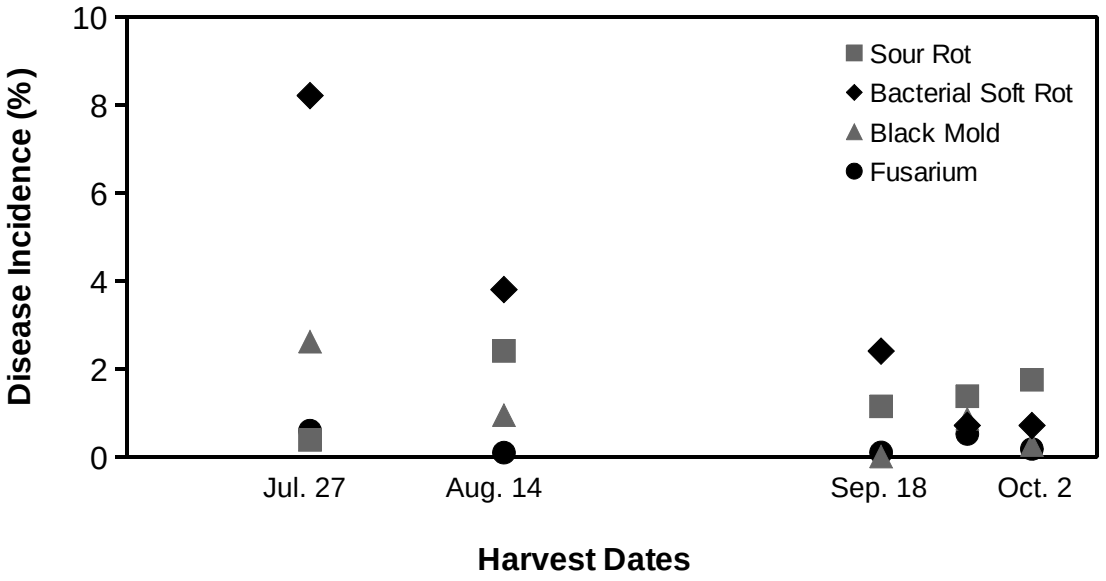

Table 4.4: Multivariate analysis (Spearman's rank correlation coefficient, Spearman's ρ) of postharvest sour rot incidence to humidity, rainfall, and degree days during the summer and fall harvest periods in 2012. No values were significant (α = 0.05).

Cumulative Days Before Sampling	Relative Humidity	Rainfall	Degree Days
1	-0.10	0.16	0.05
2	-0.10	0.36	0.10
3	-0.10	0.00	0.00
4	-0.10	0.00	-0.40
5	-0.10	0.00	-0.40
6	0.30	0.00	-0.30
7	0.30	-0.10	-0.30

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CHAPTER 5: CULTIVAR SUSCEPTIBILITY

Abstract

The susceptibility of common tomato cultivars to sour rot was assessed in order to help Eastern Shore, Virginia growers reduce losses. Ten cultivars were evaluated, namely round cultivars BHN602, BHN669, Crista, Florida 47, Florida 91, Phoenix, and Solar Fire, and Roma cultivars BHN685, Plum Crimson, and Picus. Commercially standardized wounding technique and newly established vacuum infiltration were both utilized to inoculate fruit with *Geotrichum candidum* spores. Initially, a concentration of 10^6 spores/mL was used, resulting in extremely high disease incidence and no significant differences among cultivars. Lower concentrations of spores were then used to reflect levels more commonly found throughout tomato production to re-assess cultivar susceptibility. Picus with 10^2 spores/mL and Plum Crimson with 10^4 spores/mL resulted in the lowest disease incidence, which were significantly less than the disease incidence of corresponding cultivar's fruit treated with higher inoculum concentration. The data do not suggest that the tested tomato cultivars exhibit any resistance to sour rot when inoculated with the method used in these tests. Cultivar selection should be based on other factors and not resistance to sour rot.

Introduction

Geotrichum candidum is the causal agent of sour rot of tomato fruit (*Solanum lycopersicum*) and other fresh produce. It is also present in the dairy industry and acts as a spoilage pathogen in produce and processed foods. Healthy tomato fruit are infected by *G. candidum* through microcracks, wounds, infiltration, and contact with decaying fruit (Bartz, 2009). This disease is a major limiting factor of tomato production in all tomato growing regions, including the Eastern Shore of Virginia (ESV), Florida and California. It can cause losses in the field, but the primary concern is during postharvest handling. *Geotrichum candidum* infections are prevalent during wet harvest conditions, abrupt drops in temperature due to rainfall, and improper post-harvest handling procedures (Bartz, 2012), although some of these relationships may be uncertain.

Tomato fruit producers rely heavily on cultural controls to decrease sour rot incidence. Improper harvest and post-harvest handling of tomato fruit can increase chance of infection due to injury. Load vibration and load compression during transportation from field to packinghouse add additional stress. Increased bruising, made worse if fruit are puffy due to poor seed-set and air in locular cavities, can increase fruit susceptibility to sour rot infections (Bartz, 2007; Bartz, 2012). It is also recommended that plant canopies and fruit must be completely dry during harvest. Once harvested, preventative steps can be employed to reduce disease pressure if *G. candidum* spores are present on the fruit surface. Proper dump tank water temperature must be monitored and maintained at 2.8-5.6°C higher than tomato pulp temperature; if not, the temperature differential causes the interior fruit tissue to shrink, creating a vacuum and pulling contaminated water into fruit (Bartz, 1988). It is also advised that dump tanks be less than 122 centimeters deep because water pressure at greater depths causes rapid infiltration of contaminated water into fruit (Bartz, 1982).

There are no in-field fungicides targeted to prevent sour rot infections and only one post-harvest fungicide is available to prevent further losses, leaving cultural controls an important step of disease management. Growers have seen less sour rot occur on Roma cultivars, leading to more Romas grown during fall harvest seasons, which is the time of year with higher sour rot incidence. The round tomato cultivar BHN 669 (BHN Seeds, Immokalee, FL) was developed for its resistance to tomato bacterial wilt (*Ralstonia solanacearum*), a common problem on the ESV. Though bacterial wilt problems are severe, growers do not prefer growing this cultivar because they believe softer fruit is produced, making them more susceptible to *G. candidum* infections.

These growing practices are based strictly on observations by growers and the purpose of these experiments is to determine if different cultivars of tomatoes are vary in susceptibility to sour rot. Because of the limited effective chemical interventions available to prevent *G. candidum* infections, selecting cultivars more tolerant to the pathogen could result in less yield losses.

Materials and Methods

Cultivar study

Ten tomato cultivars commonly grown on the ESV were examined for relative susceptibility to sour rot. Seven round and three Roma cultivars were tested (Table 5.1). Plants were grown to maturity according to standard commercial production practices (Santos et al., 2013; Virginia Extension, 2013). Seedlings were transplanted into plastic mulch with drip irrigation, then hand staked and strung. Fungicide and insecticide maintenance sprays were applied when needed to produce harvestable fruit. Field plots were arranged in a randomized block design and replicated four times. Tomato fruit were harvested at breaker stage and stored at 20º C until orange in color. Harvested fruit were cleaned according to industry practices (Bartz, 2009; Boyette, 1994); submerged in 125 ppm chlorine for 2

minutes in water at least 5ºC above internal fruit temperature, then stored at 20º C to dry. Silwet-L77 (Momentive, Columbus, OH) was applied to the stem scar and allowed to remain for 5 minutes, and then the stem scar was inoculated with 15µL of an aqueous suspension of *G. candidum* spores. The spore suspension was prepared at a concentration of 10^6 spores per milliliter and quantified using a hemocytometer (Hausser Scientific, Horsham, PA). Surfactant and spore suspension completely covered the stem scars. Fruit were put into a vacuum chamber for 2 minutes and -0.1 MPa pressure applied, and then pressure equilibrated with ambient atmospheric pressure for 1 minute. The cycle was repeated twice (Hadjok, 2008). Tomato fruit were placed into produce trays (Monte Package Company, Riverside, MI) within plastic containers (Rubbermaid, Atlanta, GA), moist paper towel inserted to retain moisture, and stored at 20º C. Control fruit were sanitized with chlorine but with no further treatments, then held at the same incubation conditions.

Thirty tomato fruit were used for each cultivar and treatment, and arranged in a randomized block design, each block in separate plastic containers. Tomato fruit were considered infected after 7 days if water soaking and velvety white growth representing *G. candidum* sporulation were visible. Common symptoms of sour rot are water soaking around lesions and velvety white growth (Bartz et al., 2007; Bartz et al., 2009). Disease incidence was recorded and the experiment repeated three years using the same cultivars, and each tomato fruit was treated as a replicate for greater degrees of freedom. Disease incidence was analyzed using logistic regression and Likelihood Ratio test (α=0.05), which was performed using JMP (SAS Corporation, Cary, NC).

Infectious dose response

The same ten cultivars previously listed were used to determine susceptibility to *G. candidum* at lower dosages. Fruit were harvested at breaker stage and stored at 20ºC until orange. Fruit were cleaned,

inoculated, and incubated as previously described. Three concentrations of inoculum were used: 10^2, 10^4, and 10^6 spores per milliliter, the highest being the standard for *G. candidum* trials on tomatoes. Spore suspension of *G. candidum* was prepared at a concentration of 10^6 spores per milliliter and quantified using a hemocytometer, and then lower doses were made through a serial dilution. Control fruit were cleaned but not inoculated. Thirty tomato fruit were used for each cultivar and inoculum level. Disease incidence was analyzed using logistic regression and Likelihood Ratio test (α=0.05), which was performed using JMP.

Results and Discussion

Inoculation of ten tomato cultivars with *G. candidum* resulted in statistically similar levels of disease incidence, ranging from 73%-100% disease incidence, except for Picus (Figure 5.1). Picus had significantly lower disease incidence. Picus is a Roma type tomato fruit, which have been assumed to have more resistance to *G. candidum* infections. It still displayed high levels of infection each year so we cannot classify it as having a high level of resistance, but instead only a slight resistance to infection.

During initial cultivar trials tomato fruit were inoculated with 10^6 spores/mL, which is the industry standard for fungicide efficacy trials. This is a high rate of inoculum that does not normally occur in field situations and high infection rates may mask possible differences in susceptibility, leading us to conduct a dose response analysis to determine cultivar susceptibility to *G. candidum* infection at lower doses. Even with lower doses of inoculum, there were no significant differences for the interaction of cultivar and inoculum level, only significant differences among infection levels and cultivar, specifically Picus (Table 5.2). There were high levels of infection, even at the lowest dose of inoculum. Due to the assumption of random distribution of spores, the lowest dose of spore suspension would

contain 1 spore or more in only 77% of all drops. This rate can change due to lack of homogeneity or clumping of spores, which would result in lower frequency of spores per drop.

Throughout this and other studies, the disease incidence of tomato fruit inoculated with *G. candidum* spores has varied. There are many predisposing factors that lead to sour rot development in tomato fruit, including microcracks, internal bruising, water content, and internal infections initiated in the field (Bartz, 2007; Bartz, 2012; Vigneault, 2000). Cultivars resulted in extremely high rates of infection, no matter the concentration of inoculum so we conclude that the cultivars tested are highly susceptible to sour rot.

Growers' assumptions that Roma cultivars (Solar Fire, Plum Crimson, BHN685) are more tolerant of *G. candidum* infection are not supported by our data. Roma cultivars developed disease at a similar rate as rounds, though Roma fruit maintained their integrity and did not sustain high levels of sporulation and deterioration until much later in disease development than round cultivars (personal observation). This may be due to the thicker pericarp and reduced water content in Roma fruit (Barrett, 1982; Grange, 1995). Visual observations suggest collecting data on lesion size and development after inoculation to describe how the rate of infection differs among tomato cultivars.

Table 5.1: Cultivars tested for susceptibility to *G. candidum* infection.

Cultivar	Type	Source
BHN 602	Round	BHN Seed, Immokalee, FL
BHN 669	Round	BHN Seed, Immokalee, FL
Crista	Round	Harris-Moran Seed Company, Modesto, CA
Florida 47	Round	Seminis Vegetable Seeds, Inc., St. Louis, MO
Florida 91	Round	Seminis Vegetable Seeds, Inc., St. Louis, MO
Phoenix	Round	Seminis Vegetable Seeds, Inc., St. Louis, MO
Solar Fire	Round	Harris-Moran Seed Company, Modesto, CA
Picus	Roma	Seminis Vegetable Seeds, Inc., St. Louis, MO
Plum Crimson	Roma	North Carolina Agricultural Research Service, Raleigh, NC
BHN 685	Roma	BHN Seed, Immokalee, FL

Figure 5.1: Average sour rot incidence over three years (2011-2013) in ten common tomato cultivars grown on the ESV inoculated with 10^6, 10^4, and 10^2 spores/ml. Picus sustained significantly less infection at $\alpha = 0.05$ using logistic regression and Likelihood Ratio tests.

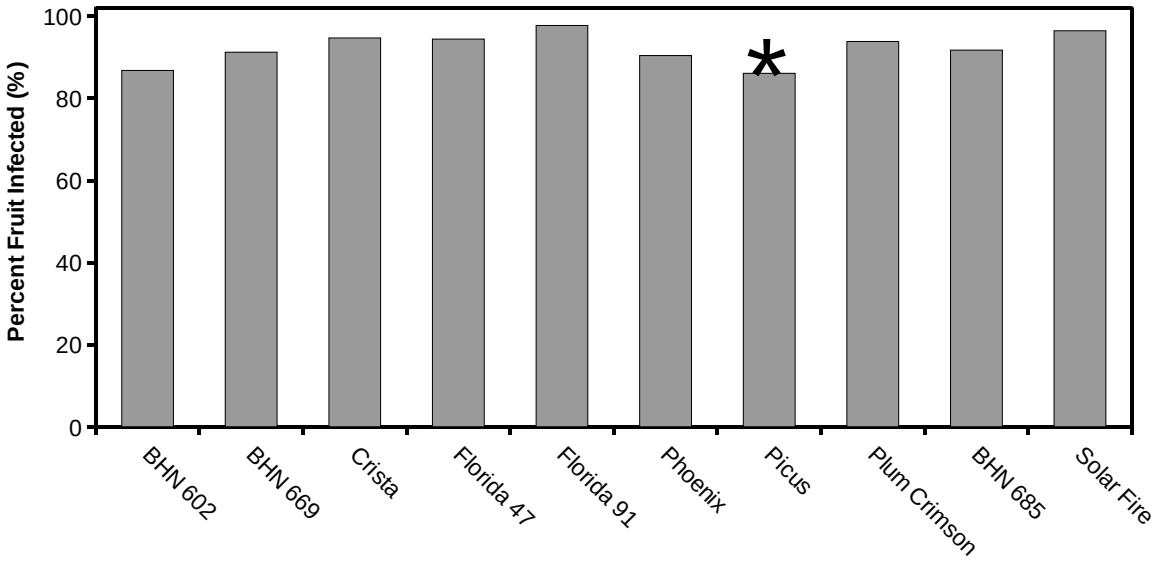

Table 5.2: Dose response of ten tomato cultivars grown on the ESV inoculated with three levels of *G. candidum*: low (10^2 spores/mL), medium (10^4 spores/mL), and high (10^6 spores/mL). Disease incidence (%) was analyzed using logistic regression and Likelihood Ratio test (α=0.05), which was performed using JMP.

	Inoculum Level			
Cultivar	**Low (10^2spores/mL)**	**Medium (10^4spores/mL)**	**High (10^6spores/mL)**	**Mean**
BHN 602	93.3	93.3	100.0	95.5
BHN 669	90.0	93.3	100.0	94.4
Crista	80.0	83.3	96.7	86.7
Florida 47	73.3	86.7	96.7	85.6
Florida 91	93.3	93.3	93.3	93.3
Phoenix	73.3	80.0	96.7	83.3
Picus	66.7	73.3	96.7	78.9
Plum Crimson	93.3	66.7	96.7	85.6
BHN 685	96.7	80.0	100.0	92.2
Solar Fire	90.0	100.0	96.7	95.6
Mean	85.0	85.0	97.4	

Source of Variation	**Chi Square**	**P > Chi Square**
Inoculum Level	812.8	< 0.0001*
Cultivar	16.9	0.0504
Inoculum Level * Cultivar	38.0	0.0784

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CHAPTER 6: INFLUENCE OF IRRIGATION AND RAIN EVENTS ON *GEOTRICHUM CANDIDUM* INFECTIONS

Abstract:

Greenhouse methods were developed to water congest tomato fruit and asses the susceptibility of water-congested fruit to *Geotrichum candidum*, causal agent of sour rot. Two studies were designed; one to test time after a rainfall fruit can be harvested without increased susceptibility, and the other to identify how varying levels of irrigation affect fruit water content and susceptibility. To simulate rainfall, plants were inundated with 5.67L water (3x normal irrigation) at the soil level for one hour. Black plastic was loosely wrapped around plants to restrict transpiration and simulate weather conditions during rainstorms. Market-ready tomato fruit were harvested 1, 4, 12, 24, and 48 hours after simulated rainfall. Half the fruit were dried in order to find total water content and the other half were inoculated with *G. candidum* spores. Fruit harvested 1 hour after inundation had significantly more water than fruit harvested after 12 and 24 hours, thought all fruit sustained 95-98% infection. The other study exposed tomato plants to three irrigation regimes (1x, 1.5x, 2x) throughout fruit development. Water content was measured for half of the market-ready fruit while the other fruit were inoculated with *G. candidum*. Irrigation levels produced significantly different water levels in fruit, though, with the vacuum-inoculation method used, susceptibility of fruit from all irrigation levels remained extremely high. Water content alone could not be assessed because other studies conducted revealed high levels of disease after inoculation with extremely low concentration of spores in suspension. If water content did play a role in tomato fruit infection rates, the data would have been clouded by the high susceptibility of tomato fruit to sour rot.

Introduction:

Geotrichum candidum is the causal agent of sour rot of tomato fruit (*Solanum lycopersicum*) and other fresh produce. It is also present in dairy industry and acts as a spoilage pathogen in produce and processed foods. *Geotrichum candidum* is a "yeast-like" fungus that grows in a mycelial form and under ideal conditions lateral branching and arthrospore formation occurs, which then break apart and act as the infectious material (Cole and Kendrick, 1969; Duran, 1972). Healthy tomato fruit are infected by *G. candidum* through microcracks, wounds, infiltration, and contact with decaying fruit (Bartz, 2009; Bartz et al., 2007). This disease is a limiting factor to tomato production on the Eastern Shore of Virginia (ESV) and other tomato production regions. It causes losses in the field, but the primary concern is during post-harvest handling.

Field observations show increased sour rot infections after rain events and other water-related environmental conditions. Extensive sour rot outbreaks in Virginia and Florida have occurred mainly late in the season and after large rainstorms (Bartz, 2006; Bartz, 2007). Fall-harvested tomato fruit on the ESV had significant sour rot infections after tropical weather systems in 2006-2009, all in September. The apparent ideal weather conditions for sour rot development include rainfall, heavy dew, fog, and abrupt temperature changes (Bartz, 1981; Bartz, 2007; Bartz et al., 2012).

Studies of citrus sour rot (*Geotrichum citri-aurantii*) found many factors that increase susceptibility, including physiological age, color changes, duration of storage, and ethylene treatments (Baudoin & Eckert, 1982). For many fungal and bacterial diseases, water uptake increases susceptibility, and fruit water loss decreases the susceptibility to infection (Johnson, 1947). Specifically in the lemon sour rot pathosystem, it was also observed that lemon fruit (*Citrus limon*) harvested after rainfall were more susceptible than those harvested during dry, sunny periods (Baudoin, 1982). Tomato fruit can also

be congested in the postharvest setting when cold water is used in dump tanks. Congestion can be measured by the weight gained by fruit after submersion. Fruit congested with at least 0.1 grams of an aqueous suspension of *Pectobacterium carotovorum* developed higher rates of infection than infected, uncongested fruit, and expressed symptoms faster than non-congested fruit (Bartz, 1982).

Scientific and anecdotal evidence suggests that heavy rainfall and high rates of irrigation may increase water in tomato fruit, making them turgid and bruise prone, thus making the tissue more susceptible to the pathogenic activity of *G. candidum* (Bartz, 2007). The objectives of this study were to test if rainfall immediately before harvest and over-irrigation of tomato plants has the ability to water congest tomato fruit, resulting in higher sour rot susceptibility. These studies were meant to develop methods to assess tomato fruit water relations and to determine the role of water during rain events without the influence of other environmental factors such as temperature, relative humidity, and air movement.

Materials and Methods

Water inundation in greenhouse

Tomato plants, cultivar BHN 602 (BHN Seed, Immokalee, FL), were greenhouse grown in 15L pots containing Pro-Mix HP planting medium (Premier Tech Horticulture, Quakertown, PA) and with a standard watering regime of 1.89 L/day for mature plants (Snyder, 2011). Plants were exposed to 12 hours of light and 12 hours of dark (ST-400-U-D, MHT Lighting, Staten Island, NY). Temperatures ranged from 18.3ºC to 26.7ºC. Plants were grown until fruit reached breaker stage and water applications were made directly before harvest. Breaker stage tomato fruit are fruit that have a definite break in color from green to tannish-yellow, pink, or red on no more than 10% of the surface (USDA, 1997). Water was applied 5.67L of water per plant (3x irrigation, common ESV rainfall) to the soil in

increments over 1 hour, with excess water escaping through drain holes. All breaker stage fruit (1-4 fruit per treatment period) were then hand harvested at 1, 4, 12, 24, and 48 hours after the simulated rain event was over. The control was harvested immediately before watering.

Tomato fruit surfaces were cleaned by wiping 70% ethanol over entire fruit, including stem scar. Half of the harvested fruit were prepared for inoculation with *G. candidum* spore suspension. Silwet-L77 (99.5% a.i., Momentive, Columbus, OH) was applied to entire stem scar (2-5 µL) and allowed to remain for 5 minutes, then an aliquot of 15 µL of *G. candidum* spore suspension (10^6 spores/mL) was inoculated onto the stem scar area. Fruit were put into a vacuum desiccator and a vacuum of -0.1 MPa was applied for 2 minutes, then pressure equilibrated with ambient atmospheric pressure for 1 minute, and this procedure was repeated twice (Hadjok, 2008). Fruit were placed into plastic produce trays (Monte Package Company, Riverside, MI) with moist paper towels, all within covered plastic containers (Rubbermaid, Atlanta, GA), and incubated at 20º C and 85% relative humidity. Number of tomato fruit displaying sour rot symptoms and signs was recorded after 7 days.

Total water content of the remaining fruit was determined. Initial fruit mass was measured by weighing, then the fruit were dried in a convection drier (Thelco Laboratory Oven, Precision Scientific) at 51.6º C for 2 days. The dry mass of each tomato fruit was used to determine total water content using the following equation:

$$[(\text{total fruit mass}) - (\text{dried fruit mass})] / (\text{total fruit mass}) = \text{water content}$$

Both disease incidence and water content data were subjected to an analysis of variance (ANOVA) and means comparison using Tukey's HSD (α=0.05), which were performed using JMP (SAS Corporation, Cary, NC).

Irrigation regime variability

Tomato plants were grown as previously described in a greenhouse setting. Three irrigation regimes, low (1x), medium (1.5x), and high (2x), were applied starting at fruit set and increasing as plant water use increased. The low watering regime resulted in no excess water run-off but still supported normal plant growth and fruit development, and the other irrigation levels were calculated based on the low volume. At full plant maturity, irrigation levels were as follows:

low (1x) = 1500 mL/day

medium (1.5 x) = 2250 mL/day

high (2x) = 3000 mL/day

Tomato fruit were harvested at breaker stage, surface cleaned with 70% ethanol, and split into two groups for further analysis. Half of harvested fruit were vacuum inoculated with *G. candidum* spores as previously described. Tomato fruit were then placed into produce trays within plastic containers, moist paper towel inserted to retain moisture, and stored at 20º C and 90% relative humidity. Sour rot symptoms and signs were observed and recorded after seven days of incubation.

The remaining half of tomato fruit were weighed then dried at 51.6º C for 2 days to find the resulting dry mass, as previously described. Total fruit water content was calculated from the initial fruit mass and resulting dry mass. Both disease incidence and water content data were analyzed using JMP, as previous described.

Results and Discussion

Tomato fruit harvested one hour after the simulated rainfall had significantly higher water content than fruit harvested 12 and 24 hours after rainfall (Table 6.1). Fruit harvested 6 hours after rainfall had intermediate water content levels that were statistically similar to all other fruits harvested

(Figure 6.1). Though there were significant differences in water content, all tomato fruit sustained 95-98% disease incidence with no significant differences.

Tomato plants grown at the lowest irrigation level (1x) produced fruit with significantly lower water content than medium (1.5x) and high (2x) rate irrigated plants (Table 6.2), and disease incidence was slightly, but statistically significantly lower as well that in fruit from medium- and high-irrigated tomato plants (Figure 6.2). In commercial tomato fields, irrigation levels are variable and rarely adjusted for environmental conditions and rainfall, leading to increased water content and fruit softness concerns. Over-irrigation of tomato plants can result in soft fruit, and it has been hypothesized that soft, watery fruit are more susceptible to postharvest infections like sour rot (Bartz, 2006; Johnson, 1947; Smith, 2006). Our data did not reveal differences in sour rot susceptibility of fruit grown on different irrigation regimes due to very high disease incidences, even at low inoculation rates (unpublished data). The low-water treatment produced very high disease incidences, it was not possible for the medium- and high-water treatment to increase disease rates. Further conclusions about irrigation's influence on susceptibility of tomato fruit cannot be made due to the consistently high rates of infection after artificial inoculation. Similar irrigation tests should be conducted if an inoculation method can be developed that results in a wider spectrum of infection rates so clear differences can be revealed.

In this study we were able to manipulate irrigation regimes to create fruit with small differences in water content. However, significant differences in fruit water content did not translate into differences in sour rot incidence. Conducting these studies in the greenhouse setting allowed us to eliminate other factors that may play a role in making tomato fruit more susceptible to disease, such as rainfall, temperature and relative humidity. It is our hypothesis that fruit water content is not the sole factor and other weather conditions such as relative humidity, temperature, and evapotranspiration are important

contributors to disease susceptibility of tomato fruit to *G. candidum*. Susceptibility may include fruit water status, but in combination with other factors.

The goal of future studies is to improve water delivery methods so there are significant differences in water content among the levels of treatments. Other measurements of water within the fruit, such as water potential and water activity, could be of great significance, though are more difficult to measure in a structure such as a tomato fruit. Future studies could include variation of nutrients and water amounts to produce tomato fruit with significantly different levels of water content. Red tomato fruit have an average water content of 94% of total mass (Pennington & Spungen, 2010), which is mostly higher than the 90.5-94.3% found in my experiments, so the goal is to have low and high irrigated tomato plants produce significantly lower and higher water percentages, respectively. Electrical conductivity (EC) assesses the quality of available nutrients in planting soil, and higher EC has been shown to increase fruit firmness, improve texture, and extend shelf life (Satti and Lopez, 1994). Adding sodium chloride to increase EC can improve tomato fruit shelf life. Another study found the effect of ammonium/nitrate ratio had an effect on the quality (including firmness) of tomato fruit (Feigin, 1980). High levels of nitrogen applied to tomato plantings produced the firmest fruit with the highest total soluble solids and dry matter content (Huett, 1989). With careful testing, application of specific nutrient levels to tomato plants could result in different levels of water content that can be used for sour rot incidence studies. Irrigation and nutrient application levels are both areas of concern for tomato growers on the ESV.

Table 6.1: Water content of tomato fruit after one hour of water application at 5.67L of water per plant (3x normal irrigation). Values with different letters are significantly different (α=0.05).

Hours After Water Application	Water Content (%)
1	91.44 a
6	91.31 ab
12	90.47 b
24	90.95 b

Figure 6.1: Rate of fruit infection harvested after a one-hour water application. All treatments had statistically similar disease incidence at 0.05 (Tukey HSD).

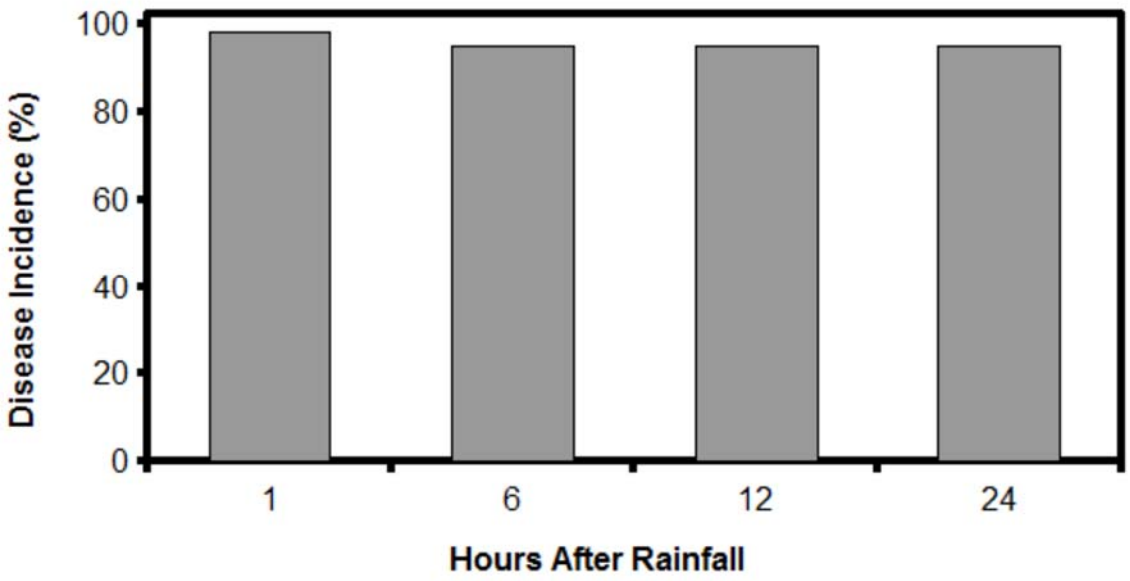
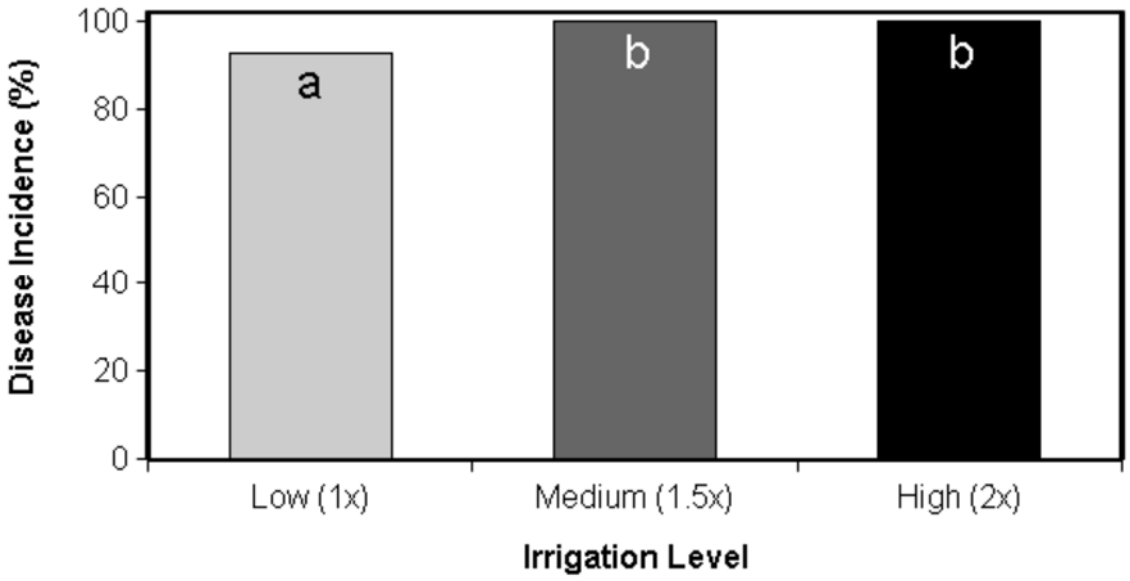


Table 6.2: Resulting water content of tomato fruit grown at three rates of irrigation. Values with different letters are significant (α=0.05).

Irrigation Level	Water Content (%)
Low (1x)	93.45 a
Medium (1.5x)	93.92 b
High (2x)	94.29 b

Figure 6.2: Rate of infection of fruit from plants grown at three rates of irrigation. Values with different letters are significantly different (α=0.05).



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