

UNDERSTANDING THE ROLE OF ENVIRONMENT AND THE USE OF FUNGICIDES  
FOR IMPROVED CONTROL OF SOUTHERN BLIGHT OF TOMATO

by

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(Under the Direction of David B. Langston, Jr.)

ABSTRACT

Southern blight (SB) caused by the fungal pathogen *Sclerotium rolfsii* is a serious soilborne disease of tomato in the southeastern United States. The use of fungicides and the most effective application methods for their delivery were investigated. Field and greenhouse experiments demonstrated the efficacy of flutolanil, penthiopyrad and fluazinam against *S. rolfsii*. At-planting drenches of fungicides were determined to be the most efficient application since subsequent in-season fungicide applications did not improve disease control in the field. Separate field studies were conducted to investigate the influence of the soil environment across three plastic mulch colors used in tomato production. Final disease incidence was affected in each mulch color (white, black, and reflective) by differences in soil temperature. Results from this study will help manage SB with the efficient use of fungicides and improve the understanding of SB epidemics in plasticulture tomatoes.

INDEX WORDS: Flutolanil, Penthiopyrad, Fluazinam, *Sclerotium rolfsii*, Southern blight, Tomato, Plastic mulch, Soil temperature, Soil moisture

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## CHAPTER 1

### INTRODUCTION AND LITERATURE REVIEW

#### **Background and Reasons for Undertaking Work**

In 1892, Peter Henry Rolfs discovered a new pathogen of tomatoes in Florida where some fields showed > 70% loss (Mullen, 2001). This new pathogen was named *Sclerotium rolfsii* by Saccardo in 1911 and has come to be known by several common names including southern wilt, southern stem rot, white mold, and Sclerotium stem rot. However, this disease is commonly known as southern blight in tomatoes. By 1944, this disease had been reported in 24 states and losses of 25-50% were common in peanut production (Mullen, 2001). It was not until the mid-1900's that growers had chemical controls for this pathogen with the introduction of pentachloronitrobenzene in the 1940's and adoption of methyl bromide (MeBr) fumigation a short time later. MeBr is a powerful biocide that has been used for the control of soilborne diseases, nematodes, and weeds. This pathogen has been adequately controlled using MeBr fumigation in plasticulture vegetable production. However, research demonstrates that MeBr is an ozone depleting substance. Therefore, its discontinuation is mandatory per the Montreal Protocol, a United Nations treaty created to phase-out the use of substances that can deplete the ozone layer. The phase out of MeBr was to be completed by 2005, but the compound is still being used through critical use exemptions (CUEs). These CUEs allow the use of MeBr in pepper, eggplant, squash, tomato, melon, and cucumber since acceptable alternatives were unavailable at the time the phaseout occurred. All CUEs will expire in 2013, and growers will be left with few chemical management alternatives that are much less effective than MeBr. Thus, it

is suspected that this disease will become much more difficult to manage. This work attempts to address the loss of MeBr through research by evaluating the efficacy of certain fungicides and their use patterns. The effect of plastic mulch color on soil temperature and moisture and how they affect the onset and severity of southern blight will also be evaluated.

## **Literature Review**

Tomato (*Solanum lycopersicum* L.) is one of the most economically important vegetable commodities in the United States. Much tomato production occurs in California, Florida, Virginia, and Georgia. Georgia tomato acreage and production value can fluctuate greatly from year to year. In 2010 the Georgia Farm Gate value and acreage for fresh market tomatoes were at a 9 year low of \$24,796,742 and 2,983, respectively (Wolfe and Luke-Morgan, 2011). An abundant production year was observed in 2003 when the Georgia Farm Gate Value and acreage were \$122,159,355 and 5,785, respectively (Boatright and McKissick, 2004). However, production value and acreage are often between these numbers.

Southern blight, caused by the soilborne fungal pathogen *Sclerotium rolfsii*, is one of the most devastating diseases of tomatoes in tropical and sub-tropical areas and in the southern and southeastern United States (McCarter, 1991). *Sclerotium rolfsii* does not produce asexual spores but overwinters in the soil as hard, tan sclerotia (Backman and Brenneman, 1997). The sexual state of *S. rolfsii* is the basidiomycete *Athelia rolfsii* (Curzi) Tu & Kimbrough, but it is rarely observed under field conditions (Backman and Brenneman, 1997). *S. rolfsii* was originally observed infecting tomato plants in a Florida field that experienced > 70% loss (Mullen, 2001).

When soil conditions are favorable, sclerotia will germinate and grow saprophytically on decaying organic matter or parasitize a host plant (Aycock, 1966). Sclerotia can survive in the soil for as long as 3 years and are affected by a number of factors including soil temperature,

moisture, burial depth, and oxygen content (Beute and Rodriguez-Kabana, 1981, Punja and Jenkins, 1984, Punja, 1985). Sclerotial survival is greatest in soils that were continuously drier, have lower soil temperatures, greater oxygen content, and when buried < 5cm deep (Beute and Rodriguez-Kabana, 1981, Punja and Jenkins, 1984).

Mycelium from germinating sclerotia can infect a host in the absence or presence of an exogenous nutrient source by two forms of germination: hyphal and eruptive (Punja and Grogan, 1979, Punja and Grogan 1981, Punja, 1985). Hyphal germination has been described as the germination of hyphae from the rind of a sclerotium with little mycelial growth unless a nutrient source is available, whereas eruptive germination occurs when aggregates of mycelium rupture the rind of the sclerotium (Punja and Grogan, 1981, Punja, 1985). Eruptive germination can be triggered by several factors including drying, plant volatile compounds, and treatment with sodium hypochlorite (Punja and Grogan, 1981).

Symptoms appear on plant parts on or in the soil on plants of any size or age. Initially a dark lesion will form at the base of the plant where it comes into contact with the soil. This infection results in girdling of the base of the tomato plant followed by wilting and yellowing of the entire plant which eventually results in plant death. When conditions are favorable, white mycelial growth and sclerotia can be observed at the soil line on infected plants (McCarter, 1991). Host tissue death results in areas of contact between host and mycelium (Aycock, 1966). Tissue death is the result of oxalic acid produced by *S. rolfsii*, which is toxic to plant tissue (Punja and Jenkins, 1984). In the host, oxalic acid binds to calcium resulting in calcium oxalate (Punja and Jenkins, 1984). The formation of calcium oxalate lowers the pH of the plant tissue and allows for cellulases and polygalacturonasaes to degrade the host tissue (Bateman, 1970, Bateman, 1972, Bateman and Beer, 1965).

The optimal environmental conditions that drive southern blight epidemics are not well understood in the plasticulture environment. Research shows that environmental parameters such as relative humidity, temperature, soil moisture and pH influence the level of disease caused by *S. rolfsii* (Punja, 1985). This pathogen can survive at temperatures ranging from 8-40°C, but its ability to infect is greatly reduced at temperatures below 20°C and above 35°C (Aycock, 1966, Mehan *et al.*, 1995). In most cropping systems, the optimal temperature for infection has been cited as 30°C (Aycock, 1966). A study conducted by Grinstein *et al.* (1979) demonstrated that mulching of the soil reduced levels of *S. rolfsii* compared with non-mulched soils. Maximum soil temperatures were 7-12 degrees warmer (45-53°C) at 5 cm depth in mulched soils (Grinstein *et al.*, 1979).

In peanut, another important host of *S. rolfsii*, the canopy microclimate influences stem rot development especially with respect to relative humidity (Rideout, 2002). Stem rot epidemics are usually associated with row closure of peanut which causes an increase in relative humidity and soil moisture in the canopy (Mehan *et al.*, 1995, Davidson *et al.*, 1991). Soil moisture plays an important role in epidemics caused by *S. rolfsii*. Much research has been conducted in peanut to investigate the relationship between soil moisture and stem rot development. In a study performed by Shew and Beute (1984) moisture level was more important than level of inoculum. Severe outbreaks of southern stem rot have been reported in peanut during long periods of moisture or rainfall (Mehan *et al.*, 1995). Additionally, disease levels were more severe following a rain event that was preceded by a dry period (Beute and Rodriguez-Kabana, 1979). Punja and Grogan (1981) propose that increased levels of stem rot observed during these drying and wetting cycles could be due to eruptive germination of sclerotia being enhanced by sclerotial drying and that disease development is favored by the following moist environment.

The effect of pH on growth of *S. rolfsii* has been well studied in the laboratory environment. Mycelial growth can occur on artificial media between pH values of 1.4 to 8.8, with an optimal pH range of 3.0 to 6.4 (Aycock, 1966, Punja, 1985, and Mehan *et al.*, 1995). Both mycelial growth and sclerotial germination are severely reduced when pH is > 7.0 (Punja and Grogan, 1982). However, stem rot can be severe in areas of Texas and Oklahoma where soil tend to be more alkaline (pH > 7.0) (Shim and Starr, 1997).

The texture and type of soil can also have a profound influence on growth and subsequent damage caused by *S. rolfsii*. Several studies report that stem rot incidence is greater in well-drained, lighter, sandier soils compared to heavier soils (Dubey, 1958, Weerapat and Schroeder, 1966, Backman and Brenneman, 1997). Shew *et al.* (1984) observed a significant positive correlation between percent sand and number of lesions present on peanut plants. This is likely due to the fact that *S. rolfsii* requires high levels of oxygen compared with some other fungi (Griffin and Nair, 1968). It is likely that sandier soils support higher oxygen concentrations at deeper depths than heavy soils, thus allowing for increased growth for *S. rolfsii* (Punja and Jenkins, 1984, Mehan *et al.*, 1995). It is also suspected that heavier soils exert more physical pressure on sclerotia, in turn reducing their ability to germinate (Punja and Jenkins, 1984).

As with all plant pathogens, *S. rolfsii* is most effectively controlled by implementing an integrated management program. Crop rotation and deep plowing are recommended cultural controls that are most effective. *Sclerotium rolfsii* can overwinter in the soil for as long as 3 years and has a wide host range of over 500 plant species in 100 plant families, making this pathogen difficult to control by crop rotation (Aycock, 1966, Punja and Jenkins, 1984). However, Brenneman *et al.* (1995) demonstrated a relationship between number of years production in a non-host crop and stem rot incidence. Bahaiagrass (*Paspalum notatum*) is an exceptionally



effective rotational crop in the Southeast for reducing levels of *S. rolfii* (Rodriguez-Kabana *et al.*, 1991a, Bowen *et al.*, 1994, Brenneman *et al.*, 1995, Timper *et al.*, 2001). Other rotational crops that fit into production practices in the southern United States effective at reducing inoculum levels include: cotton (*Gossypium hirsutum*), wheat (*Triticum* spp.), and corn (*Zea mays*) (Rodriguez-Kabana *et al.*, 1991b, Bowen *et al.*, 1994, Garren, 1961). Deep plowing is also effective in reducing inoculum by depriving *S. rolfii* of a nutrient base and burying sclerotia below infection zones (Garren, 1961, Bowen, *et al.*, 1994).

Much research has been conducted in an attempt to find effective biological controls as well as host resistance. The efficacy of both bacterial and fungal biocontrol agents for the control of *S. rolfii* have been thoroughly investigated in the past decade. Those that have been most consistent in suppressing *S. rolfii* include: *Bacillus subtilis*, *Gliocladium virens*, and *Trichoderma harzianum* (Aycock, 1966, Ristaino *et al.*, 1994, Stevens *et al.*, 2003, Curtis *et al.*, 2010).

Progress in finding and developing host resistance to *S. rolfii* has been slow due to the non-specific mode of infection observed with this pathogen (Mehan *et al.*, 1994). However, resistance has recently been bred into some peanut cultivars such as GA-07W. Resistance to southern blight was first identified in *Lycopersicon pimpinellifolium* (Mohr and Watkins, 1959). In 1992, southern blight resistance was found in six tomato breeding lines but no resistance has been found in commercially available cultivars (McCarter, 1991, Leeper *et al.*, 1992, Zitter and McGrath, 2006). Most recently, Rivard *et al.* (2010) demonstrated resistance by grafting tomato with interspecific rootstock but the mechanism of resistance is still unknown.

Damage caused by *S. rolfii* can be greatly influenced by planting date. Brenneman and Hadden (1996) found that stem rot incidence was higher in Georgia peanuts planted 21 April

than in those planted on 10 May or 20 May. In North Carolina, Gurkin and Jenkins (1985) observed higher incidence of southern blight in carrot when planted 15 February-15 March than when plots were planted on or after March 29. Epidemics of both crops are usually associated with canopy overlap which can result in a conducive environment for infection if ambient conditions are also permissive. Later plantings in both studies resulted in canopy microclimate conditions that were not optimal for disease development because ambient temperatures were outside of optimal ranges.

Due to the persistence of *S. rolfsii* chemical controls are an essential component in managing this pathogen. Both fumigants and fungicides have been readily used since the mid-1900's when they first came available (Aycock, 1966). Many fumigants have been effective in reducing southern blight incidence in tomato: metam sodium (Vapam), 1,3-dichloropropene (Telone II), methyl bromide, and chloropicrin (Jenkins and Averre, 1986). However, methyl bromide, chloropicrin, and metam sodium are the most practical and control a number of soil-borne pathogens (Mullen, 2001).

The use of fungicides to control southern blight in vegetables will likely become more prevalent as the availability of methyl bromide becomes more limited. Although other fumigants provide suppression similar to methyl bromide, they are difficult to apply, under intense environmental scrutiny, and require long pre-plant intervals. Little research has been conducted on the efficacy of fungicides to control southern blight, and even fewer compare the efficacy of fungicides to fumigants. In a recent study conducted by Langston and Sanders (2009), azoxystrobin provided a level of suppression that was comparable to fumigants demonstrating the potential for their use in controlling southern blight.

Pentachloronitrobenzene or PCNB (Blocker, Terraclor) was the first commercially available fungicide to control southern blight (Aycock, 1966). Research on the benefits of this product focused on applications prior to planting, but inconsistent results and high cost made researchers hesitant to recommend its use (Harrison, 1961, Aycock, 1966). With few alternatives, PCNB is still recommended and recent reports show efficacy in reducing disease incidence (Xie and Vallad, 2010).

Since the phase-out of methyl bromide was announced, three strobilurin fungicides have been labeled for southern blight control in tomato including: azoxystrobin (Quadris, Syngenta Crop Protection, Greensboro, NC), pyraclostrobin (Cabrio, BASF, Research Triangle Park, NC), and fluoxastrobin (Evito, Arysta LifeScience, Cary, NC). Strobilurin QoI fungicides were derived from the chemicals produced by the wood-decay mushroom *Strobilurus tenacellus* and are a single-site, multi-step inhibitor of electron transport in cytochrome bc (Anon., 1996). Pyraclostrobin and fluoxastrobin are generally recommended over azoxystrobin as they are less phytotoxic. Although fungicidal activity of strobilurins is well documented against *S. rolfsii*, the best way to apply them to tomatoes for disease control has not been extensively investigated.

Rotating fungicides with specific modes of action or tank mixing them with multi-site fungicides is an integral part of responsible fungicide resistance management. However, the most effective fungicides to control southern blight in tomato have the same specific mode of action. PCNB is an aromatic hydrocarbon with a non-specific mode of action involving lipid peroxidation. Therefore, combining the use of PCNB with the aforementioned strobilurins is an option given its non-specific mode of action. However, reduced sensitivity and tolerance to PCNB has been reported in many areas of the United States including Georgia, Oklahoma, and Texas (Damicone and Jackson, 1994. Franke *et al.*, 1998, Shim *et al.*, 1998). The fact that

tolerance has developed to PCNB's non-specific mode of action suggests that tolerance or resistance could develop to the available strobilurins (Franke *et al.*, 1998).

New chemistries of carboxamide fungicides are being investigated for southern blight control. Carboxin was one of the early carboxamide chemistries used to control *S. rolfsii* in peanuts and vegetables (Jenkins and Averre, 1986). Newer examples of carboxamide fungicides include: flutolanil, boscalid, penthiopyrad, and fluopyram. Carboxamides are generally very systemic and provide a high level of control within the fungal kingdom. Flutolanil has been used in peanut to control the basidiomycete fungal pathogens *S. rolfsii* and *Rhizoctonia solani* (Kuhn) (Csinos, 1987). However, flutolanil does not control the ascomycete leaf spot diseases in peanut (Culbreath *et al.*, 1992). Penthiopyrad is a new carboxamide fungicide with a broader spectrum of activity. It is effective in controlling both basidiomycete and ascomycete pathogens of peanut and tomato, including *S. rolfsii* (Culbreath *et al.*, 2009, Vallad, 2009). Penthiopyrad is currently registered for use in peanuts and other crops but not tomato.

Little research has been conducted in the last 50 years on southern blight of tomatoes that advances the understanding and management of this disease. The overall purpose of this research is to better understand the etiology and epidemiology of how *Sclerotium rolfsii* behaves in the plasticulture environment and how this pathogen can be more effectively managed with the use of fungicides. This research will investigate the role of certain environmental factors contributing to disease severity and onset in the southern blight pathosystem and how plastic mulch color may affect them. Fungicides will be an important component in effective southern blight management when MeBr CUEs expire. However, little information is available on how these fungicides should be applied in order to be most effective. This research will help identify additional fungicides to control *S. rolfsii* in tomato by testing them via detached plant part bioassays and

proving their efficacy in the field. An extension of this work will also focus on application methods.

Based on the above considerations, the specific objectives of this thesis were as follows:

1) Determine activity and systemicity of fungicides for control of *Sclerotium rolfsii* on tomato.

Considering the systemic distribution of fungicides is important when controlling soilborne fungal diseases, especially the basipetal distribution. Research exploring the uses of systemic fungicides has greatly improved southern stem rot control in peanut. This has not been extensively explored in tomato.

2) Determine fungicide field efficacy against southern blight using different application methods.

Often greenhouse and field efficacy of pesticides differ due to the more controlled environment in the greenhouse as opposed to the degrading environment of the field. Greenhouse studies tell us what products are promising, but their true efficacy can only be observed in the field environment. For this objective, field efficacies of the most effective fungicides from greenhouse experiments as well as some others that have shown field efficacy against *S. rolfsii* were tested using two application methods: transplant drenches followed by three subsequent applications of foliar sprays or drip-applied chemigation at 21-day intervals. These application regimes are likely to be the method by which growers will apply these products.

3) Investigate the effect of plastic mulch color on the soil conditions that influence southern blight incidence.

The soil environment plays an important role in southern blight disease onset and progress. Variables such as soil moisture and soil temperature are especially important in southern blight epidemics. In commercial tomato production, several colors of plastic mulch are

commonly used for their ability to alter the soil temperature at certain times of the year. Black plastic mulch is commonly used in the spring in order to warm the soil and promote plant growth early in the season. White plastic mulch is commonly used in the fall to avoid overheating of the soil around the tomato plants. Reflective plastic mulch has recently gained popularity as it has been effectively used to reduce feeding pressure by thrips, thus reducing *Tomato spotted wilt virus* (TSWV) incidence. It is suspected that the reflective mulch inhibits the thrips ability to locate the tomato plants. This research will investigate the interactions of soil temperature and moisture affected by plastic mulch color with southern blight onset and progress over time.

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## CHAPTER 2

### USING A DETACHED PLANT PART BIOASSAY WITH *SCLEROTIUM ROLFSII* TO EVALUATE FUNGICIDE SYSTEMICITY IN TOMATO

<sup>1</sup>Roberts, M.J., D.B. Langston, Jr., T.B. Brenneman, and P. Ji. 2012. To be submitted to *Crop Protection*.

## **Abstract**

Experiments were conducted under greenhouse conditions to evaluate acropetal and basipetal movement of several fungicides in tomato. Fluopyram (0.249 kg a.i./ha), penthiopyrad (0.234 kg a.i./ha), flutolanil (1.06 kg a.i./ha), prothioconazole (0.20 kg a.i./ha), fluazinam (0.584 kg a.i./ha), pyraclostrobin (0.224 kg a.i./ha), and pentachloronitrobenzene (1.70 kg a.i./ha) were applied 46 days after planting, either as a foliar spray or as a soil drench, at recommended rates to greenhouse grown plants. Crown, stem, branch, and leaflet tissues were excised 5 days after application and bioassayed with *Sclerotium rolfsii*. A high level of suppression was consistently observed by penthiopyrad, flutolanil, prothioconazole, fluazinam, and pyraclostrobin on crown tissues of drench-treated plants. Distribution into upper plant parts was also observed by these fungicides. However, flutolanil demonstrated a high level of acropetal mobility and was the only fungicide to consistently provide suppression on leaflet tissues. Penthiopyrad, flutolanil, prothioconazole, fluazinam, and pyraclostrobin consistently provided suppression of *S. rolfsii* on all tissue types when applied as a foliar spray. Suppression was inconsistent on all tissues with drench and foliar applications of fluopyram and pentachloronitrobenzene suggesting that these products are ineffective against *S. rolfsii*. These results demonstrate that several fungicide options exist that are effective in suppressing *S. rolfsii* on tomato applied as a drench or foliar spray.

## **Introduction**

Southern blight caused by the soilborne fungus *Sclerotium rolfsii* Sacc is one of the most damaging diseases of tomato (*Solanum lycopersicum* L.) in tropical and sub-tropical areas and in the southern and southeastern United States (McCarter, 1991). The fungus has a wide host range of over 500 plant species and produces sclerotia which can remain viable in the soil for several

years, making it persistent and thus difficult to manage (McCarter, 1991, Backman and Brenneman, 1997). Since the adoption of methyl bromide (MeBr) as a soil fumigant, this disease has been easily controlled in plasticulture tomato production. Based on MeBr's potential to deplete the ozone layer, the chemical's discontinuation is mandatory under the Montreal Protocol. All use of MeBr is to be discontinued in 2013 leaving growers with few chemical management alternatives.

The widespread use of alternative fumigants has been hindered mostly by their high cost and increasing environmental scrutiny. Several other fumigant alternatives such as Paladin (dimethyl disulfide), Telone II (1,3-dichloropropene), and chloropicrin are available. However, several factors prevent their widespread adoption. Telone II and chloropicrin are readily available but provide inadequate suppression of *S. rolfsii*. Paladin is not as readily available and its efficacy against *S. rolfsii* is unknown. In a recent study conducted by Langston and Sanders (2009), azoxystrobin applied once through the drip tape provided a level of control comparable to MeBr. These results demonstrate the potential to use this and other fungicides to control southern blight of tomatoes using conventional fungicides.

Pentachloronitrobenzene or PCNB (e.g., Blocker, Terraclor) was the first commercially available fungicide to control southern blight (Aycock, 1966). Research on the benefits of this fungicide focused on applications prior to planting, but inconsistent results and high costs made researchers hesitant to recommend its use (Harrison, 1961, Aycock, 1966). With few alternatives, PCNB is still recommended and recent reports show efficacy in reducing disease incidence (Xie and Vallad, 2010).

Since the phase-out of MeBr was announced, three strobilurin fungicides have been labeled for southern blight control in tomato including: azoxystrobin (Quadris, Syngenta Crop

Protection, Greensboro, NC), pyraclostrobin (Cabrio, BASF, Research Triangle Park, NC), and fluoxastrobin (Evito, Arysta LifeScience, Cary, NC). Since all newly available fungicides have the same mode of action, concerns about fungicide resistance have been raised. Reduced sensitivity and tolerance to PCNB has been reported in many areas of the United States including Georgia, Oklahoma, and Texas (Damicone and Jackson, 1994. Franke *et al.*, 1998, Shim *et al.*, 1998). Tolerance to PCNB indicates that strobilurins could be at high risk of resistance development in this pathogen as well (Franke *et al.*, 1998).

Two recent fungicide trials against southern blight of tomato have been particularly informative. Vallad *et al.* (2010) observed a significant reduction in final disease incidence with the use of flutolanil (Convoy, Nichino America, Wilmington, DE) compared with several biological products and PCNB. In a later trial, Vallad and Xie (2012) examined the efficacy of several biological products and fungicides. Flutolanil, fluoxastrobin, pyraclostrobin, azoxystrobin, and PCNB all significantly reduced final disease incidence compared with the check and all other treatments. More research is needed to determine the efficacy of other fungicide modes of action that are known to have activity against *S. rolfisii* compared with those currently available. In addition, information needs to be developed on how to apply them most effectively.

Raised-bed plasticulture production of vegetables is a unique system in which irrigation, fertigation, and chemigation can be utilized. Chemigation can be used to the advantage of producers to achieve better southern blight control with systemic fungicides. Fungicides can be delivered to the soil in transplant water or through drip irrigation, or they can be delivered as a foliar application with tractor mounted sprayers. Therefore the systemic movement, whether

acropetal or basipetal, should be considered when making fungicide applications for soilborne diseases such as southern blight.

Basipetal movement of fungicides, or their active derivatives, occurs in the phloem sap of the symplast sieve tubes (Brudenell *et al.*, 1995, Chollet *et al.*, 2004, Quimette and Coffey, 1990.). The fungicide must diffuse through the leaf cuticle and penetrate the xylem apoplast and symplast membrane before moving into the sieve tubes (Kleier, 1988, Kleier, *et al.*, 1998). However, most fungicides are not transported this way. Most systemic fungicides are transported upward in the plant through the xylem apoplast. The ability of fungicides to penetrate into the xylem apoplast is related to their lipophilicity. Fungicide lipophilicity is defined as the partition coefficient between a plant membrane and an aqueous environment and can be measured by determining its octanol-water coefficient ( $K_{ow}$ ). Lipophilic fungicides generally have  $\log K_{ow}$  values of 4 to 6.5. Whereas a hydrophilic or low lipophilic fungicide may have  $\log K_{ow}$  values of -7.5 to  $< 2$  (Edington, 1981, Wright *et al.*, 1994, Augusto and Brenneman, 2012).

Once a fungicide has reached the xylem apoplast, it can then be transported into the phloem by penetrating the sieve tubes. Concentration of fungicide in the phloem can fluctuate as it moves between the symplast membranes and xylem apoplast (Kleier, 1988). Fungicides with the least efflux back into the xylem apoplast will move longer distances in the phloem. While lipophilicity determines the extent of a pesticide's apoplastic systemicity, basipetal systemicity is related to the acid strength of the compound especially when considering long distance movement in the plant (Bromilow *et al.*, 1990, Devine, 1989, Grimm *et al.*, 1995, Neumann *et al.*, 1985, Rigitano *et al.*, 1987, Augusto and Brenneman, 2012). Acid strength is measured as the negative logarithm,  $pK_a$ , of acid dissociation constant  $K_a$  (Rigitano, *et al.*, 1987). The xylem

apoplast is weakly acidic to neutral in pH (approximately 6) while the phloem symplast is slightly alkaline (approximately 8) (Bromilow *et al.*, 1990, Grayson and Kleier, 1990).

Acidic fungicides that are able to penetrate into the sieve tubes will be dissociated and unable to move back into the xylem apoplast allowing them to be highly phloem mobile (Neumann *et al.*, 1985). However, this dissociation could decrease the biological activity against the intended fungal pathogen. Most fungicides are neither acidic nor basic and do not always exhibit the expected systemic properties. For example, fosetyl-Al, a weakly acidic and low lipophilic phosphonate fungicide ( $pK_a = 4.7$  and  $\log K_{ow} = -2.1$ ), is phloem mobile (Augusto and Brenneman, 2012, Brudenell *et al.*, 1995, Quimette and Coffey, 1990).

The objective of this study was to determine movement and activity of several fungicides applied as foliar sprays or soil drenches to tomatoes. All studies were conducted on plants grown in the greenhouse. Fungicide activity was quantified using a bioassay technique with *S. rolfsii*. Physical properties of fungicides will also be discussed to explain acropetal or basipetal distribution of some fungicides based on previous systemicity studies (Augusto and Brenneman, 2012).

## **Materials and Methods**

*Plant preparation.* Trials were conducted in a greenhouse at the University of Georgia Coastal Plain Experiment Station, Tifton, in 2011 and 2012. Seedling tomato plants of the cultivar Bella Rosa were transplanted into 3.79 L plastic pots filled with a 4:1:1 sand: vermiculite: peat potting media. Experiments were conducted separately by application method. Plants receiving soil drench applications were transplanted on a separate date from those receiving foliar fungicide applications due to limited bioassay facility. Separate transplanting dates allowed that all plants were the same number of days after planting (DAP) when fungicides



were applied and ensured sufficient space to conduct the bioassay. Five plants were used per treatment with each plant being one replication in each experiment.

*Fungicide applications.* Fungicide applications were made separately as either soil drenches or foliar sprays to determine retention and systemic distribution in the plant by application method. All fungicides were applied at field rates based on 935.4 L/ha application volume at 46DAP. Soil drenches were applied to each plant as 150 mL of fungicide suspension. Plants receiving foliar applications were removed from the greenhouse and arranged in a row to receive fungicide treatment and returned to the greenhouse after the foliage was dry. A CO<sub>2</sub> backpack sprayer calibrated to deliver 467.7 L/ha with three 8006 tips (TeeJet, Springfield, IL) spaced 0.48 m apart was used to direct foliar application to both sides of the tomato plants. Fluopyram (Luna Privilege S, Bayer CropScience, Research Triangle Park, NC) was applied at 0.250 kg a.i./ha, penthiopyrad (Fontelis SC, E.I. du Pont de Nemours, Wilmington, DE) was applied at 0.235 kg a.i./ha, flutolanil (Convoy SC, Nichino America, Wilmington, DE) was applied at 1.065 kg a.i./ha, prothioconazole (Proline 480 SC, Bayer CropScience) was applied at 0.200 kg a.i./ha, fluazinam (Omega 500 F, Syngenta Crop Protection, Greensboro, NC) was applied at 0.583 kg a.i./ha, pyraclostrobin (Cabrio EG, BASF, Research Triangle Park, NC) was applied at 0.225 kg a.i./ha, and pentachloronitrobenzene (Blocker 4 F, AMVAC, Los Angeles, CA) was applied at 4.203 kg a.i./ha. Physical properties of all fungicides are displayed in table 2.1.

*Detached tissue sampling and bioassay.* Systemic activity was determined using a bioassay technique 5 days after application. Four plant parts were selected per plant: a lower stem section at the crown, an upper stem section adjacent to the lower stem section, a branch at mid-canopy extending approximately 10 cm from the petiole, and the terminal leaflet from the

previously selected branch. Remaining leaflets on the branch were removed before the bioassay was performed. Excised plant tissues were placed in plastic freezer bags for transport back to the laboratory.

Plastic containers (59cm L X 43cm W X 15cm H) layered at the bottom with moist paper towels were used as a humidity chamber. Plant tissues were raised above this surface by a plastic diffuser grid. The diffuser grid was covered by a mesh screen to stabilize tissues and improve inoculation efficiency. Two-day-old *S. rolfsii* cultures used for inoculations were incubated on potato dextrose agar at 30°C and grown in the dark. A randomized complete block design was used where each humidity chamber was one replication for a total of five replications per treatment. All plant parts were inoculated with an 8-mm diameter agar plug from the margin of an actively growing culture of *S. rolfsii*. Crown, stem, and branch sections were placed on top of the agar plug with the mycelial side up while the leaflets were inoculated by placing the mycelial side of the agar plug down on the leaf surface. Containers were placed in a large incubator at 30°C for 48 hours. After incubation, lateral lesion measurements extending from the edge of the agar plug to the end of the lesion were recorded with a digital caliper and used to compare fungicidal activity. A total of four experiments were conducted with two isolates: Sr-1 obtained from tomato in Decatur County, GA and Sr-2 obtained from tomato in Tift County, GA. Each experiment was repeated with the same isolate.

*Statistical Analyses.* Lesion measurements were subjected to analysis of variance using the statistical package in ARM software (Gylling Data Management, Brookings, SD). Means were compared using Fisher's protected LSD to identify significant ( $P \leq 0.05$ ) differences among treatments.

## Results

The statistical analysis of all dependent variables indicated a significant experiment-treatment interaction for experiments conducted with isolate Sr-1, whereas no significant experiment-treatment interaction was observed for experiments conducted with isolate Sr-2. Therefore, data are not combined for any of these experiments. Data are displayed separately in four tables that are grouped by isolate and application method.

*Bioassay with Sr-1 of drench applications.* Colonization was significantly reduced at the crown by all fungicides tested with the exception of fluopyram and PCNB (Table 2.2). Fluopyram did not significantly reduce colonization of any tissue in either trial. PCNB significantly reduced colonization at the crown compared with the non-treated control in 2011. When the trial was repeated in 2012, a significant increase in colonization was observed on crown tissues of plants treated with PCNB. Penthiopyrad, flutolanil, prothioconazole, fluazinam, and pyraclostrobin provided a high level of suppression on crown tissues in both trials. These fungicides also displayed acropetal distribution by providing suppression at tissues higher on the plant. However, flutolanil was the only fungicide tested that provided a significant reduction in colonization at the leaflet in both trials. No infection was detected on crown and branch tissues of plants treated with flutolanil in either trial (Table 2.2).

*Bioassay with Sr-2 of drench applications.* Penthiopyrad, flutolanil, prothioconazole, fluazinam, and pyraclostrobin significantly reduced colonization on crown tissues in trial A (Table 2.3). In trial B, penthiopyrad and flutolanil were the only two treatments to significantly reduce colonization on crown tissue. Additionally, penthiopyrad and flutolanil were the only two treatments in these trials with significant acropetal distribution. Penthiopyrad significantly reduce colonization of *S. rolfsii* on branch tissue in trial B; however, flutolanil was the only

fungicide in both trials with significant acropetal activity in stem, branch, and leaflet tissues. No activity was observed with fluopyram or PCNB in either trial (Table 2.3).

*Bioassay with Sr-1 of spray applications.* All fungicides tested significantly reduced colonization of *S. rolfsii* at the leaflet in both trials with the exception of fluopyram and PCNB (Table 2.4). Applications of PCNB failed to reduce colonization of *S. rolfsii* at all tissue types in both trials. Significant colonization reductions on fluopyram treated plants were only observed in 2011 at leaflet and branch tissues. The highest levels of suppression in both trials were achieved with applications of penthiopyrad, flutolanil, prothioconazole, fluazinam, and pyraclostrobin on the leaflet. In the trial conducted in 2011, a significant reduction in colonization was also observed on branch, stem, and crown tissues with applications of flutolanil, prothioconazole, fluazinam, and pyraclostrobin. However, penthiopyrad did not provide a significant level of suppression at the crown tissue compared with non-treated plants. When the trial was repeated in 2012, significant suppression was observed with penthiopyrad, flutolanil, prothioconazole, fluazinam, and pyraclostrobin only on leaflet and branch tissues. Flutolanil provided complete suppression on stem tissues (Table 2.4).

*Bioassay with Sr-2 of spray applications.* Penthiopyrad, flutolanil, fluazinam, and pyraclostrobin significantly reduced colonization by *S. rolfsii* on leaflet tissues in both trials (Table 2.5). Significant activity of these fungicides was also observed on branch, stem, and crown tissues in both trials. Inconsistent results were observed with applications of fluopyram and prothioconazole. Fluopyram provided a low level of suppression on crown tissues in trial B. Prothioconazole significantly reduced colonization on all tissues in trial A. In trial B, however, a significant reduction in colonization by prothioconazole was only observed on branch and crown tissues. No significant activity was observed with PCNB in either trial (Table 2.5).

## Discussion

Penthiopyrad, flutolanil, prothioconazole, fluazinam, and pyraclostrobin provided suppression at the crown when applied as soil drench applications. This is most likely because the crown tissue is closest in proximity to the point of application. Thus, a higher concentration of fungicide in the plant can be found there. High lipophilic compounds have log  $K_{ow}$  values between 4 and 6.5 and are able to rapidly penetrate the plant cuticle (Edgington, 1981, Hsu *et al.*, 1995, Wright *et al.*, 1994). This is consistent with these results since all of these fungicides are within or just outside this range. Significant activity at the leaflet was only observed when flutolanil was applied. This is likely because flutolanil is lipophilic and nonionizable, therefore it is able to travel long distances in the slightly acidic xylem without being subject to dissociation.

Significant reductions in colonization were observed on crown tissue when penthiopyrad, flutolanil, prothioconazole, fluazinam, and pyraclostrobin were applied as a foliar spray. This could be attributed to phloem mobility, especially for prothioconazole. The optimum phloem mobility for weakly acidic compounds occurs with  $pK_a$  values of 2 to 6.5 and log  $K_{ow}$  between 1 and 3 (Brudenell *et al.*, 1995, Smith *et al.*, 1995, Wright *et al.*, 1994). Prothioconazole is a weakly acidic compound ( $pK_a = 6.9$ ) with log  $K_{ow}$  values in the cited optimal range. It is also likely that since all aerial parts of the plant were treated, fungicide also contacted stems and crown tissues and was distributed locally. Some fungicide would have also contacted the soil. Although care was taken to avoid washing fungicide from foliage to the soil, any fungicide that contacted the soil could have been washed further into the soil and taken up by the plant.

Based on the results from these experiments, penthiopyrad, flutolanil, prothioconazole, fluazinam, and pyraclostrobin will likely provide suppression of southern blight in the field. Suppression at the infection court was achieved with these fungicides regardless of application

method for most experiments though some results were inconsistent. Although not statistically compared, numerically greater inhibition was observed on crown tissues of fungicide drenched plants compared with sprayed plants. It is reasonable to speculate that more fungicide active ingredient would be present both in the crown tissue of drenched plants as well as in the soil to be taken up by the plant later. This could result in a higher level of disease control in the field and a longer protection interval. However, care should be taken when drawing these conclusions as further studies into protection interval and field efficacy of these fungicides should be conducted.

If soil drench applications are in fact more effective than foliar sprays in controlling southern blight, a problem becomes evident. Realistically, drench applications are only feasible once in a growing season, at transplanting. It is more efficient to make drench applications at this time because growers are already doing it. Subsequent applications, if necessary, can be made as foliar sprays or applied through the drip tape. Chemigation through the drip tape is a cost efficient means of delivering fungicide compared to foliar sprays since no tractor is needed. Conversely, foliar applications may be a preferred method of delivery for these fungicides in order to achieve better control of foliar fungal pathogens while still controlling southern blight. Penthiopyrad and pyraclostrobin, two effective fungicides in this study, have been used alone and in combination with contact fungicides to control target spot caused by *Corynespora casiiicola* and early blight caused by *Alternaria solani* (McGovern *et al.*, 2000, Miller and Mera, 2012, Pernezny *et al.*, 2006, Vallad, 2011a). However, resistance/reduced sensitivity has been reported in isolates of both pathogens to carboxamide and strobilurin chemistries (Vallad, 2011b).

This research demonstrates that several fungicides can be used to control southern blight of tomato. Penthiopyrad, flutolanil, and fluazinam consistently provided the highest level of suppression of *S. rolfisii* on crown tissues regardless of application method. Future studies should focus on the use these and other fungicides in the field with clear distinctions between application methods. At transplanting drenches will likely be very effective as demonstrated in this work. However, the method of delivery for subsequent applications and timing may affect disease control. Flutolanil was highly mobile in this study when applied as a soil drench which could imply activity against foliar fungal pathogens. Information on its efficacy against foliar fungal pathogens would be useful in formulating fungicide recommendations for tomatoes in the future. It was difficult to discern from this study whether significant basipetal distribution was due to phloem mobility. From a practical standpoint it may be unimportant; however, a more detailed study would be informative since results with foliar applications were inconsistent.

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**Table 2.1.** Physical properties of seven fungicides used in this study.

<b>Fungicide</b>	<b>Lipophilicity (logK<sub>ow</sub>)<sup>x</sup></b>	<b>Acidity(pK<sub>a</sub>)<sup>y</sup></b>	<b>Reference</b>
Fluopyram	3.3	0.5	Cheng, 2011
Penthiopyrad	4.36 (pH 4), 4.62 (pH 9), 4.54 (pH 10)	10	Dotson, 2011
Prothioconazole	4.16 (pH 4), 3.82 (pH 7), 2.0 (pH 9)	6.9	O’Keefe <i>et al.</i> , 2007
Fluazinam	4.03	7.22	Bailey <i>et al.</i> , 2007
Pyraclostrobin	3.99 <sup>z</sup>	N/A	Tomlin, 2004
Pentachloronitrobenzene	4.22 <sup>z</sup>	N/A	Hansch and Hoekman, 1995
Flutolanil	3.7 <sup>z</sup>	N/A	Ottley <i>et al.</i> , 2008

<sup>x</sup> Log K<sub>ow</sub> is the logarithm of concentration of a fungicide added to the octanol-water system partitioning into the octanol phase relative to the water phase.

<sup>y</sup> pK<sub>a</sub> is the acid strength expressed as the negative logarithm of the respective acid dissociation constant, K<sub>a</sub>. N/A = not applicable. Pyraclostrobin, pentachloronitrobenzene, and flutolanil have neither acidic nor basic properties.

<sup>z</sup> Log K<sub>ow</sub> values of pyraclostrobin, pentachloronitrobenzene, and flutolanil are independent of pH.

**Table 2.2.** Colonization of tomato tissue by *Sclerotium rolfsii* for seven fungicide drenches following inoculation with isolate Sr-1 in 2 years.

Year	Isolate	Treatment <sup>X</sup>	Lesion length (mm)							
			Crown		Stem		Branch		Leaflet	
2011	Sr-1	Fluopyram	7.36	ab <sup>Z</sup>	12.81	a	13.39	a	8.52	a
		Penthiopyrad	1.51	c	7.36	bc	4.50	cd	4.60	bc
		Flutolanil	0.00	c	0.22	d	0.00	d	1.50	c
		Prothioconazole	3.40	bc	9.73	abc	10.52	ab	10.12	a
		Fluazinam	0.00	c	4.87	cd	6.65	bc	8.88	a
		Pyraclostrobin	1.78	c	8.95	abc	10.54	ab	7.84	ab
		PCNB	4.41	bc	10.97	ab	13.49	a	10.21	a
		Nontreated	11.11	a	12.05	ab	14.99	a	8.17	ab
2012	Sr-1	Fluopyram	3.05	bc	4.88	ab	5.90	bc	5.57	ab
		Penthiopyrad	0.32	d	1.04	c	0.64	d	4.15	ab
		Flutolanil	0.00	d	0.33	c	0.00	d	0.33	c
		Prothioconazole	0.80	cd	2.49	bc	7.44	ab	6.15	a
		Fluazinam	0.68	d	2.67	bc	5.46	bc	2.86	bc
		Pyraclostrobin	0.75	d	2.65	bc	4.26	c	6.31	a
		PCNB	6.57	a	7.46	a	10.26	a	6.95	a
		Nontreated	3.69	b	7.17	a	9.40	a	6.31	a

<sup>X</sup> Treatments and fungicide rates were as follows: fluopyram (0.250 kg ai/ha), penthiopyrad (0.235 kg ai/ha), flutolanil (1.065 kg ai/ha), prothioconazole (0.200 kg ai/ha), fluazinam (0.583 kg ai/ha), pyraclostrobin (0.225 kg ai/ha), and PCNB (4.203 kg ai/ha).

<sup>Z</sup> Data are not combined between years because of significant treatment-year interactions. Means are compared within columns and trial. Means with the same letter(s) do not differ significantly according to Fisher's protected LSD ( $P \leq 0.05$ ).

**Table 2.3.** Colonization of tomato tissue by *Sclerotium rolfsii* for seven fungicide drenches following inoculation with isolate Sr-2 in two trials.

Year	Trial	Isolate	Treatment <sup>X</sup>	Lesion length (mm)							
				Crown		Stem		Branch		Leaflet	
2012	A	Sr-2	Fluopyram	12.11	a <sup>Z</sup>	18.54	a	19.14	a	14.10	ab
			Penthiopyrad	5.22	c	10.78	b	14.27	b	11.85	ab
			Flutolanil	2.51	d	1.56	c	0.24	c	6.32	c
			Prothioconazole	8.12	b	15.96	ab	15.21	ab	10.38	bc
			Fluazinam	8.79	b	13.73	ab	14.85	ab	11.97	ab
			Pyraclostrobin	9.28	b	12.76	ab	16.49	ab	15.81	a
			PCNB	10.52	ab	15.20	ab	16.45	ab	11.93	ab
			Nontreated	12.50	a	12.30	b	18.50	ab	12.94	ab
2012	B	Sr-2	Fluopyram	14.96	a	11.97	a	20.43	a	11.60	a
			Penthiopyrad	6.21	cd	6.37	b	10.31	c	13.87	a
			Flutolanil	2.31	d	1.17	c	0.53	d	2.99	b
			Prothioconazole	10.79	abc	14.41	a	15.29	b	10.33	a
			Fluazinam	9.22	bc	14.29	a	17.19	ab	10.55	a
			Pyraclostrobin	9.44	bc	11.40	ab	14.91	b	12.34	a
			PCNB	11.62	ab	10.10	ab	17.00	ab	12.88	a
			Nontreated	12.84	ab	10.35	ab	16.96	ab	12.99	a

<sup>X</sup> Treatments and fungicide rates were as follows: fluopyram (0.250 kg ai/ha), penthiopyrad (0.235 kg ai/ha), flutolanil (1.065 kg ai/ha), prothioconazole (0.200 kg ai/ha), fluazinam (0.583 kg ai/ha), pyraclostrobin (0.225 kg ai/ha), and PCNB (4.203 kg ai/ha).

<sup>Z</sup> Data are not combined between trials because of significant treatment-trial interactions. Means are compared within columns and trial. Means with the same letter(s) do not differ significantly according to Fisher's protected LSD ( $P \leq 0.05$ ).

**Table 2.4.** Colonization of tomato tissue by *Sclerotium rolfsii* for seven fungicide sprays following inoculation with isolate Sr-1 in 2 years.

Year	Isolate	Treatment <sup>X</sup>	Lesion length (mm)							
			Crown		Stem		Branch		Leaflet	
2011	Sr-1	Fluopyram	27.85	bc <sup>Z</sup>	26.51	bc	22.44	b	16.99	b
		Penthiopyrad	27.37	bc	11.62	def	8.18	de	6.41	cd
		Flutolanil	7.12	e	5.78	f	0.00	e	1.85	d
		Prothioconazole	23.92	c	21.06	cd	21.75	bc	16.18	b
		Fluazinam	9.79	de	8.11	ef	12.97	cd	2.02	d
		Pyraclostrobin	18.44	cd	18.19	cde	10.16	d	12.51	bc
		PCNB	45.88	a	38.10	a	36.75	a	28.53	a
		Nontreated	36.38	ab	36.56	ab	34.79	a	32.91	a
2012	Sr-1	Fluopyram	4.51	a	7.12	a	9.84	a	4.95	ab
		Penthiopyrad	3.75	a	3.48	bc	1.70	d	0.47	c
		Flutolanil	1.70	a	0.00	d	0.35	d	0.21	c
		Prothioconazole	2.71	a	5.25	ab	6.31	bc	3.48	b
		Fluazinam	2.94	a	1.51	cd	1.36	d	0.95	c
		Pyraclostrobin	2.11	a	2.85	bcd	4.38	c	0.60	c
		PCNB	4.00	a	5.35	ab	8.19	ab	6.32	a
		Nontreated	4.04	a	4.39	abc	8.90	a	6.50	a

<sup>X</sup> Treatments and fungicide rates were as follows: fluopyram (0.250 kg ai/ha), penthiopyrad (0.235 kg ai/ha), flutolanil (1.065 kg ai/ha), prothioconazole (0.200 kg ai/ha), fluazinam (0.583 kg ai/ha), pyraclostrobin (0.225 kg ai/ha), and PCNB (4.203 kg ai/ha).

<sup>Z</sup> Data are not combined between years because of significant treatment-year interactions. Means are compared within columns and trial. Means with the same letter(s) do not differ significantly according to Fisher's protected LSD ( $P \leq 0.05$ ).

**Table 2.5.** Colonization of tomato tissue by *Sclerotium rolfsii* for seven fungicide sprays following inoculation with isolate Sr-2 in two trials.

Year	Trial	Isolate	Treatment <sup>x</sup>	Lesion length (mm)							
				Crown	Stem	Branch	Leaflet				
2012	A	Sr-2	Fluopyram	16.14	a <sup>z</sup>	16.88	a	21.50	a	11.69	a
			Penthiopyrad	6.38	c	5.48	b	7.21	d	2.82	bc
			Flutolanil	0.69	d	2.98	b	0.00	e	0.00	c
			Prothioconazole	5.84	c	7.29	b	10.78	cd	5.61	b
			Fluazinam	3.27	cd	3.20	b	2.77	e	1.74	c
			Pyraclostrobin	4.59	c	3.42	b	7.55	d	6.13	b
			PCNB	11.02	b	15.33	a	13.77	bc	12.68	a
			Nontreated	11.54	b	18.55	a	17.10	b	13.91	a
2012	B	Sr-2	Fluopyram	9.02	b	11.73	ab	13.12	bc	12.2	a
			Penthiopyrad	7.25	b	6.69	cd	9.93	d	6.46	bc
			Flutolanil	0.33	e	1.29	e	0.18	f	1.27	d
			Prothioconazole	7.00	bc	10.20	bc	10.91	cd	9.46	ab
			Fluazinam	3.84	d	5.03	de	3.59	e	3.33	cd
			Pyraclostrobin	4.03	cd	5.13	de	8.95	d	5.22	c
			PCNB	14.99	a	14.66	a	19.65	a	10.21	a
			Nontreated	12.16	a	12.97	ab	14.29	b	12.38	a

<sup>x</sup> Treatments and fungicide rates were as follows: fluopyram (0.250 kg ai/ha), penthiopyrad (0.235 kg ai/ha), flutolanil (1.065 kg ai/ha), prothioconazole (0.200 kg ai/ha), fluazinam (0.583 kg ai/ha), pyraclostrobin (0.225 kg ai/ha), and PCNB (4.203 kg ai/ha).

<sup>z</sup> Data are not combined between trials because of significant treatment-trial interactions. Means are compared within columns and trial. Means with the same letter(s) do not differ significantly according to Fisher's protected LSD ( $P \leq 0.05$ ).

CHAPTER 3

INVESTIGATING THE USE OF FUNGICIDES TO IMPROVE SOUTHERN BLIGHT  
MANAGEMENT IN PLASTICULTURE TOMATOES WITH EMPHASIS ON  
APPLICATION METHOD

<sup>1</sup>Roberts, M.J., D.B. Langston, Jr., T.B. Brenneman, and P. Ji. To be submitted to *Crop Protection*.



## Abstract

Tomato (*Solanum lycopersicum* L.) is an economically important vegetable crop in the United States, especially in the Southeast. The subtropical climate and sandy soils of the Coastal Plain of Georgia make for ideal conditions for damaging epidemics of southern blight caused by *Sclerotium rolfsii* Sacc. Southern blight epidemics have become more common as methyl bromide use has been reduced. The objective of this research was to determine the field efficacy of several fungicides that have been effective in controlling this disease in other crops and how they can be applied for southern blight management. Two field trials were conducted in the spring and fall of 2011 and one in the spring of 2012. Flutolanil (1.065 kg ai/ha), penthiopyrad (0.351 kg ai/ha), difenoconazole (0.128 kg ai/ha), azoxystrobin (0.109 kg ai/ha), fluazinam (0.560 kg ai/ha), and fludioxonil (0.280 kg ai/ha) were applied as at-planting drenches followed by three applications made either by drip chemigation or as foliar sprays in 21-day intervals. Drench-only treatments were added in the spring of 2012 to discern their effect on final disease incidence. Interestingly, drench-only treatments at planting provided the same level of disease control as the other application methods. Yield data was only obtained in the spring of 2012. Yields were not statistically different between fungicide-treated and untreated plots. However, statistically significant ( $P \leq 0.05$ ) differences were observed between application methods of individual fungicides. Yields in plots treated with flutolanil drenches and sprays were significantly reduced compared with plots receiving drenches and drip chemigation. Additionally, yields in fludioxonil-treated plots were significantly higher when the fungicide was applied drench only as opposed to drenches and sprays. These results demonstrate the efficacy of fungicides in controlling southern blight.

## Introduction

Tomato (*Solanum lycopersicum* L.) is an economically important vegetable crop especially in the tropical and subtropical areas of the southeastern United States. Much tomato production occurs in California, Florida, Virginia, and Georgia. The climatic conditions combined with the sandy soils of the Coastal Plain in Georgia create an optimal environment for southern blight epidemics, caused by the soilborne fungus *Sclerotium rolfsii* Sacc. This disease has plagued producers of many crops since its discovery in 1892. *Sclerotium rolfsii* has a host range of over 500 plant species in over 100 plant families making the management of this pathogen by rotation difficult (Aycock, 1966).

Chemical management of *S. rolfsii* is essential in order to reduce losses in areas infested with the pathogen. In recent years, methyl bromide (MeBr) fumigation and raised-bed plasticulture have been the principal way that tomato growers have managed this pathogen. MeBr is a powerful biocide that has been used for the control of soilborne diseases, nematodes, and weeds. MeBr has come under intense environmental scrutiny because it is an ozone depleting substance. MeBr is scheduled to be completely phased out in the year 2013.

It is expected that fungicides will be relied upon more heavily to manage southern blight in crops where MeBr was once the principal chemical management tactic. However, little research is available on the efficacy of fungicides to control southern blight in tomato. Pentachloronitrobenzene or PCNB (Blocker, Terraclor) was the first commercially available fungicide to control southern blight (Aycock, 1966). Research on the benefits of this product focused on applications at or prior to planting, but inconsistent results and high cost made researchers hesitant to recommend its use (Harrison, 1961, Aycock, 1966). With few alternatives, PCNB is still recommended and recent reports demonstrate its efficacy in reducing

disease incidence (Xie and Vallad, 2010). However, reduced sensitivity and tolerance to PCNB has been reported in many areas of the United States including Georgia, Oklahoma, and Texas (Damicone and Jackson, 1994. Franke *et al.*, 1998, Shim *et al.*, 1998). Since tolerance has developed to PCNB's non-specific mode of action, it is possible that tolerance or resistance could develop to the available strobilurins (Franke *et al.*, 1998).

Since the phase-out of methyl bromide was announced, three strobilurin fungicides have been labeled for southern blight control in tomato: azoxystrobin (Quadris, Syngenta Crop Protection, Greensboro, NC), pyraclostrobin (Cabrio, BASF, Research Triangle Park, NC), and fluoxastrobin (Evito, Arysta LifeScience North America, Cary, NC). Strobilurin QoI fungicides were derived from the chemicals produced by the wood-decay mushroom *Strobilurus tenacellus* and are a single-site, multi-step inhibitor of electron transport in the cytochrome bc complex (Anon., 1996). Pyraclostrobin and fluoxastrobin are generally recommended over azoxystrobin as they are less phytotoxic. In a recent study conducted by Langston and Sanders (2009), azoxystrobin provided a level of suppression comparable to MeBr.

New carboxamide fungicides are being investigated for southern blight control. These fungicides are succinate dehydrogenase inhibitors inhibiting succinate dehydrogenase at complex II (FRAC, 2011). Carboxin was one of the early carboxamide chemistries used to control *S. rolf sii* in peanuts and vegetables (Jenkins and Averre, 1986). Newer examples of carboxamide fungicides included in this study are flutolanil and penthiopyrad. Carboxamides are generally very systemic and provide a high level of activity on peanut against fungal pathogens (Culbreath *et al.*, 2009). Flutolanil has been used in peanut for many years to control the basidiomycete fungal pathogens *S. rolf sii* and *Rhizoctonia solani* (Kuhn) (Csinos, 1987). Flutolanil has also demonstrated potential for use in tomato to control *S. rolf sii* (Vallad *et al.* 2010, Vallad and Xie,

2012). Penthiopyrad has been shown to be effective in controlling both basidiomycete and ascomycete pathogens of peanut and tomato, including *S. rolfsii* (Culbreath *et al.*, 2009, Vallad, 2009). Flutolanil and penthiopyrad are currently registered for use in peanuts and other crops, but not in tomato.

Although fungicidal activity against *S. rolfsii* is well documented across multiple fungicide classes, the best way to apply them to tomatoes for disease control has not been extensively investigated. The raised-bed plasticulture system of vegetable production is a unique system, especially with respect to delivering pesticides. This can be used to the advantage of producers to achieve better southern blight control with systemic fungicides. Fungicides can be delivered to the soil in transplant water or through drip irrigation, or they can be delivered as foliar applications with tractor-mounted sprayers. Preliminary studies on application methods in the greenhouse demonstrate the efficacy of soil and potential for foliar fungicide applications in tomato (Chapter 2).

The purpose of this research was to determine the field efficacy of a range of fungicides in the raised-bed plasticulture system of tomato that have demonstrated efficacy against *S. rolfsii*. Three delivery methods were investigated to determine the most effective way of applying these fungicides. The data provided is intended supply information on fungicide efficacy by application method to advance the understanding of how to most effectively manage southern blight in tomato with the use of fungicides.

## **Materials and Methods**

*Inoculum preparation.* Inoculum for field trials was grown on oat media. Media was mixed by combining 500 mL of oat seed (with husk) and 500 mL of tap water in 2 L, polycarbonate, Erlenmeyer style cell culture flasks with vented closures (Thermo Fisher

Scientific). The 0.22 µm polypropylene vented closure of these flasks allowed air but not contaminants to pass its barrier. Media was autoclaved for one hour, allowed to cool overnight and autoclaved for an additional hour the next day. The media was allowed to cool for several hours and inoculated with 5, 8 mm agar plugs obtained from the growing margins of pure, 2-day-old cultures of *S. rolfsii* on potato dextrose agar. The inoculated flasks were incubated for 4-6 weeks at 30°C while being exposed to 12 h of light and 12 h of darkness each day. Prior to field inoculations, media was extracted from flasks, placed in autoclave bags and weighed to calculate the amount of inoculum per plot.

*Field trials.* Three field trials were conducted to determine the efficacy of six fungicides delivered using three techniques. Two trials were conducted in the spring and fall of 2011 and one in the spring of 2012 at the Horticulture Farm at the University of Georgia's Tifton Campus. Sweet corn was planted in 2010 preceding these trials. Different tomato cultivars were planted in each trial. Red Bounty and Redline were planted in the spring and fall of 2011, respectively. The *Tomato yellow leaf curl virus* (TYLCV) resistant cultivar Tribute was planted in the spring of 2012 after experiencing 100% incidence in the fall 2011 trial. In 2011, plots were 6.1 m long and arranged in a randomized complete block design with four replications. Plots were 3.7 m long in the spring of 2012. There were nine plants per plot spaced 0.6 m apart in 2011 and eight plants per plot spaced 0.5 m apart in 2012. Rows were 1.8 m apart and the final raised beds were 0.8 m wide for all trials. 1,3- dichloropropene was applied at 112.2 L/ha on 23 March 2011.

Plots were inoculated by handv with *S. rolfsii* grown on oat medium for all trials; however, the amounts and methods varied. All plots were inoculated with 55.0 g of *S. rolfsii* in the spring of 2011 and the inoculum was immediately incorporated into the soil using a rototiller. Inoculation method the fall of 2011 used infected oat kernels placed on opposite sides of each

tomato plant approximately 3.8 cm from the stem. The inoculation process in the spring of 2012 was similar to that of the spring of 2011 trial except 137.8 g of *S. rolfsii* grown on oat media was used. Irrigation and fertility requirements in addition to insect, nematode, and weed control were consistent with extension recommendations of the University of Georgia. Foliar sprays of manganese, zinc, ethylenebisdithiocarbamate ion, and copper hydroxide (ManKocide at 3.36 kg/ha, E.I. du Pont de Nemours, Wilmington, DE) were applied weekly until harvest for suppression of bacterial spot (*Xanthomons euvesicatoria*). Trials were planted 20 April and 7 July in the spring and fall of 2011, respectively. Tomatoes were planted on 23 March in the spring of 2012.

A randomized complete block design was used in the spring and fall of 2011 with four replications per treatment. Fungicide treatments and rates per application were as follows: flutolanil (1.065 kg ai/ha, Convoy; Nichino America, Wilmington, DE), penthiopyrad (0.351 kg ai/ha, Fontelis; E I du Pont de Nemours, Wilmington, DE), difenoconazole (0.128 kg ai/ha, Inspire; Syngenta Crop Protection), azoxystrobin (0.109 kg ai/ha, Quadris; Syngenta Crop Protection), fluazinam (0.560 kg ai/ha, Omega 500; Syngenta Crop Protection), and fludioxonil (0.280 kg ai/ha, Cannonball; Syngenta Crop Protection). Fungicides were applied as at-planting drenches, drip chemigation, and foliar sprays in 2011. All fungicide-treated plots received at-planting drenches of fungicide solution. Untreated plots received water drenches. Drench treatments were applied at a rate of 1337 L/ha by slowly pouring 150 mL of fungicide solution around the base of each plant. Three subsequent fungicide applications were made in 21-day intervals [21, 42, and 63 days after planting (DAP)] either as foliar sprays or as drip chemigation. The first foliar sprays were applied 21 DAP with a CO<sub>2</sub>-pressurized backpack sprayer and calibrated to deliver 374.2 L/ha using ConeJet TX-26 hollow cone tips (TeeJet Technologies,

Springfield, IL) spaced 48.3 cm apart. The second and third foliar applications were applied 42 and 63 DAP with a Lee Spider (LeeAgra, Lubbock, TX) research sprayer using ConeJet TX-18 hollow cone tips (TeeJet Technologies) calibrated to deliver 561.2 L/ha with drop nozzles. Plots receiving drip-applied treatments were chemigated separately using a CO<sub>2</sub>-pressurized system in 3 L of water. The amount of fungicides applied was calculated based on the treated area (plot length x plot width x number of plots to be treated). A split-plot design with four replications per treatment was used in the spring of 2012. Main plots were the application methods and sub-plots were the fungicides. All of the aforementioned fungicides and application methods were evaluated once more with the addition of drench only treatments of all fungicides. This was added in spring 2012 to identify the effect on disease control resulting from a single at-planting drench application.

Disease incidence was recorded in each trial weekly by counting the number of plants showing signs or symptoms of infection by *S. rolfsii*. When necessary, plants were removed from the field and isolations were conducted to confirm the presence of *S. rolfsii*. Plant heights were recorded in each trial 21 DAP to identify potential phytotoxicity of fungicides. Heights of the first and every third subsequent plant were measured from the soil line to the tallest new growth and averaged for each plot. Herbicide injury that occurred prior to planting in the greenhouse in the spring of 2011 and 100% incidence of TYLCV in the fall of 2011 prohibited the formation of mature fruit making yield data impossible to obtain in both trials. However, yield data was recorded in the spring of 2012. Mature red fruit and pink fruit were harvested three times in all plots over three harvest dates. Data are displayed as the total weight in kg per hectare of these fruit from all three harvests.

*Statistical Analyses.* Plant height, yield, and southern blight incidence values were subjected to analysis of variance using ARM data management software (Gylling Data Management, Brookings, SD). Statistical comparisons were made using Fisher's protected LSD test ( $P \leq 0.05$ ). R statistical software (R Project, University of Auckland) was used to identify significant trial-treatment interactions. Combined final disease analysis was performed for trials conducted in 2011 because no significant trial-treatment interactions were present. However, disease incidence data are also displayed separately for all trials.

## **Results**

*Disease Incidence.* Combined analyses were performed for both trials conducted in 2011 because no significant ( $P \geq 0.05$ ) trial-treatment interaction was detected. All treatments significantly reduced southern blight incidence compared with untreated plots (Fig. 3.1 and Tables 3.1-3.2). Disease control was significantly reduced in azoxystrobin-treated plots compared with those treated with either delivery method of flutolanil and pentiopyrad and fluazinam drenches and drip chemigation (Fig. 3.1). Disease levels were highest end-of-season between 72 and 85 DAP in the spring of 2011 when disease incidence in untreated plots increased from 20% to 44.4% (Table 3.1). Conversely, disease levels were greatest early mid-season for the trial in fall 2011 when disease incidence in untreated plots increased from 3.33% to 22.2% between 32 and 47 DAP, respectively (Table 3.2).

Similar results were observed in the spring of 2012 when all treatments with the exception of azoxystrobin drenches and drip chemigation significantly reduced southern blight incidence compared with untreated plots (Fig. 3.2 and Table 3.3). Disease incidence was significantly lower in azoxystrobin-treated plots than untreated plots when the fungicide was applied as a drench only and as drenches and sprays (Fig. 3.2 and Table 3.3). However, drench-



only azoxystrobin was not significantly different from azoxystrobin drenches and drip chemigation or drenches and sprays (Fig. 3.2 and Table 3.3). In the spring of 2012, the largest increase in disease incidence in untreated plots was observed between 67 and 74 DAP when disease incidence increased from 3.75% to 16.3% (Table 3.3).

*Effects of fungicides on early season plant growth.* No significant ( $P>0.05$ ) differences in plant heights between treatments were observed 21 DAP for trials conducted in 2011. Drench-only treatments were used in plant height comparisons in the spring of 2012 since that was the only application that had occurred at the time. Significant differences in plant height were observed, whereby drench-only azoxystrobin applications significantly reduced plant heights compared to the untreated plots (Table 3.4).

*Effect of fungicides on yield.* Yield data was unobtainable for the trials conducted in 2011. Yield data was recorded for the trial conducted in the spring of 2012 and is displayed in Table 3.5. No significant ( $P>0.05$ ) yield differences were observed between fungicide-treated plots and untreated plots. However, plots treated with the at-plant drench of flutolanil followed by drip chemigation demonstrated significantly higher yields than plots receiving flutolanil drenches and sprays. Additionally, plots receiving fludioxonil as a drench application demonstrated significantly higher yields than fludioxonil plots receiving the drench followed by foliar sprays (Table 3.5).

## **Discussion**

Consistent results were observed with respect to fungicide and application method in both years. Flutolanil, penthiopyrad, and fluazinam typically demonstrated more consistent efficacy. Timing and severity of southern blight epidemics varied between growing seasons and years, emphasizing the importance of initiating fungicide applications before epidemics begin. The

performance of individual fungicides was independent of application regimens. This may be due to the at-planting drench applications providing most of the fungicidal activity. Drench-only treatments in 2012 provided the same statistical level of disease control for each individual fungicide as the treatments with additional spray or drip applications. Additionally, disease control was not significantly improved whether applied foliar or by drip chemigation for any individual fungicide in any trial. Drench applications are more direct and concentrated than applications through the drip chemigation or foliar sprays. Applying fungicides directly to the root system allows more of the fungicide to be absorbed into the plant and transported in the apoplast since that is how many fungicides are distributed in plant tissues (Kleier, *et al.*, 1998). Fungicide that is not absorbed into the plant (outside of the transplant size root system) forms a protective fungicide barrier around the crown of the plant. At-planting drenches were likely the most important fungicide applications for improving disease control in 2011. The results obtained in the spring of 2012 support this and even suggest that at-planting drench application may be the only fungicide application needed for control of southern blight. At-planting drenches in the spring 2012 provided the same level of disease control as fungicides applied as drenches and drip chemigation and drenches and sprays. Yield was typically higher when fungicides were applied as drenches and drip chemigation in 2012. This could be due to a suppression of other weak root pathogens with follow-up fungicide applications. However, care should be taken in drawing this conclusion as these differences were usually not statistically significant and were only obtained in one growing season. Further investigation should be done to understand the relationship between application method of each fungicide and yield.

The results of this study demonstrate that combining the best fungicides and application methods can result in levels of disease control that are comparable to those achieved with MeBr.

The IR-4 project is currently conducting studies evaluating flutolanil and fluazinam to be labeled for southern blight control in tomatoes. Future studies should continue to explore other fungicides and their effects on yield, timing of applications and number of applications.

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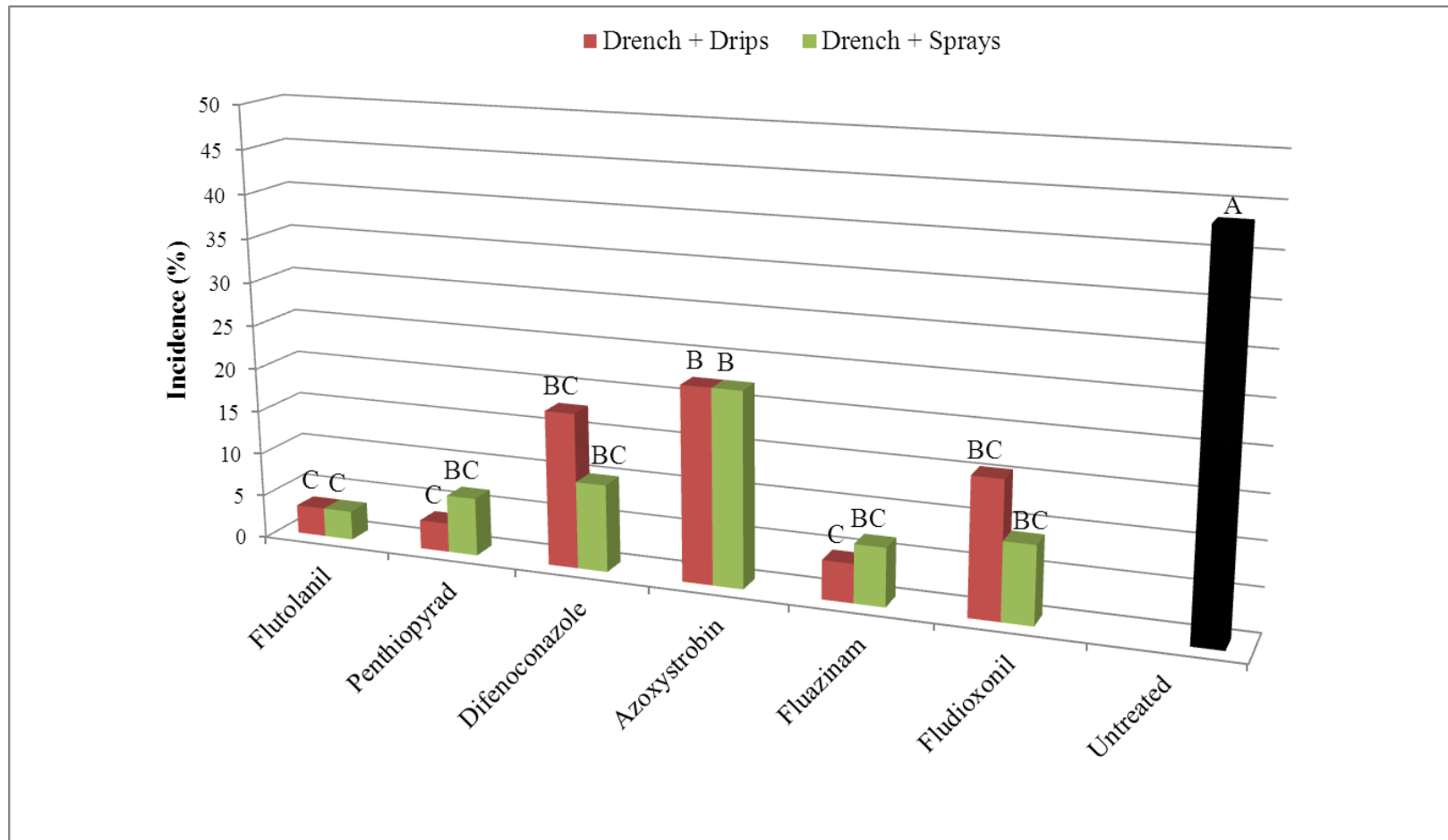
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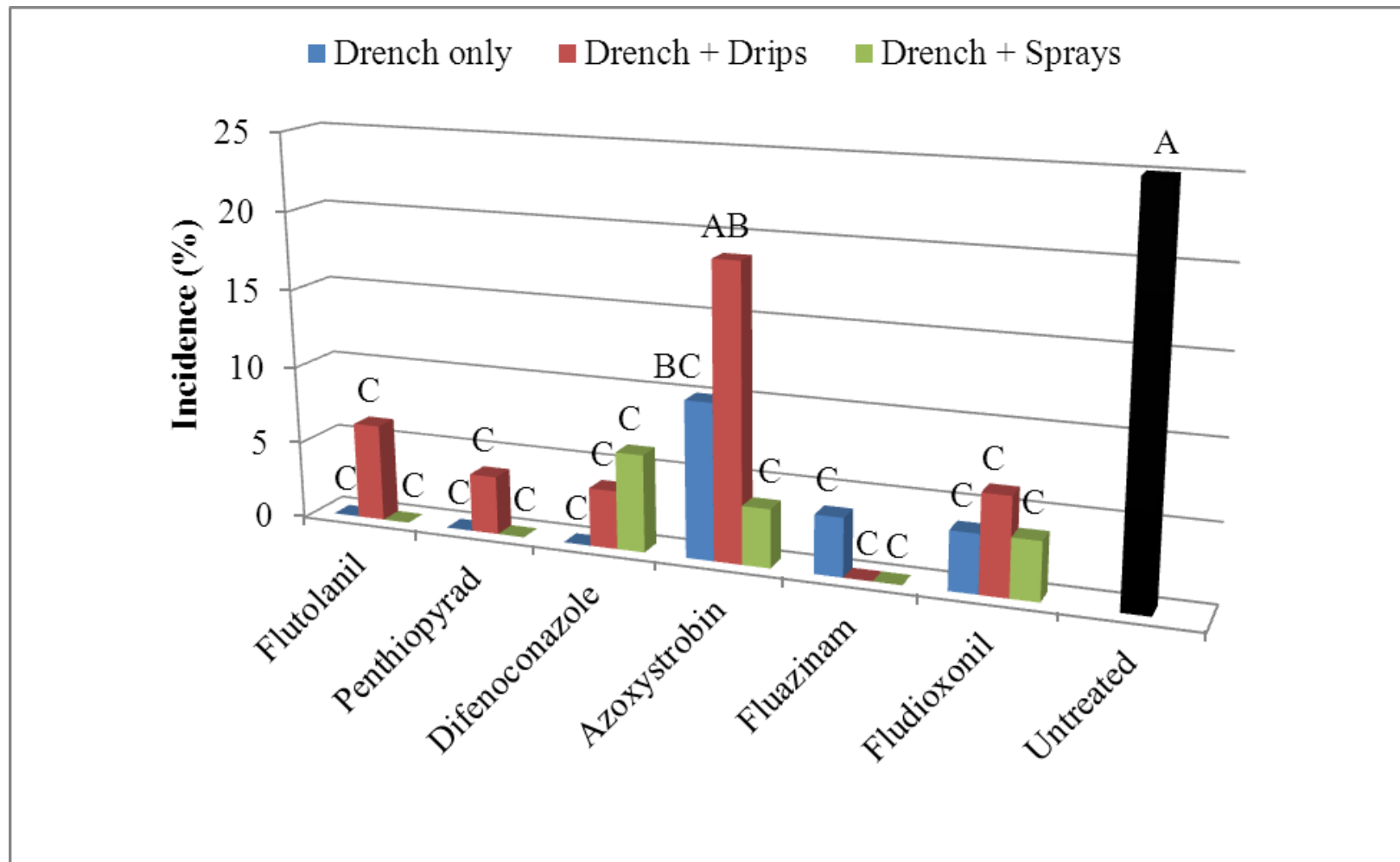
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**Fig. 3.1.** Southern blight incidence in untreated and fungicide-treated tomato plots by application method for combined trials in spring and fall of 2011. Significant differences between treatments were identified according to Fisher's protected LSD test ( $P \leq 0.05$ ). Treatments containing common letters are not significantly different. Fungicide applications were made as at planting drenches followed by three subsequent applications by drip chemigation or as foliar sprays.



**Fig. 3.2.** Southern blight incidence in untreated and fungicide-treated tomato plots by application method in a field trial conducted in spring 2012. Significant differences were identified according to Fisher's protected LSD test ( $P \leq 0.05$ ). Treatments containing common letters are not significantly different. Fungicides were applied in three ways: drench-only at planting, at-planting drenches and three subsequent drip chemigation treatments, or at-planting drenches and three subsequent foliar sprays.

**Table 3.1.** Disease incidence of *Sclerotium rolfii* on tomato in spring 2011 for fungicide-treated and untreated plots grouped by application method.

Application method <sup>W</sup>	Treatment <sup>X</sup>	Disease incidence (%)																	
		6 DAP <sup>Y</sup>	23 DAP	37 DAP	44 DAP	51 DAP	58 DAP	64 DAP	72 DAP	85 DAP									
Drench + Drip	Flutolanil	0.00	a <sup>Z</sup>	0.00	a	0.00	a	3.33	a	3.33	a	3.33	a	5.56	a	5.56	a	5.56	c
	Penthiopyrad	0.00	a	0.00	a	0.00	a	0.00	a	0.00	a	0.00	a	0.00	a	0.00	a	3.33	c
	Difenoconazole	0.00	a	3.33	a	3.33	a	5.56	a	5.56	a	5.56	a	8.89	a	8.89	a	20.00	abc
	Azoxystrobin	0.00	a	0.00	a	0.00	a	3.33	a	3.33	a	3.33	a	14.44	a	16.67	a	38.89	ab
	Fluazinam	0.00	a	0.00	a	0.00	a	0.00	a	0.00	a	0.00	a	0.00	a	0.00	a	3.33	c
	Fludioxonil	0.00	a	0.00	a	0.00	a	0.00	a	3.33	a	8.89	a	20.00	a	20.00	a	25.56	abc
Drench + Spray	Flutolanil	0.00	a	3.33	a	3.33	a	3.33	a	3.33	a	3.33	a	3.33	a	5.56	a	5.56	c
	Penthiopyrad	0.00	a	0.00	a	0.00	a	0.00	a	0.00	a	0.00	a	3.33	a	5.56	a	11.11	bc
	Difenoconazole	0.00	a	3.33	a	3.33	a	3.33	a	3.33	a	3.33	a	3.33	a	8.89	a	16.67	abc
	Azoxystrobin	0.00	a	0.00	a	0.00	a	0.00	a	8.89	a	11.11	a	16.67	a	20.00	a	42.22	a
	Fluazinam	0.00	a	0.00	a	0.00	a	0.00	a	0.00	a	0.00	a	0.00	a	3.33	a	5.56	c
	Fludioxonil	0.00	a	3.33	a	3.33	a	3.33	a	3.33	a	3.33	a	5.56	a	8.89	a	11.11	bc
Untreated		3.33	a	3.33	a	3.33	a	3.33	a	5.56	a	11.11	a	14.44	a	20.00	a	44.44	a

<sup>W</sup> Fungicide-treated plots received treatments as at-planting drenches followed by three subsequent applications either through the drip irrigation or as foliar sprays.

<sup>X</sup> Treatments and rates per application were as follows: flutolanil (1.065 kg ai/ha), penthiopyrad (0.351 kg ai/ha), difenoconazole (0.128 kg ai/ha), azoxystrobin (0.109 kg ai/ha), fluazinam (0.560 kg ai/ha), and fludioxonil (0.280 kg ai/ha).

<sup>Y</sup> DAP= Days after planting.

<sup>Z</sup> Means are compared within columns. Means followed by the same letter are not significantly different according to Fisher's protected LSD test ( $P \leq 0.05$ ).

**Table 3.2.** Disease incidence of *Sclerotium rolfsii* on tomato in fall 2011 for fungicide-treated and untreated plots grouped by application method.

Application method <sup>W</sup>	Treatment <sup>X</sup>	Disease incidence (%)													
		21 DAP <sup>Y</sup>	32 DAP	47 DAP	54 DAP	62 DAP	72 DAP	81 DAP	90 DAP						
Drench + Drip	Flutolanil	0.00	a <sup>Z</sup>	0.00	a	0.00	b	0.00	c	0.00	c	0.00	c	0.00	c
	Penthiopyrad	0.00	a	0.00	a	0.00	b	3.33	c	3.33	c	3.33	c	3.33	c
	Difenoconazole	3.33	a	3.33	a	8.89	b	14.44	b	16.67	b	16.67	b	16.67	b
	Azoxystrobin	0.00	a	0.00	a	3.33	b	5.56	bc	5.56	c	5.56	c	5.56	c
	Fluazinam	0.00	a	0.00	a	0.00	b	0.00	c	0.00	c	3.33	c	5.56	c
	Fludioxonil	0.00	a	0.00	a	0.00	b	3.33	c	5.56	c	5.56	c	5.56	c
Drench + Spray	Flutolanil	0.00	a	0.00	a	0.00	b	0.00	c	0.00	c	0.00	c	0.00	c
	Penthiopyrad	0.00	a	0.00	a	0.00	b	0.00	c	0.00	c	0.00	c	3.33	c
	Difenoconazole	0.00	a	3.33	a	3.33	b	3.33	c	3.33	c	3.33	c	3.33	c
	Azoxystrobin	0.00	a	0.00	a	0.00	b	3.33	c	3.33	c	3.33	c	3.33	c
	Fluazinam	0.00	a	0.00	a	5.56	b	5.56	bc	5.56	c	8.89	bc	8.89	bc
	Fludioxonil	0.00	a	0.00	a	0.00	b	0.00	c	0.00	c	0.00	c	0.00	c
	Untreated	0.00	a	3.33	a	22.22	a	27.78	a	31.11	a	36.67	a	38.89	a

<sup>W</sup> Fungicide-treated plots received treatments as at-planting drenches followed by three subsequent applications either through the drip irrigation or as foliar sprays.

<sup>X</sup> Treatments and rates per application were as follows: flutolanil (1.065 kg ai/ha), penthiopyrad (0.351 kg ai/ha), difenoconazole (0.128 kg ai/ha), azoxystrobin (0.109 kg ai/ha), fluazinam (0.560 kg ai/ha), and fludioxonil (0.280 kg ai/ha).

<sup>Y</sup> DAP= Days after planting.

<sup>Z</sup> Means are compared within columns. Means followed by the same letter are not significantly different according to Fisher's protected LSD test ( $P \leq 0.05$ ).



**Table 3.3.** Disease incidence of *Sclerotium rolfsii* on tomato in spring 2012 for fungicide-treated and untreated plots grouped by application method.

Application method <sup>W</sup>	Treatment <sup>X</sup>	Disease incidence (%)									
		27 DAP <sup>Y</sup>	74 DAP	84 DAP	90 DAP	102 DAP	111 DAP				
Drench Only											
	Flutolanil	0.00	b <sup>Z</sup>	0.00	b	0.00	b	0.00	b	0.00	c
	Penthiopyrad	0.00	b	0.00	b	0.00	b	0.00	b	0.00	c
	Difenoconazole	0.00	b	0.00	b	0.00	b	0.00	b	0.00	c
	Azoxystrobin	0.00	b	0.00	b	0.00	b	3.75	b	6.25	bc
	Fluazinam	0.00	b	0.00	b	0.00	b	0.00	b	3.75	bc
	Fludioxonil	0.00	b	3.75	b	3.75	b	3.75	b	3.75	bc
Drench + Drip											
	Flutolanil	0.00	b	0.00	b	0.00	b	0.00	b	3.75	bc
	Penthiopyrad	0.00	b	0.00	b	0.00	b	0.00	b	3.75	bc
	Difenoconazole	0.00	b	0.00	b	0.00	b	0.00	b	0.00	c
	Azoxystrobin	0.00	b	0.00	b	0.00	b	0.00	b	10.00	b
	Fluazinam	0.00	b	0.00	b	0.00	b	0.00	b	0.00	c
	Fludioxonil	0.00	b	3.75	b	3.75	b	3.75	b	3.75	bc
Drench + Spray											
	Flutolanil	0.00	b	0.00	b	0.00	b	0.00	b	0.00	c
	Penthiopyrad	0.00	b	0.00	b	0.00	b	0.00	b	0.00	c
	Difenoconazole	0.00	b	3.75	b	3.75	b	3.75	b	3.75	bc
	Azoxystrobin	0.00	b	0.00	b	3.75	b	3.75	b	3.75	bc
	Fluazinam	0.00	b	0.00	b	0.00	b	0.00	b	0.00	c
	Fludioxonil	0.00	b	3.75	b	3.75	b	3.75	b	3.75	bc
	Untreated	3.75	a	16.25	a	18.75	a	22.50	a	25.00	a

<sup>W</sup> Fungicide-treated plots received treatments as at-planting drenches followed by three subsequent applications either through the drip irrigation or as foliar sprays.

<sup>X</sup> Treatments and rates per application were as follows: flutolanil (1.065 kg ai/ha), penthiopyrad (0.351 kg ai/ha), difenoconazole (0.128 kg ai/ha), azoxystrobin (0.109 kg ai/ha), fluazinam (0.560 kg ai/ha), and fludioxonil (0.280 kg ai/ha).

<sup>Y</sup> DAP= Days after planting.

<sup>Z</sup> Means are compared within columns. Means followed by the same letter are not significantly different according to Fisher's protected LSD test ( $P \leq 0.05$ ).

**Table 3.4.** Tomato plant heights recorded in a southern blight fungicide trial in the spring of 2012.

<b>Treatment<sup>W</sup></b>	<b>Average plant heights (cm)<sup>X</sup></b>	
	<b>Drench only<sup>Y</sup></b>	
Flutolanil	18.2	c-f <sup>Z</sup>
Penthiopyrad	20.1	a-d
Difenoconazole	19.2	b-e
Azoxystrobin	16.5	ef
Fluazinam	20.2	a-d
Fludioxonil	21.6	ab

*Average height of untreated plots = 20.6 abc*

<sup>W</sup> Treatments and rates per application were as follows: flutolanil (1.065 kg ai/ha), penthiopyrad (0.351 kg ai/ha), difenoconazole (0.128 kg ai/ha), azoxystrobin (0.109 kg ai/ha), fluazinam (0.560 kg ai/ha), and fludioxonil (0.280 kg ai/ha).

<sup>X</sup> Plant heights recorded in cm by measuring the first and every third subsequent plant in each plot.

<sup>Y</sup> Data for drench-only treatments were used in plant height comparisons as these were the only applications made prior to the date of measurements.

<sup>Z</sup> Means followed by the same letter are not significantly different according to Fisher's protected LSD test ( $P \leq 0.05$ ).

**Table 3.5.** Tomato yield for a southern blight fungicide trial conducted in the spring of 2012 by application method.

Treatment <sup>W</sup>	Total fruit yield (kg/ha) <sup>X</sup>					
	Drench only <sup>Y</sup>		Drench + drip		Drench + spray	
Flutolanil	13197.9	cde <sup>Z</sup>	18928.0	abc	11680.6	e
Penthiopyrad	16885.2	a-e	17300.5	a-e	12307.9	de
Difenoconazole	22337.3	ab	17273.4	a-e	17444.6	a-e
Azoxystrobin	16058.7	cde	18393.9	a-d	13070.8	cde
Fluazinam	16156.2	b-e	18665.2	abc	16291.8	b-e
Fludioxonil	22979.8	a	18224.5	a-d	14710.9	cde

*Average yield of untreated plots = 17588.7 a-e*

<sup>W</sup> Treatments and rates per application were as follows: flutolanil (1.065 kg ai/ha), penthiopyrad (0.351 kg ai/ha), difenoconazole (0.128 kg ai/ha), azoxystrobin (0.109 kg ai/ha), fluazinam (0.560 kg ai/ha), and fludioxonil (0.280 kg ai/ha).

<sup>X</sup> Total yield was combined from three harvest dates.

<sup>Y</sup> Data are grouped by fungicide and application method. Applications were made as at-planting drenches only or as at planting drenches followed by three subsequent applications either through the drip irrigation or as foliar sprays.

<sup>Z</sup> Means followed by the same letter are not significantly different according to Fisher's protected LSD test ( $P \leq 0.05$ ).

## CHAPTER 4

# USING THREE PLASTIC MULCH COLORS TO INVESTIGATE THE INFLUENCE OF SOIL TEMPERATURE AND MOISTURE ON SOUTHERN BLIGHT EPIDEMICS OF PLASTICULTURE TOMATOES

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## **Abstract**

Epidemics of southern blight, caused by the soilborne fungus *Sclerotium rolfsii*, vary greatly with environmental conditions from year to year. However, the relationship between these environmental conditions and disease is not well understood. Field trials were conducted across three growing seasons to better understand the role of soil temperature and soil moisture in southern blight epidemics of plasticulture tomatoes grown in three plastic mulch colors (black, white, and reflective). Disease incidence was recorded weekly, and logit-transformed increases in disease incidence between weekly assessments were used for correlations with averages of selected environmental variables in the preceding time between assessment periods. Soil temperature was significantly ( $P < 0.05$ ) correlated with disease incidence. Differences in disease progress were observed between plastic mulch colors, whereby warmer growing seasons resulted in more severe southern blight epidemics in white and reflective mulches. Cooler growing seasons resulted in more severe southern blight epidemics in black-colored mulch. Stepwise regression models were constructed using the collected environmental variables and demonstrated a quantitative relationship between soil temperature and disease incidence. These and other variables may be useful to construct a disease forecasting model or advisory in future studies to help growers more effectively manage this disease.

## **Introduction**

Tomato (*Solanum lycopersicum* L.) is one of the most economically important vegetable commodities in the United States. Much tomato production occurs in California, Florida, and Virginia. The humid climate, frequent rainfall, and mild winters of Georgia provide excellent growing conditions for tomatoes, but also for the development of pathogens that affect them (Langston, 2012). One of the most destructive pathogens of tomatoes is *Sclerotium rolfsii* Sacc.

causing southern blight, one of the most damaging diseases of tomatoes in tropical and subtropical areas and in the southern and southeastern United States (McCarter, 1991).

Disease onset can occur at any time. The fungus attacks the stem of the plant at or just below the soil line. In advanced stages, white mycelium can be observed growing around the base of the infected plant. Symptoms occur suddenly and appear as a wilting and yellowing of the entire plant. Infected plants will slowly die and the pathogen will form small, round, tan colored sclerotia around the crown of the plant that allow the fungus to overwinter (McCarter, 1991).

Due to the persistent nature and wide host range of *S. rolfsii*, chemical control is an essential practice in managing the diseases caused by this pathogen. Both fumigants and fungicides have been used widely since the mid-1900s when they first became available (Aycock, 1966). Methyl bromide (MeBr) has been used widely since its introduction to control southern blight and other soilborne diseases of vegetables because it is a broad-spectrum biocide. However, MeBr will soon be phased out and chemical management is expected to rely more heavily on the use of fungicides. More information on the environmental factors that influence southern blight epidemics will be useful in providing better timing of fungicide applications.

*Sclerotium rolfsii* is highly influenced by environmental conditions making epidemics variable across geographical locations and growing seasons (Mehan *et al.*, 1995). Most of the information regarding the relationship between *S. rolfsii* and environmental conditions comes from experiments performed in the laboratory. In the field, much about the influence of environment on this pathogen has been gained from studies in peanut and carrot (Hari *et al.*, 1991, Mehan *et al.*, 1995, Onkarayya and Appa Rao, 1970, Jenkins and Averre, 1986, Rideout,

2002). These studies provide a good base of knowledge, but no similar studies have been conducted in a plasticulture environment.

In other hosts, research to better define the optimal environmental conditions for epidemics caused by *S. rolfsii* have shown that environmental variables such as air humidity, temperature, soil moisture, and pH influence the level of disease caused by this pathogen (Punja, 1985). *Sclerotium rolfsii* can survive in a wide range of temperatures from 8 to 40°C. However, the number of infections is reduced greatly at temperatures below 20°C and above 35°C (Aycock, 1966, Mehan *et al.*, 1995). The optimal soil temperature for infection by *S. rolfsii* has been cited as 30°C for most cropping systems (Aycock, 1966). Hari *et al.* (1991) observed that *S. rolfsii* cultured on peanut shoot extract medium grew most rapidly at 30°C. Ginstein *et al.* (1979) demonstrated that applying plastic mulch during the off-season for soil solarization reduced levels of stem rot in the following season compared with non-mulched soils. Maximum temperatures in mulched soils were 7 to 12°C higher (45 to 53°C) than in non-mulched soils.

Extensive research has been conducted on stem rot and moisture relationships in peanut. The peanut canopy microclimate influences stem rot development especially with respect to air humidity (Rideout, 2002). Stem rot epidemics are usually associated with row closure of peanut which causes an increase in humidity within and soil moisture beneath the canopy (Mehan *et al.*, 1995, Davidson *et al.*, 1991). Soil moisture also plays an important role in epidemics caused by *S. rolfsii* and much research has been conducted in peanut to investigate the relationship between soil moisture and stem rot development. Onkarayya and Appa Rao (1970) reported that soil moisture of 50% was optimal for peanut stem rot development. In a study performed by Shew and Beute (1984) moisture level was more important than level of inoculum. Severe outbreaks of southern stem rot have been reported in peanut during long periods of moisture or rainfall

(Mehan *et al.*, 1995). Additionally, disease levels were observed to be more severe following a rain event preceded by a dry period (Beute and Rodriguez-Kabana, 1979). Punja and Grogan (1981) proposed that increased levels of stem rot observed during these drying and wetting cycles could be due to eruptive germination of sclerotia being enhanced by sclerotial drying and that disease development is favored by the following moist environment. Little is understood about soil moisture-disease relationships in the plasticulture vegetable production environment.

The aforementioned studies have investigated the independent effects of temperature and moisture on *S. rolfsii*. However, these factors are confounded in the field in that one variable has an effect on the other. Therefore, it is important to understand their relationships. Soil has a considerable capacity for storage of water, which can be held with considerable force by capillary action (Or and Wraith, 2000). Several studies investigated the diurnal amplitude effects of volumetric soil water and soil surface temperature fluctuations. These experiments demonstrated smaller soil surface temperature amplitudes for soils with higher water contents (Idso *et al.*, 1975b, Reginato *et al.*, 1976). This can be explained by the thermal inertia of the water in the soil. Diurnal temperature fluctuations also have an effect on soil dielectric permittivity. Dielectric permittivity measures the amount of energy stored in a material after it has been electrically charged. In soil, the higher the dielectric permittivity, the higher the water content. The relationship between soil temperature and water content is also affected by soil type. Wraith and Or (1999) studied these relationships using time domain reflectometry (TDR) in four different soils: two silt loam soils, an Oxisol, and one sandy loam soil. Temperature was increased stepwise in samples of these soils while measuring dielectric permittivity and electrical conductivity. Dielectric permittivity increased regardless of water content for one silt loam and the sandy loam soil. The dielectric permittivity for the other silt loam and the Oxisol soils



increased when water contents were relatively low. However, a decrease in dielectric permittivity was observed in the soils when water content was relatively high. It was concluded that these irregular observations were due to an interaction between the reduction in dielectric constant of bulk water with increased temperature and the increase in dielectric permittivity with increased temperature due to a release of bound water (Wraith and Or 1999, Or and Wraith, 1999).

The raised-bed plasticulture system of vegetable production employed by most tomato growers is a unique system that is much different from the peanut cropping system which has been studied most extensively with *S. rolf sii*. The addition of the plastic mulch to the soil affects certain soil environmental variables, namely temperature and moisture, among others, which would influence how this pathogen behaves in this cropping system. Three colors of plastic mulch are used throughout the year for their capacity to warm the soil: black, white, and reflective. Black mulch is used to warm the soil in early spring and promote plant growth, whereas white mulch is used in the fall to avoid overheating the soil and damaging the plants. Reflective mulch has gained popularity for its ability to deter feeding by thrips, the vector of *Tomato spotted wilt virus* (TSWV) (Riley and Pappu, 2000, 2004). It is the purpose of this work to investigate the influence of these three plastic mulch colors on soil temperature and moisture while observing differences in southern blight onset and severity.

## **Materials and Methods**

*Inoculum preparation.* Inoculum for field trials was grown on oat media. Media was mixed by combining 500 mL of oat seed (with husks) and 500 mL of tap water in 2 L, polycarbonate, Erlenmeyer-style cell culture flasks with vented closures (Thermo Fisher Scientific). The 0.22  $\mu\text{m}$  polypropylene closure of these flasks allowed air but not contaminants to pass its barrier. Media was autoclaved for 1 hour, allowed to cool overnight and autoclaved for

an additional hour the next day. The media was allowed to cool for several hours and inoculated with five 8-mm agar plugs obtained from the growing margins of pure, 2-day-old cultures of *S. rolf sii* on potato dextrose agar (PDA). The inoculated flasks were incubated for 4-6 weeks at 30°C while being exposed to 12 h of light and 12 h of darkness each day. Prior to field inoculation, media was extracted from flasks, placed in autoclave bags and weighed to calculate the amount of inoculum per plot.

*Field trials.* A total of three field trials were conducted in the spring and fall of 2011 and in the spring of 2012 at the University of Georgia's Stripling Irrigation Research Park in Camilla, GA to investigate the influence of plastic mulch color on southern blight epidemics. The tomato cultivar Red Bounty was planted for trials conducted in 2011. In 2012, the cultivar Tribute was planted due to high incidence of *Tomato yellow leaf curl virus* (TYLCV) in the preceding year. Prior to laying plastic in 2011 and 2012, all plots were inoculated with *S. rolf sii* grown on oat media by hand and immediately rototilled into the soil. Plots received 92 g of inoculum in 2011 and 489 g of inoculum in 2012. Trials were arranged in a randomized complete block design with four replications per treatment. Treatments included three plastic mulch colors (black, white, and reflective). Plots were 10.0 m long with 1.8 m between rows. The width of raised-bed top was 0.76 m and plants were spaced 0.6 m apart. Foliar sprays of manganese, zinc, ethylenebisdithiocarbamate ion, and copper hydroxide (ManKocide at 3.36 kg/ha, E.I. du Pont de Nemours, Wilmington, DE) were applied weekly until harvest following the detection of foliar pathogens.

*Assessing disease and plant growth.* Disease incidence was recorded weekly in each trial and used to compare disease progress over time. Plants were considered diseased when symptoms first developed. These plants were noted and monitored for pathogen presence by

observing mycelial growth and sclerotial development at the soil line. This generally did not occur in smaller plants which were removed and transported back to the lab where the pathogen could be isolated on PDA to confirm the presence of *S. rolfsii*.

Plant height measurements were recorded 21 days after planting (DAP) in each trial to compare differences in early season plant growth. Measurements were recorded for the first and every third plant in each plot. The height of each plant measured was recorded in cm as the distance from the soil line to the tallest new growth foliage.

Yield data was only obtained for the trial conducted in the spring of 2012. Yield was unobtainable in the spring of 2011 due to herbicide injury and lack of fruit. In the fall of 2011, 100% incidence of TYLCV in each plot inhibited the maturation of fruit just before harvest. In the spring of 2012, pink and red fruit were harvested in each plots three times at 76, 83, and 90 DAP. Yield was combined across harvest dates for each plot and used to calculate yield in kg/ha.

*Statistical analyses of field trials.* Disease incidence, plant height, and yield of field trials were subjected to analysis of variance using ARM data management software (Gylling Data Mangement, Brookings, SD). Treatment means were compared using Fisher's protected LSD test ( $P \leq 0.05$ ).

*Soil environmental conditions.* Soil temperature (ECT temperature sensor, Decagon, Pulman, WA) in °C, volumetric water content (10HS soil moisture sensor, Decagon) in  $m^3/m^3$ , and matric water potential (MPS-1 dielectric water potential sensor, Decagon) in kPa were measured for each mulch color in all trials conducted. All sensors were buried at a depth of 5 cm. The aforementioned variables were recorded every 30 min using Decagon EM50 dataloggers. One datalogger and set of sensors were placed in one plot of each mulch color. Environmental data was downloaded weekly to a laptop computer using ECH2O utility Version 1.51 and

analyzed using Datatrak 3 software Version 3.2 (Decagon). These variables were used in correlation and regression analyses.

*Correlations between southern blight incidence and environment.* Pearson's correlation coefficients between weekly increases in southern blight incidence (based logit-transformed disease incidence) and soil environmental variables (soil temperature, soil moisture, and water potential) were conducted to determine the influence of environment on southern blight using R statistical software command  $\text{cor}(x, y)$  (R Project, University of Auckland). Means of environmental variables were from the preceding period between assessment dates.

*Stepwise regressions.* Stepwise multiple regressions were conducted using R statistical software to determine if environmental variables jointly contributed to southern blight development. Weekly increases in logit-transformed disease incidence were used in this analysis as well. Environmental variables were only added and retained in the model if their level of significance was less than or equal to 0.15.

## **Results**

*Field trials.* Epidemics of southern blight varied across growing seasons. Mulch color affected southern blight incidence (Fig. 4.1). Soil temperatures were much warmer in 2011 than 2012, which could explain the differences observed between epidemics in these years (Fig. 4.2). Final disease incidence was recorded 91 DAP in the spring of 2011. Incidence of southern blight was significantly higher when plants were grown in white plastic mulch compared with black plastic mulch. Disease incidence of plants in reflective plastic mulch fell between black and white and was not significantly different from either. Disease onset of plants in white plastic mulch was observed at 7 DAP but disease incidence did not increase again until 56 DAP. Disease onset of plants in black and reflective plastic mulches was recorded at 56 DAP. Large

increases in southern blight incidence occurred between 70 and 78 DAP for all mulch colors indicating favorable environmental conditions during this period (Fig. 4.1A).

In the fall of 2011, mulch color affected final disease incidence recorded at 106 DAP. Final disease incidence was significantly higher when tomatoes were grown in white and reflective mulches compared with plants in black plastic mulch. Tomatoes planted into black plastic mulch were severely stunted during the trial and died just before the termination of the trial. This was likely caused by overheating of the plants by the black mulch since black mulch absorbs much more heat than white or reflective colored mulches (Fig. 4.1B). Daily maximum temperatures in black mulches were often greater than 40°C during the fall of 2011.

Significant differences in disease incidence between plastic mulch colors were only observed at 112 DAP in the spring of 2012. When these data were recorded, disease incidence was significantly higher for tomatoes grown in black plastic mulch than those planted into reflective mulch. Disease incidence of tomatoes planted in white plastic mulch was not significantly different from either black or reflective mulches. When final disease incidence was recorded at 119 DAP, no significant differences in disease incidence between mulch colors was noted. Disease incidence increased for tomatoes planted in all mulch colors from 112-119 DAP with the largest increase observed for tomatoes planted into reflective colored mulch (Fig. 4.1C).

Differences in plant growth were observed across plastic mulch colors and are displayed in Fig. 4.3. In the spring of 2011, the heights of tomatoes planted in black colored mulch were reduced significantly compared with tomatoes planted in either white or reflective mulches. In the fall of 2011, plant heights were significantly greater for tomatoes planted in white mulch compared with reflective or black mulch. Additionally, plant heights of tomatoes planted in reflective mulch was significantly greater than heights of tomatoes planted in black mulch. As

time progressed, however, differences in plant heights were no longer visually discernible in the case of the aforementioned trials. No significant differences in plant heights were observed among mulch colors in the spring of 2012. However, yield data was recorded in this trial and is displayed as yield totals in Fig 4.4. Yield was recorded three times at 76, 83, and 90 DAP. No significant differences were observed for any harvest date (Fig. 4.4).

*Correlations between southern blight incidence and environmental variables.*

Correlations between environmental variables and weekly increases in logit-transformed incidence of plants infected with *S. rolfsii* are presented in Table 4.1. A significant ( $P \leq 0.05$ ) correlation was identified for soil temperature. No significant correlation was identified for volumetric water content or water potential. However, the  $P$ -value of water potential was 0.079 (Table 4.1), indicating a statistical trend.

*Stepwise multiple regression.* Results from stepwise regressions are provided in Table 4.2. Soil temperature was the only variable to contribute significantly ( $P \leq 0.15$ ) to the regression model. The  $P$ -value for water potential alone was less than 0.15. However, this variable was no longer significant when added as a second variable in the regression model (Table 4.2).

## **Discussion**

Disease onset and severity were variable across plastic mulch colors and growing seasons. This illustrates the potential value of an advisory or forecasting system for this disease. Fungicide applications could be made in a more timely manner to improve disease control but also save producers time and money. More specific information is needed on the epidemiological association of southern blight epidemics with the environment.

The information provided by this study demonstrates that plastic mulch color affects disease progress and severity. In fact, color of plastic mulch could potentially be used as a

disease management strategy. Most vegetable producers plant spring crops into black mulches to promote early plant growth. This enhancement of early-season plant growth and heating of the soil could be causing the epidemic to begin sooner, thus making it more severe. The results from the trial conducted spring 2012 demonstrate this scenario. This trial was an ideal scenario for vegetable producers. A mild winter made early planting permissible and the trial was planted 22 March. A larger increase in disease progress occurred between 49 and 56 DAP for plants in black plastic mulch compared with those planted in white or reflective colored mulches. The results also illustrate the length of time that disease progress is reduced in the white and reflective mulches compared with the black mulch. Additionally, no significant differences in plant height or yield were observed between mulch colors. Conversely, using lighter colored mulches that reduce soil temperatures and early-season plant growth would not always be effective. This scenario was observed during the trial conducted in the spring of 2011. A late frost occurred that year causing the planting date to be delayed until 19 April. Final disease incidence for tomatoes planted in white plastic mulch was significantly higher than tomatoes planted in black plastic mulch. Final disease incidence for tomatoes in reflective plastic mulch was not significantly different from either black or white colored mulches.

These observations are most likely attributed to the differences in soil temperature among the mulch colors. Soil temperatures in the black plastic mulch could have been inhibitory compared with the soil temperatures within white and reflective colored mulches in 2011. Soil temperatures were much higher in the spring of 2011 on the same calendar dates compared with the soil temperatures observed in the spring of 2012. In general, higher disease incidence was observed when soil temperatures were around 30°C, the optimal temperature for infection by *S. rolfsii* in most cropping systems (Aycock, 1966). White mulch had soil temperatures that were

more conducive for infection in 2011, whereas black mulch had soil temperatures that were more conducive for infection in 2012 because soil temperatures were cooler that year.

No difference in final disease incidence between white and reflective plastic mulch colors was observed in the fall of 2011. However, both mulch colors had significantly higher final disease incidence compared with tomatoes in black mulch only because all of these plants died from overheating. Growers do not plant fall crops into black mulch but rather into white mulch. This is done to reduce the temperature of the soil so that plants are not injured from extreme heat. Disease incidence was much lower compared with the other two trials. This trial was not repeated because of the impracticality of growing tomatoes in black mulch for fall trials. Additionally, no major differences were observed between white and reflective mulches with respect to the environmental and disease incidence variables recorded. However, data from this trial suggests that disease pressure in fall-grown tomatoes is reduced compared with those grown in the spring. More research should be conducted on fall-grown tomatoes and southern blight especially with respect to varietal selection, yield, and market quality.

The results from the correlations and stepwise regression analysis identify soil temperature as an influential variable in southern blight epidemics and provide an explanation of how mulch color influences southern blight epidemics. Pearson's correlations only identified a significant correlation between soil temperature and weekly increases in logit-transformed disease incidence. Similar results were observed from stepwise regression analyses as soil temperature was the only variable to significantly contribute to the model. However, the nearly significant *P*-value of water potential in the correlation analysis suggests that water availability could have a significant influence on epidemics that was not detected by the relatively small data set in this study. Placing sensors in every plot could have helped to discern this relationship more



accurately. However, it is also likely that water potential could be more important in warmer years since increases in soil temperature result in more water availability (i.e., increased water potential/volumetric water content) (Wraith and Or 1999, Or and Wraith, 1999).

Epidemics caused by *S. rolfsii* are highly variable and differ across growing seasons and plastic mulch colors. This study demonstrates that southern blight is influenced significantly by soil temperature in the plasticulture environment. Similar observations have been made in peanut (Rideout, 2000). Selection of mulch color could be used to manage this disease by manipulating the variables identified in this study. The potential also exists to use soil temperature and other variables to identify critical times and conditions more favorable for southern blight epidemics so that growers can make fungicide applications at the optimal time to control this disease. More research should be conducted to obtain a better understanding of how these and other variables influence southern blight epidemics in plasticulture tomatoes, especially across years and locations.

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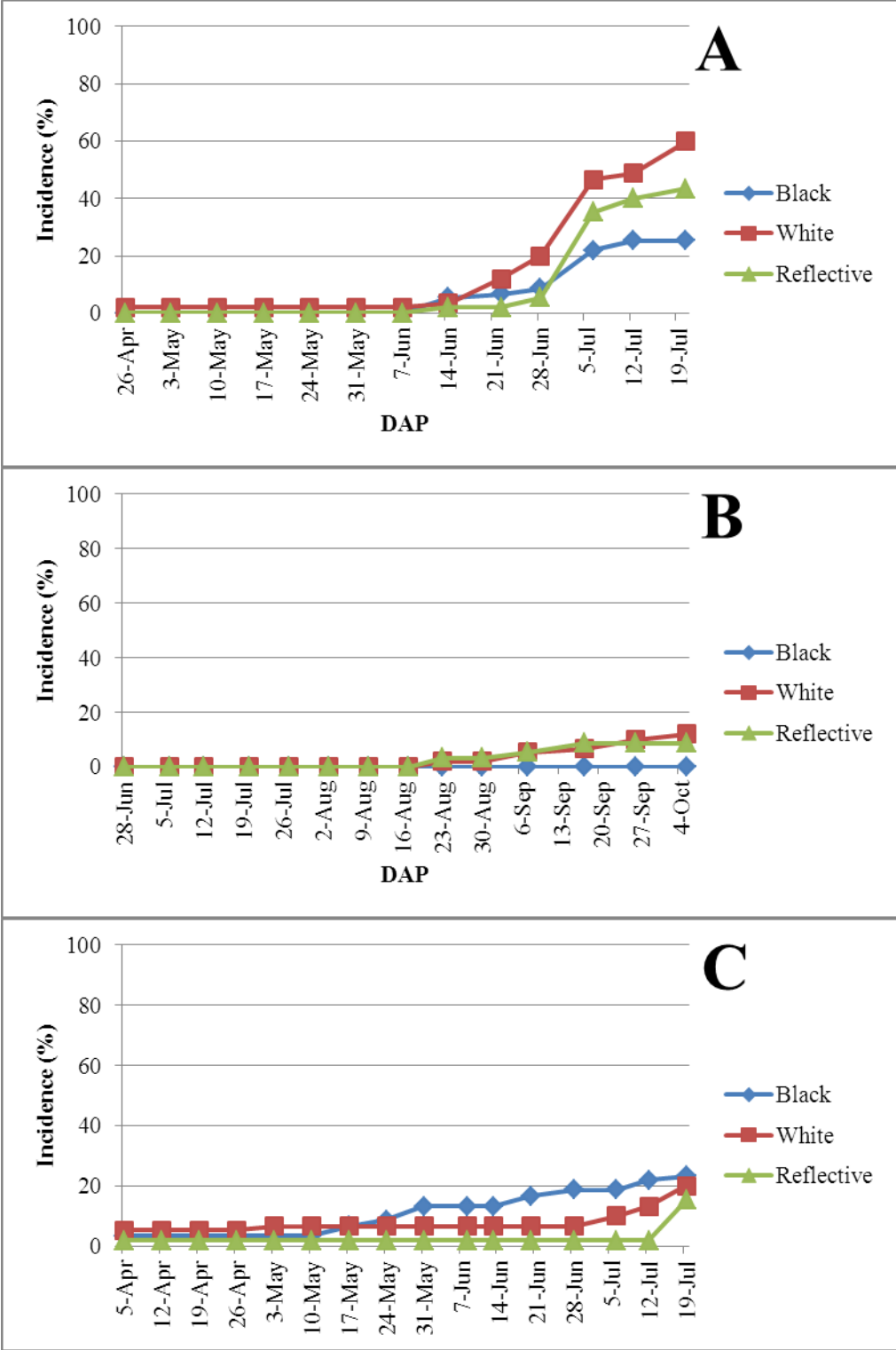
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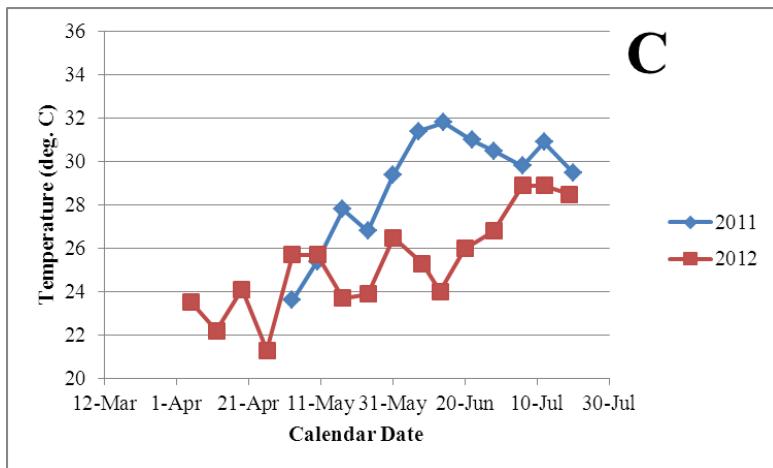
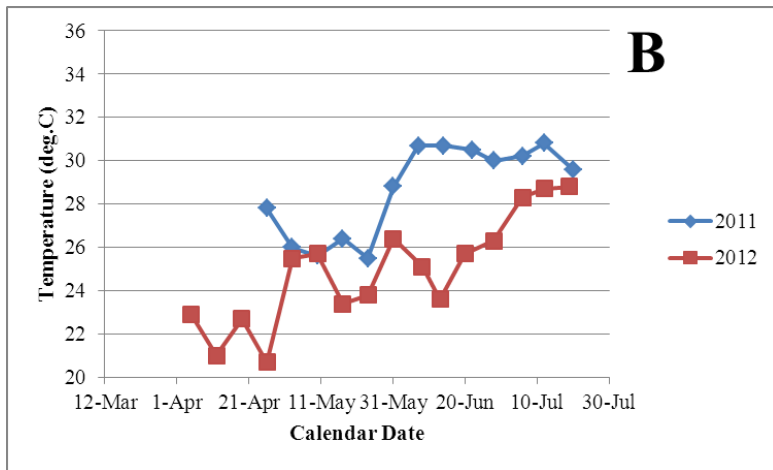
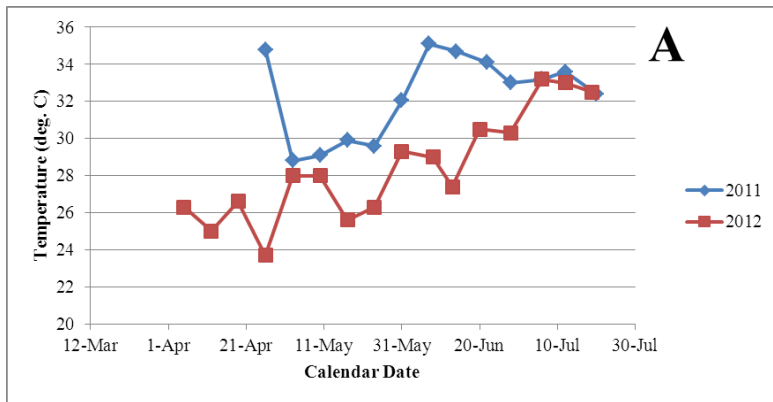
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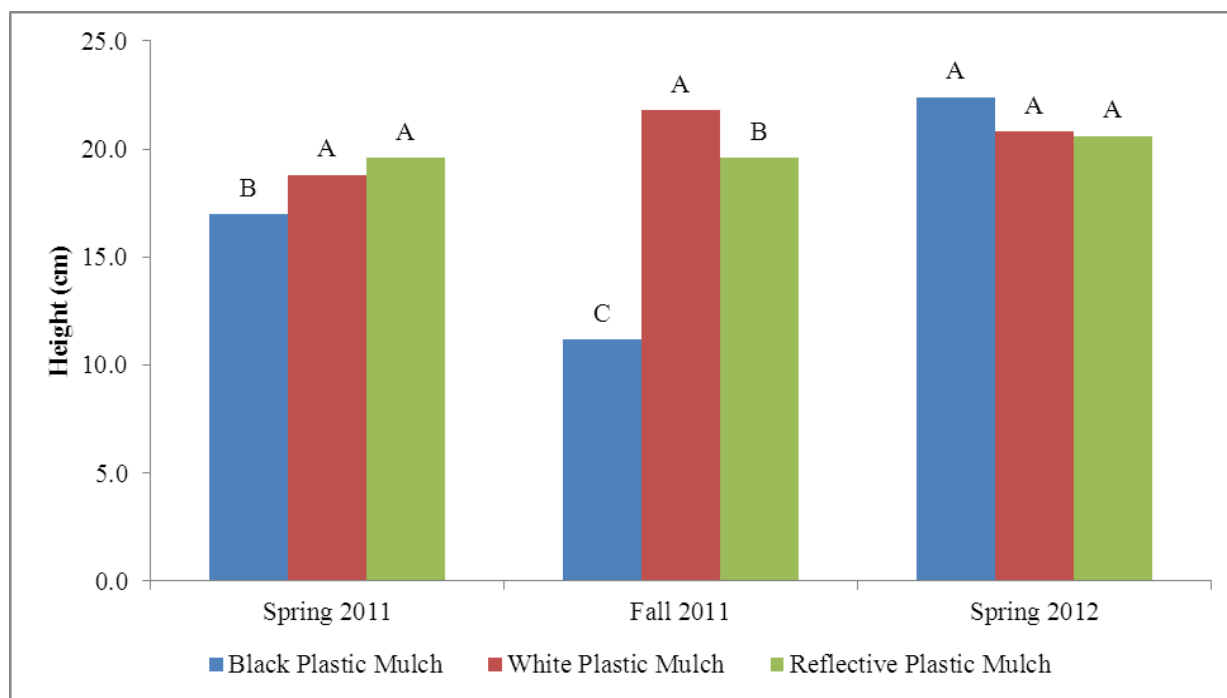
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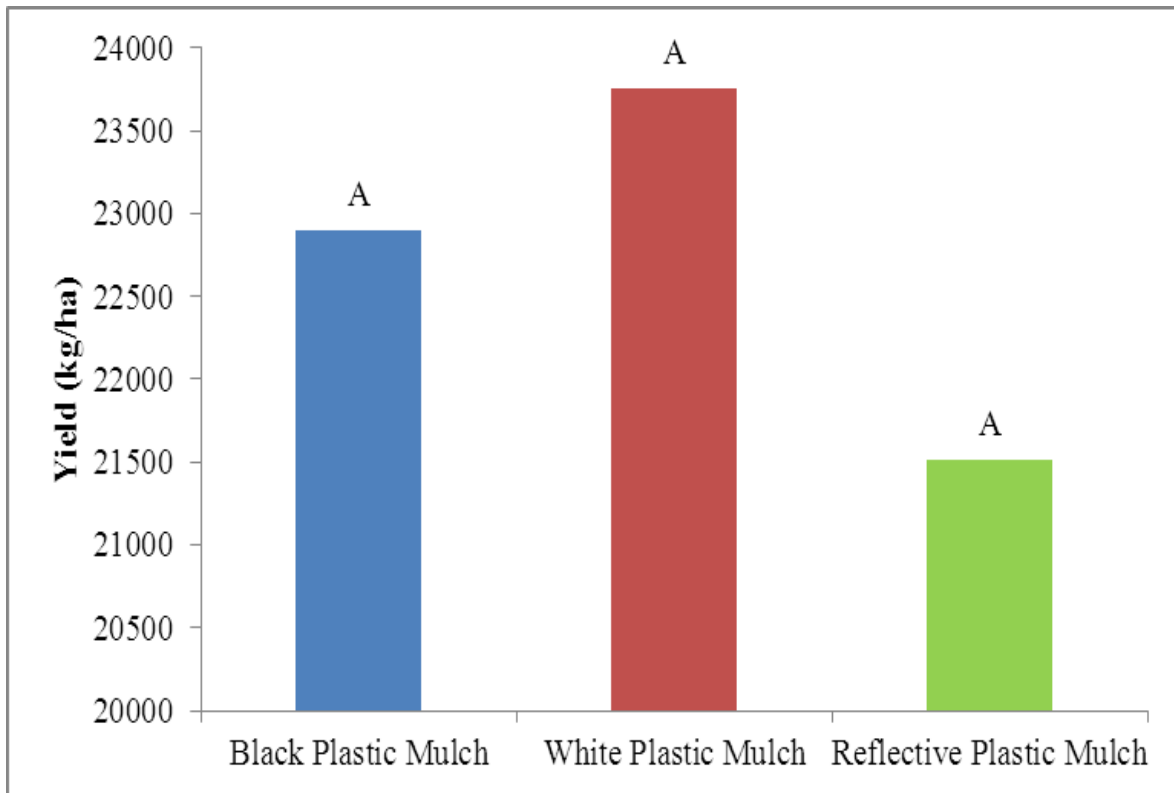
**Fig. 4.1.** Temporal progress of southern blight epidemics in plasticulture tomatoes in three trials according to plastic mulch colors and growing seasons. Data presented is for percentage of plants that exhibit any symptoms resulting from infection by *S. rolfsii* for spring 2011 (A), fall 2011 (B), and spring 2012 (C).



**Fig. 4.2.** Average weekly soil temperatures in plasticulture tomatoes in three trials according to plastic mulch colors and growing seasons. Data presented is the average soil temperatures for the period between disease assessment dates. Calendar dates are presented without the year to illustrate differences between years for mulch colors black (A), white (B), and reflective (C).



**Fig 4.3.** Heights of tomato plants grown in three mulch colors and inoculated with *Sclerotium rolfsii* across three growing seasons. Means are compared within growing seasons. Means containing the same letter do not differ significantly according to Fisher's protected LSD test ( $P \leq 0.05$ ).



**Fig. 4.4.** Yield totals of tomato plants grown in three mulch colors and inoculated with *Sclerotium rolfsii* in the spring of 2012. Data are presented cumulatively from three harvest dates. Means were not significantly different according to Fisher's protected LSD test ( $P \leq 0.05$ ).

**Table 4.1.** Correlations between weekly averages of environmental variables and weekly increases of logit-transformed incidence of southern blight.

	Soil temperature	Volumetric water content	Water potential
Pearson's coefficient	<b>0.2862</b>	0.0604	0.2054
<i>P-value</i>	<b>0.013</b>	0.608	0.079

<sup>Z</sup> Pearson's correlation coefficient (upper number) and level of significance for correlation (lower number). Bold values indicate significant correlations ( $P \leq 0.05$ ).



**Table 4.2.** Results from stepwise multiple regression analyses conducted on weekly averages of environmental variables and weekly increases of logit-transformed southern blight incidence.

Step	Variable added <sup>Y</sup>	Variable removed	Model R-squared	Variable P-value <sup>Z</sup>	Parameter estimate
1	ST		0.0819	0.0134	0.0175
2	WP		0.1036	0.1948	0.0085
3		WP	0.0819		
	Intercept			0.0562	-0.3712

Formula: Incidence= 0.0175(ST)-0.3712

<sup>Y</sup> Variable abbreviations are as follows: ST= soil temperature (°C), WP= water potential (kPa).

<sup>Z</sup> Variables were entered and retained in the model if level of significance was  $P \leq 0.15$ .

## CHAPTER 5

### CONCLUSIONS

Southern blight, caused by the fungus *Sclerotium rolfsii* is a serious disease of tomatoes in the southeastern United States. Effective disease management is best achieved through the implementation of an integrated disease management program. Cultural practices are beneficial, but the application of fungicides is the most effective method of controlling this disease (McCarter, 1991). With the impending loss of the fumigant methyl bromide in 2013, research into the use of fungicides is being conducted. However, there are few fungicides labeled to control this disease in tomato. Available fungicides include pentachloronitrobenzene (PCNB), azoxystrobin, pyraclostrobin, and fluoxastrobin. Research on the use of PCNB demonstrated inconsistent results (Jenkins and Averre, 1986, Xie and Vallad, 2010). The fact that the other labeled fungicides are all strobilurins underscores the need for additional fungicide classes for resistance management. Therefore, several different fungicides were evaluated in greenhouse conditions using a bioassay technique with *S. rolfsii* inoculation. Results demonstrated that flutolanil, fluazinam, penthiopyrad, pyraclostrobin, and prothioconazole distributed systemically in the plant to potentially provide suppression of *S. rolfsii* whether applied to soil or foliar. However, flutolanil, penthiopyrad, and fluazinam provided the highest level of suppression at the crown tissue (typical infection court site) when applied as a soil drench. Additionally, flutolanil and penthiopyrad were observed to be highly systemic when applied as a soil drench since suppression of *S. rolfsii* was observed on leaflet tissues. These findings could imply control of some foliar fungal pathogens using soil drench applications.

Results from field trials supported these observations. Flutolanil, penthiopyrad, and fluazinam provided consistent disease suppression in all field trials. Effective disease control was achieved using a single at-planting drench application with all fungicides evaluated in field studies. Therefore, it was determined that no subsequent fungicide applications were needed to improve disease control. Care should be taken when drawing conclusions based on the yield data from this work as they were only recorded in one trial and disease levels were low.

Epidemics caused by *S. rolfsii* are different across years with respect to onset and severity. Therefore, factors contributing to epidemics in the plasticulture system were investigated in this study. It was observed that the effect of plastic mulch color on soil temperature has a significant influence on final disease incidence. White mulch had significantly ( $P \leq 0.05$ ) higher final disease incidence in the spring of 2011 compared with black mulch. In the spring of 2012, black mulch had significantly higher disease incidence than the reflective mulch 112 days after planting. These observations were attributed to the differences in soil temperature across the mulch colors since Pearson's correlations identified a significant correlation between weekly increases in logit-transformed disease incidence and soil temperature averages during the preceding week. Additionally, stepwise regression analysis identified soil temperature as the only variable to significantly contribute to the regression model for increases in logit-transformed disease incidence. In general, higher disease incidence was observed when soil temperatures were around 30°C, the optimal temperature for infection by *S. rolfsii* in most cropping systems (Aycock, 1966). White mulch had soil temperatures that were more conducive for infection in 2011, whereas black mulch had soil temperatures more conducive for infection in 2012 because soil temperatures were much cooler that year.

A disease forecasting model for southern blight epidemics that could help in making more timely fungicide applications may be desirable in some cropping systems. However, the results from this work indicate that a forecast system would not be useful in tomatoes since disease control can be achieved using a single at-planting fungicide drench. In summary, the results from this study will be essential in developing effective disease management programs for southern blight using fungicides.

### **Literature Cited**

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