Evaluation of rootstocks for grafted plants as a strategy to manage Southern Blight in tomato (*Solanum lycopersicum*)

Roy William Langlois

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Approved by

Dr. Joseph M. Kemble, Professor, Department of Horticulture

Dr. Edward J. Sikora, Professor, Department of Entomology and Plant Pathology

Dr. Elina Coneva, Professor, Department of Horticulture

Thesis Abstract

Evaluation of rootstocks for grafted plants as a strategy to manage Southern Blight in tomato (*Solanum lycopersicum*)

Roy William Langlois

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Sclerotium rolfsii is a soil-borne fungus responsible for the disease Southern blight. With few effective chemical controls, this disease is a continuing problem in the southeastern U.S. Vegetable grafting has gained momentum as a method to manage soil-borne diseases. An experiment was conducted to evaluate 6 lines of tomato (*Solanum lycopersicum*) and two near relatives (*Solanum pimpinellifolium)* for varying levels of resistance to *S. rolfsii.* The intraspecific rootstocks *Multifort* and *Maxifort* were also analyze for Southern blight resistance. These six lines are processing tomatoes from Texas A&M University: *5635M, 5707M, 5719M, 5737M, 5876M, 5913M* with reported resistance to *S. rolfsii*. In 1992, Leeper and others released six processing tomato breeding lines with resistance to *S. rolfsii* (Leeper et al., 1992). Leeper (1992) specifically states that there are 2 PI's (*S. pimpinellifolium*) with noted resistance, however, they were not specifically used in the breeding program. The original source of resistance was unknown but following research from Mohr (1955) was shown that the method of incurred resistance was cited as secondary stem thickening as the plant matured which

provided a greater barrier against the pathogen. To evaluate disease resistance, plants were grown under greenhouse conditions in Auburn, AL.

These eight lines plus two susceptible controls were organized in a RCBD with two plants of each line per block with four replicates repeated 3 times in Aug, Sept, and Oct 2016. One isolate of *S. rolfsii* was used to inoculate half of the plants in each block when the plants were 8-weeks-old. The plants were evaluated and graded on a 0-5 scale over the next 7-10 days. All plants displayed signs of infection three to four days after inoculation. Greenhouse screening resulted in 100% disease pressure in an environment that favored the disease. Statistical evaluation using R software showed that although the disease progressed differently among the eight lines and controls, the results were the same for each line. No significant difference in resistance was observed among the lines screened. *5635M*, *5876M*, PI 126432, *5635M*, *5719M* performed significantly worse than the interspecific rootstocks *Maxifort* and *Multifort*, which had the overall lowest disease rating. The intraspecific rootstocks showed the greatest resistance to the greenhouse screening. Additional research should be conducted under field conditions to evaluate disease progression as past research with these six lines showed resistance to Southern blight under field conditions.

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Chapter 1: Literature Review

Introduction

Sclerotium rolfsii is a soil-borne fungus that is causal agent of the disease Southern blight. Over 500 plant species from most dicot families show susceptibility to *S. rolfsii* making this disease a serious horticultural and economic issue (Mullen, 2001). Disease caused by *S. rolfsii* is common in the tropics and subtropics on every continent. In the southeastern U.S., temperatures are sufficiently high to permit the growth and survival of the fungus (Aycock, 1966). Despite continuous research over more than 100 years since the pathogen was first identified on tomato by Rolfs in 1892 (Punja, 1985), this pathogen continues to plague growers and cause economic loss (Bowen et al., 1992). Combining the aggressive nature of the pathogen with its wide host range makes controlling the disease difficult (Punja, 1985). Chemical controls are expensive and are commonly species and geographic location specific (Chellemi et al., 1997). With the identification of tomato germplasm with resistance to *S. rolfsii*, grafting might provide a possible solution allowing growers to utilize disease resistance in rootstocks as a non-chemical control method. To create possible control solutions, however, the disease needs to be fully understood.

Literature Review

Sclerotium rolfsii

S. rolfsii is a destructive soil-inhabiting fungus found throughout much of the U.S. A wide range of cultivated and wild plants are susceptible to this disease. Southern blight is a common cause of decay and rot in stored roots, vegetables, and other produce that rest upon the ground (Aycock, 1961). In the field, once plants are infected they are usually killed. In

many disease screening reports such as in Bowen (1992), data is gathered through evaluation of disease incidence, not on a disease severity scale.

The main visible symptom of Southern blight is vascular wilt but unlike the wilt producing fungal pathogen, *Fusarium*, the attack of *S. rolfsii* may be irregular (in no pattern) (Aycock, 1961; Punja, 1985). In an infected area, the fungus may be present and the range of infection can vary drastically from losing a few plants to losses as high as 100% (Higgins, 1927; Bowen et al., 1992; Fery and Dukes, 2011). In soil containing high disease pressure, every plant may be killed. In some cases, susceptible plants have been grown in areas with considerable amounts of sclerotia, bundles of hyphal cells that form the survival structures of the fungus, with no loss (Higgins, 1927). This could be attributed to environmental conditions that do not favor pathogen growth. To describe the differences of infection, the physiology and parasitism of the fungus were reviewed to understand how the pathogen initiates and continues infection cycles throughout the growing season.

S. rolfsii infects herbaceous plants, woody plants, and fleshy roots, bulbs, and fruits (Higgins, 1927; Aycock, 1961). *S. rolfsii* can have an irregular infection pattern in the field. Identification of potential "hotspots" for infection are common and synonymous with cultural control (Bowen et al., 1992; Hagan, 1999; Punja, 1985). Control strategies such as chemical spray programs, debris removal from fields, plowing and crop rotation all help with reduced disease incidence. In identifying possible areas of infection, the symptoms and signs of the disease need to be understood.

S. rolfsii infects the lower stem near or at the soil surface, but it can also infect other susceptible parts of the plant near or touching the soil. Early damage might not be visible until

the disease has progressed through several stages of the lifecycle (Mohr, 1955; Phatak et al., 1983). Once visible symptoms are presented the stem is quickly girdled and the tissue rots. Symptoms of the disease vary from damping off in seedlings to vascular wilt in mature plants. The following figure displays a mature *Rutgers* tomato (*Solanum lycopersicum*) infected by the disease (Fig. 1A). The first noticeable symptom of Southern blight is vascular wilt. Upon closer inspection, mycelial growth is present on the surface of the growing media (Fig. 1B).

Fig. 1A ***Fig. 1B***

Figure 1. *A) Image of mature 'Rutgers' tomato (Solanum lycopersicum) that is in advanced stage of infection from Southern blight. Wilting of foliage is severe and upon closer inspection, mycelial growth is present at stem line B) Fungus on 5635 processing tomato breeding line.*

Wilting typically occurs before any sign of the disease is present such as mycelium or sclerotia (Mohr et al., 1959; Rivard et al., 2008; Rivard et al., 2010). As the plant wilts, small water-soaked lesions form on the lower stem at the soil line (Mullen, 2001). The lesions will spread to completely girdle the stem and the plants lodge with older foliage turning chlorotic or necrotic and falling off the plant. Lower sections of the stem often rot with cortical tissues

rotting several centimeters above and below the soil line (Mullen, 2001). As the stem decays a white mat of fuzzy mycelium forms on the lower stem and spreads across the surface of the soil. Shortly after the mycelium develops, small white sclerotia develop from bunched hyphal cells. As the sclerotia mature, they become smooth and harden while they obtain a tan to black pigmented rind. Sclerotia are the main survival structures of the fungus and they can be disseminated by water movement, mechanical practices (tillage), and by animals.

Physiology

Isolates of *S. rolfsii* have been collected from diverse regions around the world such as fallow soils, mangrove mud, and poorly drained soils (Canullo et al., 1992). These diverse regions suggest the fungus can develop and survive in many different environments, however, poor development has been recorded in fine-textured soils (Gondo, 1962). High pH soils slow *S. rolfsii* development while acidic conditions favor its growth (Higgins, 1927). *S. rolfsii* is a saprophytic parasite surviving on living tissue and organic matter in the soil (Mordue, 1974). Colonies of the fungus are fast growing reaching about 9 cm in diameter after three days at 23^{o} C (Mordue, 1974).

During the initial growth stages, white mycelium is produced from three types of hyphal cells visible under a light microscope. Primary conductive hyphal strands are colorless and relatively large, 5-9 µm wide, with infrequent cross walls and clamp connections (Chet and Henis, 1967). Secondary and tertiary hyphal strands are narrower, 1-2 µm wide, which arise from and penetrate plant tissue (Chet and Henis, 1967). A week into growth the hyphae begin to form sclerotia. Sclerotia are produced near the colony's margin and are initially fuzzy from the individual hyphal cells bunching together. As the sclerotia mature they harden and become

smooth with a pigmented thickened rind (Mullen, 2001). Sclerotia range from 0.5-2 mm in size and consist of a thick outer rind, which surrounds a cortex of thin walled cells. The center of the sclerotia contains loosely arranged filamentous hyphae (Chet et al., 1969). The principal function of sclerotia is survival over long periods of low food supply and unfavorable environmental conditions.

S. rolfsii can overwinter as mycelium in plant tissue or in plant debris but most commonly survives as sclerotia in the soil. Sclerotia can persist for up to 20 years in areas that have mild winters. Studies have shown that sclerotia can survive being buried 15 cm to 30 cm below the surface of the soil (Elad et al., 1980; Goldschmidt, 2014; Punja, 1985).

Sclerotia germination occurs in two separate ways, hyphal and eruptive (Higgins, 1927). Hyphal germination is the growth of individual cells from the surface of the sclerotium. These hyphal cells originate from the medulla of the cortex layer but their growth is not extensive unless there is an external source of nutrients (Higgins, 1927). In contrast, eruptive germination is characterized by aggregates of mycelium breaking through the rind of the sclerotia (Higgins, 1927). Eruptive germination uses the internal nutrients of the sclerotia to stimulate growth of the mycelium. Germination is induced by moisture level, temperature, and in some cases exposure to volatile compounds, specifically alcohols and aldehydes (Chet et al., 1967). Mature sclerotia germinate readily and have a brief, if any, required dormancy period. Both the amount of mycelial growth and the energy available for infection are affected by germination. Thus, germination type has a significant impact on infection and disease incidence (Chet et al., 1969).

Once germination occurs, the progression of the disease depends upon environmental factors such as temperature, pH, moisture, and nutrient availability. The fungus grows slowly at temperatures below 20^{o} C and growth terminates at temperatures of more than 40^{o} C (Singh et al., 1991). The optimal temperature for growth occurs between 32^{o}-37^{o} C which correlates well to the sub-tropic and tropic home of the disease. Soil pH is also significant in the development of the disease. For example, soils high in organic matter with an acidic pH between 2 and 6 sustain growth of the fungus (Higgins, 1927). The fungus does not grow on cultures more basic than 8.3 or more acidic than 1.4 (Higgins, 1927). This wide pH range for fungal development overlaps with the native pH's of most soils in the southeastern U.S. providing one of the key factors needed to stimulate and sustain growth of *S. rolfsii*.

Soil moisture and nutrient content effect fungal development. The effect of solute water potential on mycelial growth is difficult to interpret (Punja, 1985). In various situations, soil moisture is a complex tool for growth because it depends on the solute potential of the soil (Punja, 1985). Inconclusive research has shown decreased growth with increased moisture content and increased growth with well-drained sandy soils (Punja, 1985). Other research has shown however, increased disease activity in soils with high silt and moisture content (Punja, 1985). Unlike temperature and pH, the effect of soil moisture is more challenging to explain with soil factors and other environmental factors having a significant impact on hyphal growth.

S. rolfsii grows on a remarkably wide array of carbon sources (Punja, 1985). The fungus can use numerous organic and inorganic nitrogen compounds for growth (Punja, 1985). Research has showed that the fungus can use various sources of carbohydrates and organic acids for growth with lactose and glycerin being the worst and citric acid being one of the best

(Higgins, 1927). Using different sources for growth changes the metabolic products produced by *S. rolfsii*. This in turn alters how aggressive a pathogen attacks plants. Incorporating these factors: temperature, pH, soil moisture, and nutrients all significantly change the way the fungus grows and how it affects potential hosts.

Although the environment can affect the rate of infection, the mechanism by which the fungus attacks host plants is the same. The hyphal strands of *S. rolfsii* cannot directly penetrate the exterior living epidermal cells of the plant (Edmunds et al., 2003). Research has shown that *S. rolfsii* produces high amounts of organic acids, specifically oxalic acid (Higgins, 1927). Oxalic acid is toxic to plant cells and the fungus uses it to create entry points into epidermal cells. High amounts of this acid are produced as a metabolic by-product and its toxicity kills the outer plant cells. Once the epidermal plant cells are dead, the hyphal strands can enter and use the remaining nutrients of the dead cells to sustain its growth, producing secondary and tertiary hyphal strands. Research on the parasitism of the fungus found high amounts of oxalate in dead cells before the fungus hyphae entered (Higgins, 1927). Understanding this mechanism for attack helps to explain the prolific host range of the fungus. Any plant that has epidermal tissue that can be permeated by organic acid solutions is susceptible to this disease (Mordue, 1974).

Disease Control

Control of *S. rolfsii* has often been met with limited success, due in part, to the extensive host range, prolific growth, disease mechanism, and Southern blight's ability to produce large numbers of sclerotia (Elad et al., 1980; Goldschmidt, 2014). Furthermore, control measures effective for a crop or geographical area may not be adaptable elsewhere due to regulatory or

economic constraints. Control strategies have varied with the large host range of the fungus. Chemical controls are most commonly utilized in the agricultural industry for Southern blight management.

Dating back at least 30 years, Southern blight was managed using soil fumigation with methyl bromide, chloropicrin, or metam-sodium (Elad et al., 1980). Methyl Bromide was the main means of control of *S. rolfsii* as a soil fumigant until the global ban of the chemical went into effect for developed countries in 2005 per the Montreal Protocol (Garner, 2013). Methyl Bromide was a biocide used to control weeds, insects, and diseases with the ability to kill a large group of organisms. Current fumigant and fumigant combinations used to manage soil-borne diseases of vegetables are listed in the table below (Table 1).

Table 1. *Efficiency of Chemical Fumigants for Managing Soilborne Diseases*

Efficiency of Fumigants of Fumigant Combinations for Managing Soilborne Disease Pressure			
Scale ranges from "0" = not effective to "5" = highly effective			
	Rate per treated Acre[3]		
Product	**Volume (gal)**	**Weight (lb)**	**Disease[1]**
Telone II (1,3-D)	15 to 27	153 to 275	1
Telone EC[3]	9 to 24	91 to 242	1
Telone C17 (1,3-D + chloropicrin)	32.4 to 42	343 to 445	3
Telone C35 (1,3-D + chloropicrin)	39 to 50	437 to 560	5
InLine (1,3-D + chloropicrin)[3]	29 to 57.6 (See Label)	325 to 645 (See Label)	5
Pic-Clor 60 (chloropicrin + 1,3-D)	48.6	588	5
Pic-Clor 60 EC[3]	42.6	503	5
Metam Potassium	30 to 62	318 to 657	3
Metam Sodium (MS)	37.5 to 75	379 to 758	3
Chloropicrin + MS	19.5 to 31.5 + 37.5 to 75	275 to 444 + 379 to 758	5
Chloropicrin	48	150 to 350	5
Tri-Pic 100EC[3]	8 to 24	100 to 300	5
Paladin (dimethyl disulphide)	35 to 51.3	310 to 455	4
Paladin EC[3]	37 to 54.2	326 to 479	4
Dominus (ally isothiocyanate)[4]	25 to 40	212 to 340	3

[1] Soilborne disease in general, not specifically effectiveness against Southern blight
[2] Rates can sometimes be reduced if products are applied with VIF or TIF
[3] Product is formulated for application through drip lines under plastic mulch; efficiency is dependent on good distribution through bed
[4] Dominus is registered but there is limited experience with the product through University or independent trials in the Southeastern U.S; growers might want to use this as experimental

(*adapted from Kemble et al., 2017*)

Although these chemicals cannot be applied in all cases, they provide a basis for varying levels of management of Southern blight so that other chemicals can be used in conjunction with these to slow the germination of sclerotia and mycelium (Elad et al., 1980). From Table 1, all chemicals/combinations of fumigants, except Telone II and Telone EC, provide acceptable (3) or greater disease management of soilborne diseases. These chemicals rarely work as stand-alone solutions but instead are used in mixtures to provide management or suppression of diseases and are generally applied days to weeks before planting (Mullen, 2001). Although these chemicals can provide efficient management, they are expensive and often require specialized machinery for their application (Hagan and Olive, 1999). Varying levels of effectiveness of currently labeled fumigants, costs, and increased improvements in farming methods such as using plasticulture, which creates an ideal growing environment for the *S. rolfsii*; managing Southern blight is still a major concern as currently labeled fumigants are not completely effective.

With *S. rolfsii's* wide host range, chemicals are specifically designed to suppress the pathogen on one or a limited number of susceptible plant species. Several fungicides exist that can be used to help manage this disease (Table 2). Table 2 lists fungicides that are labeled for management of Southern blight on tomato (*S. lycopersicum*) in the Southeastern U.S. Table 2 provides information to help growers with suggested rates, application methods, and scheduling use of these fungicides.

Table 2. *Chemical Control for Southern blight in Tomatoes*

Chemical Management for Southern blight in Tomatoes					
Material	**FRAC**	**Rate**	**Minimum Days**		**Details**
			Harvest	**Reentry**	
Difenoconazole + benzovindiflupyr (Aprovia Top)	7 + 3	10.5 to 13.5 fl oz/ acre	0	0.5	No more than 2 applications before changing to non-Group 7 fungicide
Fluoxastrobin (Aftershock, Evito 480 SC)	11	2.0 to 5.7 fl oz/acre	3	0.5	Begin applications when environment favors disease, 7-10 day intervals. Do not apply more than once without rotation of MOA; No more than 22.8 fl oz per acre/season
Fluxapyroxad + pyraclostrobin (Priaxor 500SC)	7	4 to 8 fl oz/acre	0	0.5	**Disease Suppression Only**. Do not apply more than once without rotation of MOA; No more than 24 fl oz per acre/season
PCNB (blocker 4F) (transplanting)	14	4.5 to 7.5 pt/100 gal; 0.5 pt solution/plant	N/A	0.5	Transplanting: Apply a time of transplant for **disease suppression**. Stir mixture often for uniform dispersal; No more than 7.5 lb a.i. per acre/season
PCNB (blocker 4F) (in furrow)	14	1.2 to 1.9 gal; 10.6 to 16.7 fl oz/1000 ft row	N/A	0.5	Apply in 8 to 10 gals of water per acre (36" row spacing). Apply as in-furrow sprays to the open "V" trench prior to planting. No more than 7.5 lb a.i. per acre/season
Penthiopyrad (Fontelis 1.67SC)	7	1 to 1.6 fl oz/ 1000 ft row	N/A	0.5	Apply as a soil drench to seedling trays or at time of transplant
Pyraclostrobin (Cabrio 20EG)	11	12 to 16/ fl oz/acre	0	4 hours	**Disease Suppression Only**. Do not apply more than once without rotation of MOA; No more than 96 fl oz per acre/season

(*adapted from Kemble et al., 2017*)

From Table 2, fluoxastrobin (Aftershock) can be used to manage Southern blight in tomatoes (*S. lycopersicum*) (Kemble et al., 2017) and peppers (*Capsicum annuum*) (Culbreath et al., 2009). Pentachloronitrobenzene (PCNB) can effectively limit disease incidence when applied prior to planting (in furrow) and is registered for use on multiple vegetable crops (Kemble et al., 2017; Culbreath et al., 2009; Woodward et al., 2007). Some chemicals such as pyraclostrobin (Cabrio), only suppress Southern blight and can be ineffective if disease pressure

is severe (Kemble et al., 2017). Labels must be checked to ensure that the chemical has been approved for the crop of interest as well as being approved for the geographical location of its intended use. Chemical controls can manage Southern blight, however, environmental conditions and physical management can result is significant differences in successful use of these chemicals. Chemical applications must be organized and their use rotated based on their modes of action (MOA's) (Kemble et al., 2017). Rotating MOA's is critical in preventing the development of resistant population of pathogens (Hagan and Olive, 1999).

Although no chemical resistant strains of this fungus have been reported, the necessity to avoid patterns in chemical usage are important. Because *S. rolfsii* sclerotia can persist in the soil for as long as 20 years, tolerance to the chemicals used will likely build up over repeated exposures. With chemical control being a short-term treatment, other more long-term management strategies are desirable.

Crop rotation for Southern blight management is a planned physical strategy that has been shown to reduce disease pressure from nematodes and certain bacterial and fungal pathogens (Elad et al., 1980; Bowen et al., 1992; Grecher, 1995; Bulluck and Ristaino, 2002). Rotating plant species in field production allows for natural suppression of a disease by reducing the pathogen's exposure to a susceptible host, which limits the pathogen's ability to survive and reproduce. By alternating susceptible and resistance species of plants, disease pressure can be reduced through good planning and field management (Larkin et al., 2016).

In the case of *S. rolfsii* most dicots are susceptible. Short-term rotations have limited effectiveness because sclerotia can persist in the soil. The average rotation cycle is 3-4 years with growers rotating between families of crops (Kemble et al., 2016; Kemble et al., 2017). For

example, a grower might plant tomatoes (*S. lycopersicum*) this season and then over the next four years rotate between corn (*Zea mays*) and wheat (*Triticum* spp) before planting watermelon (*Citrullus lanatus*) or another member of the Nightshade family (*Solanaceae*) (Kemble et al., 2009). To increase the effectiveness of shorter rotations, a plan should involve growing monocots (grasses) regularly or more ancient types of plants such as turmeric or ginger that have shown less disease susceptibility to Southern blight (Punja, 1985; J.M. Kemble, personal communication). This sample rotation could be effective for management of Southern blight, however this might not be economically viable.

In conjunction with crop rotation, other physical strategies such as deep plowing of crop residues, conservation tillage, and debris removal are all used to limit soilborne disease pressure (Larkin et al., 2016). By the removal of crop residues before plowing, many soilborne diseases can be more effectively managed and limited (Flanders et al., 2016). This strategy can help to limit sclerotia of *S. rolfsii* as well. The saprophytic nature of the disease, however, allows it to survive on organic matter that is buried in the soil for an extended time. Using deep plowing can provide some temporary relief by burying the sclerotia. Since *S. rolfsii* can survive 20 years or more in the soil, however, repetitive plowing will eventually bring sclerotia back to the surface (Punja, 1985). Continuous management by plowing will increase the amount of sclerotia by burying them instead of limiting their presence. Although physical strategies provide some temporary relief, these techniques will eventually lead to increased problems without the intervention of other management strategies (Larkin et al., 2016). Adding genetically resistant plants to any management system gives growers another technique to help manage this disease.

Genetic resistance is another method that growers can use to obtain various levels of control to specific biotic and abiotic disorders without relying on chemicals (Latunde-Dada, 1993). With resistance, the plant withstands level of infection, and still produces at an acceptable level. The practically of genetic resistance depends on whether the trait for resistance is a single gene or multigenic. Single genes are generally simply inherited traits influenced by genes at one or few loci (Fery and Dukes, 2011). There are several single genes that are commonly used in tomato cultivar development such as "Ve1" gene for resistance to Verticillium species race 2 and "Mi" gene for resistance to *Meloidogyne javanica* (Acquaah, 2012). At present, no single genes have been identified for resistance to *S. rolfsii*.

Multigenic traits are affected by genes at many loci. Each gene has only a small effect on the specific trait, and the overall trait can be significantly affected by the environment (Fery and Dukes, 2011). Traits such as "salt tolerance" and "heat tolerance" (associated with the ability to set fruit under super optimal temperatures) are complicated and controlled by several genes.

Current long-term control using genetic resistance is effective for many diseases but there are several drawbacks. The primary problem is identifying germplasm with a useful level of resistance. Once identified, research needs to be conducted to determine whether this resistance is a single gene or multigenic. Next, this resistance needs to be incorporated into commercially acceptable cultivars. In a typical vegetable breeding program, it takes anywhere from 6-7 years to develop a new cultivar (Punja, 2005). One possible solution to circumvent the extended period needed to develop resistant cultivars is by using vegetable grafting, and the key to this is identifying resistant tomato lines to use as a rootstock.

In 1992, Leeper and others released six processing tomato breeding lines with resistance to *S. rolfsii* but there is no indication as to the number of genes involved with their resistance (Leeper et al., 1992). Leeper (1992) specifically stated that there are two *plant introductions* (PI's) of a wild type tomato (*S. pimpinellifolium*) with noted resistance, however, these were not specifically used in the breeding program that produced the processing lines he released. This source of resistance, however, provides an opportunity for later integration.

The integration of sources of resistance into desirable cultivars is a long-term management practice (Cushman, 2006). In the short-term, these processing tomato lines resistant to *S. rolfsii* could be used as rootstocks to manage Southern blight by combining a desirable cultivar without resistance with these resistant rootstocks (Garner, 2013). Using these rootstocks with desirable scions is a direct disease management technique that can circumvent, and in some cases, enhance current management options for Southern blight. The combination of multiple strategies for management of this disease is the best approach in the short and long term.

In this specific case, the mechanism of resistance to Southern blight has been associated with secondary stem thickening as the plant ages (Mohr, 1955; Mohr et al., 1959). As the plant matures, resistance develops from precocious development of secondary tissue on the basal meristem. This results in an overlapping phellem barrier that is five to six cells deep. This thickening creates a physical barrier that the fungus cannot penetrate. The oxalic acid produced by the fungus cannot breakdown the thicker layers of tissue and the plant resists the pathogen. Other mechanisms of resistance must exist, such as how monocots like corn (*Z. mays*) defend against *S. rolfsii*. It is less clear why monocots, specifically corn (*Z. mays*), is

immune to the disease, but it is different from secondary stem thickening. This mechanism is a physical defense that can be used in cultural management techniques such as grafting without any breeding required.

Grafted vegetables provide an opportunity for a more simplified and immediate answer to disease management. Grafting allows plants to maximize nutrient and water uptake as well as adapt to changing soil conditions (Cushman, 2006; Garner, 2013; Goldschmidt, 2014; Lee, 1994). Grafted vegetables are also used for resistance to biotic stresses such as disease and insect pests (Garner, 2013; Goldschmidt, 2014; Kubota et al., 2008; Lee and Oda, 1994). Grafted vegetables have been also shown to increase yield (Cushman, 2006; Garner, 2013; Goldschmidt, 2014; Lee, 1994).

Vegetable Grafting: Past, Present and Future

Vegetable grafting of herbaceous seedlings is a technique that has been practiced throughout history. Grafting, in the horticultural sense, is combining a piece of plant tissue (scion) with an established plant (stock), which supports and nourishes the resulting plant. From this definition, a grafted vegetable seedling is a one-time hybrid between two selected plants to emphasize benefits from both the rootstock and the scion.

Vegetable grafting was described in an ancient book written in China in the 5th century (Lee et al., 2003). The first account of interspecific grafting to increase yield and minimize pests/disease was for watermelon (*Citrullus lanatus*) grafted onto a squash rootstock (*Cucurbita moschata*) in Japan (Tateishi, 1927). This technique passed throughout most of Asia through research programs in Japan and Korea in the late 1920's and early 1930's. The first recorded grafting experiment of the Solanaceae family did not occur until the 1950's. Eggplant (*Solanum*

melongena) was grafted onto Scarlet eggplant (*Solanum integrifolium*) for its ability to effectively grow in soils with high levels of salinity (Oda, 1999).

Following eggplant *(S. melongena)* in the Solanaceae was the grafting of tomatoes (*S. lycopersicum*) in the late 1960's (Lee et al., 2003). The use of grafted vegetables continues to increase due to the benefit of disease resistance and the ability of grafted vegetables to grow more efficiently under adverse soil conditions (Kubota et al., 2008). By the 1990's in Japan, it was reported that nearly 60% of all open field and greenhouse production of melons (*Cucumis melo*), cucumbers (*Cucumis sativus*), tomatoes (*S. lycopersicum*), and eggplants (*S. melongena*) relied on using grafted seedlings (Lee, 1994). Data reported in 1990 showed South Korea producing 81% of their open field and greenhouse vegetable production using grafted vegetable seedlings (Lee, 1994).

Despite the increased costs from grafting, by the early 2000's over 500 million grafted seedlings were produced annually in Japan alone (Kobayashi, 2005). The use of grafted vegetables is more commonplace in Asia as compared to North America and Western Europe. In North America and Western Europe, vegetable grafting tends to be reserved for use in greenhouse vegetable production and in specialized field uses.

Although vegetable grafting has been practiced in Asia for 1,500 years it was not until the late 20th century that the technology was introduced to Europe and other countries. As vegetable grafting spread across Europe, it was eventually introduced to North America, South America, and Africa. Some sources state tomato grafting was first practiced in North America in the early 1900's (Lowman et al., 1946). This early account discusses tomatoes (*S. lycopersicum*) being grafted onto Jimson weed (*Datura stramonium*) as the rootstock for its resistance to root-

knot nematodes. This method was used primarily by home gardeners from 1935 to 1943 for tomatoes (*S. lycopersicum*), eggplant (*S. melongena*), and bell pepper (*Capsicum annuum*) (Isbell, 1944). This method was discontinued because of the potential transport of small amounts of alkaloids to the fruit from the rootstock (Lowman et al., 1946). From that point on vegetable grafting was not utilized mainly due to the labor-intense process associated with economically producing enough plants for use in widespread production.

Over the last 10 years, vegetable grafting technology has continued to increase in North America with most plants being used for hydroponic tomato production (Bausher, 2011; Goldschmidt, 2014). Greenhouse tomato production constitutes 90% of the grafted seedling production in North America (Kubota et al., 2008) with the focus of research being on evaluation of rootstock cultivars for increased yield. Solanaceae grafting has dominated North and Central American interests due to the relative ease of grafting procedure and healing methods compared to those required for Cucurbit grafting. Despite the increase in production cost, the benefits provided by grafting were found to offset cost. Higher yields, earlier maturity, and disease resistance conferred by use of vegetable grafting outweighed added initial costs.

The tomato (*S. lycopersicum*), specifically, is a major crop grown in North and Central America with a high demand for grafted vegetable seedlings developed for field production (Kubota et al., 2008). The use of grafted tomatoes for field production is still advancing with the biggest strides taking place in Mexico. After successful trials, tomato growers in Mexico began using grafted seedlings to manage Fusarium wilt race 3 (Kubota et al., 2008).

Although field production and use of grafted plants is increasing in North and Central America several current issues pose major limitations that need to be addressed in future work.

One issue is the large number of possible rootstocks, specifically for tomatoes, that exists (Goldschmidt, 2014). With many possible rootstock cultivars, it is surprising that most seedlings are grafted with only a handful of common choices. In many cases, companies have developed propriety lines that are not available for general use. The changing information on rootstocks for disease resistance, nutrient and water effectiveness, and stress tolerance can make rootstock selection challenging. As a result, propagators and growers must take it upon themselves to experiment to determine which rootstocks work the best.

The other current issues involving vegetable grafting systems are labor and cost. In Asia, the labor cost is not an issue and large grafting establishments have no difficulty producing hundreds of thousands of grafted seedlings annually (Goldschmidt, 2014). In North America, labor cost is a major limiting factor. An experienced grafter can produce anywhere from 300-500 plants an hour depending on the grafting technique and quality of the seedlings (Kubota et al., 2008). At this rate, current labor costs in North America are a major limitation when looking at large scale production facilities (Goldschmidt, 2014). This has resulted in a push to develop machinery and robotics that can complete the grafting process quicker and more efficiently (Kobayashi, 2005). As with most advances in technology the current machines work well and are efficient, however, they are expensive (Kobayashi, 2005). Eventually, as the technology improves and the costs are reduced, automation will take over. These machines will allow propagators to produce thousands of seedlings efficiently and at low cost resulting in a full emergence of grafted vegetables in to the market place over the coming years (Kubota et al., 2008).

How and Why does Grafting Work

There are several different methods that are used currently in grafting operations. The method used depends first on species of plant and then on grafter's preference. The main styles for grafting vegetables are cleft, splice, side, approach, and whole insertion (Lee, 1994; Garner, 2013). Depending on the species being grafted, for example the difference between Cucurbits and Solanaceae crops, the desired grafting style can vary. For Cucurbits: side, approach, and whole insertion are most commonly used with approach grafting as the top selection for its high success rates (Lee, 1994). Approach grafting is a technique in which a notch is cut into the stem of two plants at the same height, making sure not to cut entirely through the stem (Garner, 2013). The two plants are then twisted into each other and held in place with a clip or piece of Parafilm (Cushman, 2006). The plants are given time to heal resulting in them growing together. After healing the undesired rootstock and scion are cut away leaving the resulting grafted plant (Cushman, 2006).

In comparison, grafting Solanaceae crops can be done by cleft or splice method both being common in application (Garner, 2013). Splice grafting is a simple technique in which a diagonal cut (at least 45^0) is made through two plants completely separating the scions from their rootstocks (Bausher, 2011). The scions of each plant are exchanged and placed on the opposite rootstock by matching up the slice angles and holding the connection in place with a clip. The two most important parts to a splice graft are to select plants that have similar stem diameters; and to make the cut angles as similar as possible as this will increase the probability of the graft's survival (Bausher, 2011).

With a basic understanding of the grafting process, it is also important to know what is

physiologically happening to the plant. Success in forming a permanent graft union depends mainly on two factors, plant affinity, also called compatibility, and contact between cambia or other meristematic tissue (Garner, 2013). Graft compatibility can be described as the establishment of a successful graft union as well as the extended survival and proper function of the composite plant (Goldschmidt, 2014). A major factor in compatibility is taxonomic affinity.

"Autografts", meaning a graft between the same individual plants, is presumed always compatible. In "heterografts", broadly speaking, intraspecific grafts (rootstocks and scions belonging to plants from the same botanical species) are nearly always compatible. Interspecific grafts (rootstock and scion belonging to different species of the same genus) are usually compatible, while intrafamilial grafts are rarely compatible and interfamilial grafts are essentially incompatible (Goldschmidt, 2014).

Physiologically speaking a successful graft is the establishment of connected vascular tissues between the rootstock and scion (Aloni et al., 2008; Goldschmidt, 2014). Vascular elements are regenerated by cell differentiation of parenchymatous tissue into xylem and phloem (Aloni et al., 2008). Anatomical changes also occur with the formation of necrotic tissue as a 'scar' forms to protect the internal tissue before a full connection is made (Aloni et al., 2008). The connection of the scion and rootstock occurs from the development of callus cells at the graft location before vascular differentiation can occur (Aloni et al., 2008). Once differentiation begins, vascular tissues are formed and the graft interface is breached from both sides allowing for a connection to be made and the union can heal. The difficulty associated with grafting comes not from the actual process but on how well the desired plants interact.

As of yet there is no evidence for a specific biochemical-immunological recognition/rejection mechanism between graft components in plants (Goldschmidt, 2014). In heterograft compatibility, however, chances of a successful graft union decreases with genetic distance of the components indicating the presence of a physiological mechanism for rejection of some kind (Aloni et al., 2008). Yet, at the same time, incompatibility occurs even among related genera of the same family (Kubota et al., 2008). Within the Solanaceae family, reciprocal grafts of tomato (*S. lycopersicum*) and bell pepper (*C. annuum*) are considered severely incompatible whereas tomato and eggplant (*S. melongena*) are only moderately compatible (Lee, 1994; Kubota et al., 2008). In terms of understanding degrees of compatibility, it can be viewed as all varieties of gray, with some working very well, others completely failing, and many combinations somewhere in between.

A common observation of graft failure is the disturbance of vascular element orientation. The induced-wound of grafting can result in changes to the normal flow of endogenous auxin, which plays a key role in vascular cell differentiation (Yin et al., 2012). Auxin is present and involved in early stage graft union formation of tissue before xylem fibers can bridge the graft interface to form successful connections.

Other grafting effects can be viewed better from a broader context with root-top relations. This root-top relation refers to the observed effect that using invigorating expansive rootstocks have on their grafted scions (Martinez-Ballesta et al., 2010). Vigorous rootstocks are chosen for their ability to efficiently take up water and nutrients, however, they can also negatively impact their new scions. Root-top interactions in a grafted plant are complex because they undergo a drastic change in union/healing process followed by lifelong

interactions between different genomes (Martinez-Ballesta et al., 2010). In most cases, unfavorable grafts result in the buildup of carbohydrates, starch, or even biochemical products that slowly 'poison' the composite plant (Martinez-Ballesta et al., 2010). The general 'poisoning' mechanism is an impairment of phloem transport at or around the graft location resulting in no vascular connection and the plant dies (Moing et al., 1990; Martinez-Ballesta et al., 2010).

In Summary

Sclerotium rolfsii is a soil-borne fungus responsible for the disease Southern blight. The disease caused by *S. rolfsii* is common in the tropics, subtropics and on every continent including the southeastern U.S. In the southeastern US, temperatures are sufficiently high to permit the growth and survival of the fungus (Aycock, 1966). Combining the aggressive nature of the pathogen with its wide host range makes control methods difficult (Punja, 1985). Chemical controls are expensive and are commonly species and geographic location specific (Chellemi et al., 1997). Chemical usage for Southern blight management depends on the varying modes of action (MOA) from the chemistry applied. The more specific a mode of action for a chemical, however, the faster the pathogen gains resistance (Hagan and Olive, 1999).

To avoid creating resistant strains of the fungus growers must alternate between classes of chemicals. Although no chemical resistant strains of this fungus have been reported, the necessity to avoid patterns in chemical usage are important. Because *S. rolfsii* sclerotia can persist in the soil for as long as 20 years, tolerance to the chemicals used will likely build up over repeated exposures. With chemical control being a short-term treatment other more long-term management strategies are desirable. With the identification of tomato germplasm

with resistance to *S. rolfsii*, grafting may provide a possible solution that allows growers to utilize disease resistance in rootstocks as a non-chemical control method.

In a long-term management system, disease resistance through breeding is the most effective strategy available. Although problems with breeding can result when money, time, and politics are considered. Breeding is expensive and time-consuming requiring dedicated resources before any possible sources of resistance are advanced into commercially acceptable cultivars. Considering these problems, vegetable grafting provides a research opportunity for a more rapid adaptation of a cultural management technique. Vegetable grafting provides a cultural management technique that allows growers to quickly utilize sources of disease resistance when paired with desirable scions. This experimental research considered advanced rootstocks for grafting for management of Southern blight.

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Chapter 2: Disease Severity Screening and Graft Compatibility

Introduction

Sclerotium rolfsii is a soil-borne fungus responsible for the disease Southern blight. Over 500 plant species, mostly dicots have shown to be susceptible to this disease. Southern blight is a serious horticultural and economic issue. Southern blight is common in the tropics and subtropics of countries on every continent including the southeastern U.S. In the southeastern U.S., temperatures are sufficiently high to permit the growth and survival of *S. rolfsii* (Aycock, 1966). Despite continued research over more than 100 years since the pathogen was first identified on tomato (*S. lycopersicum*) by Rolfs in 1892 (Punja, 1985), this pathogen continues to plague growers and cause economic loss. Combining the aggressive nature of the pathogen with its wide host range makes control methods difficult. Chemical controls are expensive and are commonly species and geographic location specific. Genetic resistance is currently not available in any commercial cultivars, however, breeding lines have been developed with reported resistance to *S. rolfsii*. Using these lines, grafting might provide a potential tool in managing Southern blight in tomatoes.

Grafting of vegetable seedlings is a horticultural technology that involves creating a one-time hybrid between two selected specimens, allowing the grower to take advantage of multiple qualities specific to either plant. This hybrid is the result of selecting two plants that distinguish themselves for different reasons. In rootstocks, the desired characteristics involve the ability to express biotic resistance to pests and diseases, develop vigorous root systems, maximized water and nutrient up take, and manage abiotic stresses such as soil quality and salinity. In scions, desired characteristics focus on fruit quality and yield, leaf area and pest

resistance. In combining the selected plants through grafting, a one-time hybrid is created with no genetic modification that can effectively grow in spite of abiotic and biotic stresses while still producing an acceptable mature yield.

Materials and Methods

Overview

Several steps were conducted to complete this research. Initially, isolates of *S. rolfsii* were collected from several locations around the U.S. Isolate identity was then confirmed using Polymerase chain reaction (PCR). Next, the isolates were used to infect *Rutgers* tomato plants (*S. lycopersicum*), a variety susceptible to *S. rolfsii*, to identify the virulence of each isolate. Virulence testing was analyzed by visual evaluation and the most aggressive isolate was selected. At the same time germplasm with reported resistance to *S. rolfsii* were collected and a seed number increase was done for the experimental processing tomato breeding lines (*S. lycopersicum*) and two *plant introductions* (PI's) from wild type tomatoes (*S. pimpinellifolium*) obtained from the USDA. The details of the seed increase are included in Appendix 1. After isolate selection, screening of the experimental germplasm was conducted for susceptibility or resistance to *S. rolfsii*. Following disease screening, the experimental lines and sources of resistance were used in vegetable grafting to determine graft compatibility between rootstocks and selected scions. All the following controlled environment research was conducted in the Plant Science Research Complex in Auburn, AL at 32.609^0 N, 85.480^0 W.

Sclerotium rolfsii isolates

For this experiment, several isolates of the fungus were collected from Alabama, Georgia, California, and New York. Fourteen isolates were received and PCR conducted to

confirm purity and identity of each isolate resulting in 10 positive isolate identifications of *S. rolfsii*.

After confirmation of isolate by PCR, virulence testing was started on 1 March 2016 when 30 *Rutgers* tomato plants were 6-weeks-old. Seed were sown on 19 January 2016 in 48-cell trays (cell dimensions: 3.8 cm x 3.8 cm x 6 cm) using a peat-based substrate, Faford 3B (Sungro Horticulture, MA) and grown in the greenhouse under normal light with the temperature set at 28^0 C day/22^0 C night. The seedlings were transplanted into 15.25 cm (1.33 L) greenhouse pots three weeks after seeding. Plants were fertilized with 200 ppm nitrogen from 20-10-20 twice a week starting at two weeks of age for four weeks before inoculation with *S. rolfsii*. The 20-10-20 fertilizer contained 20% nitrogen from 8% ammoniacial nitrogen and 12% nitrate nitrogen, 10% phosphate from P_2O_5, and 20% potassium from soluble potash (K_2O). Three plants were inoculated with each of the 10 *S. rolfsii* isolates by using infected oats (*Avena sativa*) in a method that will be described in the next section. Inoculated plants were arranged in a CRD and placed back into a greenhouse under normal light with temperatures set at 28^0C day/22^0C night. The plants were placed on Redi-Heat Heavy Duty heated propagation mats (Phytotronics Inc., MO). The virulence of the isolates was evaluated with the mats set at 32^0 C to mimic the disease progression at the planned experimental temperature. The plants were visually evaluated to determine which isolates progressed most rapidly. Evaluations were done by comparing wilting, stem girdling, and cortex rotting.

Inoculation Technique

Several inoculation techniques were previously tested to determine the most effective method of infection (Mohr, 1955). It was important to use an inoculation technique that gave

close to 100% infection rate as to eliminate the variable of infection development. Mohr showed that the most effective method was using *S. rolfsii*-infected oats. With this method >99% infection rate occurred on the tested plants in this trial (data not shown). This method involved soaking the oats in water overnight then autoclaving at 137.9 kPa and 93.3^0 C for 35 minutes (Mohr, 1955). The oats were then mixed with mycelium from 10 individual cultures of *S. rolfsii*, covered, and placed in a dark incubator with a temperature of 30^0 C and 85% RH for 48 hours. After removing the oats from the incubator, they were mixed daily for the next three days while the fungus continued to grow. After five days, the oats were completely covered with mycelium and ready as inoculum.

Disease Screening

An experiment was conducted to evaluate six lines of tomato (*S. lycopersicum*) and two wild type near relatives (*S. pimpinellifolium)* of tomato (*S. lycopersicum*) for resistance to *S. rolfsii.* Of these eight, six processing tomato lines from Texas A&M University: *5635M, 5707M, 5719M, 5737M, 5876M, and 5913M* were selected due to reported resistance to *S. rolfsii*. Development of these lines started in the late 1950's and was completed nearly 40 years later (Mohr, 1955; Leeper et al., 1992). These lines were bred originally for heat tolerance in processing tomatoes while subsequently inheriting Southern blight resistance. The original source of their resistance to *S. rolfsii* was unknown. Four other lines: *Rutgers*, *Celebrity,* and two intraspecific rootstocks *Maxifort* and *Multifort,* were included in the study. Of these four selections, *Rutgers* and *Celebrity* were susceptible controls and *Maxifort* and *Multifort* were common intraspecific grafting rootstocks. The final two lines were *PI 126932* and *PI 126432* (*S.*

pimpinellifolium) both with reported resistance to *S. rolfsii* (Leeper et al., 1992). To evaluate disease resistance, plants were grown under greenhouse conditions.

The selected lines were grown in a peat-based substrate, Faford 3B (Sungro Horticulture, MA), in a greenhouse. Plants were seeded into 48 cell flats, (cell dimensions: 3.8 cm x 3.8 cm x 6 cm), and grown under normal greenhouse conditions with no supplemental lighting. Daily temperature settings were 31^0 C day/21^0 C night. Plants were watered daily until substrate was saturated and about 10% volume drained out and fertilized with 150 ppm nitrogen from 20-10-20 every third day starting one week after seeding. Seedlings were transplanted into standard 15.25 cm pots (1.33 L) four weeks after seeding and the fertilizer rate was increased to 200 ppm nitrogen from 20-10-20 every third day. Plants were grown until 8-weeks-old allowing for development of secondary stem tissue before inoculation.

Screening was done using *S. rolfsii*-inoculated oats as described above (Mohr, 1955). The most aggressive isolate from the virulence testing was selected and used throughout the experiment. An isolate obtained from a location in Georgia (GA-9/4) was selected and used for infection in the disease screening and the age experiments. Inoculum was created as previously described. The day before inoculation, the plants were placed in a RCBD with four blocks. Each block was on two; 0.304 m x 3.04 m Redi-Heat Heavy Duty heated propagation mats (Phytotronics Inc., MO) placed in a straight line. The resulting blocks were four parallel lines 6.09 m long on the greenhouse bench. The heated propagation mats were connected to four separate controllers (Redi-Heat Digital thermostat), (Phytotronics Inc., MO) set at 32^o C to provide an optimal temperature for fungus growth (Higgins, 1927). Inoculation occurred in the

greenhouse between 1300 to 1500 hours with the internal greenhouse temperature set at 28^{o} C day/22^{o} C night.

Each block contained 24 plants, 2 each of the 12 lines, with a 1:1 ratio of inoculated: control. To start the infection procedure, the selected plants were prepared by removing the substrate at the stem line to create a space for the inoculum. Inoculation was done using 5-10 oats per plant placed on the loosened substrate touching the stem. After inoculation 2.5 cm of substrate was placed on top of the infected oats. All the plants were watered and remained on the propagation mats. Beginning three days after infection the inoculated plants were rated on a 0-5 scale based on disease severity. Ratings were taken of all infected plants resulting in four ratings per line. These four values, one from each block, were averaged to determine the disease rating for that specific line daily. This severity scale was adapted from an article on severity ratings for Southern blight on cowpea (Frey and Dukes, 2002) and is listed below:

<u>Disease Severity Scale Employed:</u>

"0" healthy plant with no sign of the disease

"1" no visible lesions, plant beginning to wilt with no obvious signs of mycelial growth

"2" small lesions, <25% of stem diameter, white mycelial growth present with the oldest foliage wilting (See **Fig 2A** below)

"3" moderate lesions, 26-50% stem diameter, mycelial growth abundant, white sclerotia forming, younger foliage beginning to wilt

"4" large stem lesions, >50% of the stem diameter, cortex decay becoming visible, sclerotia change color from light to dark, severe wilting with older foliage changing color (See **Fig B** below)

"5" dead plant, completely wilted, fully girdled stem (See **Fig 2C** below)

Fig. 2A

Fig. 2B

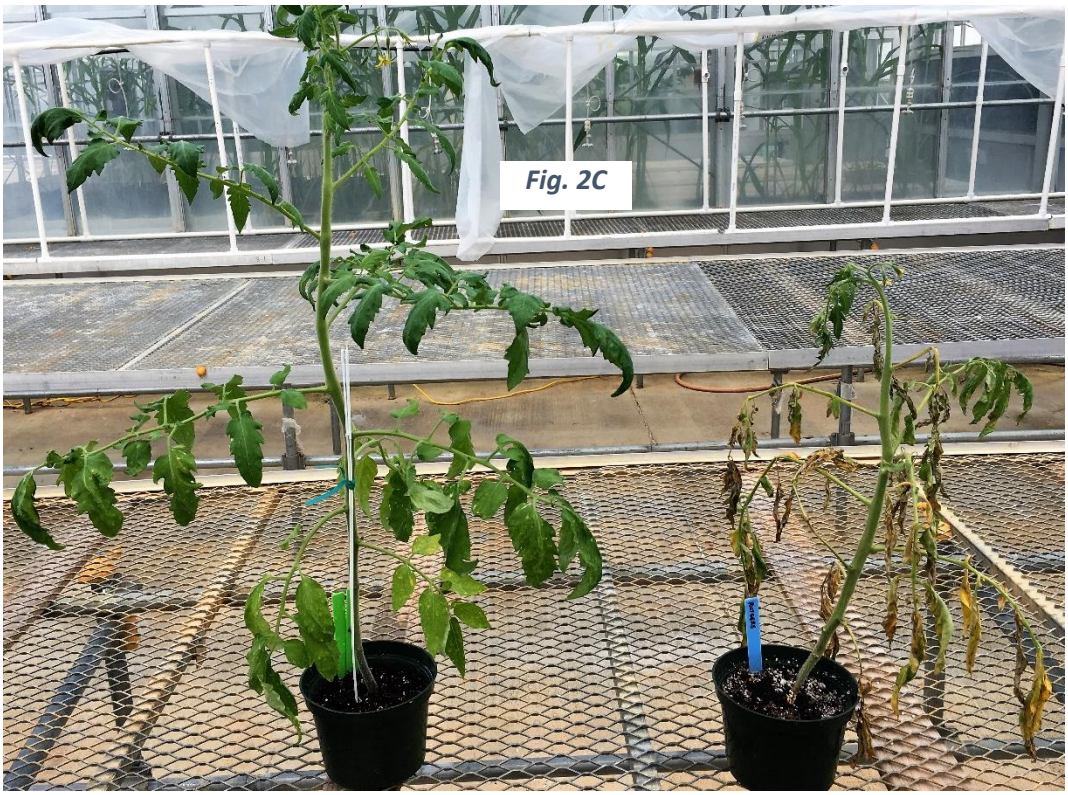


Fig. 2C

Figure 2. *Four Images depicting the stages of evaluation for Southern blight from the greenhouse screening. A) Disease rating of 2, initial mycelium mat produced on the soil surface, B) Disease rating of 4 with sclerotia changing color from white to brown with a hardened rind, C) Severe wilting equaling a 5, dead plant, on the right and a healthy control plant on left, D) stem cuttings from infected (on left) and healthy (on right) plants showing the difference in interior rotted tissue.*

The plants were evaluated once a day for five days starting three days after inoculation. Evaluations were conducted for one week between 1300-1500 hours every day to avoid any possible interactions from temperature fluctuations in the greenhouse. This experiment was repeated three times in Aug., Sep., and Oct. 2016. Average soil temperatures were measured using an Extech IR thermometer (FLIR Systems Inc., OR) and the greenhouse conditions were recorded. After each run the plants were disposed of and the propagation mats were cleaned and sterilized using a 20% bleach solution (1:5 v/v).

Age Experiment

The reported mechanism for resistance being evaluated in this experiment is secondary stem thickening that develops as plants mature. A study to evaluate any possible interaction

between plant age and temperature on disease progression was designed. Previous research from Mohr described the interaction of plant age and temperature (Mohr, 1955).

Four plant ages (8-, 7-, 6-, and 5-weeks-old) and four soil temperatures (32^0, 27^0, 21^0, 15^0 C) were tested with two plants from each age at each temperature interval. Of these two plants, one was inoculated and one not (control). 4 plant ages * 4 temperature treatments * 2 plants = 32 total plants. For this experiment, there were 32 total plants that were broken into 4 different groups with one group containing 8 plants at each temperature level. Of these 8 plants at each temperature, 4 were inoculated and 4 were healthy controls. Each of the four temperature levels were on one, 0.304 m x 3.04 m Redi-Heat Heavy Duty heated propagation mats (Phytotronics Inc., MO). The mats were set at one of the four temperatures (32^0, 27^0, 21^0, and 15^0 C) with each mat controlled using a separate controller (Redi-Heat Digital thermostat), (Phytotronics Inc., MO). Plants were inoculated on 11 Oct. 2016 using the *S. rolfsii*-infected oat method described previously. Beginning three days after inoculation, the plants were rated on the same 0-5 scale based on disease severity as described previously. Evaluations were taken beginning for five consecutive days after inoculation until seven days after inoculation for a total of five ratings per infected plant.

Grafted Seedlings and Plant Care

In the final element of this research, we evaluated the graft compatibility of the six processing tomato (*S. lycopersicum*) lines. The six breeding lines were tested for graft compatibility with the two intraspecific rootstocks being used as commercial standards to provide goal survival rates. The two PI's were included as rootstocks to visualize the compatibility between the different species (interspecific graft). The tomatoes (*S.*

lycopersicum) *Rutgers* and *Celebrity* were used as the scions. For the grafting experiment, seeds of the six processing tomato (*S. lycopersicum*) breeding lines, the two intraspecific rootstocks, and the two PI's were sown in a peat-based substrate, Faford 3B (Sungro Horticulture, MA), using 48-cell plastic market trays (cell dimension: 3.8 cm x 3.8 cm x 6 cm).

Several trays of each rootstock and scion were sown to account for the different germination time required for each line in order to assure a range of stem diameters for flexibility in the grafting procedure. After germination, seedlings were watered and checked daily. The seedlings were maintained at 28^{o} C day/22^{o} C night in the greenhouse. Seedlings were fertilized with 150 ppm nitrogen from 20-10-20 starting one week after being sown and applied one more time, ten days after seeding. After the emergence of the second set of true leaves (10-14 days after sowing) the plants were ready for grafting. A digital-caliper (Mitutoyo – Absolute Digimatic, Aurora, IL) was used to measure the stem diameter of the plants to evaluate suitability for grafting. When plants reached a stem diameter of 1.75 mm no water was applied for the next two days in preparation for grafting.

Grafting Procedure

Grafting was done using the six processing tomato (*S. lycopersicum*) breeding lines, the two intraspecific rootstocks, and the two PI's as rootstocks. The tomatoes (*S. lycopersicum*) *Rutgers* and *Celebrity* were used as the scions. The grafting experiment was conducted in a greenhouse with computer controlled evaporative cooling pads and fans in Auburn, AL. at 32.609^{o} N, 85.480^{0} W. Grafting took place at a specific time of day, early morning (before 1000 hours), or evening (after 1700 hours), to minimize transpirational loss of water through the leaves. As an added preparation, plants were not watered for two days prior to grafting to

reduce the water pressure in the rootstocks to increase the survival rate after grafting (Fernandez-Garcia, 2003).

Stem diameters between 1.5–2.0 mm were optimal for grafting. Similar sizes of the graft components (scion and rootstock), increased the odds of a successful graft (Bausher, 2011). After size matching, the plants were grafted using the splice grafting technique. The splice graft is preferred due to its simplicity and high success rate. The splice graft was performed by making a 45^{o} minimum angle cut through both the scion and rootstock (Barrett et al, 2012). Once the cuts were made the matched scion was placed on the rootstock matching the cuts. Both pieces were held in place with a silicon clip (Silicon top-grafting clips, 1.5-2.0 mm).

The grafting experiment was conducted three times in Dec. 2016, Jan. 2017, and Feb. 2017. Six grafted plants with each rootstock and scion combination were produced. 10 rootstocks * 6 plants * 2 scions = 120 grafted seedlings. The premise being to evaluate each processing tomato (*S. lycopersicum*) line against the two intraspecific rootstocks and the two interspecific PI's. It was expected the tomato rootstocks (*S. lycopersicum*), *Multifort* and *Maxifort*, would have high success rates because this was an intraspecific graft and these lines were bred specifically for grafting purposes. The two PI's were expected to have lower success rate due to plant physiology and genetic difference (interspecific grafts). Grafted plants were placed in the healing chamber immediately after grafting and the chamber was closed and covered with black plastic. The plants were checked daily and the bottom pool was filled with water as necessary to keep humidity high.

Healing Chamber

Once the plants were grafted, they begin to wilt immediately as the scions were severed from their water source. The key was to complete the grafts as quickly as possible and transfer grafted plants into the healing chamber. The healing chamber was an enclosed structure that allowed for a consistent relative humidity (>85% R.H.), temperature, and light. For this experiment, the healing chamber was constructed of 1.905 cm polyvinyl chloride (PVC) pipe (dimensions: 1.524 m 0.762 m x 0.914 m) (Fig. 3). The top of the structure had a peaked roof to allow condensation to collect and reduce the likelihood of water dripping onto the plants (Rivard, et al, 2013). The exterior of the chamber consisted of two pieces clear polyethylene plastic, 3 mm, completely enclosing the structure, however, the top piece could be easily pulled back for access to the interior. The plastic was held to the frame with duct tape with holes strategically cut to fit extensions of the top connecting pipes. The base of the chamber sat on another sheet of 3 mm clear plastic that was raised along the edges using bricks to create a pool. This pool was filled with water to keep the relative humidity at a minimum of 85% in the chamber while the plants were recovering (Rivard et al, 2013).

Figure 3. *Healing chamber, constructed of PVC piping and polyethylene plastic, containing grafted plants (left) and empty (right)*

The healing process was critical to the success of the grafts. Before grafts were placed into the chamber the interior walls were sprayed with water as well as filling the bottom pool with about 3 mm of water. The grafted seedlings were then placed into the chamber onto the center pipes to keep them out of the water. The exterior clear plastic was closed and black plastic was spread over the chamber to eliminate all light (Rivard, et al, 2013). The grafted plants were deprived of all light for two days. On the third day, the black plastic was removed and the interior of the chamber was checked to see if water needed to be added to the pool at the base, if necessary refilled to 3 mm level. The plastic was then reclosed and a shade cloth was placed over the chamber, which replaced the black plastic. The shade cloth allowed 50% sunlight into the chamber. After two days, the shade cloth was removed and the interior checked but this time leaving the plastic open for 2-4 hours allowing the plants some extra light and lower humidity. After the allotted time, the chamber was closed for 1-2 more days depending on the progress of the plants making sure each day to allow the chamber to remain open for 2-4 hours. Finally, the grafted seedlings were removed from the chamber after a total of 5-6 days.

Statistical Analysis

Statistical analysis was done using R computer software (https://www.r-project.org/). Disease screening analysis data was originally pooled for averages and analyzed before accounting for the interaction of time. The decisions was made through research and consulting of a statistician to analyze the data using repeated measures for the disease severity screening. The runs were evaluated separately using AOV and ANOVA procedures. The

individual runs were analyzed using pairwise comparison t-tests to evaluate differences between lines in each run.

The data for the age experiment was collected from a single experiment. This data was evaluated using AOV and ANOVA procedures to analyze any differences between the age of the plants and the interaction between temperature and disease progression. %Graft Survival comparison data was collected from the three grafting runs completed. This data was pooled and analyzed as one set because using the healing chamber for the plants provided a single environment that did not allow for any further interactions. This data was evaluated using AOV procedures to examine the differences in %graft survival rates. All statistical analyses were done using a significance factor of 95% ($P \leq 0.05$).

Results

Disease Screening

The experimental lines can be broken into four categories: breeding lines, intraspecific rootstocks, interspecific cousins (PI's), and the susceptible controls. Comparisons were made between the individual breeding lines as well as between categories. Throughout Run 1, the ratings across the 12 lines in the test were not statistically different (P=0.31) indicating that each line responded similarly to infection from *S. rolfsii*. Three days after inoculation the disease evaluations among the six processing tomato lines averaged 3.7 with a low value of 3 and a high value of 4 (Table 3).

After four days, the disease ratings averaged 4.4 with at low value of 3.75 and a high value of 5 across all lines (Table 3). Five days after inoculation, the ratings averaged 4.6 with a low value 4 and a high value of 5. Six days after inoculation, the ratings averaged 4.8 with a low value of 4.25 and a high value of 5. The final day of evaluation, day seven, the ratings averaged 4.9 with a low value of 4.25 and a high value of 5.

Over the entire evaluation period, the six processing tomato lines did as well as the two intraspecific rootstocks but these results were not statistically different from each other or the susceptible controls (Table 3).

Table 3. *Disease Severity from Run 1 conducted in August 2016*

	Run 1					
	Day 3	**Day 4**	**Day 5**	**Day 6**	**Day 7**	***Average***
5876M[1]	3.75	4.25	4.75	5.00	5.00	*4.55*
5913M[1]	3.75	4.25	4.50	4.75	5.00	*4.45*
5737M[1]	3.75	4.25	4.50	4.75	5.00	*4.45*
5719M[1]	4.00	4.75	5.00	5.00	5.00	*4.75*
5635M[1]	3.25	4.25	4.25	4.50	4.50	*4.15*
5707M[1]	3.00	3.75	4.00	4.25	4.25	*3.85*
PI 126432[2]	3.75	4.25	4.50	4.75	5.00	*4.45*
PI 126932[2]	4.00	4.75	5.00	5.00	5.00	*4.75*
Multifort[3]	3.75	4.50	4.75	5.00	5.00	*4.60*
Maxifort[3]	4.00	5.00	5.00	5.00	5.00	*4.80*
Rutgers[4]	3.75	4.25	4.50	4.75	5.00	*4.45*
Celebrity[4]	3.75	4.75	4.75	4.75	4.75	*4.55*
	N.S.	N.S.	N.S.	N.S.	N.S.	
Average	*3.71*	*4.42*	*4.62*	*4.79*	*4.87*	

[1]breeding lines, [2]PI's (*S. pimpinellifolium)* source of resistance, [3]intraspecific rootstock (commercial grafting standard), [4]Southern blight susceptible control

In comparing the groups of lines, the processing tomato breeding lines had the highest average severity ratings across Run 1 although not statistically significant. A cumulative disease severity rating was calculated for each of the 12 lines using their daily averages (Table 3). None of these lines differed from one another (P=0.07). The best performing of the processing tomato lines was *5707M* with an overall disease severity rating of 3.85 (Table 3). The worst performing line for Run 1 was *Maxifort,* which had an overall disease severity rating of 4.8 (Table 3). No lines differed statistically and none performed better than the controls, *Rutgers* and *Celebrity* (P=0.12).

Run 2 was conducted from 21-26 Sep. 2016 where the average ambient temperature outside the greenhouse was 28^0 C and internal greenhouse temperature was set at 28^0 C. The

heated propagation mats maintained the soil temperature at 32^0 C. Disease severity ratings were taken between 1300-1500 hours daily starting three days after inoculation for a total of seven days.

Throughout Run 2, the disease severity ratings across all 12 lines were not significantly different at any evaluation point (P=0.06). Since there were no significant differences between any of the lines evaluated, we looked for patterns in the data (Table 4). Three days after inoculation the disease severity among the six processing tomato lines averaged 1.5 with a low value of 1 and a high value of 1.75 (Table 4).

Four days after inoculation, the disease severity averaged 2 with a low value of 1.25 and a high value of 2.75 (Table 4). Five days after inoculation, the severity ratings averaged 2.5 with a low value 1.5 and a high value of 3.5. Six days after inoculation, the ratings averaged 3.2 with a low value of 2.25 and a high value of 4.25. The final day of evaluation, day seven, the severity ratings averaged 3.5 with a low value of 2.75 and a high value of 4.25.

As in Run 1, over the entire severity evaluation period, the six processing tomato lines did as well as the two intraspecific rootstocks but these results were not statistically different from each other or the susceptible controls (Table 4). As a group, the six processing tomato breeding lines performed as well as the intraspecific rootstocks. Each had a disease severity rating of 2.4 (Table 4). The difference between the severity rating of the six processing tomato breeding lines, 2.4, and the PI's, 2.9, were also not significant (P=0.26). The average disease severity ratings among the breeding lines, 2.4, and the susceptible controls, 2.6, were also not significant (P=0.79).

In comparing the groups of lines, the processing tomato breeding lines performed as well as the intraspecific rootstocks in Run 2 with no significant difference. As with Run 1, the best performing of the processing lines for Run 2 was *5707M* with an overall disease rating of 1.75 (Table 4). The worst performing of the processing line for Run 2 was *Rutgers*, which had an overall disease rating of 3.2 (Table 4).

Table 4. *Disease Severity from Run 2 conducted in September 2016*

	Run 2					
	Day 3	**Day 4**	**Day 5**	**Day 6**	**Day 7**	***Average***
5876M[1]	1.75	2.25	2.75	3.25	3.75	*2.75*
5913M[1]	1.50	2.25	2.75	3.25	3.75	*2.70*
5737M[1]	1.50	2.25	2.75	3.25	3.50	*2.65*
5719M[1]	1.75	2.25	2.75	3.25	3.75	*2.75*
5635M[1]	1.25	1.25	1.75	2.75	3.25	*2.05*
5707M[1]	1.00	1.25	1.50	2.25	2.75	*1.75*
PI 126432[2]	1.75	2.50	3.50	4.00	4.25	*3.20*
PI 126932[2]	1.75	2.00	2.50	3.25	3.75	*2.65*
Multifort[3]	1.75	2.25	3.00	3.50	4.00	*2.90*
Maxifort[3]	1.25	1.50	1.75	2.75	3.00	*2.05*
Rutgers[4]	1.75	2.75	3.50	4.25	4.25	*3.30*
Celebrity[4]	1.25	1.25	1.50	2.50	2.75	*1.85*
	N.S.	*N.S.*	*N.S.*	*N.S.*	*N.S.*	
Average	*1.52*	*1.97*	*2.50*	*3.18*	*3.56*	

[1]breeding lines, [2]PI's (*S. pimpinellifolium)* source of resistance, [3]intraspecific rootstock (commercial grafting standard), [4]Southern blight susceptible control

The disease ratings across the 12 lines were not statistically different from one another (P=0.67) indicating that each line responded similarly to infection from *S. rolfsii* for the entire evaluation period in Run 3. Since there were no significant differences between any of the lines evaluated, we looked for patterns in the data (Table 5). There were no significant differences between any of the lines at any evaluation period (Table 5).

Table 5. *Disease Severity from Run 3 conducted in October 2016*

	Run 3					
	Day 3	**Day 4**	**Day 5**	**Day 6**	**Day 7**	***Average***
5876M[1]	1.25	2.25	2.25	3.50	4.25	*2.70*
5913M[1]	1.25	2.25	2.50	3.25	4.00	*2.65*
5737M[1]	1.25	2.50	2.75	3.75	4.50	*2.95*
5719M[1]	1.50	2.00	2.75	4.00	4.25	*2.90*
5635M[1]	1.75	2.75	2.50	4.50	5.00	*3.30*
5707M[1]	1.75	2.25	2.75	4.00	4.75	*3.10*
PI 126432[2]	1.25	2.25	3.00	3.50	4.00	*2.80*
PI 126932[2]	1.25	2.50	2.75	4.50	4.75	*3.15*
Multifort[3]	1.50	2.25	3.00	3.75	4.25	*2.95*
Maxifort[3]	1.50	2.50	2.75	4.75	5.00	*3.30*
Rutgers[4]	1.25	2.50	2.50	3.50	4.00	*2.75*
Celebrity[4]	1.50	2.50	2.50	4.75	5.00	*3.25*
	N.S.	N.S.	N.S.	N.S.	N.S.	
Average	*1.41*	*2.37*	*2.66*	*3.97*	*4.47*	

[1]breeding lines, [2]PI's (*S. pimpinellifolium)* source of resistance, [3]interspecific rootstock (commercial grafting standard), [4]Southern blight susceptible control

Three days after inoculation the disease severity evaluations over the six processing tomato lines averaged 1.4 with a low value of 1.25 and a high value of 1.75. Four days after inoculation, the disease severity evaluations averaged 2.4 with a low value of 2 and a high value of 2.75 (Table 5). Five days after inoculation, the severity ratings averaged 2.6 with a low value 2.25 and a high value of 3. Six days after inoculation, the severity ratings averaged 4 with a low value of 3.25 and a high value of 4.75. The final day of evaluation, day seven, the severity ratings averaged 4.5 with a low value of 4.25 and a high value of 5.

As in Runs 1 and 2, over the entire evaluation period, the six processing tomato lines did as well as the two intraspecific rootstocks but these results were not statistically different from each other or the susceptible controls (Table 5). As a group, the six processing tomato breeding

lines performed as well as the intraspecific rootstocks. The average disease rating for the six processing lines was 2.9 and 3.1 for the intraspecific rootstocks, respectively. The disease ratings for the breeding lines, 2.9, and the susceptible controls, 3.0, were also not significant (P=0.81).

An overall disease rating was calculated for each of the 12 lines using the daily averages. The averages for Run 3 were not statistically different across all lines for the entire evaluation period. The best performing line for Run 3 was *5876M* with an overall disease rating of 2.7 (Table 5). The worst performing lines for Run 3 were *Maxifort* and *5635M,* which each had an overall disease rating of 3.3 (Table 5). *5707M* had had the lowest rating for the first two experimental runs and in this final run it performed in the middle of the group with a rating of 3.1.

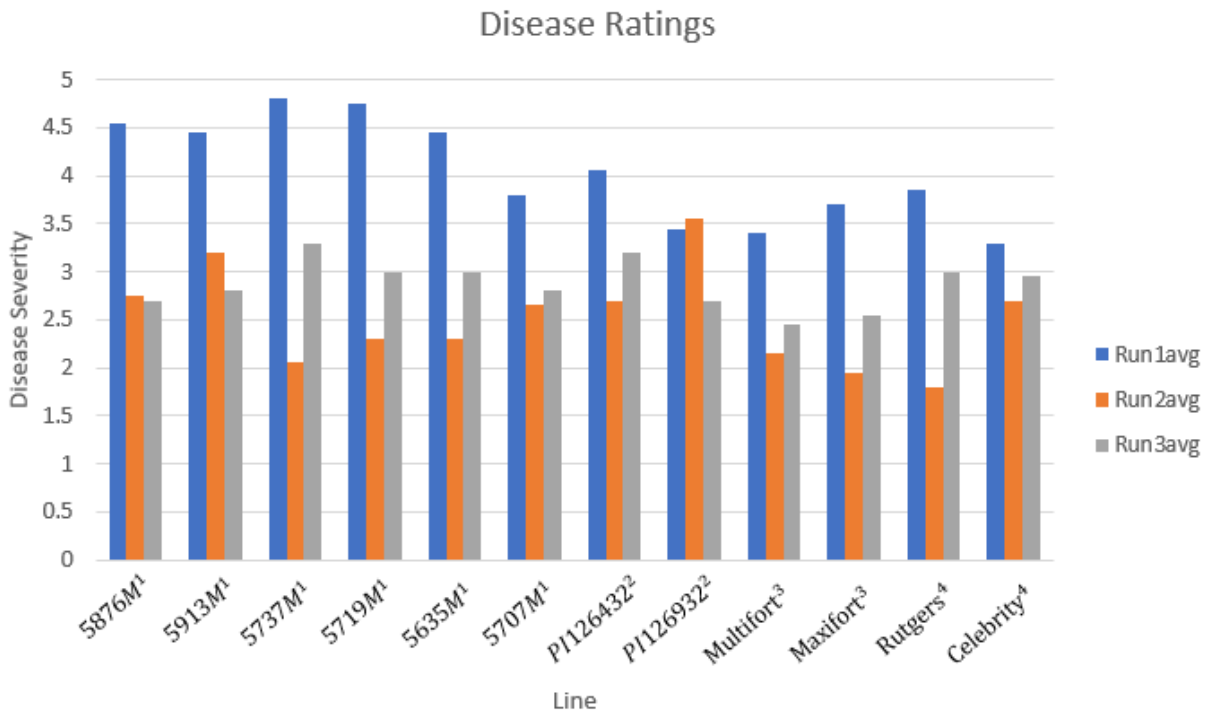
When the data from each run were pooled for the initial ANOVA procedure, the interaction between disease ratings and time was statistically significant (P=0.035). The factor, time, influenced the rate that the disease progressed between each run. The impact of the changing seasons affected how the lines performed during each run. For example, in comparing Runs for the line *5913M,* the disease progressed at different rates from Run 1 though Run 3 (Figure 4). The disease ratings were lower as the season moved from summer to fall through Aug. to Oct. Time was the only difference between each Run.

Southern blight progressed at different rates between the three runs. Figure 4 shows the average disease rating for each line divided up into the three individual runs. Although time showed statistical significance in comparing all three runs, the real difference came from comparing Run 1 with the other two runs. The impact of temperature on *S. rolfsii* is well

documented (Higgins, 1927; Punja, 1985; Singh et al., 1991) and was discussed earlier. Figure 4 provides a visual example of how temperature and thus time are important factors in Southern blight development. In comparison, the disease ratings in Run 1 and Run 2 were significantly different from one another (P=0.038) as well as the disease ratings from Run 1 and Run 3 (P=0.042) (data not shown). The disease progressed more quickly in Run 1 than in Run 3 due to higher ambient temperatures. Although there seemed to be a pattern concerning time and disease rating, this pattern cannot be verified because the disease ratings between Run 2 and Run 3 were not statistically different (P=0.24). The suspected relationship of time with rating was influenced mainly by greenhouse ambient temperature and changing light conditions. In theory, this is understood but as time progressed from Run 2 to Run 3 the temperature did not change at the same rate. From Sep. to Oct. to temperature change was less than that of summer (Aug.) to fall (Sep., Oct.).

The impact of time is the changing variable, which accounts for changes in external temperature and light duration/intensity. These factors resulted in significantly different disease rating over the first two Runs as well as the first and third Runs. The experimental data could not be pooled because of this time interaction so an overall average comparison was not statistically viable. Plotting the results in Figure 4, however, does show the significant interaction of time and provides a visual to see how the disease ratings progressed as well as the differences between the lines.

Figure 4. *Disease Severity Ratings from Southern blight Evaluations Comparing the Three Experimental Runs*



*Disease severity rating taken by averaging ratings for the 4 plants per line over the evaluation period for a total of 20 values

[1]breeding lines, [2]PI's (*S. pimpinellifolium)* source of resistance, [3]interspecific rootstock (commercial grafting standard), [4]Southern blight susceptible control

Age Experiment

The age vs. temperature experiment was conducted on the same heated propagation mats used for the disease screening. The plan was to test four different ages at four different temperatures, however, the experiment was conducted in Oct. 2016 and the internal greenhouse temperature did not drop below 21°C. The two lower temperatures of 21° C and 15° C were never reached and the digital controllers for the pads were never triggered to turn on. Thus, only the two temperatures, 32° C and 27° C, were accurately measured and maintained for the duration of the evaluation period (Table 6). As a result, the two lower temperature treatments were dropped from the analysis.

Table 6. *Age and Temperature Experiment using Rutgers Tomatoes*

		Time vs. Age				
Age (weeks)	*Temp*	*Day 3*	*Day 4*	*Day 5*	*Day 6*	*Day 7*
8	32° C	3^{AB}	3.5	3.5	3.5	4.5
8	27° C	3.5^{AB}	4	4	5	5
7	32° C	3.5^{AB}	4.5	4.5	5	5
7	27° C	2.5^{A}	3	4	4.5	4.5
6	32° C	2.5^{A}	4	4	4.5	4.5
6	27° C	4^{BC}	4.5	4.5	5	5
5	32° C	5^{BC}	5	5	5	5
5	27° C	5^{BC}	5	5	5	5
		***0.044*	*N.S.*	*N.S.*	*N.S.*	*N.S.*

*All plants were *Rutgers* and disease severity ratings were the average of 2 plants
**Only Day 3 showed any statistical difference at both temperatures between plant ages. Numbers followed by the same letter were not significantly different. No further statistical differences were found

The plants were evaluated using the same 0-5 scale of rating for disease severity used in the disease screening study. Analysis of data showed that as the plants matured the disease progressed more slowly with lower ratings regardless of temperature (Table 6). Although the

disease progressed more slowly in the older plants, the disease ratings were not statistically different in the overall experiment (P=0.33). The only day that the ratings showed a statistical difference was Day 3 when older plants showed statistically lower disease ratings as compared to younger plants (Table 6). This pattern did not hold, however, and the disease ratings progressed quickly over the next four days. At the youngest age, five-weeks-old, the plants were all killed three days after infection (Table 6). This was expected and concurred within previous research from Mohr (1955).

The 27^0 C treatments showed slightly slower disease progression than the 32^0C but these ratings were not statistically different overall (P=0.55). Disease ratings across the four ages were all similar between the two temperature treatments (Table 6). Comparing plant maturity showed that as the plants matured they had a better ability to survive the fungus longer, but by the end of the experiment, each age transplant rated either 4.5 or 5 (Table 6). There were no significant differences between the four different aged transplants as previously mentioned for either temperature treatment. The same was true for the comparison between plant age and temperature. The lower disease ratings at the oldest age and the lowest temperature were not significantly different (Table 6).

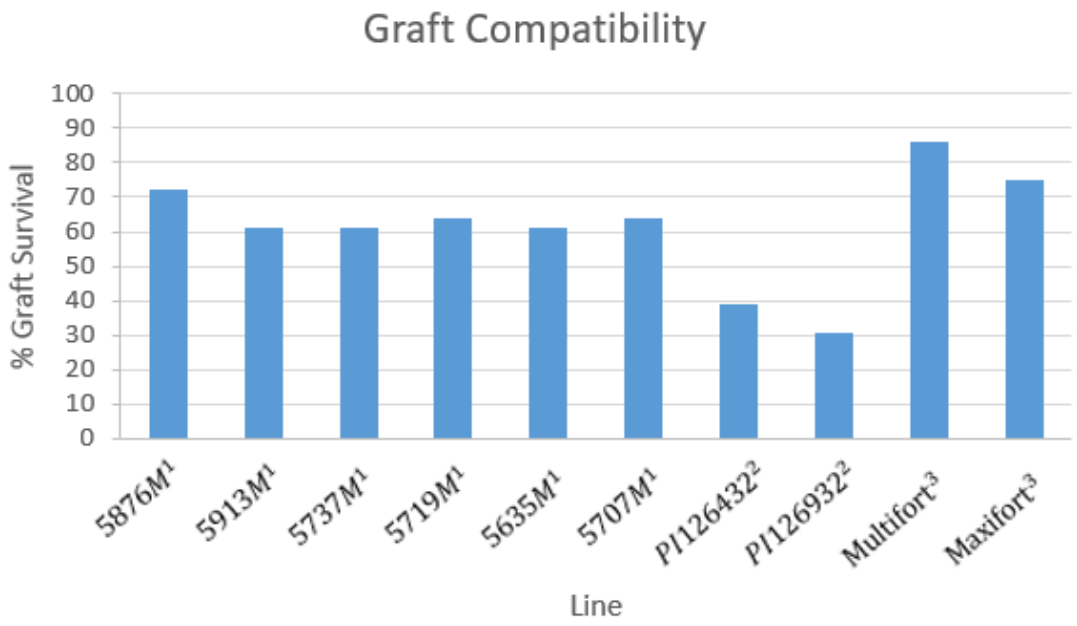
Graft Compatibility

Graft compatibility was evaluated to determine usefulness of the processing tomato (*S. lycopersicum*) breeding lines as rootstocks. Each plant graft combination was evaluated three times in Dec. 2016, Jan.2017, and Feb. 2017. The grafting process described earlier was used in each run. The grafted plants were evaluated the day after being removed from the healing

chamber. Due to the nature of the splice graft, graft success or failure was distinctly visible. Data from the three grafting runs were pooled for each of the ten lines that were grafted.

Celebrity and *Rutgers* were grafted onto each of the six processing tomatoes breeding lines as well as onto *Maxifort* and *Multifort* (common intraspecific tomato rootstocks), and onto PI 126432 and PI 126932 (*S. pimpinellifolium* wild types with reported resistance to *S. rolfsii*). Unlike the disease evaluation, data was pooled because the plants healed in the closed environment of the healing chamber.

Figure 5. *Percent Graft Survival of the Six Breeding Lines, Two Intraspecific Rootstocks, and the Two PI's*



[1]breeding lines, [2]PI's (*S. pimpinellifolium)* source of resistance, [3]intraspecific rootstock (commercial grafting standard)

The relationship between time and the %graft survival were not evaluated because the consistent environment in the healing chamber eliminated influences from outside light intensity/duration. %Graft survival ranged from 30.5% for PI 126932 (lowest) to 86.1% for *Multifort* (highest) (Fig. 4). %Graft survival was expected to be lower for *S. pimpinellifolium*

lines PI 126432 and PI 126932 as these were interspecific grafts. These grafts had the lowest success rate with both below 40% (Fig. 4). *Multifort* and *Maxifort*, the two intraspecific tomato (*S. lycopersicum*) rootstocks had the highest success with both %graft survival rates over 75% (Fig. 4). The higher survival rate of the intraspecific rootstocks was expected because these rootstocks have been bred specifically for grafting onto the cultivated tomato (*S. lycopersicum*).

The six processing tomato breeding lines all had similar survival rates between 60-70% with the two scions (Fig. 4). There was no statistical difference between the six processing tomato (*S. lycopersicum*) lines when using either scion, *Rutgers* or *Celebrity* (P=0.77). Although these six lines all performed similarly with these scions, the %graft survival of the processing tomato breeding lines differed significantly from the %graft survival using the two PI's (P=0.003) and the intraspecific rootstocks (P=0.047).

The intraspecific rootstocks were expected to perform well and they did with both values over 75% survival rate and this was significantly different from that of the PI's (P=0.0002). In comparing the breeding lines with the PI's, the PI's performed poorly which was expected due to different plant physiology and genetic differences (interspecific grafts). The breeding lines performed better than the PI's, which both had %graft survival rates below 40% (P=0.003).

Discussion

The experimental lines can be broken into four categories: breeding lines, intraspecific rootstocks, sources of resistance (PI), and the susceptible controls. Comparisons were made for the individual breeding lines and between these categories. Disease ratings were interpreted as the progression of the disease over time. Different ratings reflected the progression of *S. rolfsii* over time. Time showed a significant interaction between the three experimental runs of the disease screening analysis. Thus, the evaluations were analyzed individually instead of pooling the data. The interaction of time and disease rating was likely significant because of the effect temperature has on the growth of the fungus. Disease ratings averaged highest values in the month of August when the average outside temperature was highest of the three evaluations. Higher temperature along with increased light allowed for the most advanced development of *S. rolfsii* amongst the evaluations.

Although the greenhouse temperatures were continuous for all three runs the outside temperature and light still had a significant effect on how the disease progressed. This was demonstrated in the comparison between the Run 1 and the other 2 runs. In Aug. the disease developed more quickly than in Sep. and Oct. (Fig. 4). As the temperature and light intensity decreased, the disease ratings were lower because the disease progressed more slowly even with a continuous soil temperature.

Comparing the runs separately showed differences in the rate that the disease progressed. The impact of temperature on *S. rolfsii* is well documented (Higgins, 1927; Punja, 1985) and was discussed earlier but Figure 4 provides an example of how temperature and thus time are important factors in development of Southern blight.

In comparison, disease ratings from Run 1 and Run 2 were significantly different (P=0.038) as well as the ratings from Run 1 and Run 3. There seemed to be a pattern connecting time and disease rating, however, this pattern could not be verified because the disease ratings between Run 2 and Run 3 were not statistically different. The suspected relationship between time and disease rating was influenced mainly by greenhouse ambient temperatures. In theory, this is understood but as time progressed from Run 2 to Run 3 the temperatures did not change at the same rate (data not shown). From Sep. to Oct. temperature change was less than that of summer (Aug.) to fall (Sep., Oct.) (data not shown).

This difference in time of year and how the seasons progressed affected the ambient temperatures and the day length as well as light intensity in the greenhouse. Taking these factors into consideration the growth of the fungus was altered which in turn changed the progress of the disease. This was seen in the ratings over the course of the disease screening. In considering time as a significant factor, an interesting note to point out is that this research was completed over the course of one year. If this research were to be replicated at a different time, or at a different location, the data might reveal other interactions.

The initial premise of this study was to determine how well the six processing tomato breeding lines with reported resistance to *S. rolfsii* would work as rootstocks to manage Southern blight. Graft compatibility of these lines was evaluated to determine the effectiveness of these six rootstocks plus two PI's (wild type tomatoes *S. pimpinellifolium* with reported resistance to *S. rolfsii*), and two intraspecific rootstocks commonly used for grafting cultivate tomatoes (*S. lycopersicum*). Graft survival ranged from 30.5% for PI 126932 (lowest) to 86.1% for *Multifort* (highest). The six processing tomato lines all had similar survival rates

between 60-70%. There was no statistical difference between the six breeding lines with either scion. Although the breeding lines all performed similarly, there were statistical differences among these lines as well as between the two intraspecific rootstocks and the two PI's.

Disease resistance and graft compatibility were evaluated in these experiments. In this research, the six processing tomato breeding lines were screened for resistance to the disease Southern blight. Plants were evaluated on a severity scale that was adapted from previous research (Fery and Dukes, 2011). The six processing lines, as well as two intraspecific rootstocks, and two plant introductions (PI's) were including in this screening. Results from the severity screening showed the aggressive nature of the fungus *S. rolfsii*. All the infected plants in the screening were severely damaged from the disease under greenhouse conditions.

This severity screening was completed in three runs through Aug., Sep., and Oct. The disease ratings from the three runs could not be pooled as one data set because there was a significant interaction between time and the disease ratings. This analysis showed that the three runs had to be analyzed separately because Run 1 was statistically different from the other two. There was no pattern with how the disease progressed through time, however, because Run 2 and Run 3 were not statistically different. The change of the season from summer to fall showed an affected how the disease progressed, however, in the two fall months (Sep. and Oct.) the disease ratings were not statistically different. This showed that the disease severity ratings did not progress on a linear route from month to month. By understanding the epidemiology of the disease, the interaction of time on disease rating can be explained.

This relationship was evaluated as the impact of time on the disease rating, however, the cause of this interaction was expected due to changing ambient temperatures as well as changing light conditions as the season progressed from summer to fall (Aug. - Oct.). Temperature is a serious factor for fungus growth and disease progression. Under optimal greenhouse conditions, *S. rolfsii* thrived. As time advanced, the impact of these variables altered the ANOVA and showed that the ratings were significantly altered. In analyzing these runs separately, comparisons between daily evaluations and lines were made. Statistically, there were no significant differences between any of the six processing tomato lines over the daily disease evaluations for each run.

After the disease screening was complete, an age experiment was conducted to examine the relationship between plant age and temperature on Southern blight development. The specific mechanism for resistance in this experiment was secondary stem thickening that developed as the plants matured. Previous research had shown that this stem thickening does not occur until the plants were close to 8-weeks-old. This age experiment was conducted to examine the progress of the disease on plants of different ages as well as how temperature affected the fungus. Four different ages (8-, 7-, 6-, and 5-weeks-old) were infected with the fungus and were maintained at two different soil temperatures (32^0 C and 27^0 C).

The plants were evaluated using the same 0-5 scale of rating used in the disease screening. Analysis of data showed that as the plants matured, the disease progressed at a slower rate yielding lower disease ratings regardless of the temperature regime (Table 6). Although the disease moved more slowly in the older plants, these disease ratings were not statistically different. Disease ratings across the four ages were all similar between the two

temperature treatments regimes. Comparing plant maturity showed that as the plants matured they had a better ability to survive the fungus longer. The age difference did not show any significant difference in disease ratings as previous mentioned for either temperature treatment.

The last part of this experiment evaluated graft compatibility between the six processing tomato breeding lines using the two selected tomatoes (*S. lycopersicum*) scions, *Celebrity* and *Rutgers*. The intraspecific rootstocks and plant introductions (PI's) were included as rootstocks to visualize desirable and undesirable combinations. *Celebrity* and *Rutgers* were grafted onto each of the six processing tomatoes breeding lines as well as onto *Maxifort* and *Multifort* (common intraspecific tomato rootstocks), and onto PI 126432 and PI 126932 (*S. pimpinellifolium* wild types with reported resistance to *S. rolfsii*). Unlike the disease evaluation, data was pooled because the plants healed together in the closed environment of the healing chamber.

Graft survival ranged from 30.5% for *PI 126932* (lowest) to 86.1% for *Multifort* (highest) (Figure 5). The PI grafts (interspecific grafts) had the lowest success rate with both below 40%. *Multifort* and *Maxifort*, the two intraspecific rootstocks had the highest success with both %graft survival rates over 75%. The six processing tomato breeding lines all had similar success rates between 60-70% with the two scions. There was no statistical difference between the six breeding lines when using either scion, *Rutgers*, or *Celebrity*. Although these six lines all performed similarly with these scions, the %graft survival of the processing tomato breeding lines differed significantly from the %graft survival of the other four lines. The %graft survival

was significantly better using the processing tomato lines than the PI's. The PI's produced significantly worse %graft survival than the intraspecific rootstocks.

Conclusions

This research evaluated possible rootstocks for grafted plants as a strategy to manage Southern blight in tomatoes. Through greenhouse severity screenings and graft compatibility tests the six processing tomato breeding lines were evaluated for their disease resistance and graftability. The greenhouse disease screening was a harsh system that favored the development of the disease and produced results that were not statistically different for any of the lines screened. Although there were observable differences in the way the lines reacted to the infection, statistically all lines behaved similarly.

The graft compatibility part of the experiment showed that the six processing tomato breeding lines were adequate rootstocks with %graft survival rates all above 60%. Although this was not as high as the intraspecific rootstocks (grafting standards), these results showed the possibility in using these lines as potential rootstocks or advancing them in a breeding program to enhance new cultivars.

To continue this research the next steps should focus on field trials where disease severity screenings are conducted in field locations with known Southern blight pressure. Previous research showed that these six processing tomato breeding lines do have resistance under field conditions (Mohr, 1992). Taking this same experiment and applying it in a field location containing *S. rolfsii* may show better results of how the plants hold up. Field evaluations would likely be more conducive in determining how these grafted plants will react to Southern blight.

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Appendix 1

Seed Increase

Seed obtained from the USDA was delivered in limited quantities unless a larger amount is specifically requested and available. Originally, 50 seed of each of the six lines (5635, 5678, 5913, 5719, PI 126432, PI 126932) was obtained from the USDA germplasm site in Geneva, NY. This was not enough to complete the desired experiments and a seed increase was started. Eight seeds of the six lines were planted in a 48-cell tray using Faford 3B (Sungro Horticulture, MA) substrate on 18 January 2016. The seedlings were fertilized with 100 ppm nitrogen 20-10-20 every three days starting two weeks after seeding for two weeks before they were transplanted. At four-week-old 48 seedlings were transplanted into 15.25 cm (1.33 L) plastic nursery pots using the same substrate. The fertilizer rate was increased to 150 ppm nitrogen every three days. The plants were watered daily and treated for insect and diseases as required. At 7-weeks-old, six transplants of each line were transplanted into standard 1-gallon nursery containers and staked with bamboo poles for support. The fertilizer rate was increased to 200 ppm nitrogen 20-10-20 every three days for the next three weeks. At 10-weeks-old the fertilizer rate was increased for the last time to 250 ppm nitrogen from the same fertilizer every three days until harvest started. The applications of fertilizer were rotated between 20-10-20 and calcium nitrate at the same 250 ppm nitrogen concentration to avoid blossom end rot.

Over the growing period several of the plants died from various problems that were never determined. It was possible that white fly damage severely injured some of the plants, a chemical was accidently introduced to the environment, or improper fertilization technique resulted in abnormal growth resulting in death of the plant.

Before harvest was started, five of the lines had five plants each except for 5635M which only had two plants survive. The first harvest of was done on 9 May 2016 when the plants were 16-weeks-old. The initial harvest produced tomatoes for all lines with 5635M having the least. The plants were continuously watered every day and fertilized with 250 ppm nitrogen 20-10-20 or calcium nitrate twice a week. The plants produced for the next 6 weeks until June 20, 2016. It is important to note that more than 50% of harvested tomatoes had blossom end rot. This was assumed to be from the plants drying out between watering and not a lack of calcium. Calcium nitrate application ensured sufficient amounts the available mineral, however in the 1 gallon pots the plants used an excessive amount of water and the water schedule was not timed. This variable made some plants dry out more quickly than others and blossom end rot was very common.

Once tomatoes were harvested the seed was removed by slicing them in half and scraping/squeezing the seeds and pulp into a Styrofoam cup. Once all seeds from an individual line were separated, the cup was filled with water so the contents were floating and left to ferment in sunlight. The amount of water varied based on the amount of pulp collected. The cups were filled so that the mixture was sufficiently a liquid, not a slurry, making sure that the amount of water was not excessive. If too much water was placed in the cup, the pulp mixture would not start to ferment. The steps to incur fermentation were repeated for each line and the cups were covered and stirred daily for 7 days to encourage mold growth and allow the sugars to ferment and to clean the seeds naturally. As the mixture fermented, more water was occasionally added to keep the pulp mixture a liquid and not a slurry. After a week of fermentation, each cup was filled 3/4 of the way with a 5:1 bleach to water solution and stirred

for 2 minutes. After 2 minutes the cup, containing the seeds and remaining pulp, was poured through a mesh strainer where the liquid was discarded and the seeds were caught. The strainer was placed under cold running water for 3 minutes to wash off the bleach. The clean seeds were placed on paper towels on top of screens on a lab bench to allow them to dry. The seeds were left to dry for 10-14 days to allow for sufficient removal of all liquid. Once dry, the seeds were picked clean by hand of remaining tissue. As the seeds dried they were placed in envelopes and labeled to keep the lines separate. Seeds of each individual line were not separated based on harvest date, instead seeds from an entire line were bulked together as they were harvested and cleaned. These “seed increase” seeds were kept separate from other seed obtained from the USDA.