**Epidemiology of Target spot of cotton and tomato**

by

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## Abstract

Target spot is a foliar disease of cotton caused by *Corynespora cassiicola*. This disease was first reported in Alabama in 2011. The overall goal of this study was to address the epidemiology of the target spot of cotton and tomato. The specific objectives were: 1) to determine the effect of the temperature on conidial germination of *C. cassiicola* causing target spot of cotton; 2) to determine the effect of temperature and leaf wetness duration on target spot of cotton caused by *C. cassiicola*; and 3) to evaluate the if there is a difference in epidemiology of tomato and cotton isolates of *C. cassiicola* causing target spot of tomato. For the first objective, spores of cotton isolate of *C. cassiicola* were incubated in wet water agar at six different temperatures (12, 16, 20, 24, 28, and 32°C) and conidial germination was counted at 4, 8, and 12 hrs. Highest conidial germination percentage occurred at 24°C with 8 and 12 hrs incubation in water. To address the second objective, cotton plants were inoculated with each of three cotton isolates of *C. cassiicola* (CA1, CC1, and CM18). The inoculated plants were incubated at different temperatures (16, 20, 24, 28, and 32°C) with varying leaf wetness durations (8, 12, 16, 24, 32, 40, and 48 hrs). Early onset, i.e., 1 day after inoculation, was observed at 28°C with ≥24 hr leaf wetness. Lengthening leaf wetness durations lead increased numbers of lesions at all tested

temperatures (16, 20, 24, 28, and 32°C). Highest lesion numbers were observed at 28°C with 48 hr leaf wetness for all three isolates used in this study. Low lesion numbers were observed at 16 and 32°C for all three isolates (CA1, CC1, and CM18). For the third objective, cotton and tomato isolates of *C. cassiicola* were inoculated on tomato plants. These inoculated plants were incubated at three temperatures (20, 24, and 28°C) with varying leaf wetness durations (8, 12, 16, 24, 32, 40, and 48 hrs). Highest lesion numbers on tomato was observed at 28°C with 48 hr leaf wetness for cotton and tomato isolates of *C. cassiicola*. Low temperature (20°C) required ≥40 hr leaf wetness for onset of disease on tomato caused by cotton isolate, while tomato isolates required ≥16 hr wetness for disease onset on tomato. Lowest lesion numbers among the three tested temperatures were observed at 20°C for tomato and cotton isolates. Lengthening leaf wetness duration led to increase in lesion development for both cotton and tomato isolates on tomato.

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# I. Introduction and literature review

## Cotton

Cotton (*Gossypium hirsutum*), which is also known as "white gold", is an important fiber crop worldwide. Fiber of this plant is used as raw material in the textile, pulp and paper industries, and oil extracted from the cotton seed is used in food, cosmetics, chemicals, pharmaceuticals, etc. Cotton seed cake is also used as a cattle feed (Proto et al. 2000). The United States Department of Agriculture-Economic Research Service (USDA-ERS 2017) states that cotton contributes 30% to the total world fiber used. The National Cotton Council of America (NCC 2017a) reports that since 1834 (except 1985/86), the United States has been the largest exporter of cotton. United States cotton exports are approximately 10 million bales every year (NCC 2017b). India, China, the United States and Pakistan were the top four cotton producing countries in 2015 (Table 1). India, China, the United States and Pakistan together produce two-thirds of the world's cotton (USDA-ERS 2017).

## Origin of cotton

Biogeographical distribution of types of cotton indicates that domestication of different species of *Gossypium* occurred in four different areas of the world (Stewart 2001).

*Gossypium arboreum* and *G. barbadense* were probably domesticated in the Yucatan Peninsula, *G. hirustum* was domesticated in Central America, and *G. herbaceum* was domesticated in southeastern Africa (Stewart 2001).

On the basis of the existing degree of diversity of cotton, it seems that four genomic affinity groups (A, B, E, F) of *Gossypium* have originated in Africa (Stewart 2001). Also, some species in the E genomic group originated in the Horn of Africa and east along the coast of Arabia and the Indian Ocean (Stewart 2001). Three additional related genomic groups (C, G, K), including 17 species, originated in Australia (Stewart 2001).

**Taxonomy of Cotton (GRIN)**

Kingdom: Viridiplantae

Phylum: Streptophyta

Division: Tracheophyta

Subdivision: Spermatophyta

Class: Rosidae

Order: Malvales

Family: Malvaceae

Genus*: Gossypium*

**Botanical description**

The cotton plant by nature is a perennial but cultivated as an annual shrub. The shape of a cotton seed is ovoid and pointed at one end. The seed coat is covered by lint. Seed is acid-delinted before planting (Oosterhuis and Jernstedt 1999). The cotton seed

germinates by epigeal germination in which the hypocotyl moves downward while the epicotyl and cotyledons are pulled upward through the soil (Oosterhuis and Jernstedt 1999). The root system of the cotton plant consists of a taproot with secondary or lateral roots (Oosterhuis and Jernstedt 1999). The shoot system has a main axis stem, leaves, buds, branches, floral buds, flowers, and bolls (Oosterhuis and Jernstedt 1999). There are two different types of branches: monopodial, i.e. vegetative, and sympodial, i.e. reproductive branches (Oosterhuis and Jernstedt 1999). Leaves are spirally arranged on a stem. Each node has one leaf, and each leaf and branch is 3/8 of a turn away from another leaf and branch respectively (Oosterhuis and Jernstedt 1999). Cotton plants have a complete flower, i.e. flowers have sepals, petals, stamens, and carpels (Oosterhius and Bourland 2001). The flowering pattern of cotton plant is unique. Flowers open spirally upward among the branches known as vertical flowering, and outward on the same branch known as horizontal flowering. The first flower to open is at 6 or 7 main-stem nodes and at first position on that sympodial branch. The next flower opens after 3 days, on the same relative position on the next higher sympodial branch. In horizontal flowering, the flower which is on the outer side of the predecessor opens after approximately 6 days (Oosterhius and Bourland 2001). Cotton fibers are basically epidermal trichomes which emerge from the primary meristem of the plant (Oosterhius and Bourland 2001).

**Types of cotton**

There are many wild species of *Gossypium* but most do not have fibers and are not economically feasible to cultivate for commercial purposes. These wild species are used as

genetic resources and provide genes for resistance to biotic and abiotic stresses. There are four principle cultivated species of cotton. These species include *G. arboreum*, *G. herbaceum, G. hirsutum* and *G. barbadense* (Lewis and Richmond 1968; Mundro 1987). New world cottons include *G. hirsutum* and *G. barbadense*; whereas *G. arboreum* and *G. herbaceum* are considered old world cottons. The two main species grown in The United States are *G. hirsutum* and *G. barbadense*, with largest planted area under *G. hirustum* followed by *G. barbadense* (Table 2).

Cotton fiber quality is determined by micronaire, fiber length, fiber strength, and color (Luo et al. 2016). Micronaire represents fineness and maturity of the cotton fiber, and is considered an important component of fiber quality. Coarse fiber possesses has too high of a micronaire value (> 4.5) and is undesirable for spinning (Luo et al. 2016). Immature fiber possesses too low micronaire (<3.8), cause breaks in the yarn and is difficult to dye while processing (Luo et al. 2016). Discoloration of the fiber is undesirable and can cause dyeing problems (Luo et al. 2016). The micronaire values between 3.7 and 4.2 are considered as premium and are most desirable. Micronaire values between 3.5 and 3.6 or between 4.3 and 4.9 are considered as base, which are better than discount values but less desirable than premium values. Micronaire values either below 3.4 or above 5.0 are considered as discount and are least desirable. Discount means an amount by which price is reduced from the base rate in order to purchase low grade cotton fiber. Micronaire values are decreased by potassium deficiency, dense stand, and excess nitrogen, but increased by

poor boll set due to high temperatures and water stress (Hake et al. 1990). Micronaire can vary with cultivar selection (Ramey 1999).

*Gossypium hirsutum*: This tetraploid cotton species is also known as American upland cotton because when it was domesticated by colonists and adapted to upland sites (Lewis and Richmond 1968). *Gossypium hirsutum* accounts for 90% of the world's cotton crop (Singh and Kairon 2000). *Gossypium hirsutum* is desired for its wide adaption, high lint percentage, and high yield (Kohel et al. 2001). These are short staple cottons (Mundro 1987) with fiber length averaging 2.92 cm (Benedict et al. 1999). The micronaire value is approximately 4.50 (Benedict et al. 1999). Fiber diameter of *G. hirsutum* is greater than that of *G. barbadense* (Benedict et al. 1999).

*Gossypium barbadense*: This tetraploid cotton species is usually known as Sea-Island cotton or Egyptian cotton. These are long staple cottons (Mundro 1987) with fiber length averaging 3.25 cm (Benedict et al. 1999). Bracts of *G. barbadense* cover the whole flower bud due to bract's large size (Singh and Kairon 2000). Fiber diameter is the lowest among all cotton species. Bundle fiber strength is stronger than that of *G. hirustum* and *G. arboreum*. The micronaire value is approximately 4.2 (Benedict et al. 1999). Fiber of this species is of high quality.

*Gossypium arboreum*: This diploid cotton species is produced in India, China and Pakistan (Mundro 1987) and is also known as Indian cotton (Singh and Kairon 2000). Fiber is coarse and yields of this species are low (Mundro 1987; Singh and Kairon 2000). The

micronaire value of this cotton species is 7.50, and the fiber length is approximately 1.88 cm (Benedict et al. 1999).

*Gossypium herbaceum*: This species is short staple cotton. *G. herbaceum* and *G. arboreum* are together known as Desi cotton or Asiatic cotton (Mundro 1987). Yields of this diploid species are low (Singh and Kairon 2000). Diploid species possess good resistance to biotic and abiotic stresses whereas tetraploid species are high yielding (Singh and Kairon 2000).

**Cotton as a crop**

For crop management of cotton plants, growth and development can be divided into stages. These stages are (Oosterhius 2001): germination, emergence and establishment of seedling; development of leaf area canopy; flowering and development of boll; and maturation.

Ideally, radicals start germinating 2 to 3 days after sowing. Cotyledons provide food for the developing seedling and help in the absorption of sunlight and are further used for photosynthesis leading to plant growth. In 2 to 4 weeks, true leaves take over the task of photosynthesis (Oosterhius and Bourland 2001). The growth of the plant is upright and indeterminate (Eaton 1955; Oosterhius 2001, Oosterhius and Bourland 2001). Leaves of cotton are of three types: kidney-shaped cotyledons; prophylls, which are the first leaves that develop on a branch and develop without petioles; and true leaves (Oosterhius and Bourland 2001). True leaves are broad with 3 to 5 lobes. The plant has a tap root which can grow about 25 cm deep and branch roots can grow 2 m outward (Oosterhius and

Bourland 2001). In 5 to 7 weeks, the cotton plant produces small green flower buds with a pyramidal structure, commonly known as squares (Oosterhius and Bourland 2001). Over the next 3 to 4 weeks, these squares develop and buds swell and bracts exert pressure until squares open to produce a flower (Oosterhius and Bourland 2001). Due to adverse weather conditions, pest damage, water or nutrient deficiencies, 60% of all squares and juvenile bolls may shed, but flowers are not affected (Oosterhius and Bourland 2001). Both self-pollination and cross-pollination occur in cotton plants (Oosterhuis and Jernstedt 1999). The flower usually opens in morning and stays open for a single day (Oosterhuis and Jernstedt 1999). Flower color changes from white to pinkish red during the day after pollination, and the petals wither exposing a green immature boll (Oosterhuis and Jernsedt 1999). The boll is a segmented pod with an average of eight seeds per locule attached to its central column (Oosterhuis and Jernstedt 1999). Numbers of locules can vary from three to five (Oosterhuis and Jernstedt 1999). The boll starts increasing its size as the primary growth substance, i.e. cellulose, of the fibers start growing and thickening. The formation of fibers is initiated just before anthesis in *G. barbadense*, and just after anthesis in *G. hirsutum* (Oosterhuis and Jernstedt 1999). The boll takes 40 to 45 days after anthesis to mature and split open (Oosterhuis and Jernstedt 1999). The carpels, when fully dry, are known as burs and are responsible for holding locks of fully matured fruit. The cotton is ready to pick at this time.

The growth rate of cotton plants follows a sigmoid curve. During emergence and seedling development, the growth rate is slow and then an exponential increase in growth

rate can be seen during canopy development to flowering and boll development. Eventually, the growth rate becomes slow at boll maturation (Oosterhius 2001).

**Production practices**

(a) Cultivar selection: Selecting a good cultivar is an important decision. While choosing any variety, its yield potential, pest resistance traits, cold tolerance, fiber quality traits, seedling vigor, heat tolerance, maturity, and technology traits should be kept in mind. Moreover, a cultivar should be chosen according to location and irrigation facilities (Whitaker et al. 2015). A cultivar is said to be more stable if it performs well across a wide range of environments (Silvertooth et al. 1999; Whitaker et al. 2015).

(b) Planting dates: Usually late April to early May is considered a good time for planting cotton (Norfleet et al. 1997; Whitaker et al. 2015). When planted early, soil may have sufficient moisture for the crop in rain fed areas but also faces problems such as increased cold weather stress, poor stand and seedling diseases due to cool and wet weather conditions (Silvertooth et al. 1999; Whitaker et al. 2015). Very late planting leads to greater vegetative growth, management difficulties and poor yield (Silvertooth et al. 1999). Soil temperatures should be greater than 18˚C for 3 consecutive days up to a depth of 4 inches at planting (Silvertooth et al. 1999; Whitaker et al. 2015). Planting at soil temperatures below 13˚C leads to reduced seedling vigor and increased seedling disease incidence (Silvertooth et al. 1999). Warm soil temperatures should continue at least for a week to insure stand establishment (Silvertooth et al. 1999; Whitaker et al. 2015).

(c) Plant population/seeding rate: Seeding rate depends on the variety's seed vigor and soil type (Silvertooth et al. 1999; Whitaker et al. 2015). An optimum plant stand is 7 to 10 plants/m of row (Whitaker et al. 2015). If the soil quality is poor, soil is crusted, the field is infested with soil-borne fungi or the seed is of poor quality, then the seeding rate should be increased accordingly (Whitaker et al. 2015). A plant population of 114,000 to 121,000 plants/ha is optimal for high yields (Bridge et al. 1972).

(d) Fertilization: Alabama soils are inherently moderate in potassium (K) and low in phosphorus (P) due to the scarcity of these nutrients in parent material of the soils, leaching, dissolution and surface runoff (Mitchell and Huluka 2012). Soil testing is an important step that should be taken before applying fertilization. On the basis of Cation Exchange Capacity (CEC or ECEC), Alabama's soils are divided into 4 categories (Mitchell and Huluka 2012): sandy, loamy to clay, clay, and calcareous clay of Black Belt (Table 3).

If soil is acidic, with pH below 6.0, then liming is recommended. An appropriate pH for cotton production is 6.5 (Mitchell and Huluka 2012). Soils with more organic matter and clay particles require more lime to raise the soil pH compared to sandy soils with low organic matter (Mitchell and Huluka 2012). Dolomite limestone has more than 6% Mg and some percentage Ca, so it fulfills the Mg and Ca needs of cotton along with increasing the pH (Whitaker et al. 2015). Soil group 1, with 0 - 28 kg/ha extractable Mg and other soil groups with 0 - 56 kg/ha extractable Mg, are considered to be low in Mg, while Group 1 soils with extractable Mg above 28 kg/ha and other soil groups with extractable Mg above

56 kg/ha are considered soils enriched with high Mg. Application of magnesium sulfate or magnesium oxide at 28 kg/ha can correct low Mg, whereas if pH is also below 6.5, then dolomite limestone at 1121 kg/ha can be applied (Mitchell and Huluka 2012).

Phosphorus: P is an immobile nutrient; therefore, the entire required dose should be applied before planting (Whitaker et al. 2015). Soil rating and recommendation rate of P for different soil groups in Alabama are described on the basis of extractable P (Table 4).

Potassium: Application of the entire required dose of potassium is also recommended before planting. Two foliar sprays at 2.26 - 4.53 kg/$K_2O$ can be done during early bloom, if severe K deficiency persists (Whitaker et al. 2015). Appropriate applications of potassium lead to increases in seed cotton yield, size of bolls, and fiber micronaire values (Bennett et al. 1965). Soil rating and recommendation rate of K for different soil groups in Alabama are described on the basis of extractable K (Table 5).

Nitrogen: Appropriate amounts of N are very important because low N rates can lead to yield reduction, whereas high N rates can lead to boll rot, increased vegetative growth, delayed maturity and poor yield (Whitaker et al. 2015). Alabama soils are generally low in organic matter, therefore N availability for the crop is also low (Mitchell and Huluka 2012). The recommended N rate is 101 kg/ha which should be applied in splits (Mitchell and Huluka 2012; Touchton et al. 1981; Whitaker et al. 2015). An initial N application should be done at planting and at the rate of 1/4 to 1/3 of the total recommendation. Sidedress N should be applied between first square stage to first bloom stage. After the 3$^{rd}$ week of bloom, application of N is not recommended (Whitaker et al.

2015). The recommended nitrogen rate should be increased by 25% for a cropping pattern of cotton followed by cotton or with deep sandy soils (Whitaker et al. 2015).

Boron: This micronutrient plays an important role in flowering, pollination and fruiting of cotton (Mitchell and Huluka 2012, Whitaker et al. 2015). The recommended rate of B is 0.56 kg/ha in two equal splits by foliar spray during first square to first bloom (Whitaker et al. 2015).

Water needs: Cotton is a drought tolerant crop but a properly timed irrigation can enhance yield and fiber quality. Soil moisture sensors can be used to measure the moisture level of the soil. Consultants and online scheduling applications such as real time wireless smart sensor array (Vellidis et al. 2008) are also available to help growers for scheduling irrigation. Water needs of the cotton crop increase until the 3$^{rd}$ week after bloom and then declines (Bednarz et al. 2003) Cotton needs 0.38 cm of water per day at 1$^{st}$ bloom (Whitaker et al. 2015). In the 2$^{nd}$ week of bloom, water needs increase to 0.56 cm/day (Whitaker et al. 2015). The water requirements in the 3$^{rd}$ and 4$^{th}$ week after bloom are 0.76 cm/day (Whitaker et al. 2015). In the 5$^{th}$ and 6$^{th}$ week after 1$^{st}$ bloom, water needs decrease to 0.56 cm/day (Whitaker et al. 2015). Cotton requires 0.38 cm of water per day in the 7$^{th}$ week after 1$^{st}$ bloom (Whitaker et al. 2015). The crop should be examined in the 8$^{th}$ week after 1$^{st}$ bloom to decide if irrigation should be terminated (Whitaker et al. 2015). Usually, irrigation should be terminated completely when the crop reaches 10% open bolls (Whitaker et al. 2015).

**Insect pests of cotton and their management**

In 2016, insects caused 2.6% and 2.3% yield losses in the United States and Alabama, respectively (Williams 2016). Some important insect pests of cotton in Alabama are described below.

Bollworms: Cotton bollworm, *Helicoverpa zea*, and tobacco budworm, *Heliothis virescens,* are two important pests of cotton. Larvae damage apical buds, squares, blooms and bolls. Infested plants have damaged discolored bolls with open bracts and bolls are usually aborted (Leonard et al. 1999).

Spider mites: Spider mites, *Tetranychus* spp., are problematic in hot and dry weather. They leave foliage discolored after feeding on plant juices (ACES 2016). A heavily infested crop will defoliate (ACES 2016).

Armyworms: Early instar larvae of this insect feed on leaf tissue and cause defoliation. Later instar larvae feed inside squares, blooms, and bolls and reduce yield (Leonard et al. 1999). Females lay eggs in clusters of 150 inside the leaves (ACES 2016). Fall armyworms, *Spodoptera frugiperda,* can be identified due to its typical mark which is shaped like an inverted Y on the head. Another insect is beet armyworm, *Spodoptera exigua,* which is a sporadic pest (ACES 2016).

Cabbage and soybean loopers: The larvae of cabbage looper, *Trichoplusia ni*, and soybean looper, *Pseudoplusia includes,* damage interveinal leaf tissue (Leonard et al. 1999). A net-like appearance of the remaining leaf area can be seen (Leonard et al. 1999). Loopers can defoliate an entire cotton crop (ACES 2016).

Cutworms: Many cutworm species such as *Peridroma saucia*, *Agrotis ipsilon*, *A. subterranea*, and *Feltia ducens* are pests of cotton seedlings (ACES 2016; Leonard et al. 1999). Cutworms girdle stems at the soil level, reduce the plant stand which leads to delayed crop maturity and poor yields (MU 2004). Fields where the soil temperature is low and where conservation tillage practices are applied, are prone to cutworm infestation (ACES 2016).

Cotton Aphids: Aphids, *Aphis gossypii,* can be found on the underside of leaves, on stems and on terminals. Aphids are vectors of several viruses such as Potyviruses in many crops, and they can transmit *Cotton anthocyanosis virus*, and cotton blue disease in cotton. Symptoms of aphid infestation are yellowing and curling of leaves. Later, sticky and sugary liquid called honey dew can be seen in open bolls and this can contaminate lint (Leonard et al. 1999).

Grasshoppers: Cotton is most susceptible to grasshopper damage from the crook stage to the 6 true leaf stage. The crook stage is a brief period when the hypocotyl elongates from the radicle, forms an arch and begins to push up through the soil, (ACES 2016). Both adults and nymphs chew on the stem and cause destruction by reducing stands (ACES 2016).

Thrips: Some common species of thrips which attack cotton are *Frankliniella fusca*, *F. tritici*, *F. occidentalis*, and *Sericothrips variabilis* (Leonard et al. 1999). Common signs of a heavy infestation of thrips in a cotton crop are distorted leaves with brown edges that cup upward (ACES 2016).

Plant bugs: There are several plant species which attack cotton such as *Lygus hesperus*, *L. lineolaris*, and *Neuroclopus nubilus* (Leonard et al. 1999). These pests cause destruction at the bloom stage of cotton by affecting squares and young bolls resulting in hard lock of one or more locks per boll (ACES 2016).

Whiteflies: The important species of whiteflies which attack cotton are *Trialeurides abutilonea* and *Bemisia tabaci* (Leonard et al. 1999). The nature of damage due to whitefly is by sucking cell sap and secreting honey dew. Sooty mold can grow on honey dew, leading to premature severe defoliation (ACES 2016). All life stages of this insect can be found on the underside of leaves (ACES 2016). Whiteflies are vectors of many important viruses of cotton such as *Cotton leaf curl virus, Cotton leaf crumble virus,* and *Cotton yellow vein virus* (Brown 2001).

Stink Bugs: The common species of stink bugs which attack cotton are *Acrosternum hilare*, *Nezara viridula*, and *Euschistus servus* (ACES 2016). Damages due to these bugs are more likely in cotton adjacent to peanut or corn. Stink bugs cause damage to seeds in young bolls leading to reduced quantity and inferior lint (ACES 2016). Inspection of the interior of cotton bolls is required to check for infestation as sometimes as there are no external signs.

**Integrated pest management**

IPM (Integrated Pest Management) tools for management of cotton include cultural practices, selective use of insecticides, insect scouting, use of transgenic cultivars and beneficial arthropods. Insect management reduces the pest population below the economic

damage level, instead of bringing a pest population to zero, which better maintains ecological balance (ACES 2016).

Transgenic varieties: Bollgard cotton varieties which are genetically engineered to produce an insecticidal protein from *Bacillus thuringiensis* are effective tools for management of cotton bollworm, *H. zea*, and tobacco budworm, *H. virescens* (Perlak et al. 2001).

Cultural control: Some production practices, when altered, reduce pest populations. For example, practices such as increasing plant population density, excessive fertilization with nitrogen, late planting, and improper application of herbicide, can lead to delayed or lengthened fruiting period which makes the plant more susceptible to pest attacks (ACES 2016). In addition, conservation tillage in cotton can enhance cutworm infestation while reducing thrips infestation (ACES 2016). Alfalfa, as a trap crop, is an effective method to manage *Lygus* spp. in cotton (Hokkanen 1991).

Biological control: Green lacewings, lady bird beetles, syrphid flies, and spiders are important native predators of aphids, and larvae of beet armyworm, tobacco budworm, and bollworms (ACES 2016). Some major predators of whitefly are *Orius* spp., *Brumoides suturalis*, and *Coccinella septempunctata* (Gerling et al. 2001). Thrips can be managed by native predators such as *Chrysoperla carnea*, and *Orius niger* (Atakan 2006). An entomophagus fungus, *Neozygites fresenii,* which is native to the Midsouth and Southeast United States can be applied as biological control of cotton aphid, *A. gossyphii* (Steinkraus et al. 2002). Several mycoinsecticide products based on *Verticillium lecanii*, *Paecilomyces*

*fumosoroseus*, and *Beauveria bassiana* provide good control of whiteflies, *B. tabaci* (Faria and Wraight 2001). It is important to keep in mind the toxicity level of pesticides to these beneficial insects while choosing any insecticide (ACES 2016).

Selective use of insecticide: While selecting any insecticide many things should be considered, including its efficacy against target insects, the chance of insects developing resistance towards that insecticide, human safety hazards, and its effect on non-target organisms (ACES 2016). Regular scouting of the fields should be done to determine the population density of both beneficial and harmful insects. Insecticides should be applied only when the population density of the harmful insect becomes greater than an economic threshold level (ACES 2016).

**Nematodes and their management**

Some plant parasitic nematodes cause serious damage on cotton. The most damaging nematodes on cotton in Alabama are the cotton root-knot nematode (*Meliodogyne incognita* Race 3) and the reniform nematode (*Rotylenchulus reniformis*). Damage from nematodes results in losses of crop yields, plant quality, or both (Overstreet and McGawley 2001).

Nematodes damage cotton by feeding on roots of the plants. Along with direct damage to the roots, *M. incognita* also causes indirect damage by increasing susceptibility of cotton to Fusarium wilt and to fungal seedling disease complexes (Thomas and Kirkpatrick 2001). Feeding activities of nematodes on the roots cause wounding; this aids the entry of the disease-causing bacteria and fungi into plants (ACES 2016). Reniform

nematodes alter the development of the cotton plant, along with causing reduction of yields (Lawrence and McLean 2001).

The initial symptoms of plant parasitic nematode infestation are when the infected plants appear chlorotic and stunted with a weak stem (Overstreet and McGawley 2001). Roots of the cotton plants infected by root-knot nematodes develop spindle-shaped or rounded galls (Thomas and Kirkpatrick 2001).

Reniform nematodes do not produce any diagnostic aboveground symptoms. The infected plants are uniformly stunted and show symptoms typical of potassium deficiency such as lower leaves turning yellow with brown tips and leaf margins (Robinson 1999). In fields with established reniform nematode populations, secondary root systems of the infected plants are underdeveloped and have fewer roots (Lawrence and McLean 2001).

Cotton fields infested with nematodes can be managed by using IPM strategies. Cotton varieties resistant to root-knot nematode and Fusarium wilt should be planted in the fields infested with these pests (ACES 2016). Crop rotation with peanuts, small grains, millet, sudangrass, sorghum, or pasture grasses reduce the population of cotton root-knot nematodes, since root-knot nematodes cannot reproduce on these crops (ACES 2016). Cotton fields infested with reniform nematodes should be rotated with corn, grain sorghum, small grains, or peanuts (ACES 2016). Plowing the field immediately after picking cotton suppresses nematode populations (ACES 2016). Nematicides such as Velum Total (imidacloprid + fluopyram) at 1.02 – 1.32 l/ha can be applied in-furrow or below seed at planting (ACES 2016).

## Diseases and their management

**Seedling Diseases.** Seedling diseases can severely affect cotton production (DeVay 2001). Seedling diseases can cause acute disease symptoms such as seed decay, and preemergence or post emergence death of seedlings resulting in a poor stand (DeVay 2001). Another symptom pattern is chronic and involves lesions on the hypocotyl; root lesions are another type of seedling disease symptom which can lead to reduced growth and crop development delay (DeVay 2001). Seedling pathogens of cotton are mainly *Pythium* spp., *Rhizoctonia solani, Fusarium* spp., and *Thielaviopsis basicola* as well as minor fungi, all of which attack plants in the first 6 to 8 weeks after planting (ACES 2016).

Seed rot and pre-emergence damping off: These diseases are caused by *Pythium* spp. during or prior to seed germination (ACES 2016; Howell 2001). Seed rot occurs when the seed is infected by these pathogens within hrs of planting while pre-emergence damping-off occurs between seed germination and seedling emergence (ACES 2016; Howell 2001). The typical symptom of seed rot is, when the seed is squeezed, the rotten content comes out like toothpaste. Symptoms of pre-emergence damping off are a soft and watery appearance of the radicle that leads to seedling death (ACES 2016; Howell 2001). Conducive soil temperatures at planting for *Pythium* seedling diseases are 16 to 20˚C. Cool temperatures and wet soil conditions are most favorable for *Pythium* spp. (Howell 2001). Important species associated with these diseases are *P. ultimum* and *P. irregular*, which are more desctructive at low temperatures and high moisture conditions, and *P.*

*aphanidermatum,* which is more damaging in tropical areas (Howell 2001). *Rhizoctonia solani* is also often involved in these diseases as well (ACES 2016; Howell 2001).

Seedling root rot and post-emergence damping off: Symptoms first appear on hypocotyls. Plants look light green, and pale, with stunted growth (ACES 2016). *Fusarium* spp. cause dry rot, whereas *Pythium* causes light brown necrotic lesions at the soil level leading to post-emergence damping off that is more severe in cool and wet seasons (ACES 2016; Howell 2001). *Rhizoctonia solani* causes reddish-brown sunken lesions at or below the soil level on the hypocotyl (ACES 2016; Rothrock 2001). Lesions girdle the hypocotyl creating the symptoms, known as wirestem, and eventually may lead to death of the plant (ACES 2016; Rothrock 2001).

Seedling diseases lead to poor stands, which leads to significant reduction of yields (DeVay 2001). Also, poor stands can cause difficulties with the management of the other pests. After some years, fields with established soil borne plant pathogens can result in severe stand losses, which can require replanting (DeVay 2001). In Alabama, losses due to seedling diseases ranged from 3 to 11% annually over the 20-year period from 1978 to 1998 (Gazaway 1998).

**Foliar Diseases.** Leaf spots: Corynespora leaf spot or target spot, caused by *Corynespora cassiicola,* is an emerging disease of intensively managed cotton. Symptoms of this disease start in the lower canopy. Reddish-brown concentric necrotic spots on leaves are formed (Fulmer et al. 2012). A study in 2013 showed that lint loss in Alabama on an

apparently susceptible variety, due to the target spot of cotton, reached approximately 448 kg lint/ha (Hagan et al. 2015).

Other fungi pathogenic to cotton are *Stemphylum solani, Alternaria macrospora, Cercospora gossypina,* and *Ascochyta gossypii* (Baird 2001a; Oosterhius 2001). Alternaria leaf spot is a late season disease which can also be observed on cotyledons and bolls (Baird 2001b). Typical symptoms are brown lesions with purple margins which show concentric zones on expansion. Later, the center of lesions may fall out (Baird 2001b). Alternaria leaf spot causes minimal yield losses, because the crop is developed by the time the disease occurs. However, Alternaria leaf spot in a disease complex with Cercospora or Stemphylum leaf spot often results in premature defoliation, reduced fiber quality and suppressed yields (Wade et al. 2015).

Another foliar disease, common in cotton-producing regions of the United States, is Ascochyta blight caused by *A. gossypii* (Baird 2001c). Symptoms of this disease are circular, light brown lesions with dark brown borders on leaves which later coalesce and form irregular necrotic areas. On stems, reddish purple to black cankers can be seen. Girdling of stem leads to death and shredding of the infected tissue leaving behind exposed plant tissue (Baird 2001c). Crop losses due to Ascochyta blight are rare, but in favorable weather conditions have been reported up to 10% (Baird 2001c). Ascochyta blight affects fiber quality along with yields in favorable conditions (Baird 2001c).

Boll rots: Boll rots are considered the second most economically important group of diseases after seedling diseases (Batson 2001). According to the Beltwide Cotton

Disease Loss Estimates, boll rots caused an average of 2.0% losses of yield over ten years (1991-2000) in the United States (Batson 2001).

Boll rot can be caused by a number of fungi and bacteria such as *Fusarium* spp., *Diplodia* spp., *Xanthomonas* spp., *Glomerella gossypii*, *Rhizoctonia* spp., and *Alternaria* spp. (Batson 2001).

*Fusarium* spp. invade the boll from its base and continues to the top of the boll. Infected bolls turn brown black from the inside while salmon pink color can be seen outside (Batson 2001). *Diplodia* spp. invade through bracts but if enough moisture is present then the fungus can enter through the carpel. Initially, small brown lesions can be seen on the carpel and bract (Batson 2001). Further, lesions completely cover the bolls which turn black, dry and split open. *Xanthomonas* spp. also causes angular leaf spot and bacterial blight of cotton along with boll rot. Bacteria enter bolls through nectaries, stomata and sometimes wounds, and produce water soaked, dark green and greasy circular lesions on the boll. Later, these lesions coalesce, become sunken, brown colored and have a dried center with a dark red margin. The bacteria colonize the developing fiber. These locks of colonized fiber do not fluff and become hard (Batson 2001). *Alternaria* spp. invade the boll from the suture of the opening. In conditions of high humidity, the whole boll is colonized and decays but when it dries it produces hard locks, where the lint fibers do not fluff (Prostko et al. 1998).

**Disease management**. Seedling disease control: (a) A well prepared seedbed with soil temperatures 18˚C or above at 4 inch depth for three consecutive mornings before sowing

reduces risk of seedling diseases (ACES 2016). Seeds are more prone to chilling damage. The seed bed should have adequate moisture but not be wet as these conditions make seedlings more prone to diseases (ACES 2016).

(b) If the soil is acidic, lime should be applied as cotton grown in acidic soils is more susceptible to seedling diseases and has reduced seedling growth (ACES 2016)

(c) High quality seed should be preferred with greater than 80% germination (ACES 2016).

(d) Seed treatment is an important step for managing seedling diseases. Some important fungicides for seed treatment are pentachloronitrobenzene (PCNB), thiram and vitavax as these are effective against *Rhizoctonia* spp. and *Fusarium* spp., whereas metalaxyl and mefenoxam are effective against *Pythium* spp. (ACES 2016). Use of seed treated with a combination of myclobutanil and metalaxyl is effective for management of *Rhizoctonia solani*-induced damping off and *Pythium*-induced damping off and result in increased plant stand (Davis et al. 1997). Other fungicides such as Quadris Flowable (azoxystrobin), Ridomil Gold (mefenoxam) and Terramaster (etridiazole) can be applied in-furrow for management of Pythium-incited seedling diseases. Fungicides recommended for leaf spots and boll rot control include Priaxor (fluxapyroxad and pyraclostrobin), Headline (pyraclostrobin), Quadris Flowable (azoxystrobin), Twinline (pyraclostrobin and metaconzole), and Topguard (flutriafol) (Table 6).

**Methods of measuring cotton seed yield and lint yield**

There are two types of products obtained from cotton that are commercially important. One is the lint, which is used primarily in the textile industry, and another is seed, which is important in the food industry as it is used for cooking oil; the meal and hull of the cotton seed is also used as fertilizer, animal and fish feed (Proto et al. 2000). The unit of measurement used for cotton lint yield is the bale, which is equivalent to 500 pounds of lint. Cotton seed yield can be measured by two methods (Lopez and Elam 1996). One way is to measure the actual seed yield is by taking a sample at the ginning mill. The gin separates the fiber (lint) from seed of the cotton sample. The lint weight is then measured as is the actual cottonseed weight to calculate yield per acre. Another way is to randomly sample 25 to 100 bolls one week before stripping. The lint is manually sorted from the seed and the weight of lint is taken as is the weight of the seed cotton (lint plus seed). Then lint percentage can be calculated by ratio of lint weight/total seed cotton weight. The following formula is used to calculate yield in kg/ha (E):

$E= \{LY*[(100-L\%) / L\%]\}\times 1.121$ where LY= lint yield in lb. per acre and L%= lint percent of seed cotton (Lopez and Elam 1996).

Lint Yield (Prostko et al. 1998): To estimate lint yield of cotton in the field, count the number of bolls in 10 feet of row. Calculate average number of bolls/foot. This average is multiplied by the number of linear feet per acre according to row spacing. This will give an average number of bolls per acre. This average is then multiplied by 0.0033. This number will determine lint pound per acre. The lint pounds per acre are multiplied by 1.121 to obtain lint yield in kilograms per hectare.

## Introduction of *Corynespora cassiicola*

*Corynespora cassiicola* is a necrotrophic fungus well known as a leaf spotting plant pathogen (Barthe et al. 2007; Huang et al. 2010). The fungus, which was first described by Berk. & M.A. Curtis as *Helminthosporium cassiicola* in 1868 and was renamed as *Corynespora cassiicola* by C.T. Wei in 1950, is a cosmopolitan plant pathogen and is abundantly present in subtropical and tropical countries (Barthe et al. 2007; Dixon et al. 2009). It has been found on 530 plant species from 380 genera including monocots, dicots, ferns, one cycad and also on human skin (Dixon et al. 2009; Huang et al. 2010). Economically important hosts of this pathogen include *Solanum lycopersicum* (tomato), *Glycine max* (soybean), *Gossypium hirsutum* (cotton), *Carica papaya* (pawpaw), citrus, *Hevea brasiliensis* (rubber), *Vigna unguiculata* (cowpea), *Phaseolus vulgaris* (bean), *Nicotiana tabacum* (tobacco), and *Capsicum annuum* (pepper) (Barthe et al. 2007; Cutrim and Silva 2003; de Lamotte et al. 2007; Dixon et al. 2009; Fernandes and Barreto 2003; Jinji et al. 2007; Kwon et al. 2005; Oluma and Amuta 1999; Pereira et al. 2003). Isolates of *C. cassiicola* can survive as a pathogenic, saprotrophic or endotrophic fungus depending on host substrate (Kingsland 1986; Schlub et al. 2007) . It can be found on different substrates such as leaves, stems, flowers, fruits, roots, and nematode cysts (de Lamotte et al. 2007; Dixon et al. 2009; Huang et al. 2010).

## Taxonomy of *Corynespora cassiicola*

Kingdom: Fungi

Phylum: Ascomycota

Subphylum: Pezizomycotina

Class: Dothideomycetes

Order: Pleosporales

Family: Corynesporascaceae

Genus: *Corynespora*

Species: *cassiicola*

*Corynespora cassiicola* has no currently known telemorphic stage (Schlub et al. 2007). *Corynespora* has the same sort of conidial structure as *Helminthosporium*, so they are related morphologically but according to the current phylogenetic research, *Pyrenophora* relates more closely to *Corynespora* compared to *Helminthosporium* (Schlub et al. 2007).

## Description of *Corynespora cassiicola*

Colony growth of *C. cassiicola* shows concentric rings which may give a flucculose appearance with color varying from grey to brown (Conner et al. 2013; Schlub et al. 2007). Mycelium of the fungus does not produce a stroma (Schlub et al. 2007). The conidiophores produced by the fungus are simple, erect at their base, cylindrical or slightly wider at the apex, intermittently branched, smooth with 1-8 septate (Seaman et al. 1965) and subhyaline to dark brown (Conner et al. 2013; Fulmer et al. 2012; Schlub et al. 2007; Seaman et al. 1965). Length of the conidiophores is 85-100 µm long with width of 4-11 µm (Schlub et al. 2007). Enteroblastic conidiogenous cells produce conidia singly or in chains at a broad apical pore on the conidiophore (Seaman et al. 1965). Conidia are variable

in shape, i.e., obclavate to cylindrical, rarely Y-shaped, and straight to slightly curved with a slightly tapered hemispherical apex and truncate base, and subhyaline to brown or olivaceous. The fungus has conspicuous thickened dark colored hilum (Conner et al. 2013; Fulmer et al. 2012; Schlub et al. 2007; Seaman et al. 1965). Different researchers recorded different sizes of conidia and different numbers of pseudoseptation in condia. For instance, conidia of the fungus may be 36-186 µm long and 8-19 µm wide when grown on PDA for 8 days (Kwon et al. 2005). Other researchers state that conidia are 50-209 µm long and 7 to 15 µm wide with 4-15 pseudosepta when the fungus was cultured on V8 at 21˚C for 14 days at light/ dark cycle (Conner et al. 2013); approximately the same results were found when the fungus was cultured on PDA at same temperature for same period at dark/ light cycle with fluorescent light (Fulmer et al. 2012) whereas others state that conidia are 103-343 µm long and 12-25 µm wide with 3-24 pseudosepta when found on soybean host (Seaman et al. 1965). *Corynespora cassiicola* has a bipolar germination system (Kwon et al. 2005).

## Distribution of *Corynespora cassiicola*

*Corynespora cassiicola* has caused crop damage resulting in high economic losses in over 70 countries. It is abundantly found in tropical and subtropical areas (Barthe et al. 2007; Silva et al. 2003). Isolates of the fungus have been found in Canada, Japan, United States, Korea, Sri Lanka and many other subtropical counties affecting soybean seedlings (Kwon et al. 2005; Seaman et al. 1965; Silva et al. 2000). The pathogen has damaged rubber trees in 10 top rubber producing countries with the most devastation in Indonesia (Barthe

et al. 2007). The pathogen was first found in Malaysia and then in seedling nurseries of rubber trees in India in 1958 (Jinji et al. 2007; Romruensukharom et al. 2005) and causes extensive premature defoliation in Sri Lanka where it became an epidemic in 1987 (Silva et al. 2000; Silva et al. 2003). *Corynespora cassicola* is found in Brazil, West Africa, Indonesia, China, Thailand (Jinji et al. 2007; Ogbebor and Adekunle 2005; Oluma and Amuta 1999) and most rubber producing countries in Asia (de Lamotte and et al. 2007; Jinji et al. 2007). Cucumber in Japan (Miyamoto et al. 2009), Europe (Oluma and Amuta 1999), Korea (Kwon et al. 2005) and the Republic of Seychelles (Kingsland 1986) and is also in the United States in Florida (Blazquez 1967) are also subject to attack by *C. cassiicola*. Corynespora spot is caused by *C. cassiicola* on hot pepper in China and Korea (Chai et al. 2014; JinHyeuk et al. 2001; Jinji et al. 2007). Target spot is found in all tomato growing regions of the world. It was first identified by F.C. Deighton in Sierra Leone (Üretimi 2004). Later this disease appeared in Korea, India, Queensland, Australia, United States, Brazil and Southern Nigeria, Cuba, Sri Lanka, Romania, Republic of Seychelles (Dixon et al. 2009; Kingsland 1986; Kwon et al. 2005; Oluma and Amuta 1999; Silva et al. 2000; Üretimi 2004). This fungus has been reported on the noxious weed *Lantana camara* in Brazil (Cutrim and Silva 2003). Target spot by *Corynespora cassiicola* is affecting cotton in India, China and the United States (Conner et al. 2013; Fulmer et al. 2012; Lakshmanan et al. 1990; Wei et al. 2014). Another vegetable, balsam pear, is host of this fungus in the United States, Korea and Japan (Kwon et al. 2005). *Corynespora cassiicola* is causing damage to watermelon in the Seychelles (Kingsland 1986).

## Proof of pathogencity of *C. cassiicola* on cotton

A fungal suspension ($2\times10^4$ spores/ml) of two isolates was used for inoculating cotton and sterile water was sprayed on cotton seedlings instead of a fungal suspension, as a non-inoculated control. All seedlings were incubated for 72 hrs at 21˚C in a moist chamber (Conner et al. 2013). Fulmer et al. (2012) incubated seedlings for 48 hrs at 21.1˚C. All inoculated cotton seedlings showed leaf spot symptoms produced by *C. cassiicola* in 6 days (Conner et al. 2013) or in 7 days (Fulmer et al. 2012), while no symptoms were found on non-inoculated controls. Re-isolation of the fungus was done and then isolates were cultured and confirmed by morphological characteristics (Conner et al. 2013). *Corynespora cassiicola* sequences in the gene bank were 99% similar to the sequence of archived isolates of this fungus. Koch's postulates were also done for *Corynespora* leaf fall disease of rubber (Jinji et al. 2007) and *Corynespora* leaf spot of balsam pear (Kwon et al. 2005).

## Major diseases caused by *Corynespora cassiicola*

*Corynespora* leaf fall disease destroyed 4600 ha rubber tress (*H. brasiliensis*) since 1987 in Sri Lanka (Silva et al. 2003) which resulted in a 20-25% economic losses to rubber growing countries (Silva et al. 2003). Symptoms of this disease can be seen on fruits, leaves, twigs and petioles (Barthe et al. 2007; Silva et al. 2003). A characteristic symptom of leaf fall disease is a fish bone or railway track-like appearance formed by the darkening of the veins and necrotic spots with a brown or white papery center surrounded by dark brown ring (Barthe et al. 2007; Jinji et al. 2007; Silva et al. 2003). A yellow halo can also

be seen (Jinji et al. 2007). Later, rubber leaf fall causes premature defoliation and that reduces latex production (Barthe et al. 2007; Ogbebor and Adekunle 2005; Silva et al. 2003).

Another important disease is *Corynespora* leaf spot of cucumber (*Cucumis sativus*) which is also known as leaf fire disease in Europe (Blazquez 1967). Leaf fire symptoms are characterized by irregular yellow specks which later become angular or somewhat circular with a light brown colored center and surrounded by a dark brown margin. The necrotic leaves fall from the plant (Blazquez 1967).

*Corynespora cassiicola* also affects soybean. There are two symptom patterns seen on soybean: one is target spot on leaves and another is stem and root rot (Seaman et al. 1965). In some regions of the northern United States, only the latter disease is seen (Seaman et al. 1965). Dark brown streaks can be seen which later girdle hypocotyls or the root near the crown (Seaman et al. 1965). Target spot on soybean can reduce plant growth. Foliage symptoms include reddish brown pin-point lesions (Seaman et al. 1965). If free moisture becomes available then these spots turn into characteristic target spot symptoms with concentric light and dark necrotic rings with dark brown margin (Seaman et al. 1965).

*Corynespora cassiicola* is a serious pathogen on winter-grown tomatoes and caused losses of 11800 kg/ha (Pernezny et al. 2002). Leaf lesions with light brown center further surrounded by dark brown margins, sometimes a yellow halo can be seen on some cultivars (Schlub et al. 2007; Üretimi 2004). Diseased areas of leaves became desiccated and papery (Onesirosan et al. 1975). Premature defoliation occurs with continued disease

intensification. In addition, fruits showing slight sunken lesions, which later become a large darker sunken pitted area are unmarketable (Schlub et al. 2007).

Target spot disease caused by *C. cassiicola* is an emerging disease in cotton. Symptoms are seen on leaves and bracts (Fulmer et al. 2012). Initially, brick red dots expand to form circular or irregular concentric spots with tan to light brown centers on leaves (Conner et al. 2013; Fulmer et al. 2012). Symptoms can begin around 68 days after planting in susceptible varieties in combination with frequent rain/irrigation, which can lead to premature leaf fall (Conner et al. 2013). Some isolates affect the bolls. Circular water soaked lesions with dark brown margins can be seen on plants infected by these isolates (Lakshmanan et al. 1990). Afterwards, these lesions coalesce and become large necrotic spots that further lead to boll rot (Lakshmanan et al. 1990). Target spot can cause 70% premature defoliation and heavy loss of lint (Fulmer et al. 2012).

## Disease epidemiology for different hosts

In soybean, disease development is more severe at 15˚C and is less severe at 20˚C (Seaman et al. 1965), while target spot development on tomato occurs at 20 to 28˚C (Jones and Jones 1984). Target spot requires high humidity as periods of drought during August and September may decrease disease development (Seaman et al. 1965). With sesame, a leaf spot caused by *C. cassiicola*, causes higher losses in the dry season when plants are weaker and premature leaf senescence occurs under to drought conditions (Seaman et al. 1965). High humidity along with leaf wetness for 16 to 44 hrs is necessary for disease development on tomato (Jones and Jones 1984; Schlub et al. 2007). Disease development

on *Lantana camara* is severe at 20 to 30˚C with leaf wetness of 6 or more hrs (Pereira et al. 2003). Disease severity is highest at 28˚C (Pereira et al. 2003). *Corynespora* leaf spot develop on pepper requires a temperatures between 25 to 30˚C (Chai et al. 2014; Üretimi 2004).

## Host specific toxin production

*Corynespora cassiicola* produces a host-selective toxin (HST) known as cassiicolin (Barthe et al. 2007; de Lamotte et al. 2007; Onesirosan et al. 1975; Pereira et al. 2003; Üretimi 2004). These HSTs are mostly low molecular weight secondary compounds, with host specific toxicity at low concentrations (Üretimi 2004). Isolates of *C. cassiicola* produce a toxin in synthetic medium, which is able to induce cellular damage to the specific host (tomato, rubber, *Lantana camara*) similar to the damage caused by fungus to the host (Barthe et al. 2007; de Lamotte et al. 2007; Onesirosan et al. 1975; Pereira et al. 2003; Üretimi 2004). On the synthetic medium as on modified *Alternaria* medium (MAM), maximum toxin production occurs after a 12 day incubation period (Onesirosan et al. 1975). The optimum temperature for toxin production in synthetic medium is 24-28˚C. The triggers for maximal production of cassiicolin in synthetic medium are 3% glucose and an optimum pH range of 6-7 (Onesirosan et al. 1975). Due to HSTs produced by *C. cassiicola*, *C. cassiicola* has the potential to be used as a biological control agent against the noxious weeds such as *Lantana camara* (Pereira et al. 2003). Study of HSTs can help for finding detoxification genes from plants and microorganisms that can further help in identifying disease resistance mechanisms against *C. cassiicola* (Üretimi 2004).

## Isolation of the pathogen

Symptomatic tissue from freshly collected leaves are cut into small pieces with a sterilized scissor and surface sterilized with 70% alcohol for 3 minutes and these infected tissue pieces are placed onto Potato Dextrose Agar (PDA) (Silva et al. 2003). Incubation temperatures have varied from 28±2˚C for 2 days (Silva et al. 2003) or 25˚C in dark (de Lamotte et al. 2007; Pereira et al. 2003), then a subculture or single spore isolation is done to obtain a pure culture (Miyamoto et al. 2009; Silva et al. 2003). A variety of artificial media are used to see the effect on growth of the mycelium and sporulation such as 20% V-8 juice agar (Seaman et al. 1965), antibiotic V-8 juice agar (Conner et al. 2013). Alphacel agar, Difco cornmeal agar with 30 mg/liter chlortetracycline (CMA), malt agar (Seaman et al. 1965). Seaman et al. (1965) found slower mycelial growth on PDA, while submerged growth and scanty spore production were noted on malt-agar and CMA (Seaman et al. 1965). The germination percentage is higher on V-8 agar (Seaman et al. 1965). Pereira et al. (2003) used media like Vegetable-broth-agar medium (VBA; prepare by vegetable decoction of 25 g red beet, 25 g carrot, 25 g potato, 25 g tomato, 25 g pumpkin, 25 g cabbage , 25 g taro leaves, 12.5 g kale, 6.25 g shallot, and 6.25 parsley, sieve and make up to 1 liter with water and 20 g agar), Lantana leaf extract sucrose agar (LSA; decoction of 200 g of triturated lantana leaves, 30 g sucrose, and 20 g agar, final volume 1 liter with water), Potato-Carrot-Agar (PCA). Greatest mycelial growth and sporulation was observed on LSA and VBA media, respectively (Pereira et al. 2003). Other researchers use Malt-Extract-Agar (MEA), Czapek-Dox-Agar (CDA) and with additions of 6 mg of

thiamine hydrochloride or 2 g biotin or 1 mg each of pyridoxine or riboflavin in these medium to have more variation in mediums (Oluma and Amuta 1999). Growth is greatest on MEA and slowest on PDA with thiamine as an addition. Sporulation is highest on CDA with biotin and it is poorest on PDA or PDA + thiamine (Oluma and Amuta 1999).

## Enhancing sporulation of *Corynespora cassiicola*

Mycelial growth was not seen at 5˚C or 35˚C with highly restricted mycelial growth observed at 30°C (Seaman et al. 1965). Some researchers used 21˚C as an incubation temperature (Conner et al. 2013) whereas other find 25±1˚C to be an optimum temperature for growth of mycelium (de Lamotte et al. 2007; Dixon et al. 2009; Oluma and Amuta 1999; Pereira et al. 2003). Favorable temperature for sporulation of *C. cassiicola* is 20 to 28˚C but the greatest sporulation was noted at 23˚C (Pereira et al. 2003). Sporulation of *C. cassiicola* occurs after 7 days growth but conidia numbers are higher after 15 days (Pereira et al. 2003). Scraping the colony surface of this fungus with a glass slide after 3 days growth and then incubating under continuous light (25 flux) at 21 to 23˚C for the next three or more days can increase sporulation (Jinji et al. 2007; Onesirosan et al. 1975; Pereira et al. 2003).

## Storage methods

A simple method is to cover the pure culture of *C. cassiicola* with autoclaved mineral oil and store at 5˚C in the dark (Dixon et al. 2009; Miyamoto et al. 2009). Immersion in sterile water is the best method for storage with 71% revival rates (Roy et al. 2014). For this method, a disk of actively growing fungus is taken from pure culture and

transferred to a test tube containing 10 ml sterile water. The test tube is closed properly and wrapped with parafilm and stored at room temperature (de Lamotte et al. 2007; Roy et al. 2014). Continuous culturing used to preserve pure culture can reduce virulence of the fungus with time (Roy et al. 2014). Another method is to store *C. cassiicola* at -20˚C. Take a piece of mycelium with sterilized cork borer from the original culture and transfer it to quarter strength PDA plate which is covered with 3 to 4 layers of filter paper. Incubate this parafilm sealed plate at room temperature for 2 to 3 weeks in the dark, then transfer filter paper in a sterilized coin envelope with sterilized forceps. Air-dry coin envelopes overnight, then place in airtight container with desiccant and store at -20˚C (Brewer, personal communication).

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Table1. Cotton production data of top four cotton producing countries (2015).

| Country | Production (1000 bales) | Harvested Area (ha) | Yield (kg/ ha) | Imports (1000 bales) | Exports (1000 bales) |
|---|---|---|---|---|---|
| India | 26,400 | 11,899,781 | 483 | 1,076 | 5,764 |
| China | 22,000 | 3,050,115 | 1570 | 4,406 | 128 |
| United States | 12,888 | 3,267,836 | 859 | 33 | 9,153 |
| Pakistan | 7,000 | 2,800,020 | 545 | 3,300 | 250 |

Table 2. Cotton production data of the United States (2015).

| Type of cotton | Planted Area (ha) | Harvested Area (ha) | Yield (kg/ha) | Bales (millions) |
|---|---|---|---|---|
| *Gosspium hirsutum* | 3,408,262 | 3,205,110 | 846 | 12.455 |
| *Gossyium barbadense* | 64,142 | 62,686 | 1504 | 0.433 |

Table 3. Different soil groups on the basis of ECEC value (Modified from Mitchell and Huluka 2012).

| Soil Group | Soil Type | ECEC Value (cmol/kg) | Soil Series |
|---|---|---|---|
| Soil group 1 | Sandy | <4.6 | Dothan, Troup, Orange-burg, Ruston and Alaga |
| Soil group 2 | Loamy to Clay | 4.6- 9.0 | Madison, Lucedale, Hartsells, Cecil, Pacolet and Savannah |
| Soil group 3 | Clay | >9.0 | Dewey, Talbott, Boswell, Iredell, Colbert and Decatur |
| Soil group 4 | Calcareous Clay of black belt | | Houstan, Sumter, Oktibehha, Leeper and Vaiden |

Table 4. Soil rating on the basis of extractable P and recommendation rate of P (Modified from Mitchell and Huluka 2012).

| Extractable P | Soil Group 1 (P kg/ha) | Soil Group 2 (P kg/ha) | Soil Group 3 (P kg/ha) | Soil group 4 (P kg/ha) |
|---|---|---|---|---|
| Very Low | 0- 13 | 0- 13 | 0- 8 | 0- 20 |
| Low | 15- 28 | 15- 28 | 9- 17 | 21- 40 |
| Medium | 29- 56 | 29- 56 | 18- 34 | 41- 81 |
| High | 57- 112 | 57- 112 | 35- 67 | 82- 161 |
| Very High | 113- 280 | 113- 280 | 68- 168 | 162- 404 |
| Extremely High | 281 + | 281 + | 169+ | 405+ |
| Recommended Equation for $P_2O_5$ (kg/ha) where (x= extractable P value) | 134.52- 2.63x | 134.52- 2.63x | 134.52- 4.33x | 134.52- 1.83x |

Table 5. Soil rating on the basis of extractable K and recommendation rate for K (Modified from Mitchell and Huluka 2012).

| Extractable K | Soil Group 1 (K kg/ha) | Soil Group 2 (K kg/ha) | Soil Group 3 (K kg/ha) | Soil Group 4 (K kg/ha) |
|---|---|---|---|---|
| Very Low | 0- 34 | 0- 50 | 0- 67 | 0- 90 |
| Low | 35- 67 | 52- 101 | 68- 135 | 91- 179 |
| Medium | 68- 135 | 102- 202 | 136- 269 | 180- 269 |
| High | 136- 269 | 203- 404 | 270- 538 | 270- 538 |
| Very High | 270- 538 | 405- 807 | 539- 1076 | 539- 1076 |
| Extremely High | 539+ | 807+ | 1076+ | 1076+ |
| Recommended equation for $K_2O$ (kg/ha) where x= Extractable K value | 134.52- 1.10x | 134.52- 0.75x | 134.52- 0.56x | 134.52- 0.53x |

Table 6. Recommended fungicides for leaf spots and boll rots.

| Fungicide Group | Chemical name and Trade name | Pathogen | Application rate (per ha) | Application time and repetitions |
|---|---|---|---|---|
| (QoI Strobilurins) (Group 11) | azoxystrobin QUADRIS FLOWABLE | *Ascochyta blight*; *Glomerella* spp.; *Fusarium* spp.; *Alternaria* spp.; *Corynespora cassiicola* | 437 - 657 ml | This fungicide is applied before pin head square to early bloom stage. Application can be repeated after 14 to 21 days. This fungicide cannot be applied consecutively more than two times. |
| (QoI Strobilurins + Triazole) (Group 11+ Group 3) | (Pyraclostrobin + metconazole) TWINLINE | *Alternaria* spp.; *Asochyta blight*; *Cercospora* spp.; *Corynespora* spp.; *Fusarium* spp.; and *Stemphyllum* spp. | 511 - 620 ml | Application is done before disease development. Another application is repeated after 7 to 14 days. Application of TWINLINE more than two consecutive sprays must be avoided. |
| Triazole (Group 3) | Flutriafol TOPGUARD | *Corynespora cassiicola* | 511 - 1023 ml | First application is done at first bloom stage and repeated once after 7 to 14 days. Minimum 37.8 l spray solution is recommended. This product cannot be applied more than twice. |
| QoI Strobilurins (Group 11) | Pyraclostrobin HEADLINE SC | *Alternaria* spp.; *Asochyta blight*; *Cercospora* spp.; *Corynespora* spp.; *Fusarium* spp.; and *Stemphyllum* spp. | 289 - 875 ml | Application is done before disease development. Another application is repeated after 7 to 14 days. Application of HEADLINE more than two consecutive sprays must be avoided. |

## II. Effect of temperature on conidial germination of *Corynespora cassiicola* causing target spot of cotton


### Abstract

*Corynespora cassiicola* is a cosmopolitan pathogen that causes target spot of cotton. In recent years, this disease has been commonly reported in the cotton fields of southeastern and mid-southern states of the U. S. This disease can cause up to 70% premature defoliation that can cause yield losses in apparently susceptible cultivars. Conidial germination is the first step of the infection process of this pathogen. Therefore, it is important to understand the environmental conditions required for conidial germination of *C. cassiicola*. The goal of this study was to determine the effect of temperature on conidial germination of a cotton isolate of *C. cassiicola.* A spore suspension of an isolate of *C. cassiicola* was pipetted and spread onto water agar in petri plates, which were maintained at six different temperatures ranging from 12 to 32ºC, and conidia germination was assessed after 4, 8, and 12 hrs. Greatest conidial germination after 8 and 12 hrs of incubation was observed at 24ºC. Conidia failed to germinate below 24°C with a 4 hr incubation period and below 20°C with an 8 hr incubation period. Quadratic regression analysis indicates that optimum temperature for conidia germination after 8 hr incubation was 28.4ºC.



**Keywords:** Target spot, plant pathogen, cotton isolate, epidemiology

## Introduction

Target spot of cotton is an emerging disease caused by *Corynespora cassiicola*, a cosmopolitan plant pathogen (Sumabat et al. 2016). The disease was first reported in Mississippi by Jones (1961) and again in southwestern Georgia in 2005 (Fulmer et al. 2012). Since 2005, this disease has been detected in U.S. southeastern and mid-southern cotton-producing states (Mehl et al. 2017).

Cotton is an important crop in Alabama and is one of the top three agronomic crops in terms of production acreage (USDA-NASS 2016). The major diseases associated with cotton in Alabama are seedling diseases such as seed rot, seedling root rot, pre-emergence and post-emergence damping off. These seedling diseases are associated with fungi, *Pythium* spp., *Fusarium* spp., *Rhizoctonia solani*, and *Thielaviopsis basicola* (ACES 2016). Other common diseases seen in Alabama are foliar diseases such as *Stemphylium, Cercospora,* and *Ascochyta* leaf spots as well as target spot (Hagan and Sikora 2013).

Target spot is considered a substantial threat for intensively managed, irrigated ”rank” crop in the southern third of the Alabama (Hagan 2015). In 2012, target spot of cotton caused up to 70% premature defoliation in Georgia (Fulmer et al. 2012). In 2013 at a coastal southwestern Alabama study, target spot of cotton was associated with 448 kg/ha lint losses in an apparently susceptible cultivar, PhytoGen 499 WRF, and 269 kg/ha lint losses in an apparently less susceptible variety, Deltapine 1252 B2RF (Hagan et al. 2015).

It is important to know the conditions that favor disease development in order to most efficiently manage that disease. Forecasting infection periods of a pathogen can help in scheduling timely fungicide applications. Temperature and moisture duration are important environmental factors for predicting infection periods and disease development (Tarr 1972). The infection process by plant pathogens is divided into three phases: pre-entry or prepenetration, penetration and colonization (Tarr 1972). Spore germination is the first step of the pre-entry phase. Moisture duration and temperature are important factors that can affect the percentage of conidia that germinate and the rate of conidial germination (Tarr 1972). Unfavorable temperatures can delay conidial germination (Tarr 1972). Favorable temperature requirements may differ among races of a fungus (Tarr 1972). Isolates of *C. cassiicola* from different hosts appear to have different favorable temperature ranges for conidial germination. For example, a favorable temperature range for conidial germination of an okra isolate of *C. cassiicola* was shown to be 25 to 30˚C and the highest conidial germination percentage was at 25˚C (Ahmed et al. 2013). However, the ideal temperature range for conidial germination of isolates of *C. cassiicola* from rubber was 15 to 35˚C (Fernando et al. 2012). Conidial germination of soybean isolates of *C. cassiicola* in free water was favored by the temperature range of 20 to 30˚C, while on V-8 agar, and a high conidial germination percentage (above 50%) was observed over temperatures ranging from 10 to 30°C (Seaman et al. 1965).

Optimum temperatures for conidial germination can also differ from optimum temperatures for vegetative growth of the same race of a fungus (Tarr 1972). This can be

seen with *Urocystis occulta* which requires 15˚C for conidial germination, while the optimum temperature for growth of the germ tube is 24˚C (Tarr 1972). Isolates of *C. cassiicola* from some hosts such as soybean have different optimum temperatures for conidial germination and mycelial growth. As noted above, a favorable temperature range for conidial germination of an isolate of *C. cassiicola* from soybean is 20 to 30˚C in free water, while the optimum for mycelial growth is 20˚C, while at 30°C, growth was restricted (Seaman et al. 1965).

Before the environmental conditions that favor development of target spot of cotton can be properly determined, the temperature requirements for the pre-entry phase of cotton isolates of *C. cassiicola* needs to be established. Therefore, the present study was done to investigate the effect of temperature on time required for conidial germination of a cotton isolate of *C. cassiicola*.

**Methods and materials**

**Production of fungal colonies.** An isolate of *C. cassiicola* (CC1) was established from diseased cotton leaves collected at the Prattville Agricultural Research Unit, Alabama. A diseased leaf was kept in a moist chamber for 24 hrs and then lesions on the leaf were microscopically examined. Single conidia were collected from these lesions using a sterilized needle. Single conidia were placed on V8 media, prepared with 160 ml of V8 juice, 3 g of calcium carbonate and 20 g of agar per liter water and the cultures maintained at 28˚C. Colonies of *C. cassiicola* for conidia production were produced on quarter-strength potato dextrose agar (qPDA), prepared with 9.75 g of potato-dextrose-agar

powder (PDA) and 11.25 g of agar powder per liter water. Fungal colonies were sub-cultured by removing 5-mm diameter plugs from the periphery of the 12 day old colony arising from the single conidium. Plugs were placed on fresh qPDA and kept at 28°C for 12 days to obtain fungal colonies. These cultures were maintained by sub-culturing on fresh qPDA every 12 days from the preceding fungal colonies.

**Effect of temperature on conidia germination.** A conidial suspension was prepared from 12-day old colonies by filling plates with 3 ml of sterile water. The mycelium was rubbed lightly with a sterile L-shaped glass rod to release conidia. The resulting conidial suspension was filtered through 2 layers of cheesecloth. The concentration of conidia was determined with a haemocytometer and adjusted to $2\times10^4$ conidia/ml.

Two ml of conidial suspension and 2 ml of sterile water were pipetted and spread onto water agar media and the petri-dish was sealed with parafilm. Water agar medium was prepared with 20 g of agar powder per liter water. The effect of temperature on germination of *C. cassiicola* was tested at 12, 16, 20, 24, 28, and 32 °C in dark. Three replicate plates were included at each temperature. The number of germinated conidia was determined in each petri-dish by examining 40 arbitrarily selected conidia under the microscope after 4, 8, and 12 hrs of incubation at each temperature. A conidium was considered germinated when its germ tube was equal to or longer than the length of the conidia. The whole experiment was repeated twice.

**Data analysis.** Percentage of conidial germination was calculated for each plate. Conidial germination percentage at six different temperatures and three incubation periods was analyzed by PROC GLIMMIX using SAS 9.4 (SAS Institute Inc., Cary, NC, USA). Means comparisons between temperatures were done using Fisher's protected least significant difference test using $P \leq 0.05$. Regression analysis was done using conidial germination percentage as a dependent variable and temperature as the independent variable. Different regression models such as linear, quadratic, and cubic regression models were examined for conidial germination percentage data at three different incubation periods. Regression models were considered significant if $P \leq 0.05$. The regression model which had best fit statistics were used to determine regression equations.

**Results**

Temperature ($P$<0.0001), incubation period ($P$<0.0001), and the interaction of temperature and incubation period ($P$<0.0001), had significant effects on conidial germination of a cotton isolate of *C. cassiicola*. An increase in conidial germination percentage at tested temperatures was generally observed with increase in incubation hrs (Fig. 1). Low mean conidial germination percentage (<15%) was observed at 12 and 16°C across all three incubation periods (Fig. 1). Mean conidial germination percentage was greatest at 24ºC for 8 and 12 hrs incubation periods (Fig. 1). No conidia germinated below 20 and 16ºC with 4 and 8 hrs of incubation, respectively (Fig. 1). After 12 hr incubation, conidia kept at 16ºC had the lowest mean germination. At the three highest temperatures

tested (24, 28, and 32℃), mean conidial germination was above 50 and 90% after 8 and 12 hrs, respectively (Fig. 1).

The response of conidial germination to the different temperatures after 8 and 12 hrs of incubation were modeled using quadratic regression analysis. The resultant quadratic equation for 8 hr incubation was:

$G = (-0.29) \times (T)^2 + 16.49 \times T - 170.67$; $R^2 = 0.56$, $P < 0.0001$; where G represents conidia germination %, and T represents temperature (Fig. 2). This model indicates that the optimum temperature for conidial germination after 8 hr incubation was approximately 28.4℃. The quadratic equation for conidia germination percentage after 12 hr incubation was: $G = (-0.25) \times (T)^2 + 16.15 \times T - 162.89$; $R^2 = 0.81$, $P < 0.0001$ (Fig. 3). The calculated optimum temperature for conidia germination after 12 hr incubation was 32.3℃.

**Discussion**

This study was conducted with the goal to evaluate the effect of temperature on conidial germination of a cotton isolate of *C. cassiicola*. The results of this study showed that conidial germination on water agar was equally high at 24 to 32°C with 12 hr incubation. These results appear similar to the favorable temperature range of 25 to 30°C for conidial germination of okra isolates of *C. cassiicola.* High conidial germination percentage (≥ 60%) of a soybean isolate on V-8 agar was observed at a wide temperature range, 10 to 30°C, with highest observed germination percentage (96%) at 15°C (Seaman et al. 1965). In the current study, low conidial germination (≤10%) of cotton isolate was observed at temperatures <20°C. The soybean isolate used in that study was collected in

September in Ottawa, Ontario (Seaman et al. 1965), and the average minimum and maximum temperature of Ottawa, Ontario in September is generally approximately 10 and 20.5°C, respectively. Therefore, it seems that soybean isolate evaluated by Seaman et al. (1965) was adapted to lower temperatures.

The usual average low and high temperature in August in Prattville, Alabama, from where the cotton isolate (CA1) used in this study was collected, is approximately 22.5 and 33.3°C, respectively. As noted above, high conidial germination percentage (≥90%) was observed at ≥ 24°C with 12 hr of incubation. Hence, it appears the environmental temperature prevailing in the cotton growing areas in Alabama are highly favorable for the conidia germination of cotton isolates of *C. cassiicola.*

In this study, bipolar germination of conidia of this cotton isolate was most commonly observed. Sometimes bipolar germination was also accompanied with germination of intercalary cells. However, Seaman et al. (1965) usually noticed polar germination with rare germination of intercalary cells, and sometimes bipolar germination of the soybean isolates of *C. cassiicola* was also observed. The conidial germination type can be affected by time of incubation period, substrate, and temperature (Seaman et al. 1965). As commonly observed bipolar germination of conidia of the cotton isolate in our study contrasts to the usual polar germination of soybean isolates of another study, therefore, it is possible that isolates with different host origins can also affect the type of conidial germination.

Our results showed that after 4 hr incubation, there was no significant difference in conidial germination percentage at the six-tested temperatures, 12 to 32°C. Therefore, it can be concluded that conidial germination of this cotton isolate requires more than 4 hr incubation in water to obtain high conidial germination at any temperature. However, at ≤20°C with 4 hr incubation very low (≤0.27%) germination occurred but at ≥24°C, approximately 10% conidial germination was observed. These results suggest that there is a possibility of initiation of infection processes at any temperature from 24 to 32°C with 4 hr of incubation in water.

The highest conidial germination percentage was observed at 24°C but there was no significant difference in conidial germination percentage after 8 or 12 hr incubation. Therefore, it seems that at 24°C and 8 hr incubation, further increases in wetness duration will not significantly increase the conidial germination percentage. So, it can be concluded that at 24°C, 8 hr incubation period is enough for high conidial germination and initiation of infection process by the cotton isolate (CA1) of *C. cassiicola*.

The three highest tested temperatures (24, 28, and 32°C) resulted in significantly high conidial germination after 4, 8, and 12 hrs incubation. Hence, it appears that lengthening incubation period had significant effect on conidial germination at these temperatures. At lower temperatures such as <20°C, no significant increase in conidial germination percentage was noted when the incubation period was extended from 4 to 12 hrs. Therefore, it can be assumed that conidia at <20°C requires more than 12 hr incubation

for high conidial germination as numerical increase in conidial germination was observed at 12 and 16°C with the lengthening of the incubation period.

In conclusion, our results showed that temperature had a significant effect on the time required for conidial germination of our cotton isolate of *C. cassiicola*. Among the six tested temperatures, 24°C with 8 and 12 hrs resulted in greatest conidial germination. In addition, the other two temperatures tested (28 and 32°C) with 12 hr also resulted in significantly higher conidial germination than with 8 hr incubation at these temperatures. Therefore, it can be concluded that at ≥24°C, an increase in incubation period can result in a significant increase in conidial germination. Moreover, as noted above, the average environmental temperatures in the cotton growing areas of Alabama are highly favorable for conidial germination of cotton isolate of *C. cassiicola* and this isolate is well adapted to Alabama. This result can explain the high initiation of infection process of *C. cassiicola* on cotton.

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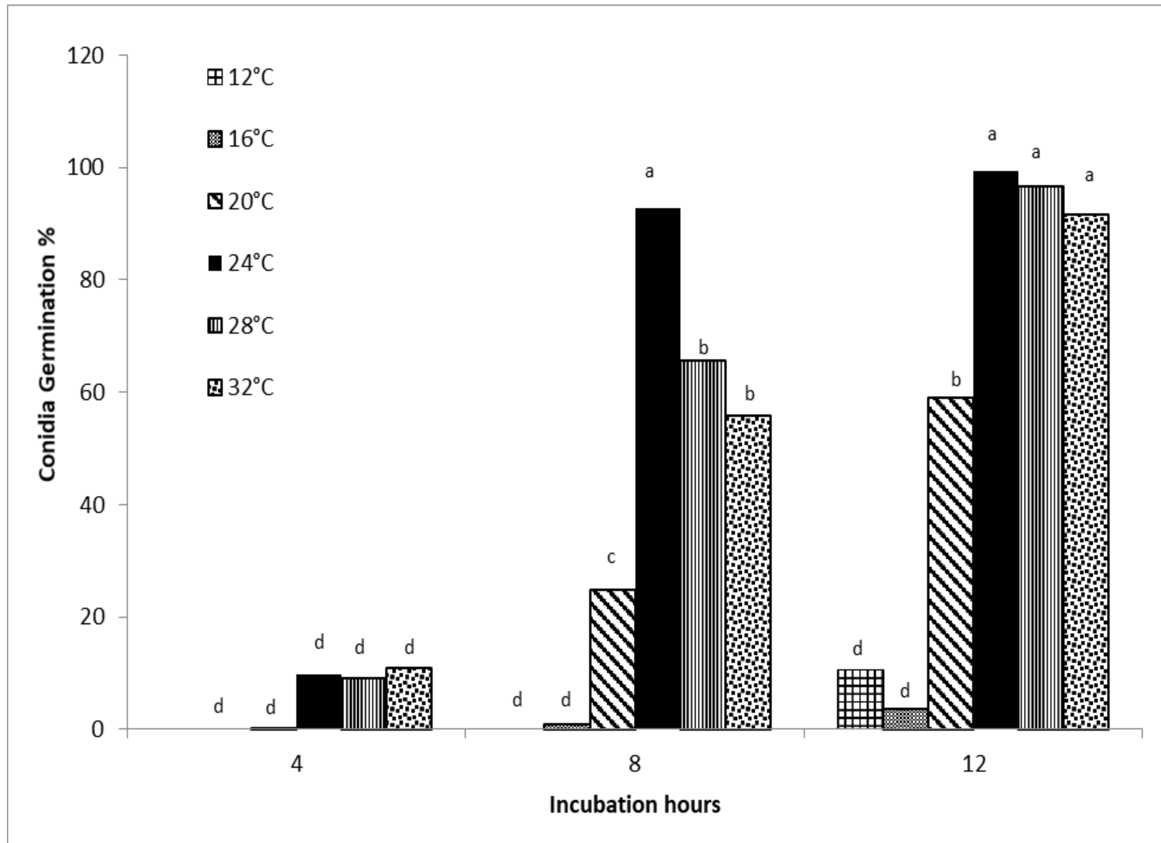


Figure 1. Conidia germination percentage with 4, 8, and 12 hrs incubation. Different letters above bars represent significant differences among different temperatures at *P*=0.05.

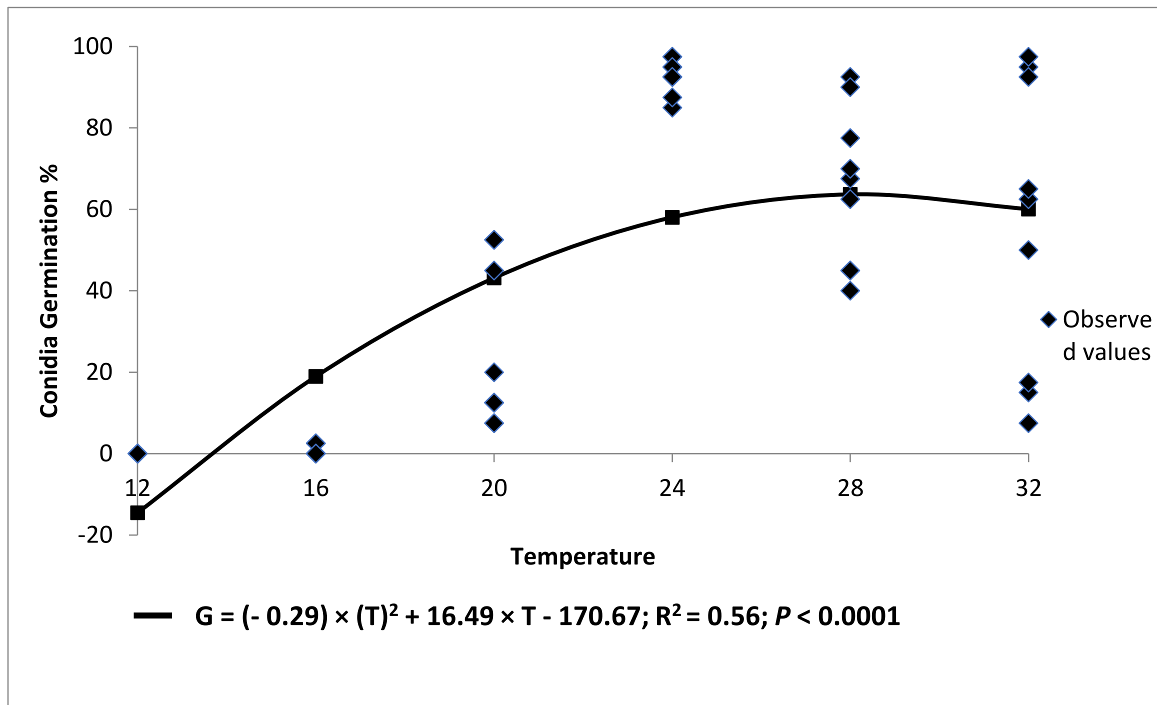


Figure 2. Effect of temperature on conidia germination after 8 hr incubation. Quadratic regression was conducted using conidia germination percentage (G) after 8 hr as the dependent variable and temperature (T) as the independent variable.

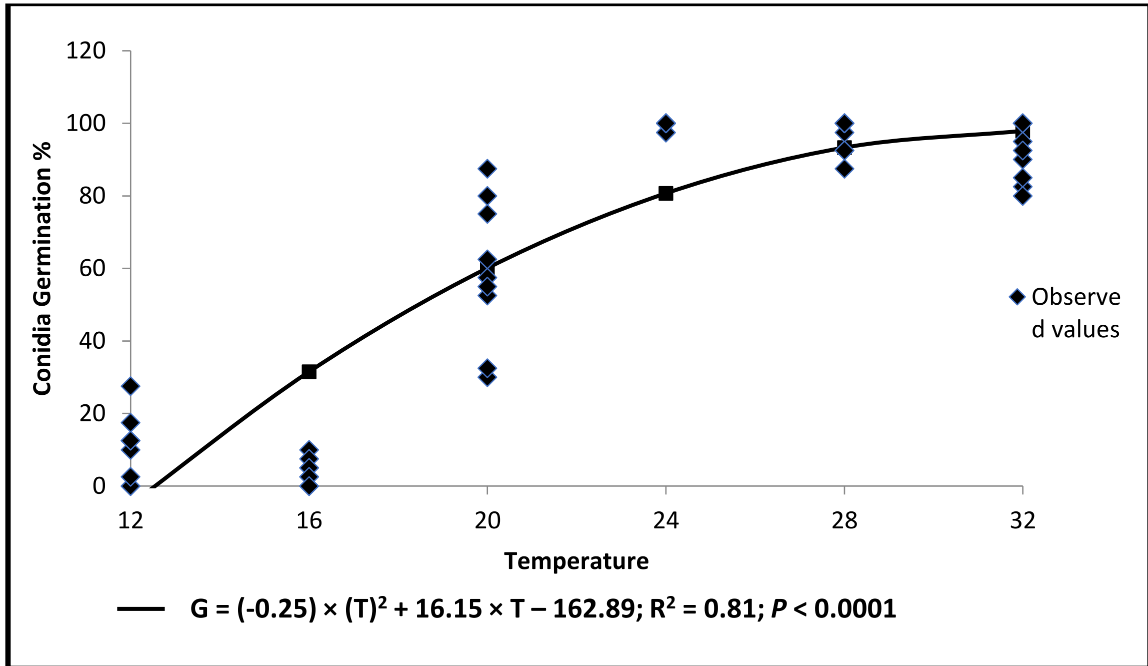


Figure 3. Effect of temperature on conidia germination after 12 hr incubation. Quadratic regression was conducted using conidia germination percentage (G) after 12 hr as the dependent variable and temperature (T) as the independent variable.

## III. Effect of temperature and leaf wetness duration on target spot of cotton caused by *Corynespora cassiicola*

### Abstract

Cotton is an important crop in the southeastern and mid-eastern U. S. *Corynespora cassiicola* is causing a foliar disease, target spot, that has the potential to cause economic losses to susceptible cotton varieties. To manage target spot of cotton, understanding the optimum environmental conditions required for disease development is needed. Therefore, the purpose of this study was to determine the favorable weather parameters, such as temperature and leaf wetness duration, that affect disease onset and lesion development. Five different temperatures (16, 20, 24, 28 and 32°C) and seven leaf wetness durations (8, 12, 16, 24, 32, 40, and 48 hrs) were tested. Plants were drop inoculated, kept in wet plastic bags as specified above, and arranged in a split plot design in growth chambers that had set temperatures. Greatest lesion numbers were observed at 28°C with 48 hr of leaf wetness for all three *C. cassiicola* isolates, while isolate CA1 and CM18 at 16°C and CC1 at 32°C resulted in lowest lesion numbers. Lengthening leaf wetness duration at each tested temperature generally resulted in increased lesion numbers for all isolates. Earliest onset of disease was 1 day after inoculation (DAI) and occurred when plants were incubated at 28°C with ≥8 hr leaf wetness for isolates CA1, and CM18 and with ≥24 hr leaf wetness duration for isolate CC1.

**Keywords**: epidemiology, onset, and lesion development.

## Introduction

Target spot of cotton is a foliar disease caused by *Corynespora cassiicola*. This disease can be responsible for premature defoliation and can cause reduction in yields of cotton (Hagan 2015). *Corynespora cassiicola* is a well-known necrotrophic, leaf spotting plant pathogen that is widely distributed in subtropical and tropical regions worldwide (Barthe et al. 2007; Huang et al. 2010). Isolates of *C. cassiicola* have also been reported to be endophytic in some hosts (Collado et al. 1999; Gond et al. 2007; Hyde et al. 2001; Kingsland 1985; Lee et al. 2004). This fungus is a cosmopolitan plant pathogen and has been found on 530 plant species from 380 genera including monocots, dicots, ferns, one cycad, as well as on human skin (Dixon et al. 2009; Huang et al. 2010). Some economically important hosts of *C. cassiicola* include *Solanum lycopersicum* (tomato) (Barthe et al. 2007; Schlub et al. 2009), *Glycine max* (soybean) (Seaman et al. 1965), *Gossypium hirsutum* (cotton) (Conner et al. 2013; Fulmer et al. 2012), *Carica papaya* (papaya) (Cutrim and Silva 2003; Oluma and Amuta 1999), *Hevea brasiliensis* (rubber) (de Lamotte et al. 2007; Jinji et al. 2007), *Vigna unguiculata* (cowpea) (Li et al. 2014), *Nicotiana tabacum* (tobacco) (Fajola and Alasoadura 1973), and *Capsicum annuum* (pepper) (Chai et al. 2014).

Target spot of cotton is an emerging disease in the southeastern United States. Target spot of cotton was first reported in Mississippi (Jones 1961). This disease was reported again by a crop consultant in 2005 in southwestern Georgia (Fulmer et al. 2012). Since 2005, target spot has been reported in most cotton producing states in the U. S. (Mehl

et al. 2017). In Alabama, target spot was detected for the first time on dryland and irrigated cotton in 2011 (Conner et al. 2013).

In Alabama, cotton is the third most important crop in terms of production acreage (USDA-NASS 2016). In the lower third of Alabama, target spot can be a damaging disease in intensively managed, irrigated 'rank' cotton (Hagan 2015). Symptoms of this disease can be seen on leaves and bracts of cotton plants (Fulmer et al. 2012). The initial symptoms are small brick red lesions, which later merge and turn to circular or irregular concentric spots with tan to light brown centers (Conner et al. 2013; Fulmer et al. 2012). Lakshmanan et al. (1990) reported that some isolates of *C. cassiicola* caused boll rot in India. Initially, circular water soaked lesions with dark brown margins can be seen on which later merge and become necrotic spots and these necrotic spots lead to boll rot.

Target spot of cotton can cause up to 70% premature defoliation (Fulmer et al. 2012) which can result in significant lint losses. In 2013 at a coastal southwestern Alabama study, target spot of cotton was associated with 448 kg/ha lint losses in an apparently susceptible cultivar, PhytoGen 499 WRF, and 269 kg/ha lint losses in an apparently less susceptible variety, Deltapine 1252 B2RF (Hagan et al. 2015).

Plant disease management is closely related to plant disease epidemiology (Tarr 1972). Understanding of the effect of environmental factors on disease development is important in order to manage the disease efficiently. Disease forecasting can help to efficiently time fungicide applications. Temperature and moisture are two important

environmental factors which affect onset and development of foliar diseases such as target spot.

Pathogens differ relative to the conditions that favor their development when on different hosts. Isolates of *C. cassiicola* from different hosts appear to have different optimum temperatures and leaf wetness durations for disease development. For example, favourable temperatures for disease development on tomato is above 20˚C and severe disease is seen at 32˚C (Schlub et al. 2009), while disease development on soybean is more severe at 15˚C and no disease is detected if temperatures remain above 20˚C (Seaman et al. 1965). The favorable temperature range for target spot development on *Lantana camara* is 20 to 30˚C (Pereira et al. 2003), as compared with 25 to 30˚C for cucumber (Kwon et al. 2003). Although target spot of tomato requires 16 to 44 hrs of leaf wetness for disease development (Jones and Jones 1984), *C. cassiicola* on *L. camara* only requires leaf wetness for 6 hours for disease development (Pereira et al. 2003) while long dew periods are required for disease development on cucumber (Kwon et al. 2003). Although a dry season in August through September reduces disease in soybean, drought conditions in sesame increases losses due to leaf spot caused by *C. cassiicola.* This suggests that plants become more susceptible to the disease due to premature senescence occurring as a result of drought (Seaman et al. 1965).

Target spot of cotton is becoming a serious threat to cotton growing in the southeast U. S. In order to efficiently manage this disease, it is important to understand the environmental conditions required for the occurrence and development of this disease.

Knowledge of favorable temperature and leaf wetness duration requirements can be used to accurately predict the disease occurrence and development.

## Materials and Methods

**Production of fungal colonies.** Two isolates of *C. cassiicola* (CM18 and CA1) were obtained from M. Brewer's laboratory, UGA, and one isolate of *C. cassiicola* (CC1) was recovered from a lesion on a diseased cotton plant at the Prattville Agricultural Research Unit, Prattville, Alabama, in 2015. The diseased leaf was kept in a moist chamber for 24 hrs and then lesions on the leaf were microscopically examined. A sterilized needle was used to dislodge a spore from the lesion, and the spore was placed on V8 media. This medium was prepared with 200 ml of clarified V8 juice, 2 g of calcium carbonate and 15 g of agar per liter of water (Jeffers 2007). These cultures were maintained at 28˚C.

**Spore production.** Colonies of the cotton isolates of *C. cassiicola* (CM18, CA1, CC1) were produced on quarter-strength potato dextrose agar (qPDA), prepared with 9.75 g of potato-dextrose-agar powder (PDA) and 11.25 g of agar powder per liter of water. Fungal plugs of 5-mm diameter were taken from the periphery of the original culture petri dishes at 12 days. These fungal plugs were sub-cultured on fresh qPDA every 12 days and maintained at 28˚C to obtain fungal colonies for spore production.

**Preparation of spore suspension**. A spore suspension was prepared from 12 day old colonies. Petri dishes containing colonies were filled with 3 ml of sterile agar water, prepared with 2 g of agar powder and 0.5 ml of Tween 20 per liter. The mycelium was lightly scraped with a sterile L-shaped glass rod to detach spores. The resultant spore

suspension was filtered through 4 layers of cheesecloth. The spore concentration was counted by using a hemocytometer and adjusted to $4\times10^4$ spores/ml.

**Plant production.** Seeds of the upland cotton variety, PhytoGen 499 WRF, were kept in a moist chamber for 2 days to initiate germination. Germinated seeds were planted in potting mixture (Sun Gro professional growing mix, Seba Beach, Canada) in $9.5 \times 9.5$ $cm^2$ plastic pots. The potting mixture was autoclaved 3 times for 60 minutes each time before planting seeds. Plants were kept at 28˚C in a growth chamber and watered as required.

**Inoculation method.** Plants were inoculated with each of three isolates (CA1, CC1, and CM18) when 22 to 25 days old (3 to 4 true leaf stage) at 5:00 p.m. Three true leaves of a plant were drop-inoculated with a pipette. Five drops of spore suspension of 40 µl each were inoculated per leaf. Inoculated leaves were covered with wet tissue (Kimwipes) and then the entire plant was enclosed in a plastic bag.

**Effect of temperature and moisture on disease development.** Bagged plants were kept at 16, 20, 24, 28 and 32˚C in individual growth chambers and inside each chamber, isolates were whole plots and leaf wetness duration were arranged as split-plot treatments. Trials were repeated in growth chambers, with different temperatures, which allowed for the larger unit of temperature. Twenty-one plants were inoculated with each isolate, for each temperature, and the inoculated plants were kept in a growth chamber. Three inoculated plants served as three subsamples for each isolate, and were removed from the plastic bags at 8, 12, 16, 24, 32, 40, and 48 hrs after inoculation. Plants were

examined at 24 hr intervals following inoculation and day of disease onset was recorded. Lesions were counted on three inoculated leaves at 3, 6, and 9 days after inoculation (DAI). The whole experiment was repeated once.

**Data analysis.** The number of days after inoculation recorded for the onset of symptoms and total number of lesions counted on three inoculated leaves for each treatment at 3, 6 and 9 DAI were used for data analysis. Generalized linear mixed model analyses (PROC GLIMMIX) were done using SAS 9.4 (SAS Institute Inc., Cary, NC, USA) to determine the effect of factors and interactions terms on onset and lesion counts. Linear and cubic regression analysis were performed using PROC REG on SAS 9.4 to understand the effect of each treatment on lesion counts.

**Results**

**Onset**. There were significant effects due to temperature, leaf wetness duration, isolate, and all interaction terms except for the interaction of temperature and isolate on disease onset (Table 1). Earliest onset, i.e., 1 day after inoculation (DAI), was observed at 28˚C for isolate CA1 with 8 to 48 hrs leaf wetness duration (Fig. 1A). Similarly, isolates CM18 (Fig. 1B) and CC1 (Fig. 1C) had the earliest onset 1 day after inoculation (DAI) at 28°C with ≥24 leaf wetness hrs. With isolate CA1, disease onset was generally observed 2 DAI at 20 and 24˚C in combination with ≥12 hr leaf wetness, while plants inoculated with isolates CM18 and CC1 needed ≥24 leaf wetness hr for disease onset at 2 DAI (Fig. 1). No disease was observed with any of the isolates at 16˚C with leaf wetness durations < 24 hr. Similarly, 32˚C with leaf wetness durations less than 16, 24 and 32 hr resulted in no

disease development with isolates CA1, CM18 and CC1, respectively (Fig. 1). No disease onset was observed for all three isolates (CA1, CM18, CC1) at four tested temperatures (16, 20, 24, and 32˚C) with 8 hr leaf wetness (Fig. 1). Disease onset was delayed with leaf wetness duration of 24 hr or less at 16 and 32˚C.

**Lesion numbers**. There were significant effects due to temperature, leaf wetness duration, isolate, and all interaction terms on lesion numbers at 3, 6, and 9 DAI (Table 1). Positive slopes of linear models showed that for each of the three isolates at all incubation temperatures (16, 20, 24, 28, and 32°C), lengthening leaf wetness duration usually resulted in increased lesion number at 3 (Fig. 2A, 3A, 4A), 6 (Fig. 2B, 3B, 4B), and 9 DAI (Fig. 2C, 3C, 4C). For example, lesion numbers with isolates CA1, CM18, and CC1 at 24˚C with 16 hr leaf wetness duration at 3 DAI were 7.3, 5.5, and 2.0, respectively, compared with 139.8, 62.5, and 19.3, respectively, when leaf wetness duration increased to 48 hr. All three isolates had highest lesion counts at 28˚C at 3 (Fig. 2A, 3A, 4A), 6 (Fig. 2B, 3B and 4B) and 9 DAI (Fig. 2C, 3C and 4C). Highest lesion numbers at 3 (Fig. 2A, 3A, 4A), 6 (Fig. 2B, 3B, 4B) and 9 DAI (Fig. 2C, 3C, 4C) was observed at 28˚C with 48 hr leaf wetness across all isolates. For example, the highest mean numbers of lesions at 3 DAI for CA1, CM18 and CC1 were 257.2, 212.3 and 42.7, respectively. Among all five tested temperatures (16, 20, 24, 28 and 32°C) at each tested leaf wetness duration, lowest lesion counts at 3, 6 and 9 DAI were observed at 16˚C for isolate CA1 and CM18, while CC1 had lowest lesion counts at 32˚C. Cubic regression suggested that optimum temperature for isolates CA1, CM18 and CC1 with 8 hr leaf wetness is 29.1, 28.1 and 25.9°C, respectively

(Fig. 5A). Similarly, cubic regression suggested that optimum temperature for isolate CA1, CM18 and CC1 with 16 hr leaf wetness (Fig. 5B) is 28.5, 28.5, 27.6°C, respectively, while with 24 hr leaf wetness (Fig. 5C) optimum temperature for isolate CA1, CM18 and CC1 is 28.1, 27.6 and 27.8°C, respectively.

Overall, at 3, 6, and 9 DAI for all *C. cassiicola* isolates, 16 and 32˚C with each tested leaf wetness durations had low lesion counts, while an increase in lesion counts were noticed with a rise in temperature from 20 to 28˚C when leaf wetness duration lengthened from 12 to 48 hrs (Fig. 2, 3 and 4). Isolate CA1 was more virulent based on lesion counts, followed by CM18 and then CC1. For example, 28°C with 48 hr leaf wetness lead to 275.5, 233.8 and 48.8 mean lesion counts for isolate CA1, CM18 and CC1, respectively, at 9 DAI.

**Discussion**

The goal of this study was to investigate the influence of environmental conditions, such as temperature and leaf wetness duration, that favors the onset and development of *C. cassiicola*-incited target spot on cotton. We used onset and lesion numbers at 3, 6, and 9 DAI as the basis for disease evaluation. Temperature, leaf wetness duration and their interaction had significant effects on target spot development on cotton. Jones and Jones (1984) also found that temperatures of 16, 20, 24, 28, and 32°C resulted in moderate disease development on tomato. Similar to Jones and Jones, we found that *C. cassiicola* was able to cause disease on cotton over the tested temperature range of 16 to 32°C, however at the extreme tested temperatures (16 and 32°C) low lesion counts resulted only with ≥ 24 hr of leaf wetness.

Fulmer et al. (2012) kept inoculated cotton seedlings for 48 hr in a moist chamber at 21.1°C and noted disease onset within 1 week of inoculation. Similarly, Conner et al. (2013) observed disease onset in 6 days when inoculated plants were kept at 21°C in a moist chamber for 72 hr. We observed disease onset at 2 DAI on plants inoculated with CA1 at 20°C with ≥16 hrs leaf wetness. Plants were incubated at constant temperature in our study that might resulted in early onset than in the above stated studies.

In our study, the highest disease severity was observed at 28°C with 48 hr leaf wetness (maximum leaf wetness duration tested). Seaman et al. (1965) found that day and night temperatures of 25 ± 2°C and 22 ± 2°C, respectively, with 48 hr moisture, resulted in numerous pinpoint lesions on the foliage of soybean. Similarly, Jones and Jones (1984) found that 20 to 28°C was the favorable temperature range, with highest disease severity observed at 24 and 28°C on tomato. Therefore, it seems like that favorable temperature for target spot of cotton coincides those observed for target spot of soybean and tomato.

During our experiments, leaf wetness was required for disease onset. No symptoms were observed in our preliminary experiments with very high relative humidity but without free moisture on leaf surfaces. The leaf wetness duration required for the onset and development of target spot of cotton is temperature dependent similar to other pathosystems such as apple scab (MacHardy and Gadoury 1988) and early blight of tomato (Madden et al. 1978). Our study showed that shorter leaf wetness durations of 8 and 12 hrs were sufficient for disease onset at 28 and 24°C, respectively. However, at temperature extremes of 16 and 32°C, *C. cassiicola* required a leaf wetness duration in excess of 24 hr

for disease onset. This trend might be explained by our previous study of incubation time required for spore germination of cotton isolates at different temperatures. In that study, it was noted that lower temperatures such as 16°C with 12 hr (wetness period) resulted in a low spore germination of approximately 10% compared with > 65% germination at 24 to 28°C with 8 hr wetness period, which shows that longer moisture periods are required for spore germination at lower temperatures. However, approximately 90% spore germination was observed at 32°C with 12 hr wetness but onset of disease was not observed < 24 hr leaf wetness. This possibly suggests that at this elevated temperature, other processes such as penetration and colonization of leaf tissues might be interrupted or delayed.

Another significant finding of our study was that lesion numbers increased linearly as leaf wetness duration lengthened from 8 to 48 hrs at each tested temperature (16, 20, 24, 28, and 32°C). Possibly, extended leaf wetness duration gives sufficient time for spore germination as above noted. This can aid in completing the infection process and result in high disease severity.

The usual average low and high temperatures prevailing in Alabama in July to September seems highly favorable for the target spot of cotton. Therefore, more numerous rain events in the months of July to September in Alabama favors rapid disease development that results in premature defoliation within a few days of target spot onset.

Our results show that with lower temperatures such as 16°C with ≥ 24 hr leaf wetness, onset of disease was observed which suggests that *C. cassiicola* isolates from cotton are adaptive to low temperatures. For isolate CA1 at 20°C to 28°C with ≥16 hr and

32°C with ≥24 hr leaf wetness, disease onset was observed 2 and 3 DAI, respectively. It seems likely that this finding can explain the recent reports of target spot outbreaks in cotton in Mid-south states such as Tennessee as favorable temperatures for disease generally prevails in months of July to September (Kelly 2016).

In our study, we usually observed vein necrosis of inoculated leaves at 24 and 28°C with ≥ 40 hr leaf wetness. Also, lesions started to coalesce after 9 DAI leading to premature defoliation at 24 to 28°C with longer leaf wetness durations. This might have happened due to cassiicolin, the toxin produced by *C. cassiicola* that triggers leaf abscission (Onesirosan et al. 1975) and only one lesion on the main vein may be sufficient to trigger this process (Chee 1988).

Results of this study described the effect of leaf wetness duration and temperature conditions on the onset and disease severity of target spot of cotton, but this does not incorporate the knowledge of the entire infection and sporulation cycle of *C. cassiicola*. Therefore, future studies should involve the study of environmental conditions that affect pathogen penetration, infection processes and sporulation on lesions. Another limitation of our study is that all experiments were performed on a single apparently susceptible cotton cultivar, PhytoGen 499 WRF, and the results have not been validated under field conditions. Therefore, our study can be the basis of future field experiments which can include the effect of moisture conditions and temperatures on infection of additional cotton varieties, including apparently resistant varieties.

We observed that all three isolates (CA1, CM18 and CC1) used in this study had similar favorable temperature and leaf wetness requirements for target spot of cotton. Two isolates (CA1 and CM18) were from Georgia, while CC1 was isolated in Alabama. Therefore, this suggests that isolates from different geographical origin have similar favorable environmental conditions for causing target spot of cotton. This finding supports that all cotton isolates of *C. cassiicola* are genotypically similar (Sumabat et al. 2016).

*Corynespora* had been known as *Helminthosporium* until the mid-1900's on the basis of similar morphological conidia characterstics (Schlub 2009). Our observation also showed that conidial structure of cotton isolates of *C. cassiicola* was similar to *Helminthosporium*. In addition, conidia of *Corynespora cassiicola* germinate in a similar manner as conidia of *Helminthosporium,* i.e., conidia germinated generally from terminal cells and sometimes from intercalary cells (Luttrell 1963). The temperature and leaf wetness relationship that we established in this study will help in efficiently managing the target spot of cotton by timely spray of fungicides. Moreover, this information can be helpful in developing risk maps and pest risk assessments of *C. cassiicola.*

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Table 1. Table of significance for the effect of temperature, leaf wetness duration, and isolate on disease severity (3 DAI, 6 DAI, 9 DAI) and onset by *C. cassiicola*.

| | **Factors** | **DF** | **F value** | **Pr > F** |
|---|---|---|---|---|
| **Onset** | Temperature (T) | 4 | 66.62 | 0.0007 |
| | Leaf wetness duration (M) | 6 | 303.34 | <.0001 |
| | Isolate (I) | 2 | 64.20 | <.0001 |
| | T×M | 17 | 45.39 | <.0001 |
| | M×I | 12 | 67.41 | <.0001 |
| | I×T | 8 | 1.42 | 0.3255 |
| | T×M×I | 29 | 2.37 | <.0001 |
| **3 DAI** | Temperature (T) | 4 | 880.16 | <.0001 |
| | Leaf wetness duration (M) | 6 | 1002.31 | <.0001 |
| | Isolate (I) | 2 | 512.48 | <.0001 |
| | T×M | 24 | 265.60 | <.0001 |
| | M×I | 12 | 139 | <.0001 |
| | I×T | 8 | 187.28 | <.0001 |
| | T×M×I | 48 | 51.43 | <.0001 |
| **6 DAI** | Temperature (T) | 4 | 705.97 | <.0001 |
| | Leaf wetness duration (M) | 6 | 1080.31 | <.0001 |
| | Isolate (I) | 2 | 685.72 | <.0001 |
| | T×M | 24 | 252.22 | <.0001 |
| | M×I | 12 | 141.14 | <.0001 |
| | I×T | 8 | 230.39 | <.0001 |
| | T×M×I | 48 | 48.21 | <.0001 |
| **9 DAI** | Temperature (T) | 4 | 837.43 | <.0001 |
| | Leaf wetness duration (M) | 6 | 1065.61 | <.0001 |
| | Isolate (I) | 2 | 691.73 | <.0001 |
| | T×M | 24 | 248.52 | <.0001 |
| | M×I | 12 | 137.58 | <.0001 |
| | I×T | 8 | 226.83 | <.0001 |
| | T×M×I | 48 | 46.67 | <.0001 |

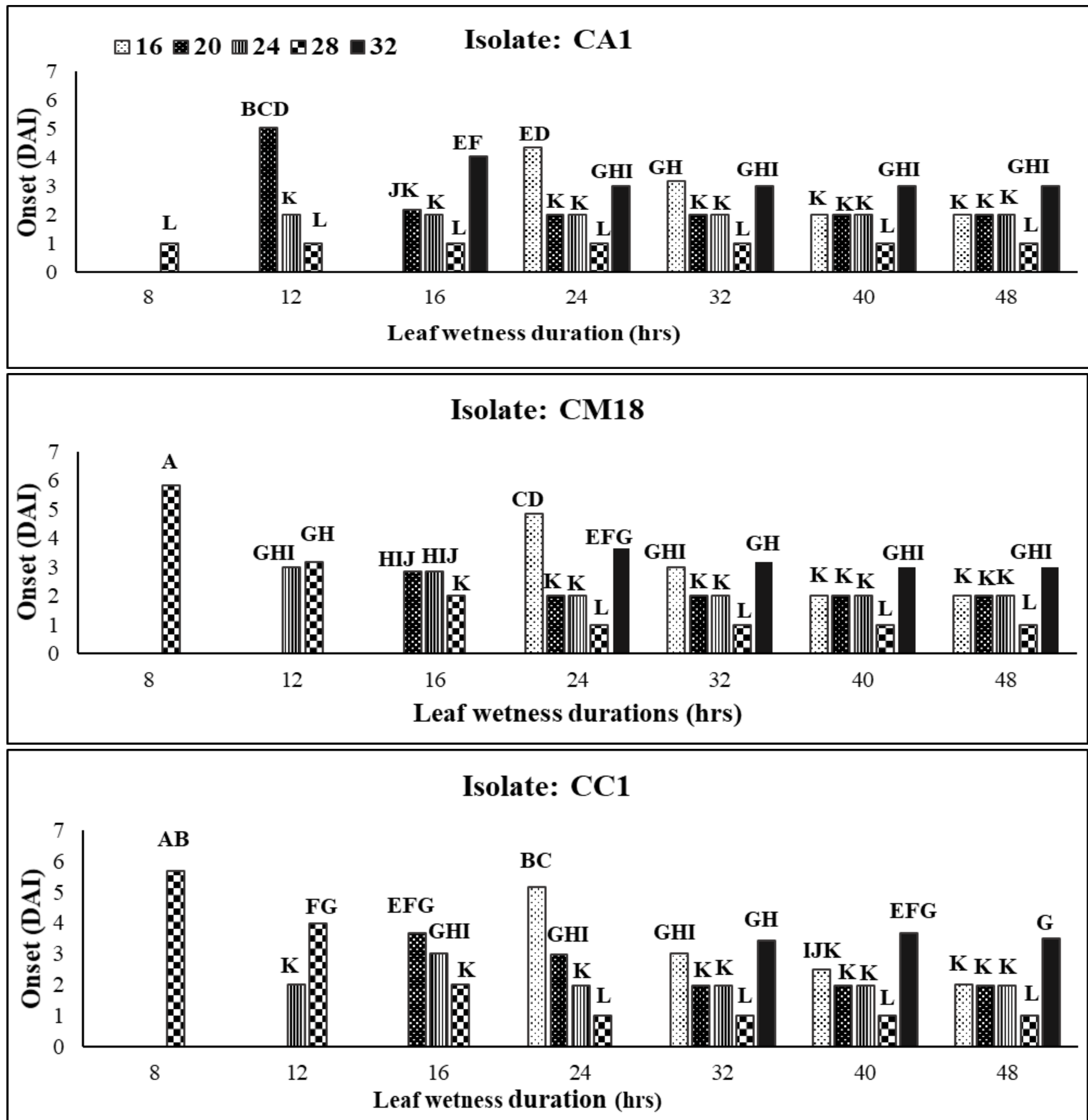


Figure 1. Effect of leaf wetness duration on days after inoculation (DAI) required for onset of target spot of cotton by *Corynespora cassiicola* for isolates CA1 (A), CM18 (B) and CC1 (C) at five different temperatures (16, 20, 24, 28 and 32°C). Different letters above bars represent significant differences among all three isolates and five temperatures at $P$=0.05.

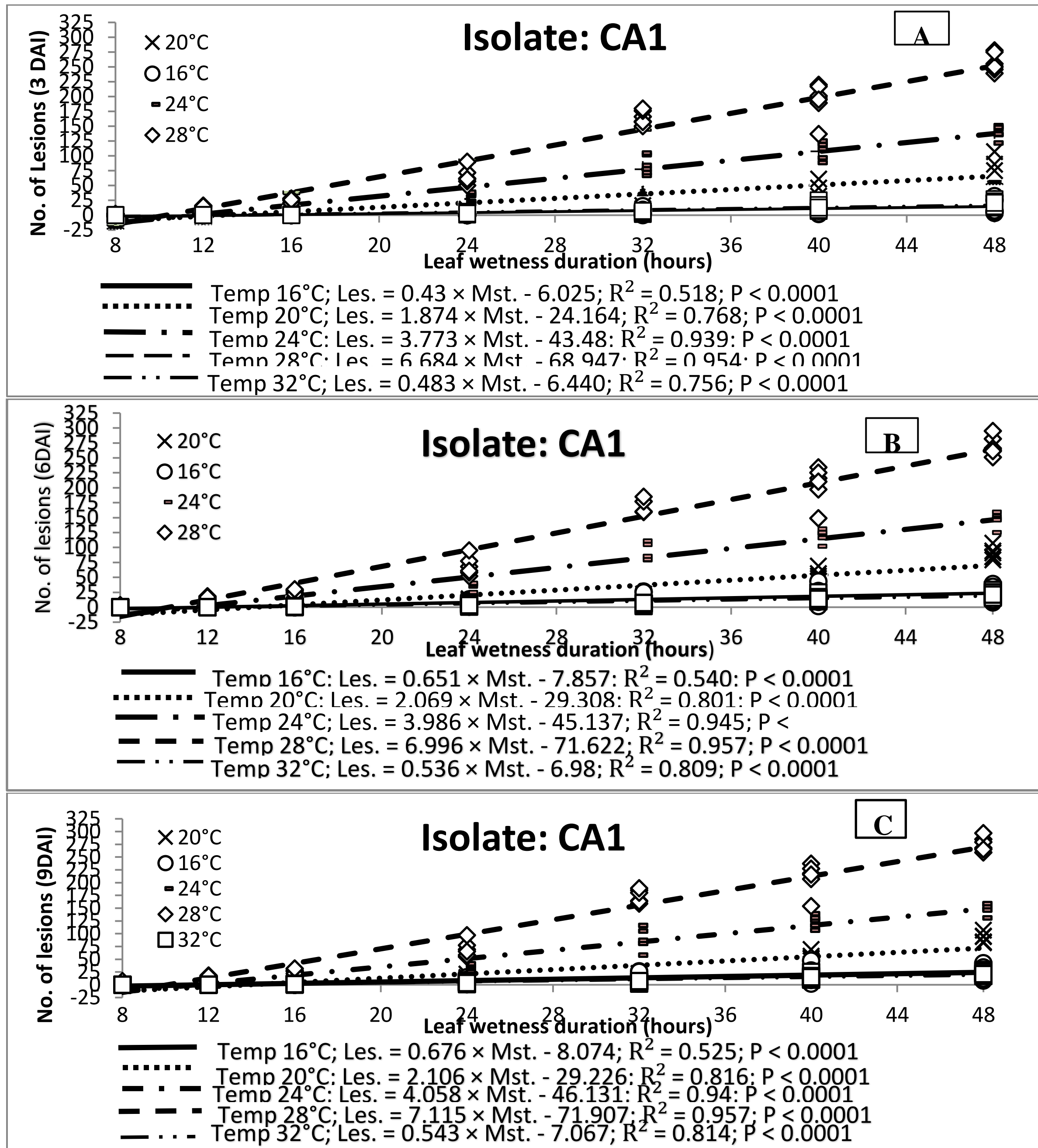


Figure 2. Effect of leaf wetness duration on number of lesions produced by *Corynespora cassiicola* (Isolate: CA1) at five different temperatures (16, 20, 24, 28 and 32°C) counted 3 (A), 6 (B) and 9 (C) days after inoculation (DAI). Linear regression was conducted for incubation temperatures (16, 20, 24, 28 and 32°C) using lesion counts as a dependent variable and leaf wetness duration as an independent variable.

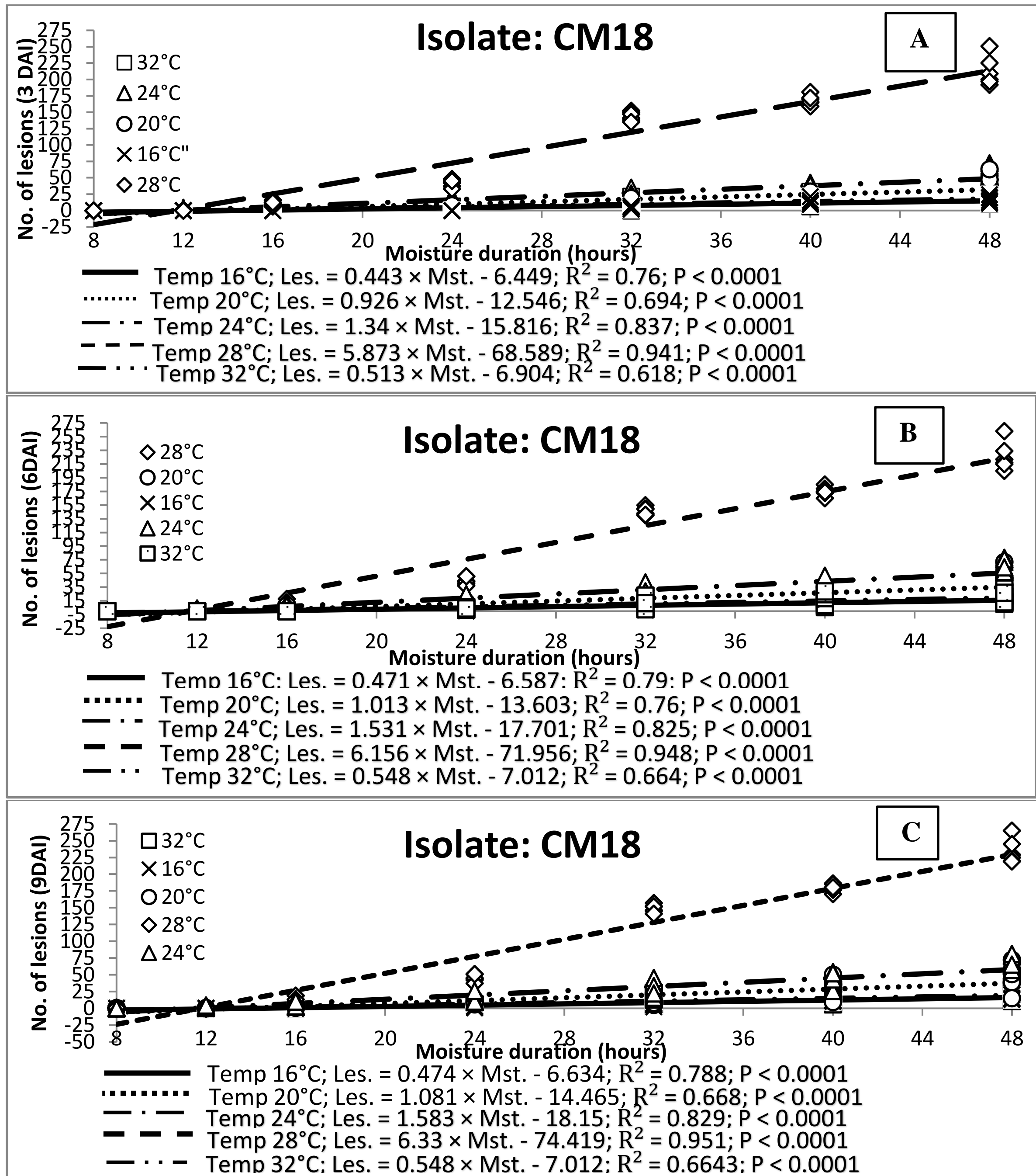


Figure 3. Effect of leaf wetness duration on number of lesions produced by *Corynespora cassiicola* (Isolate: CM18) at five different temperatures (16, 20, 24, 28 and 32°C) counted 3 (A), 6 (B)and 9 (C) days after inoculation (DAI). Linear regression was conducted for incubation temperatures (16, 20, 24, 28 and 32°C) using lesion counts as a dependent variable and leaf wetness duration as an independent variable.

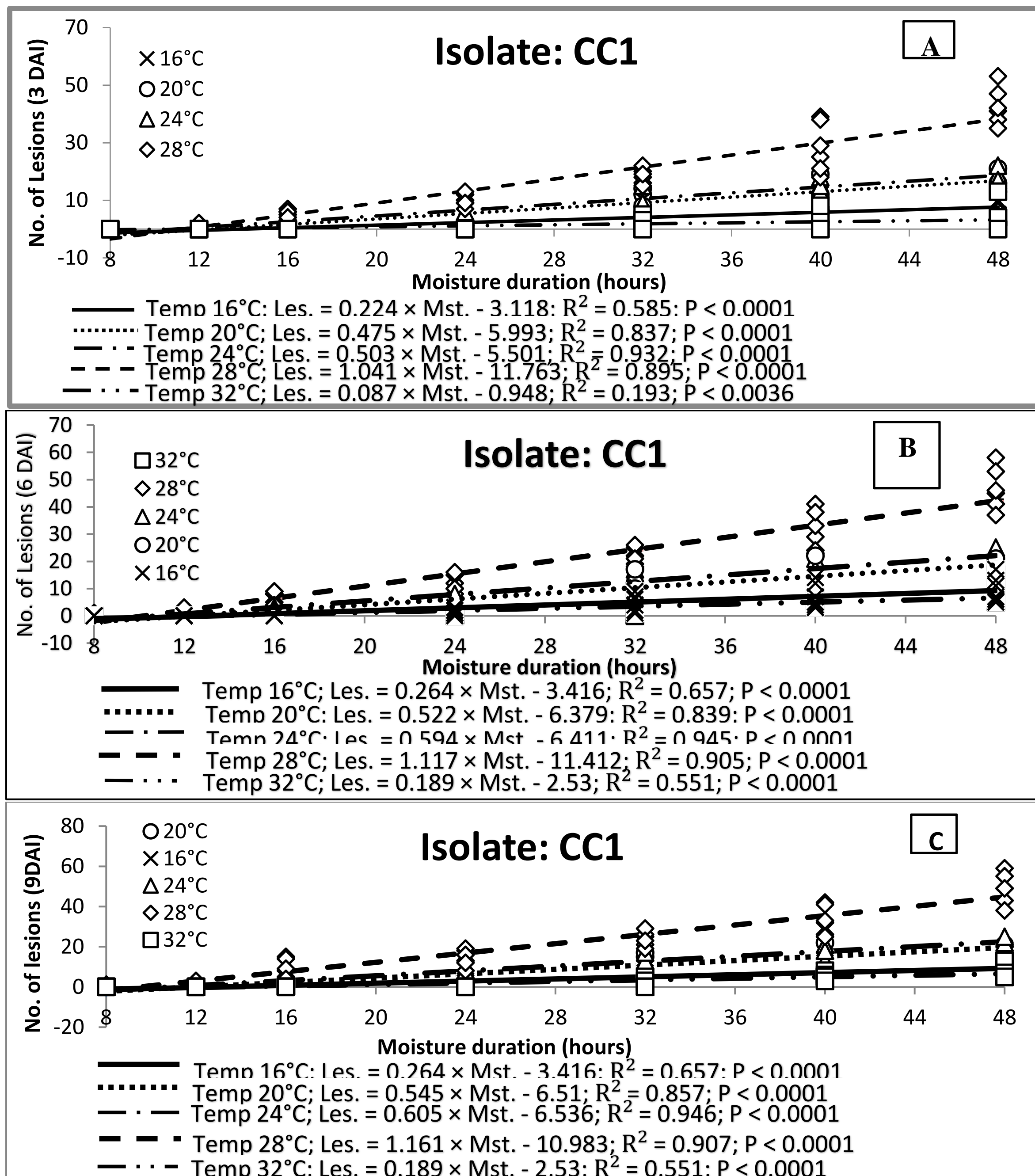


Figure 4. Effect of leaf wetness duration on number of lesions produced by *Corynespora cassiicola* (Isolate: CC1) at five different temperatures (16, 20, 24, 28 and 32°C) counted 3 (A), 6 (B) and 9 (C) days after inoculation (DAI). Linear regression was conducted for incubation temperatures (16, 20, 24, 28 and 32°C) using lesion counts as a dependent variable and leaf wetness duration as an independent variable.

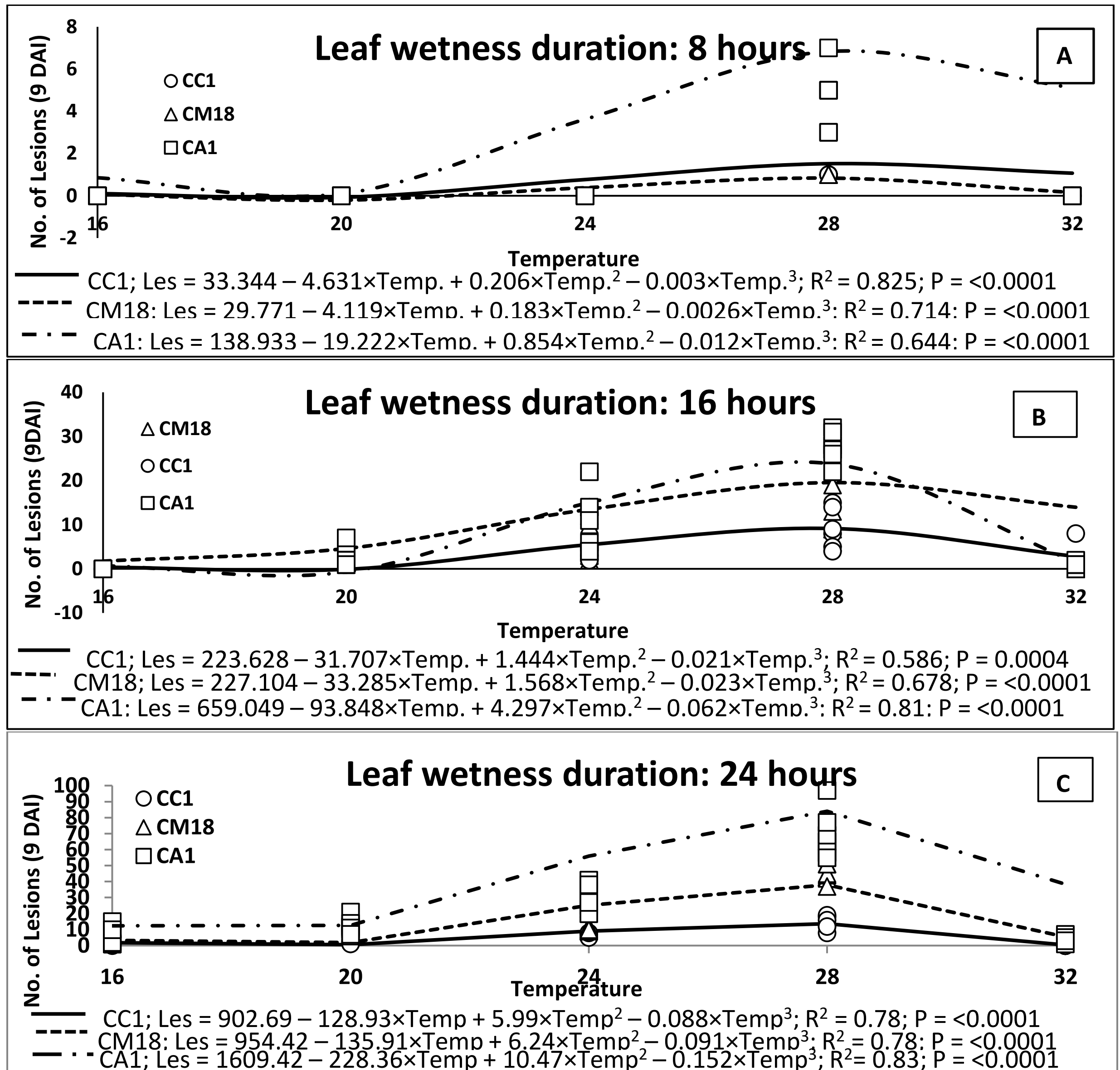


Figure 5. Effect of temperature on number of lesions produced by *Corynespora cassiicola* (Isolate: CA1) after 8(A), 16 (B) and 24 hours (B) leaf wetness duration counted 9 days after inoculation (DAI). Cubic regression was conducted for leaf wetness duration (8, 16 and 24 hours) using lesion counts as a dependent variable and temperature as an independent variable.

## IV. Difference in epidemiology of target spot of tomato caused by tomato and cotton isolates of *Corynespora cassiicola*


### Abstract

Target spot of tomato is caused by a cosmopolitan pathogen, *Corynespora cassiicola*. Cotton is another important host of this pathogen. Cotton isolates are cross pathogenic to tomato, therefore, the goal of this study was to determine if the favorable environmental conditions differ for isolates of *C. cassiicola* originating from different hosts. Three different temperatures (20, 24 and 28°C), and seven leaf wetness durations (8, 12, 16, 24, 32, 40 and 48 hrs) were tested. Plants were drop inoculated, kept in wet plastic bags, and arranged in a randomized complete block in a growth chamber at a set temperature. Highest lesion numbers were observed at 28°C with 48 hrs of leaf wetness for both tomato isolates (TCL1 and 1343) and the cotton isolate (CA1), while 20°C resulted in lowest disease severity. However, the cotton isolate caused no disease at 20°C with <40 hrs leaf wetness. Increases in leaf wetness duration at each of the tested temperatures generally resulted in increased numbers of lesions for all isolates (TCL1, 1343 and CA1). Disease onset required ≥16 hrs of leaf wetness duration at 24 and 28°C for the cotton isolate (CA1), while for tomato isolates (1343 and TCL1), 28°C with ≥12 hrs and 24°C with ≥16 hrs leaf wetness resulted in disease onset.



**Keywords.** Temperature, leaf wetness duration, host, onset and lesion numbers.

## Introduction

Target spot of tomato is an important foliar disease of field and greenhouse grown tomatoes in Florida (Pernezny et al. 2002; Schlub et al. 2009). Target spot can cause losses up to 11,800 kg/ha when the disease is not managed (Pernezny et al. 2002). This disease is caused by a cosmopolitan fungus, *Corynespora cassiicola,* which is a well-known necrotrophic leaf spotting plant pathogen abundantly present in subtropical and tropical regions of the world (Barthe et al. 2007; Huang et al. 2010). This fungus has been found on 530 plant species from 380 genera including monocots, dicots, ferns, and one cycad (Dixon et al. 2009; Huang et al. 2010). *Corynespora cassiicola* causes leaf spot diseases in economically important hosts such as *Solanum lycopersicum* (tomato) (Barthe et al. 2007; Schlub et al. 2007), *Glycine max* (soybean) (Seaman et al. 1965), *Gossypium hirsutum* (cotton) (Conner et al. 2013; Fulmer et al. 2012), *Carica papaya* (papaya) (Cutrim and Silva 2003), *Hevea brasiliensis* (rubber) (de Lamotte et al. 2007; Jinji et al. 2007), *Vigna unguiculata* (cowpea) (Li et al. 2014), *Nicotiana tabacum* (tobacco) (Fajola and Alasoadura 1973), and *Capsicum annuum* (pepper) (Chai et al. 2014).

The symptoms of target spot of tomato include small necrotic lesions on foliage that have light-brown centers and dark margins. Later these lesions merge and turn into large blighted areas and cause premature defoliation (Pernezny et al. 2002). Symptoms on tomato fruits include small, brown, sunken flecks to large deeply pitted areas which reduces the market suitability of the fruit (Pernezny et al. 2002, Schlub et al. 2009).

Development of target spot of tomato occurs over the temperature range 20 to 28˚C (Jones and Jones 1984). Disease severity is highest at 28˚C. Minimum wetness duration of 16 hrs is required for disease development and disease severity increases with as wetness duration lengthened from 16 to 44 hrs (Jones and Jones 1984).

Studies on the cross pathogenicity of isolates of *C. cassiicola* from different hosts has revealed that some isolates are cross pathogenic to a wide range of hosts, while others are host specific (Dixon et al. 2009). Onesirosan et al. (1974) stated that a cotton isolate of *C. cassiicola*, M3 from Mississippi, U. S., was weakly virulent to tomato, causing few-to-many pin-point lesions, while tomato isolate, N15 from Nigeria, was moderately virulent to cotton, causing 4 to 10 mm sized lesions on leaves. Isolates of *C. cassiicola*, originating from different hosts might have different favorable environmental requirements, which affect disease severity. Therefore, the overall goal of this study was to determine the difference in favorable conditions for target spot of tomato caused by tomato and cotton isolates of *C. cassiicola.*

**Materials and Methods**

**Production of fungal colonies.** A cotton isolate of *C. cassiicola* (CA1) and two tomato isolates (TCL1 and 1343) were obtained from M. Brewer's laboratory, UGA. Colonies of the cotton and tomato isolates of *C. cassiicola* (CA1, TCL1, 1343) were cultured on quarter-strength potato dextrose agar (qPDA), prepared with 9.75 g of potato-dextrose-agar powder (PDA) and 11.25 g of agar powder per liter of water. Fungal plugs of 5 mm diameter were taken from the periphery of 12-day-old cultures. These fungal plugs

were sub-cultured on fresh qPDA every 12 days and maintained at 28˚C to obtain fungal colonies for conidia production.

**Preparation of conidial suspension.** A spore suspension was prepared from 12 day old fungal colonies. Petri dishes containing colonies were filled with 3 ml of sterile agar water, prepared with 2 g of agar powder and 0.5 ml of Tween 20 per liter. The mycelium was lightly scraped with a sterile L-shaped glass rod to detach conidia. The resultant conidial suspension was filtered through 4 layers of cheesecloth. The conidial concentration was counted by using a hemocytometer and adjusted to $4 \times 10^4$ conidia/ml.

**Plant production.** Seeds of the tomato cultivar, San Marzano, were kept in a moist chamber for 2 days to initiate germination. Germinated seeds were planted in potting mixture (Sun Gro professional growing mix, Seba Beach, Canada) in $9.5 \times 9.5$ $cm^2$ plastic pots. The potting mixture was autoclaved 3 times for 60 minutes each time before planting. Plants were kept at 28˚C in a growth chamber and were watered as needed.

**Inoculation method.** Individual plants were inoculated with one of three isolates (CA1, TCL1, or 1343) of *C. cassiicola* when 20 to 25 days old (3 to 4 true leaf stage) at 5:00 p.m. Three true leaves of a plant were drop-inoculated with a pipette. Five drops, each of 40 µl conidial suspension were placed on each leaf of a plant. Inoculated leaves were covered with wet tissue paper (Kimwipes) and then the entire plant was enclosed in a wet plastic bag.

**Effect of temperature and moisture on disease development.** Bagged plants were kept at 20, 24, and 28°C in separate growth chambers. Each growth chamber had a

set temperature and inside the growth chamber, each isolate was kept in a different block with all seven tested moisture durations randomized within the block. Repetitions of the trials in growth chambers, with different temperatures, allowed for the larger unit of temperature. Twenty-one plants were inoculated with each isolate, for each temperature. Three inoculated plants served as three subsamples for each isolate, and were removed from wet bags and wet tissue papers at 8, 12, 16, 24, 32, 40, and 48 hrs after inoculation. Plants were examined at 24 hr intervals following inoculation and day of disease onset was recorded. Lesions were counted on all three inoculated leaves at 3, 6, and 9 days after inoculation. The whole experiment was repeated once.

**Data analysis.** The number of days after inoculation recorded for onset of symptoms and total number of lesions counted on three inoculated leaves for each treatment at 3, 6 and 9 DAI were used for data analysis. Generalized linear mixed model analyses (PROC GLIMMIX) were done using SAS 9.4 (SAS Institute Inc., Cary, NC, USA) to determine the effect of the factors and interactions on onset and lesion numbers at 3, 6, and 9 DAI. Quadratic regression analysis was performed using PROC REG in SAS 9.4 to understand the effect of each treatment on disease severity at 3, 6, and 9 DAI.

## Results

**Onset**. There were significant effects due to temperature, leaf wetness duration, isolate, all interaction terms except interaction of isolate with temperature on disease onset (Table 1). No lesions developed with 8 hr of leaf wetness, for all three tested temperatures, or with 12 hr leaf wetness at 20 or 24°C (Fig. 1). Cotton isolate CA1 did not cause disease

with <16 hr wetness at 24 and 28°C or at 20°C with <40 hr wetness. Tomato isolates 1343 and TCL1 at 20°C with ≥ 16 and ≥24 hrs, respectively, produced lesions, while the cotton isolate required ≥40 hr leaf wetness for disease onset on tomato (Fig. 1). Lesions developed at 2 DAI with tomato isolates 1343 and TCL1 at ≥24°C with ≥16 hr and ≥24°C with >16 hr leaf wetness, respectively. Similarly, cotton isolate CA1 caused lesions at 2 DAI at 24°C with ≥32 hr and 28°C with ≥16 hr leaf wetness (Fig. 1).

**Lesion numbers**. There were significant effects of temperature, leaf wetness duration, isolate, and all interaction terms except temperature × isolate × leaf wetness duration at 3, 6, and 9 DAI (Table 1). The effect of leaf wetness durations of 8, 12, 16, 24, 32, 40 and 48 hrs at the three tested temperatures (20, 24 and 28°C) on lesion numbers was well described by quadratic models. Positive slopes demonstrated that for both tomato isolate 1343 and TCL1 at each of the three tested incubation temperatures (20, 24 and 28°C), an increase in leaf wetness duration increased numbers of lesions at 3 (Fig. 2A and 2B), 6 (Fig. 3A and 3B) and 9 DAI (Fig. 4A and 4B). For example, 1343 and TCL1 at 24°C with 16 hr leaf wetness at 9 DAI resulted in mean numbers of lesions, 6.8 and 5.3, respectively, while lengthening leaf wetness to 48 hr increased the mean numbers of lesions to 127.8 and 123.6, respectively. Similar trends were observed for the cotton isolate CA1 at 20, 24 and 28°C at 3 (Fig. 2C), 6 (Fig. 3C), and 9 DAI (Fig. 4C) but the quadratic regression model was significant for 24 and 28°C only, as 20°C resulted in disease onset only with 40 and 48 hrs leaf wetness. For example, for the cotton isolate CA1 at 24°C,

lengthening leaf wetness from 16 to 48 hrs increased the mean numbers of lesions from 1.6 to 86.0 at 9 DAI.

Highest lesion numbers were observed at 28°C with 48 hr leaf wetness for both tomato isolates 1343 and TCL1 and the cotton isolate CA1 at 3 (Fig. 2), 6 (Fig. 3) and 9 DAI (Fig. 4). The highest mean lesion numbers for the tomato isolates 1343 and TCL1, and cotton isolate CA1, at 3 DAI were 189.0, 132.0, and 115.6, respectively. Tomato isolate 1343 at 24°C with 48 hr leaf wetness after 3 DAI resulted in 118.5 mean numbers of lesions, while at 20°C with 48 hr leaf wetness the mean numbers of lesions decreased to 54.7. Similarly, plants inoculated with the cotton isolate CA1 at 24°C with 48 hr leaf wetness developed 80 lesions by 3 DAI, while at 20°C with 48 hr leaf wetness, 15.5 lesions developed. The quadratic regression suggested that optimum temperature for tomato isolate TCL1 and cotton isolate CA1 with 48 hr leaf wetness (Fig. 5) is 27.1 and 30.1°C, respectively.

Numerical increases in lesion numbers were usually observed with lengthening days after inoculation for each of the three isolates (TCL1, 1343 and CA1) at each of the three tested temperatures (20, 24 and 28°C). For example, mean numbers of lesions for isolate 1343 at 20°C with 32 hr leaf wetness at 3 and 9 DAI were 29.3 and 32.3, respectively.

**Discussion**

The goal of this study was to determine the if there was a difference in the favorable temperature and moisture conditions required by cotton and tomato isolates of *Corynespora cassiicola* for causing target spot of tomato. We recorded disease onset and number of lesions at 3, 6 and 9 DAI at three tested temperatures (20, 24 and 28°C) with seven tested leaf wetness durations (8, 12, 16, 24, 32, 40 and 44 hrs).

Results of this study showed that 28°C with 48 hrs leaf wetness duration had the highest lesion numbers for the three tested isolates on tomato. This suggests that 28°C is the most favorable temperature among the three tested temperatures (20, 24 and 28°C) for target spot onset and development. This result also coincides with results of our previous study which elucidated the optimum temperature and leaf wetness duration for target spot of cotton. Similarly, Jones and Jones (1984) also showed that 28°C resulted in highest disease severity of target spot of tomato. Hence, it is possible that 28°C is highly favorable for disease development of target spot by cotton and tomato isolates of *C. cassiicola*, irrespective of the host of origin. Also, onset was observed at 28°C with ≥12 hr leaf wetness for both tomato isolates TCL1 and 1343 but no lesion formation was seen below 16 hr of wetness for the cotton isolate CA1 at 28°C. Apparently, the cotton isolate used requires a longer leaf wetness duration than the tested tomato isolates for disease onset on tomato. On the other hand, our previous study showed that onset of target spot of cotton was observed for isolate CA1 at 28°C with a minimum 8 hr leaf wetness duration, indicating

that the cotton isolate requires a longer leaf wetness duration for disease development on tomato than on cotton.

The cooler temperature (20°C) evaluated herein with >40 hr wetness did not favor disease development by the cotton isolate CA1 on tomato, but the tomato isolates, 1343 and TCL1, initiated disease at 20°C with 16 and 24 hrs leaf wetness, respectively. This shows that tomato isolates can infect tomato at cooler temperatures such as 20°C, while the cotton isolate needs warmer temperatures (>20°C) at longer wetness intervals to cause disease on tomato. Therefore, the cotton isolate had a narrow favorable temperature range (24 to 28°C) for causing target spot of tomato, while tomato isolates seem to have a broader favorable temperature range (20 to 28°C) for infection on tomato. However, the cotton isolate used in this study (CA1) caused moderate disease development on cotton at 20°C in our previous study. Therefore, it is possible that the cotton isolates might have a broad favorable temperature range on cotton as compared on other hosts and confirms host specificity of tomato and cotton isolates of *C. cassiicola* (Sumabat et al. 2015).

Another significant finding of our study was that disease severity increased linearly at each of the three temperatures (20, 24 and 28°C) with lengthening leaf wetness durations, 16 to 48 hrs. Although at 20°C, the cotton isolate could only produce visible symptoms with ≥40 hr of leaf wetness, lengthening leaf wetness from 40 to 48 hrs increased mean lesion numbers from 6.6 to 19 at 9 DAI. This finding indicates that longer leaf wetness durations at a cooler temperature (20°C) can also cause considerable disease on tomato by the cotton isolate. Also, our finding contrasts from the results of Jones and Jones (1984)

which stated that favorable leaf wetness durations for target spot of tomato was 16 to 44 hrs, as they noted a decrease in disease severity above 44 hr leaf wetness.

The cotton isolate at favorable temperatures (24 and 28°C) with ≥32 hr leaf wetness duration led to a high number of lesions and lesion merger at 9 DAI indicating that the cotton isolate was highly virulent at favorable temperatures with longer leaf wetness durations. This result contrasts with results of Onesirosan et al. (1974) who reported that a cotton isolate from Mississippi was weakly virulent on tomato and caused only a few pin point lesions that did not increase in size.

In our study, the cotton and tomato isolates of *Corynespora cassiicola* resulted in high disease severity on tomato at favorable temperatures (24 and 28°C) with longer leaf wetness durations. This indicates that prevailing temperatures in the field for spring season tomato are favorable for cotton isolate of *C. cassiicola* and can favor disease development in the field.

Our study can help in understanding that prevailing temperature and leaf wetness conditions in the fields can affect the cross pathogenicity of cotton isolates on tomato. The temperature and leaf wetness relationship that we established in this study will help in efficiently managing the target spot of tomato caused by cotton isolate by timely spray of fungicides.

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Table 1. Table of significance for the effect of temperature, leaf wetness duration, and isolate on disease severity (3 DAI, 6 DAI, 9 DAI) and onset by *C. cassiicola.*

| | **Factors** | **DF** | **F value** | **Pr > F** |
|---|---|---|---|---|
| **Onset** | Temperature (T) | 2 | 564.38 | <.0001 |
| | Leaf wetness duration (M) | 5 | 68.03 | <.0001 |
| | Isolate (I) | 2 | 262.35 | <.0001 |
| | T×M | 8 | 33.37 | <.0001 |
| | M×I | 9 | 24.66 | <.0001 |
| | I×T | 4 | 115.33 | 0.4381 |
| | T×M×I | 12 | 18.34 | 0.0381 |
| **3 DAI** | Temperature (T) | 2 | 84.88 | 0.0005 |
| | Leaf wetness duration (M) | 5 | 161.78 | <.0001 |
| | Isolate (I) | 2 | 30.12 | <.0001 |
| | T×M | 8 | 12.51 | <.0001 |
| | M×I | 9 | 3.87 | 0.0002 |
| | I×T | 4 | 3.93 | 0.029 |
| | T×M×I | 11 | 1.15 | 0.3254 |
| **6 DAI** | Temperature (T) | 2 | 97.30 | 0.0004 |
| | Leaf wetness duration (M) | 5 | 181.59 | <.0001 |
| | Isolate (I) | 2 | 34.11 | <.0001 |
| | T×M | 8 | 13.59 | <.0001 |
| | M×I | 9 | 4.01 | 0.0001 |
| | I×T | 4 | 3.72 | 0.0342 |
| | T×M×I | 12 | 1.08 | 0.3751 |
| **9 DAI** | Temperature (T) | 2 | 93.11 | 0.0004 |
| | Leaf wetness duration (M) | 5 | 183.12 | <.0001 |
| | Isolate (I) | 2 | 35 | <.0001 |
| | T×M | 8 | 13.06 | <.0001 |
| | M×I | 9 | 4.04 | <.0001 |
| | I×T | 4 | 3.51 | 0.0406 |
| | T×M×I | 12 | 0.94 | 0.5051 |

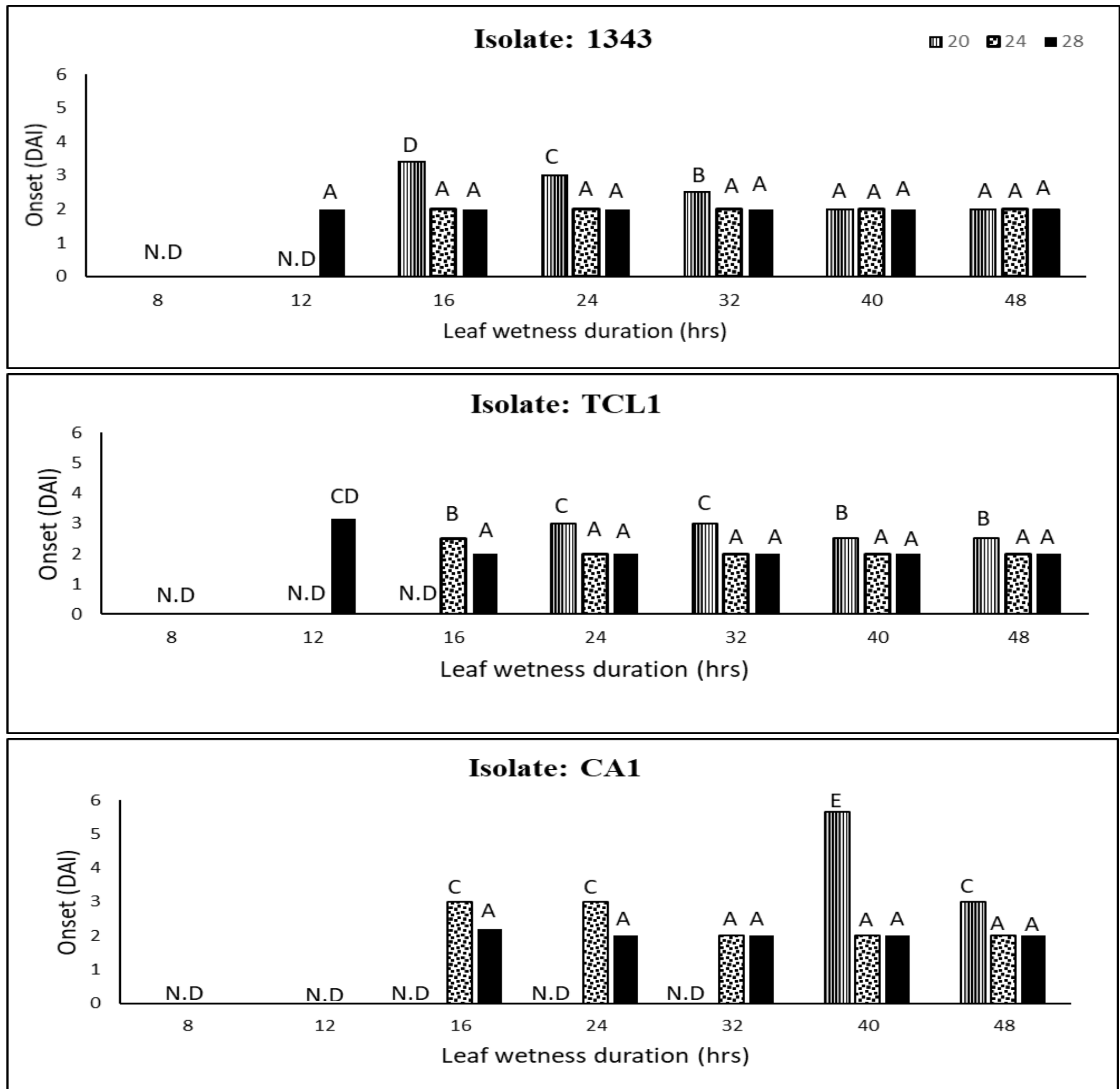


Figure 1. Effect of leaf wetness duration on days after inoculation (DAI) required for onset of target spot of tomato by *Corynespora cassiicola* (cotton isolate (CA1) and tomato isolates (1343 and TCL1) at three different temperatures (20, 24, and 28°C). Different letters above bar represent significant differences among all three isolates and three temperatures at *P*=0.05.

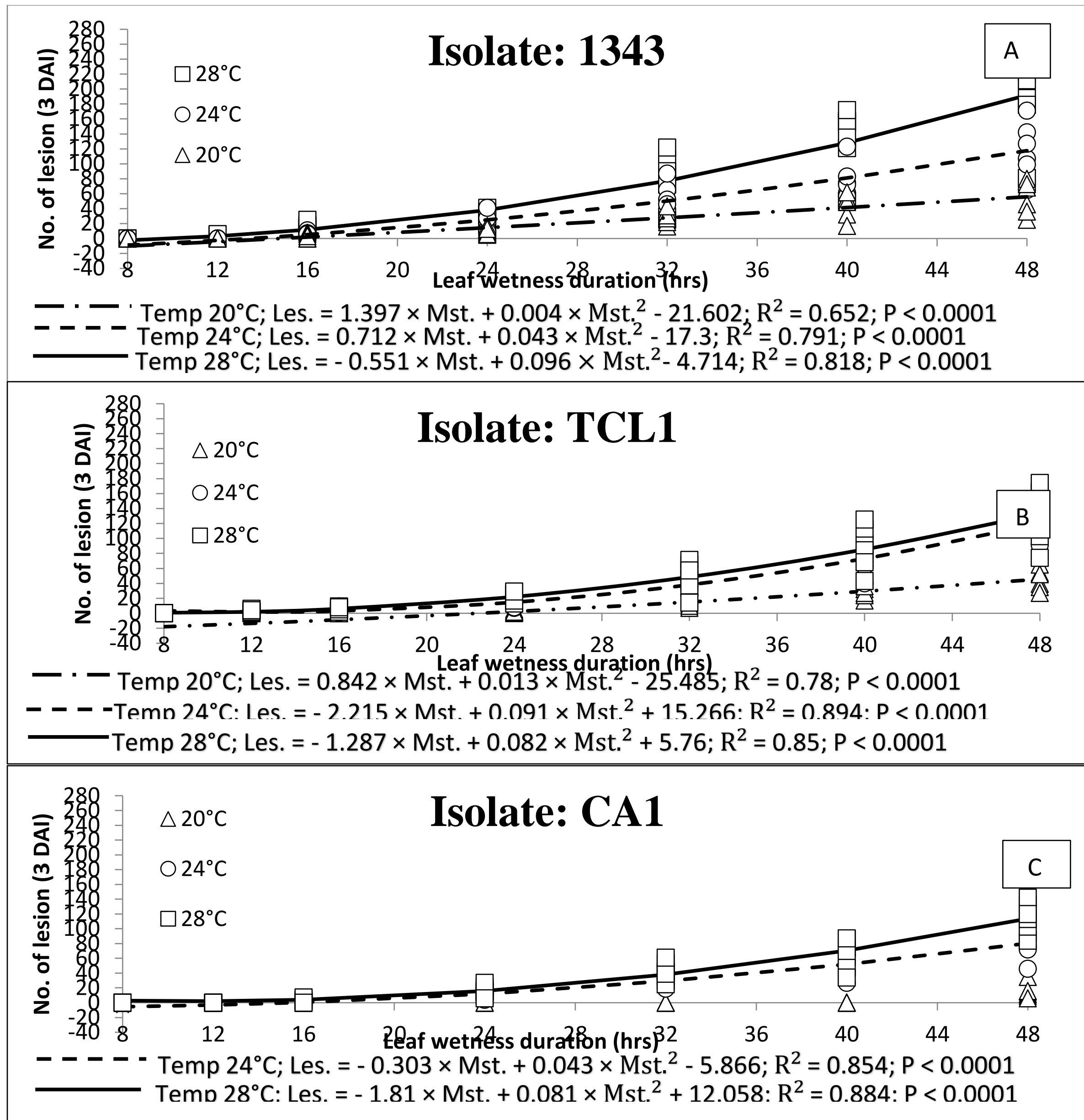


Figure 2. Effect of leaf wetness duration on number of lesions produced by tomato isolate 1343 (A), tomato isolate TCL1 (B) and cotton isolate CA1 (C) of *Corynespora cassiicola* at three different temperatures (20, 24 and 28°C) counted 3 days after inoculation (DAI). Quadratic regression was conducted for incubation temperatures (20, 24 and 28°C) using lesion numbers as a dependent variable and leaf wetness duration (Mst.) as an independent variable.

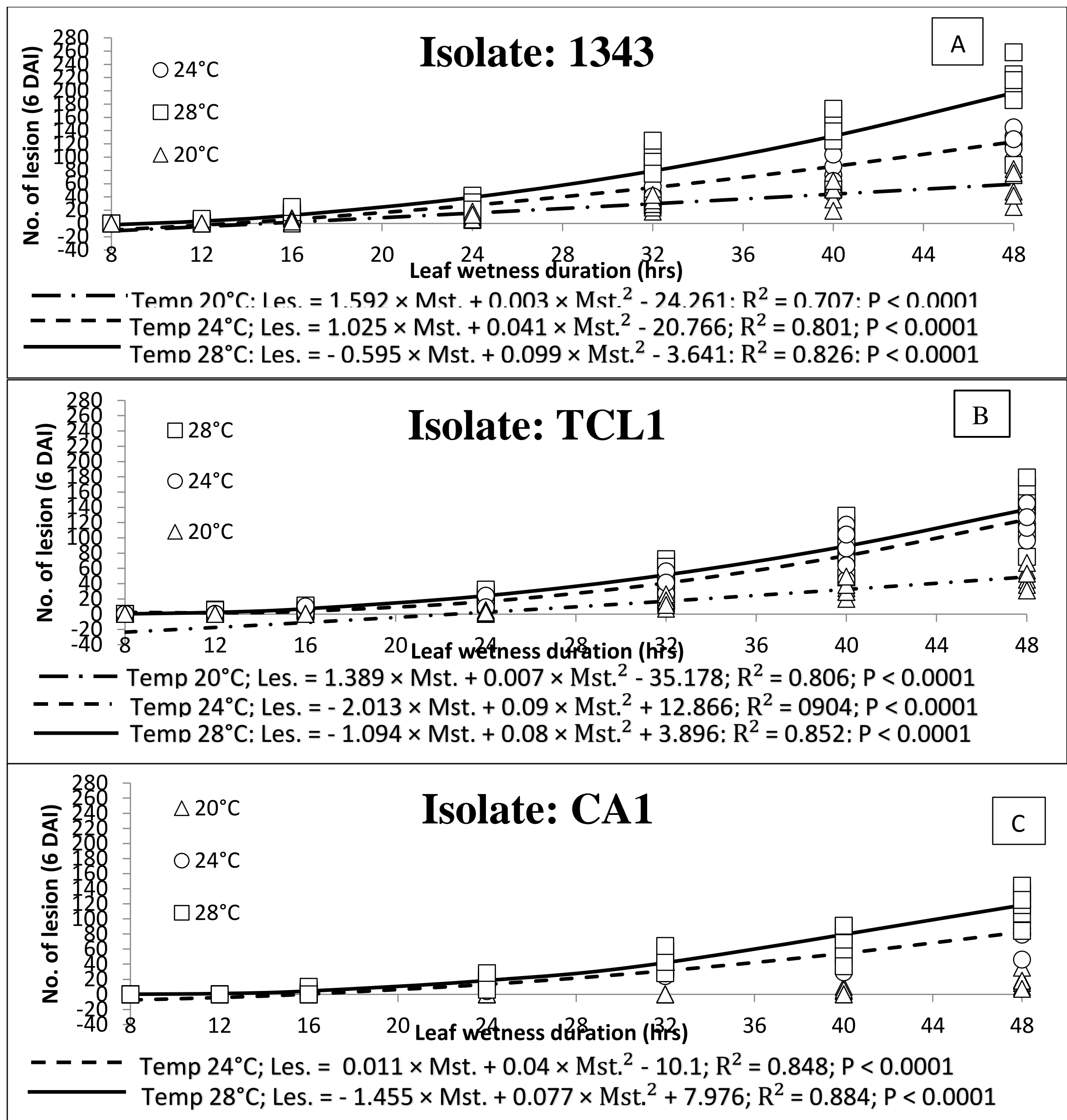


Figure 3. Effect of leaf wetness duration on number of lesions produced by tomato isolate 1343 (A), tomato isolate TCL1 (B) and cotton isolate CA1 (C) of *Corynespora cassiicola* at three different temperatures (20, 24 and 28°C) counted 6 days after inoculation (DAI). Quadratic regression was conducted for incubation temperatures (20, 24 and 28°C) using lesion numbers as a dependent variable and leaf wetness duration (Mst.) as an independent variable.

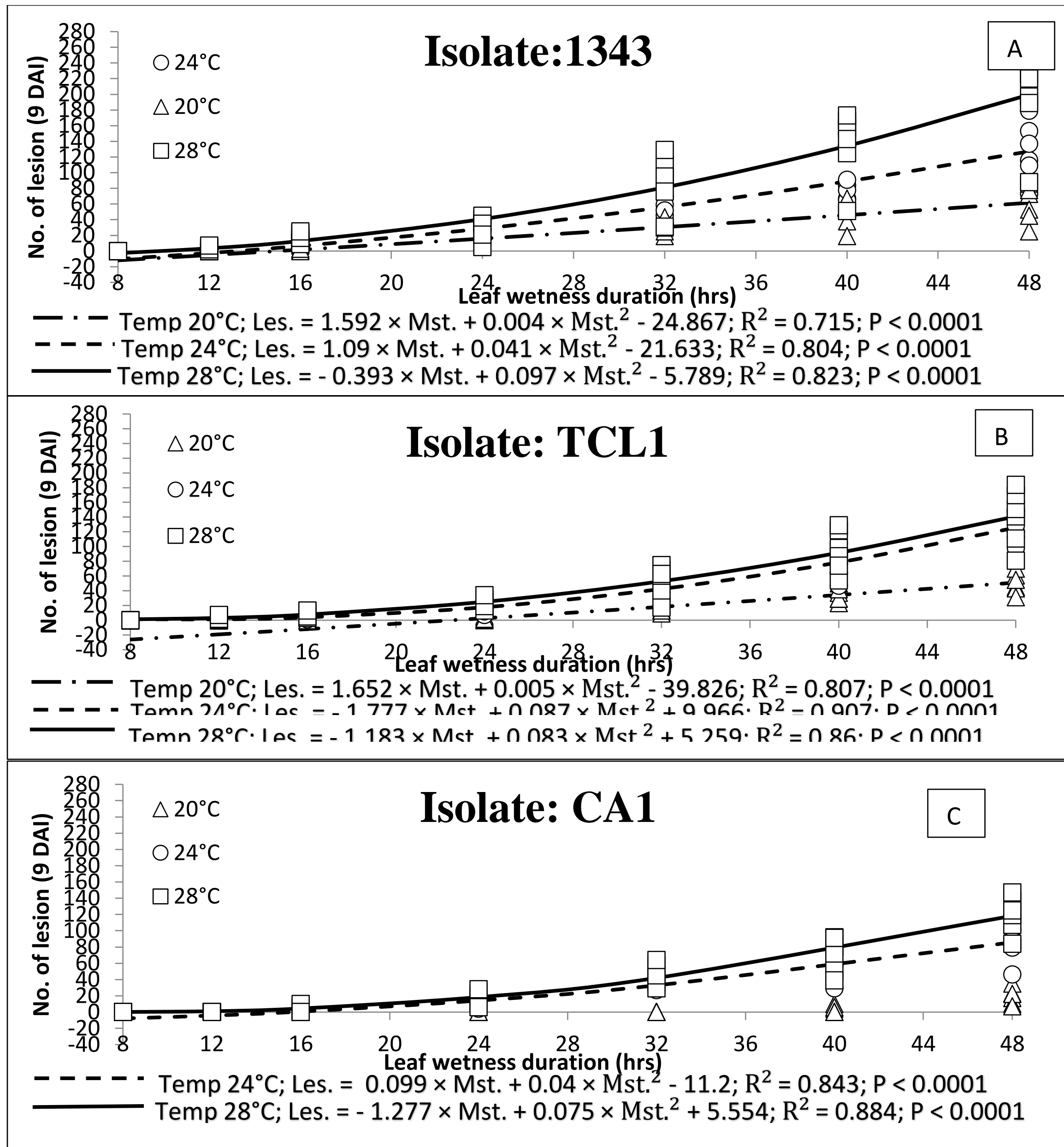


Figure 4. Effect of leaf wetness duration on number of lesions produced by tomato isolate 1343 (A), tomato isolate TCL1 (B) and cotton isolate CA1 (C) of *Corynespora cassiicola* at three different temperatures (20, 24 and 28°C) counted 9 days after inoculation (DAI). Quadratic regression was conducted for incubation temperatures (20, 24 and 28°C) using lesion numbers as a dependent variable and leaf wetness duration (Mst.) as an independent variable.

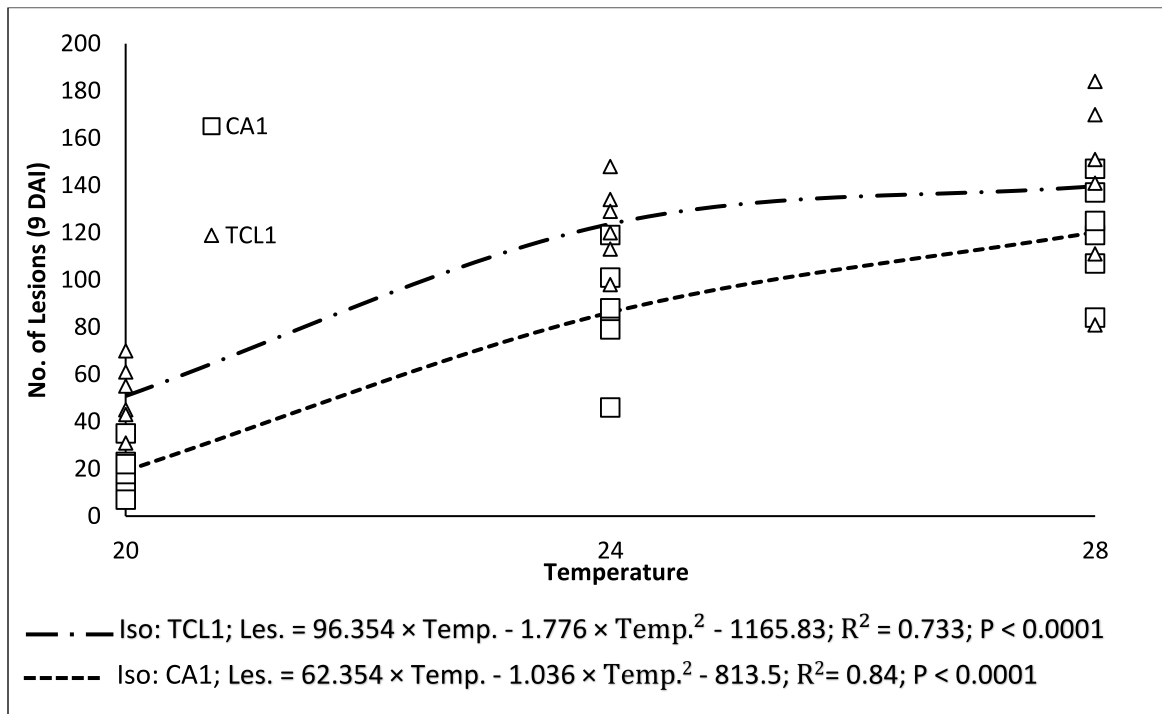


Figure 5. Effect of temperature on number of lesions produced by *Corynespora cassiicola* (Isolate: CA1 and TCL1,) after 48 hr leaf wetness counted 9 days after inoculation (DAI). Quadratic regression was conducted for leaf wetness duration (48 hr) using lesion numbers as a dependent variable and temperature as an independent variable.

## V. Summary

The study described herein was initiated with the goal of addressing the epidemiology of target spot of cotton and tomato. The main objectives of this investigation were: 1) to determine the effect of temperature on conidial germination of a cotton isolate of *Corynespora cassiicola*; 2) to determine the effect of temperature and leaf wetness duration on target spot of cotton caused by *C. cassiicola*; and 3) to determine if there is a difference in the epidemiology of target spot of cotton caused by cotton and tomato isolates of *C. cassiicola*.

For objective 1, conidial suspension of cotton isolate (CC1) and sterile water were pipetted and spread onto water agar media. Petri dishes were incubated at six different temperatures (12, 16, 20, 24, 28 and 32°) and were evaluated for conidial germination at 4, 8 and 12 hrs of incubation. Three replicate plates were included at each temperature and three trials were done for this study. Temperature and wetness period had significant effects on conidial germination. High conidial germination percentage was observed at 20 to 32°C after 12 hr of wetness, while 4 hr of wetness allowed low conidial germination percentage. Increased wetness hours at all tested temperatures increased conidial germination. These findings could be useful for understanding that the average environmental temperatures in the cotton growing areas of Alabama are favorable for spore germination and initiation of the infection process of a cotton isolate of *C. cassiicola* causing target spot.

Temperature and leaf wetness duration are important environmental factors for predicting infection periods and disease development. Forecasting infection periods of *C. cassiicola* can help in making timely applications of fungicide. Therefore, two trials were conducted in order to determine the favorable temperature and moisture conditions that support target spot of cotton caused by *C. cassiicola.* In both trials, treatments comprised a 5×7×3 factorial with five levels of temperature (16, 20, 24, 28 and 32°C), seven leaf wetness durations (8, 12, 16, 24, 32, 40 and 48 hrs) and three cotton isolates of *C. cassiicola* (CA1, CM18 and CC1). Three true leaves of individual plants were drop inoculated with each of three isolates (CA1, CM18 and CC1) of *C. cassiicola*. Inoculated leaves were covered with wet tissue paper and the entire plant was enclosed in a wet bag and incubated in separate growth chambers at 16, 20, 24, 28 and 32°C. Plants were removed from wet bags and wet tissue papers at 8, 12, 16, 24, 32, 40 and 48 hrs leaf wetness. Onset and lesion numbers of target spot of cotton were significantly impacted by temperature, isolate and leaf wetness duration. Earliest onset (1 day after inoculation) was observed at 28°C with ≥24 hr wetness. Lesion numbers were higher on plants incubated at 20, 24 and 28°C with longer leaf wetness durations. These findings could be useful for understanding the occurrence of target spot of cotton based on the varying temperatures and rainfall patterns. The temperature and leaf wetness relationship established in this study can help to efficiently manage target spot of cotton.

In order to determine the difference in favorable environmental conditions for isolates of *C. cassiicola* originating from different hosts, two trials were conducted on tomato. In both

trials, treatments comprised a 3×7×3 factorial with three levels of temperature (20, 24 and 28 °C), seven levels of leaf wetness (8, 12, 16, 24, 32, 40 and 48 hrs) and three isolates of *C. cassiicola* (Tomato isolates: TCL1 and 1343; Cotton isolate: CA1). Three true leaves of individual plants were drop inoculated with one of three isolates (TCL1, 1343 or CA1) of *C. cassiicola*. Inoculated leaves were covered with wet tissue paper and the entire plant was enclosed in a wet bag and incubated in separate growth chambers at 20, 24 and 28°C. Plants were removed from wet bags and wet tissue papers at 8, 12, 16, 24, 32, 40 and 48 hrs leaf wetness. Onset and lesion numbers of target spot of tomato caused by tomato and cotton isolates were significantly impacted by temperature, isolate and leaf wetness duration. Early onset was observed at warmer temperatures (24 and 28°C) with >16 hr leaf wetness. Lesion numbers on tomato were high for both tomato and cotton isolates incubated at 24 and 28°C with longer wetness durations, while cooler temperature (20°C) did not favor the cotton isolate on tomato and confirms that the cotton isolate is cross pathogenic on tomato only at highly favorable temperatures and longer wetness durations. These findings can help to understand that prevailing temperature and leaf wetness duration in the field can affect the cross pathogenicity of cotton isolates of *C. cassiicola.*