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I am submitting herewith a thesis written by Hillary Dawn Ross entitled “Cultural Control Methods that Effect the Development and Spread of *Corynespora cassicola* (Berk. & Curt.) Wei on African violet (*Saintpaulia ionantha* Wendl.)” I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Entomology and Plant Pathology.

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**Cultural Control Methods that Effect the Development
and Spread of *Corynespora cassiicola* (Berk. & Curt.)
Wei on African Violet (*Saintpaulia ionantha* Wendl.)**

A Thesis
Presented for the
Master of Science
Degree
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Hillary Dawn Ross
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DEDICATION

This thesis is dedicated to my family and friends.

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ABSTRACT

In recent years, a large commercial grower of African violets (*Saintpaulia ionantha* Wendl.) in middle Tennessee has experienced epidemics of Corynespora leaf spot caused by *Corynespora cassiicola* (Berk. & Curt.) Wei (Alan Windham, personal communication). Symptoms of Corynespora leaf spot include rapidly expanding circular lesions on the surface of the leaves and petioles. The disease occurs in propagation material and mature plants of *S. ionantha* which result in thousands of plants being discarded daily. The objectives of this research were to: 1) determine if irrigation methods affected disease severity, 2) to determine if fungicidal spray intervals could be extended beyond the recommended two week interval, and 3) to determine if leaf age affects the susceptibility of *S. ionantha* to *C. cassiicola*.

Three irrigation treatments (drip, mist, and ebb & flow) were evaluated for their effect on disease severity of *C. cassiicola*. Over a seven week period, plants were observed for the presence of lesions on leaves and petioles. This experiment was repeated four times with four to six replications per repetition. Disease severity was not significantly different in the three irrigation treatments in reducing the spread of *C. cassiicola*.

Three fungicides (propiconazole, thiophanate-methyl, chlorothalonil) and a water control were applied to symptomatic *S. ionantha*. Leaves were collected for eight weeks.

Overall, leaves sprayed with the thiophanate-methyl treatment produced the lowest amount of sporulation and isolate growth, but no treatment was effective in inhibiting the growth of *C. cassiicola* in *S. ionantha* tissue.

The effect of leaf age on disease susceptibility was evaluated using three stages of *S. ionantha* leaves: juvenile, mature, and senescing. Lesion size was larger on juvenile and senescing leaves than on mature leaves.

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CHAPTER I

LITERATURE REVIEW

African violets, *Saintpaulia ionantha* H. Wendl., are endemic to Tanzania and either grow near sea level or at other lowland sites (107). *Saintpaulia ionantha* belongs to the Gesneriaceae family, mainly a tropical family, which is divided into the following two subfamilies: the Gesnerioideae, which have epigynous flowers with four stamens, and the Cyrtandroideae which have hypogynous flowers with two stamens (7). *Saintpaulia* belongs to the subfamily Cyrtandroideae.

The first known collection of *Saintpaulia* was in April 1884 off “the coast opposite Zanzibar” by Sir John Kirk, the British vice-consul of Zanzibar (7). The specimen collected by Kirk was sent to the Kew Botanic Gardens in England. Kirk also sent a note describing the specimen as “a lovely blue flower that will quite equal the Sultani Balsam” (7). Unfortunately, the *Saintpaulia* specimen sent to the Kew Botanic Gardens was in poor condition and could not be identified.

By the 1890s, Tanganyika had been under German control for nearly a decade and regional commissioners, which were German military officers, were sent to the countries regional centers (7). In 1891, a regional commissioner, Baron Adalbert Emil Walter Redcliffe Le Tanneux von Saint-Paul-Illaire was stationed in the city of Tanga. He collected flowers from Tanga and the East Usambara Mountains and sent the specimens to his father, Hofmarschal Baron Ulrich von Saint Paul von Fischbach, President of the German Dendrological Society. Von Saint Paul sr. shared the specimens with Hermann

Wendland, Director of the Botanic Garden at Herrenhausen in Hannover. In 1893, Wendland placed the new specimen into a new genus called *Saintpaulia* in honor of both father and son for their introduction of a new specimen into Europe. Wendland described the new species and named it *S. ionantha*. “Ion” is Greek for violet. Wendland also proposed the German name “Usambara Veilchen,” which was later translated to “Usambara violet.” However, “Usambara violet” was later changed to what is now known as the “African violet.”

In 1893, the Austrian botanist Karl Fritsch placed *Saintpaulia* under the subfamily Crytandroideae in the tribe Ramondeae (43). The Ramondeae tribe is now referred to as the Didymocarpeae tribe (43, 78). In 1906, Charles Baron Clarke collected two species of *Saintpaulia* from the Usambara Mountains. Unfortunately, Clarke mistakenly identified *S. ionantha* as *S. kewensis* and applied the name *S. ionantha* to another taxon (7, 21).

In 1947, B. L. Burtt reduced *S. kewensis* to a synonym of *S. ionantha* and described the other taxon as *S. diplotricha* (7, 21). *Saintpaulia diplotricha* differed from *S. ionantha* because leaves of *S. diplotricha* are clothed with dual indumentums of both long and short hairs whereas the hairs of *S. ionantha* are of uniform length (20).

In 1908, Fritsch divided the genus *Saintpaulia* into two sections with the following three known species: *S. ionantha*, *S. goetzana*, and *S. pusilla* (21). Section I, *Eusaintpaulia* included the rosulate species, *S. ionantha* and *S. pusilla*. Section II, *Archisaintpaulia* included the caulescent species, *S. goetzana*. These sections were later

abandoned by Burt as new species were added to the genus *Saintpaulia* that were intermediate to these sections.

Morphology of the genus *Saintpaulia*

Characteristics that are important in identifying *Saintpaulia* include growth habit and posture, distribution, and nature of leaf hairs on the adaxial surface (21). Growth habit is rosulate and caulescent. Rosulate species have very short internodes below the leaf-crown. The stem is short, thick, and fleshy, and rooting occurs on the lower part of the stem. Caulescent plants have well marked internodes that separate the successive pairs of leaves. Stems are of even thickness and tend to be prostrate. Rooting occurs at the leaf nodes.

The most important characteristic for distinguishing *Saintpaulia* species is the eglandular hair types on the adaxial leaf surface (21). Stems of these perennial herbs are either procumbent, rooting at the nodes, with distinct internodes, or the stems can be short and thick, elongating slowly, with a dense terminal crown of leaves. Leaves are more or less fleshy, opposite, and petiolate and the blade is suborbicular to elliptic. Peduncles contain two or more flowers in a cyme that open serially at each dichotomy of the inflorescence and arise either from the leaf-axil along the stem or the crown (107, 21). Bracts are typically small and linear (21). The calyx is divided almost to the base in five segments that are linear and arranged irregularly. Lower laterals lie within the lower margins of the lateral corolla-lobes; the uppermost segments are in the median; while the upper laterals are found within the outer margin of the upper corolla-lobes (not in line with the sinus).

Sepals vary from five to seven and are green, erect, linear, and obtuse (57). The corolla is short (21). There are only two fertile stamens. Anthers are large and visible outside the mouth of the corolla tubes with a bright yellow hue and have one chamber whereas the flowers have a distinct disk (7, 107). Two to three small staminodes are exerted from the corolla tubes (107). The ovary is short and tapers abruptly into the style about three times its length (21). It is glabrous except at the base and is stiffly exerted to either side of the center of the corolla. The stigma is centrally depressed, small, papillose, and terminal. To avoid self-pollination, the stigma is receptive only three to four days after pollen is shed (7). Fruits have a cylindrical capsule or a subglobose capsule (107). The capsule structures are either long and slender or short and stout and are never twisted.

Classification of *Saintpaulia ionantha*:

Kingdom: Plantae

Division: Magnoliophyta

Class: Magnoliopsida

Order: Lamiales

Family: Gesneriaceae

Genus: *Saintpaulia*

Species: 20 known species

***Saintpaulia ionantha* morphology**

The plant is rosette and can grow up to 45 cm in diameter (107). The stem develops only below the close-packed terminal cluster of leaves (21). Leaves are always hairy on the

adaxial surface and smooth and glabrous on the abaxial surface. The indumentums of the adaxial leaf surface contains long erected hairs that are not equal in length. Flowers are self-colored (identical color) and can be 2.5 cm or more in diameter. At the base, the leaf blade is cordate (heart-shaped) and fruits develop in a short, blunt capsule that is less than 2 cm long. Leaves are suborbicular, can grow to 8 cm in length and 6 cm in width, and are dark green on the adaxial surface with a purplish hue on the abaxial surface (21, 96). Two staminodes are present, the peduncles have both long and short hairs, all of which are spreading and the petioles are pubescence (96).

In the wild, *S. ionantha* is found at both low and higher altitudes growing in moist and shaded areas along gullies or steep rocks (7). They grow best on granitic, metamorphic gneisses or acidic rocks. *Saintpaulia ionantha* prefers a moist soil, however, excessive moisture leads to root damage as roots are deprived of oxygen (66). If water is limited, then growth will be stunted and the plant will not fully recover even if water becomes available.

The American Violet Society recognizes the following four classes of *S. ionantha* based on plant size: miniature (less than 15.24 cm in diameter), semi-miniature (15.24 to 20.32 cm in diameter), standard (20.32 to 40.64 cm in diameter), and large (over 40.64 cm in diameter) (54). Leaf margins of the foliage can be serrated, spoon, plain, holly, ruffled, trumpeted, and pointed (97). Flower shape can be single, double, semi-double, star-shaped, and fringed; along with a wide variety of colors and hues ranging from purple, red-violet, white, blue, lavender, orchid, and bi-color or multi-color (54).

To date, twenty species of *Saintpaulia* have been identified in east Africa (97). There are more than 40,000 named cultivars and 7,500 of the cultivars are registered with the African Violet Society of America (58). By 1985, the U. S. trade of *Saintpaulia* reached \$30 million dollars annually. In 1988, the Western European crop was valued more than \$30 million dollars. In 1991, the Netherlands crop was valued at \$20 million dollars (7).

Corynespora cassiicola

A large greenhouse operation in Nashville, Tennessee, has had severe epidemics of *Corynespora* leaf spot on *S. ionantha* in recent years (Alan Windham, personal communication). Unfortunately, little information has been published in the relationship between *S. ionantha* and *Corynespora cassiicola* (Berk. & Curt.) Wei.

In 1896, Cooke described an unknown species of *Cercospora* found in the pit of melon and named it *C. melonis* (28). In 1906, Güssow was studying a glasshouse disease of cucumber *Cucumis sativus* L. and identified the pathogen to be *C. melonis*, but proposed the new genus *Corynespora* because the conidia were formed in chains and a hyaline isthmus was present between the conidiophore and the conidium (51). Güssow changed the pathogen name to *Corynespora mazei*. However, in 1910, Lindau named the pathogen *Corynespora melonis* (Cooke) Lindau (73).

In the early 1900s, cowpea *Vigna unguiculata* (L.) Walp. and soybean *Glycine max* (L.) Merr. plants in China were infected by a fungus that produced conidia that were large and slender, with a thick exospore and were pale olivaceous brown; as the conidia aged, the color was reported to intensify (123). In culture, the conidia were long, hyaline and

produced in short chains. In 1936 and 1939, Tai (114) and Teng (115) respectively, identified the fungus as *Cercospora vignicola* Kawamura. Liu (74) identified a fungus as *Helminthosporium vignae* Olive on cowpeas in Japan, that later would be identified as the same organism.

Helminthosporium vignae was described in 1945 by Olive et al. (86) on cowpea and soybean in the U.S. Symptoms of the disease on cowpea included reddish-purple spots that slowly enlarged into brown, circular areas with wavy margins (86). Mature spots appeared with a light brown center with reddish-brown margins. Spots ranged from 3 to 10 mm in diameter and were more prominent on the adaxial leaf surface than the abaxial surface. In severe cases, leaves became chlorotic and defoliation occurred. Elongate, dark brown conidiophores were observed microscopically in lesions on both surfaces of the leaf. The conidiophores were more prevalent in the reddish-brown margins. The conidiophores arose from “swollen basal cells and [produced] conidia singly or in chains at their tips” (86). The morphology of the conidia were compared to those of *H. vignae* (123).

Helminthosporium vignae isolates from China and Japan were compared to *Corynespora melonis* (123). Descriptions of *C. melonis* were identical to the description of *H. vignae*. Olive et al. (86) and Wei (123) reported the conidial morphology to be identical in both shape and structure, and observed a hyaline isthmus in the fungal isolates (123). They concluded *Cercospora melonis*, *Cercospora vignicola*, and *Helminthosporium vignae* were all species of *Corynespora* (123).

Another species of *Helminthosporium*, *H. cassiicola* Berkeley & Curtis, was also thought to be associated with the genus *Corynespora*. *Helminthosporium cassiicola* was found in tropical countries on eleven different hosts. Sixteen isolates of *H. cassiicola* were species collected by F. C. Deighton (32). A specimen of *H. cassiicola* was deposited with the Herb. Brit. Mus. (Nat. Hist.) and was labeled 'Fungi Cubenses Wrightiani' collected by C. Wright No. 628 (123). Wei (123) examined the specimen and concluded that *H. cassiicola* was morphologically identical to *Corynespora*. Wei proposed the following synonyms of *C. cassiicola*: *Helminthosporium cassiicola* Berk. & Curt. (11), *Cercospora melonis* Cooke (28), *Corynespora melonis* (Cooke) Lindau (73), *Corynespora mazei* Güssow (51), *Helminthosporium papayae* H. Sydow (113), *Cercospora vignicola* Kawamura (65), *Helminthosporium vignae* Olive (86).

Characteristics of *Corynespora cassiicola*

Corynespora cassiicola, which commonly causes diseases referred to as *Corynespora* leaf spot or target spot, is a pathogen that demonstrates high virulence and is characterized by rapidly expanding lesions (80).

Wei's description of *Corynespora cassiicola* is as follows (123): Mainly parasitic to leaves, but also attacks petioles, fruits, and stems. Lesions appear varying in size from 1 mm to 2 cm in diameter. Lesions are yellowish-brown with purplish-brown margins or zonations, often secondary. Conidiophores are mostly hypophyllous, perpendicular to the surface of the substratum, extending from mycelium that has emerged through the epidermis or

occasionally from aerial hyphae. The conidiophores are mostly single, straight, sparingly septate, dark brown, with or without a bulbous base, and slightly or not at all swollen at the tip which is thick walled. They are capable of reaching a length of 600 μ or more and 3.8-11.3 μ in diameter and are lighter in color towards the apex. Conidia are borne singly at the apex, forming chains of 2 to 6 spores, and are sometimes connected by a hyaline isthmus to the conidiophore or to other conidium. The conidia are obclavate, sometimes cylindrical, straight or often slightly curved, and conspicuously tapering towards the apex. Conidium are usually composed of thin-walls, that are pale olivaceous brown and darkening with age, with up to 20 or more pseudoseptate. The exospore is thick and hyaline, the pellicle is colored and the hilum measures 32-220 \times 8.4 μ . Germination occurs by polar germ-tubes.

Corynespora cassiicola has been reported as a pathogen of at least seventy hosts throughout the world including rubber tree (*Hevea brasiliensis* (Willd. ex A. Juss)), cucumber (*Cucumis sativus*, L.), tomato (*Lycopersicon esculentum*, L.), soybean (*Glycine max*, L. Merr.), tobacco (*Nicotiana tabacum*, L.), hydrangea (*Hydrangea macrophylla*, Seringe), and zebra plant (*Aphelandra squarrosa* Nees) (80, 106). When *Corynespora* leaf spot was first diagnosed on zebra plants in 1973, it marked the first time that *C. cassiicola* had been diagnosed on a foliage plant (25). On zebra plants, pathogen virulence was increased when leaves were wounded or in contact with the potting media (25, 111). Necrotic lesions expanded rapidly and were rarely surrounded by a halo (80, 25). The necrotic areas spread to form mature circular leaf spots that varied in size from

10 to 20 mm in diameter (80). Lesions were present 7-14 days after inoculation (25). The disease cycle of *C. cassicola* in *S. ionantha* is not known, but for the majority of its hosts, the fungus can survive in crop debris for up to two years, as well as in the seeds of soybean (93). Environmental conditions that favor disease development on its host include warm temperatures and high relative humidity (24). *Corynespora* leaf spot attacks propagation material and mature plants of *S. ionantha*, which may result in the destruction of thousands of plants daily. Symptoms may develop after shipping plants to retail markets.

Saintpaulia ionantha growers have used thiophanate-methyl (Cleary's 3336, W. A. Cleary, Dayton, NJ) to control the disease; however, fungicide resistance to Cleary's 3336 in the pathogen population has been observed in production but not verified. This has made thiophanate-methyl less effective as a control measure (Mark Windham, personal communication).

The objectives of these studies concerning *C. cassicola* were: 1) to determine if irrigation methods affected disease progression and if so, which irrigation method would be most effective in reducing the spread of *C. cassicola*, 2) to determine which fungicide treatment would reduce the viability and sporulation of *C. cassicola* in African violet leaf tissue, and 3) to determine if leaf age influenced susceptibility to *C. cassicola*.

CHAPTER II

EFFECT OF IRRIGATION METHODS ON THE SPREAD OF *CORYNESPORA CASSIICOLA*

Introduction

Saintpaulia ionantha is ambiguous to soil moisture and the method of watering. They require evenly moist, but not saturated soil (66). If the soil remains dry for an extended period of time, growth will be stunted and flowering will be poor (66, 54). If over watered, roots will die in a oxygen deficient environment (105). Over watering can cause brittle leaves that split open easily, as well as shorter, thicker petioles that can also become brittle (97). Root damage from over watering can lead to the development of crown rot disease caused by *Pythium* sp. and *Phytophthora* sp. (66).

If foliage of *S. ionantha* remains wet for extended periods, powdery, white residues and/or ring spots may appear and be conducive to foliar pathogens (88). Irrigation water applied to the foliage below ambient temperature (18° C) may cause chlorotic spots (66). If the surface of leaves remain wet, foliar diseases, such as botrytis blight (*Botrytis cinerea* (de Bary) Whetz) may develop. Water-soaked lesions develop on the abaxial surface of leaves infected with *Botrytis* (36).

Drip, mist, and ebb & flow are commonly used irrigation systems in the production of *S. ionantha*. Each irrigation system demonstrates strengths and weaknesses in regards to the dissemination of plant pathogens. It is important to determine which irrigation system will potentially inhibit the spread of *Corynespora* leaf spot and which irrigation

system creates a less favorable environment for other foliar and soilborne pathogens that attack *S. ionantha*.

Drip irrigation, also referred to as micro-irrigation or trickle irrigation, is comprised of a network of emitters, pipes, tubing, and valves through which water is applied slowly and uniformly to the root zone (62). As the foliage remains dry, the environment is thought to be less conducive for some foliar pathogens (84). In 2002, drip and sprinkler irrigation systems with metam sodium, a chemigation biocide, were compared to determine which would inhibit the spread of stem rot (*Sclerotium rolfsii* Sacc.) on potato (*Solanum tuberosum* L.) (19). Browne et al. (19) concluded that subsurface drip irrigation had a lower incidence of stem rot on potato tubers (13 to 23%) than sprinkler irrigation (56 to 62%). The lower incidence of the pathogen on the host was due to the surface soil remaining relatively dry. In 2004, Lanier et al. (70) also proved that subsurface drip irrigation was better than overhead sprinklers for inhibiting the growth of early leaf spot (*Cercospora arachidicola* Hori) on peanut (*Arachis hypogea* L.). In summary, humidity conditions favorable for infection and sporulation occurred with subsurface irrigation (64% of crop). Sprinkler systems provided additional periods favorable for infection and sporulation, so 95% of the crop became infected.

Mist irrigation, also known as overhead irrigation, often is dispersed with water lines suspended over the crop or nozzles on risers coming up from underneath the bench (13). The uniformity of overhead irrigation is difficult (13). Also, favorable conditions for foliar diseases may exist. *Pectobacterium carotovora* Schaad (bacterial soft rot) and *Erwinia chrysanthemi* Burkholder et al. (bacterial leaf spot) may cause serious problems

for *S. ionantha* when foliage remains wet for an extended period of time (109). Symptoms include darkening of veins, darkened water-soaked patches and wilting of leaves, stem rot, and plant death. Both bacteria enter through hydathodes and wounds and are spread from one plant to another by the splashing of water, insects, and/or contaminated equipment (3). Powdery mildew caused by *Erysiphe cichoracearum* DC ex Merat. is favored by higher humidities (109). The pathogen develops a network of hyphae that penetrates the surface of the epidermal cells. White colonies appear on the foliage, stems, calyx, flowers and pedicel.

Ebb & flow sub-irrigation, also referred to as seepage irrigation, involves artificially raising the water table in the growing medium and moistening the root zone of the plants (9). Water and nutrients are moved by a submersible pump from the holding tank to the benches. As water is applied only to the soil, the irrigation method may reduce the severity of foliar diseases (84). However, salt buildup and soilborne pathogens may become problems (8). Although minimal problems with soilborne pathogens have been reported, propagules of *Fusarium oxysporum* Schlecht have been identified on the bottom of reservoirs (95). Biernbaum (12) reported high levels of *Pythium aphanidermatum* (Edson) Fitzp. and *Pythium ultimum* Trow inoculum in reservoirs. *Xanthomonas campestris* pv. *begoniae* (Takimoto) Dye have been found in ebb & flow irrigation systems, but low transmission levels resulted in rapid pathogen death (5).

The objective of this study was to determine if irrigation methods had an effect on disease progression caused by *Corynespora* leaf spot on *S. ionantha*. The hypothesis was that irrigation methods would have an effect on the development of *Corynespora* leaf

spot.

Methods and Materials

Experimental Setup. From June to August 2006, four repetitions involving eighteen replications were conducted on each treatment of drip, mist, and ebb & flow. Repetitions 1 and 3 were conducted in separate greenhouses from repetitions 2 and 4. Each treatment consisted of eight healthy plants (cultivar 'Michelle') surrounded by one plant symptomatic of *Corynespora* leaf spot. A total of 486 African violets were used for these tests. Each plant within a treatment was placed 5.79 cm apart from the diseased plant. Treatments were randomly placed side by side and separated by 0.51 cm of Plexiglass (Keeling Co., Knoxville, TN) that stood 60.33 cm tall and 120.65 cm wide making the treatment area 2.4 meters (Figure 2-1). The temperature in the greenhouse was maintained at 27°C.

Data were collected each week for seven weeks. The eight healthy plants were observed weekly for the appearance of lesions. Once a lesion was observed on a healthy plant, the plant was marked as infected.

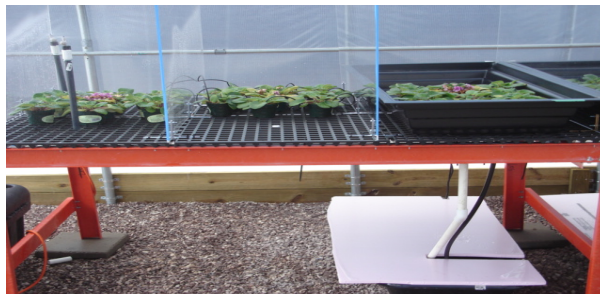


Figure 2-1. Irrigation methods used in this study separated by plexiglass partitions.

Left to right: mist, drip, and ebb flow.

Irrigation treatments. The ebb & flow treatment cycled on three days a week: Monday, Wednesday, and Friday at ten in the morning for fifteen minutes. The ebb & flow treatment consisted of Canadian Ebb & Flow trays (1.2 m x 0.6 m) (All Seasons Gardening and Brewing Supplies, Nashville, TN) and Canadian reservoirs that held 102 liters of water (All Seasons Gardening and Brewing Supplies, Nashville, TN). The water was pumped by an ECO Plus submersible pump (ECO 264) that pumped 999 LPH (National Garden Wholesale, Nashville, TN). The ebb & flow treatment was operated by an ECO Plus Digital Timer 15 AMP (National Garden Wholesale, Nashville, TN).

The mist irrigation used micronet vibro-mist nozzles, violet color, and orifice size 0.032 (30 psi: 9.59 gal/hr) (Sonne-Gro, Inc., Knoxville, TN). The timer for the mist irrigation cycled three times everyday between 8-10 a.m., 11:30-1:30 p.m., and 3-5 p.m. (Batrow Inc., Short Beach, CT). During these cycles, the mist was on for two minutes and off for ten minutes. The drip used woodpecker drippers (Sonne-Gro Inc., Knoxville, TN) and red emitters that used a half a gallon of water per hour (Sonne-Gro Inc., Knoxville, TN).

The drip tubing was the 0.64 cm soft vinyl 0.22 x 0.16 mm (Rain Bird Corporation, Inc., San Diego, CA) and the drip stakes were Above Ground Spot-Spitter® Grey Mini Flows (Roberts Irrigation Production, Inc., San Marcos, CA). The timer for the drip cycled on once a day for twenty minutes at ten in the morning (Batrow Inc., Short Beach, CT).

Statistical Analysis

The data were analyzed as a complete randomized block using SAS Proc Mixed (101). The normality of residuals was tested with the Shapiro-Wilk test with a range of 0-1, where 1 was considered normal. The homogeneity of variance was tested using the Levene's test, which is used to determine if groups or samples have equal variance, at a criterion alpha level of 0.05.

Results

During the first week no symptoms of *Corynespora* leaf spot were observed in any of the repetitions.

Repetition 1. In week 2, symptoms of *Corynespora* leaf spot were observed in all treatments (Figure 2-2). By week 3, at least 25% of the plants in each treatment were symptomatic. At week 5, disease incidence was 100% in all treatments, with the exception of the mist treatment (88%). The experiment was intended for 7 weeks of observation, but 100% infection was reached by the sixth week (Figure 2-2).

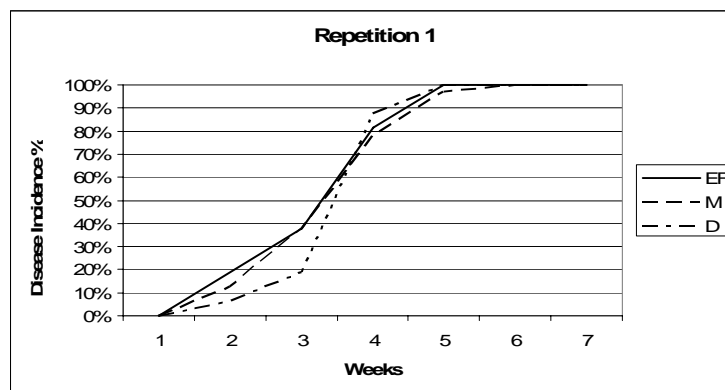


Figure 2-2. Effect of irrigation methods on disease incidence of *Corynespora* leaf spot.

(EF=ebb & flow irrigation method; M=mist irrigation method; D=drip irrigation method).

Repetition 2. Disease incidence was 7% in the mist treatment and 9% in the drip treatment by week 2. No symptoms were present in the ebb & flow treatment. Disease incidence increased to 38%, 63%, and 80% in the ebb & flow, drip, and mist treatments, respectively, by week 3. Disease incidence was 81.3% for the ebb & flow treatments, 86% for the drip treatment, and 92% for the mist treatment by week 4. By week 6, all treatments in the six replications had reached 100% infection (Figure 2-3).

Repetition 3. By week 2, disease incidence was 19% in the ebb & flow and mist treatments, and 22% in the drip treatment. At week 3, disease incidence had increased to 31% in the mist and drip treatments and 44% in the ebb & flow treatment. Disease incidence was 81.5% in all treatments by week 5. Disease incidence was 100% in all treatments by week 7 (Figure 2-4).

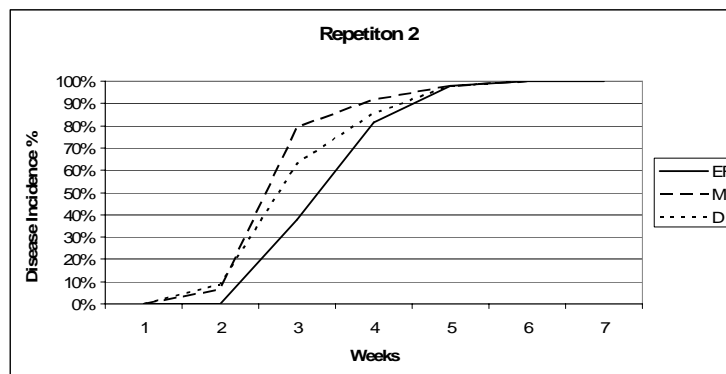


Figure 2-3. Effect of irrigation methods on disease incidence of *Corynespora* leaf spot.

(EF=ebb & flow irrigation method; M=mist irrigation method; D=drip irrigation method).

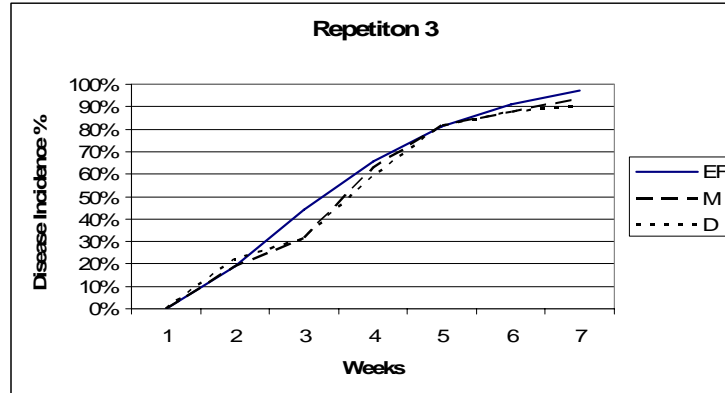


Figure 2-4. Effect of irrigation methods on disease incidence of *Corynespora* leaf spot. (EF=ebb & flow irrigation method; M=mist irrigation method; D=drip irrigation method).

Repetition 4. In week 2, disease incidence was 16% in the mist and ebb & flow treatments and 28% in the drip treatment. In week 3, disease incidence had increased to 35%, 32%, and 25% respectively in the mist, drip, and ebb & flow treatments. Disease severity was more than 50% by week 4 in all treatments. The end of week 7 resulted in 97% infection for the ebb & flow treatment, 94% infection for the drip treatment, and 88% infection for the mist treatment (Figure 2-5).

The only significant differences were between the variables Disease Incidence and Weeks at the criterion alpha level of 0.05. The other variables, Irrigation Types and the interaction between Weeks by Irrigation Type, did not play a significant role the development of the disease in these experiments (Table 2-1).

The interaction between the Disease Incidence and Weeks were significantly different between Weeks 1 through 5. Between weeks 5, 6, and 7, the disease progression of *Corynespora* leaf spot plateau at 0.9 (Table 2-2).

The Shapiro-Wilk test indicated a normality of residuals at 0.97, indicating that the residuals or errors were normally distributed (Table 2-3).

Although, symptoms increased from week to week in all treatments, the hypothesis must be rejected and the null hypothesis accepted that the irrigation treatments do not affect the development of *Corynespora* leaf spot epidemics.

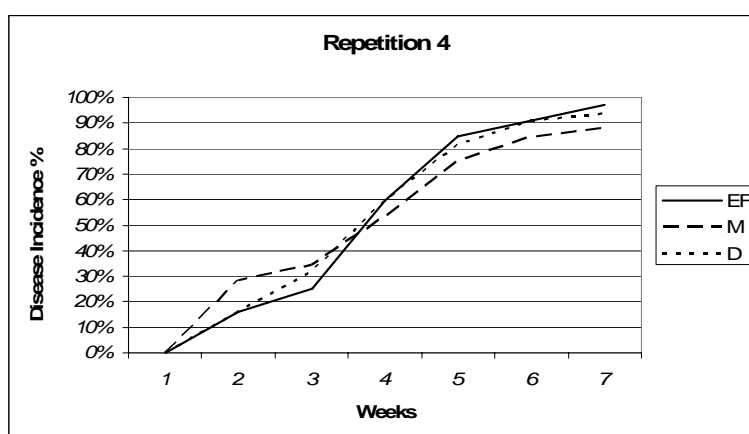


Figure 2-5. Effect of irrigation methods on disease incidence of *Corynespora* leaf spot. (EF=ebb & flow irrigation method; M=mist irrigation method; D=drip irrigation method).

Mixed ANOVA PD		
Disease Incidence	F value	Pr>F
Wk	597.10	<.0001*
IT	0.21	0.8148
Wk * IT	1.05	0.4052

Table 2-1. Variables tested for their affect on disease incidence. Variables included Wk=week, IT=irrigation type, and Wk*IT=week and irrigation type interaction.

Obs	Week	Estimate For Disease Incidence	Standard Error For Disease Incidence	Letter Group
1	1	-0.00654	0.03101	F
2	2	0.1275	0.03101	E
3	3	0.4105	0.03101	D
4	4	0.7246	0.03101	C
5	5	0.9001	0.03101	B
6	6	0.9440	0.03109	AB
7	7	0.9649	0.03109	A

Table 2-2. Disease development from week to week for all three repetitions.

For the experiment seven observations (Obs) were conducted over the seven week period. Estimate indicates the mean for disease incidence. The standard error for disease incidence represents the error that could have occurred for that week. The letter group indicates similarities or differences among the weeks. Different letters represent significant differences.

Test	-----Statistics-----		-----p value-----	
Shapiro-Wilk	W	0.971493	PR<W	<0.0001
Kolmoogorov-Smirnov	D	0.092731	PR>D	<0.0100
Cramer-von Mises	W-Sq	0.710229	PR>W-Sq	<0.0050
Anderson-Darling	A-Sq	3.777766	PR>A-Sq	<0.0050

Table 2-3. Normality of Residuals.

Discussion

No irrigation system was effective in reducing the incidence of *Corynespora* leaf spot on *S. ionantha*. Although all three irrigation methods used in this study have been used in *S. ionantha* production facilities, mist is the most commonly used irrigation method. The mist treatment, theoretically, should have produced a more conducive environment for

the spread of *Corynespora* leaf spot because as water is splashed onto the plants, conidia are disseminated from lesions to healthy tissues. Increases in disease incidence and severity of diseases due to *P. carotovora*, *E. chrysanthemi*, and *E. cichoracearum* in *S. ionantha* were attributed to conducive environments associated with mist irrigation (109). However, an increase in disease incidence and severity was not observed for *C. cassiicola*. For *Corynespora* leaf spot, disease incidence was unacceptably high (100% by week 7 in repetitions 1 and 2 and 94% and 88%, respectively, in repetitions 3 and 4) for all irrigation treatments. Because mist systems are less expensive to build and maintain and apparently do not provide a more conducive environment for *Corynespora* leaf spot, a recommendation can not be made to change from mist to another irrigation method.

The drip treatment was expected to be the most effective system for reducing the spread of *Corynespora* leaf spot because water is applied to the root zone and the wetting of foliage and possibly facilitating the movement of conidia is avoided. Unlike results reported by Browne et al. (19) and Lanier et al. (70) where the drip irrigation was found to reduce the spread of stem rot on potato and early leaf spot on peanut, the drip treatment in this study did not reduce the spread of *Corynespora* leaf spot on *S. ionantha*. Symptom development increased each week throughout the experiment resulting in an unexpected high percentage of infection for this treatment on *S. ionantha*.

Few problems have been reported concerning the spread of plant pathogens in the ebb & flow treatment. Even with the presence of *Fusarium oxysporum* propagules (95), *Pythium* sp., (12) and *Xanthomonas campestris* pv. *begoniae* in the recirculating water,

low levels of disease occurred. However, in this study, the ebb & flow treatment resulted in a high incidence of *Corynespora* leaf spot symptoms on leaves and petioles of the plants.

Although temperature and relative humidity surrounding the plants may have affected disease development, they were not properly monitored. However, by understanding how other pathogens sporulate in favorable growing conditions, insight may be obtained as to how *C. cassiicola* develops and reproduces.

Page et al. (90) found that at lower temperatures, *Helminthosporium oryzae* Breda de Haan conidia retained their viability the best. After 100 days *in vitro*, 81% of conidia were viable at 2° C, whereas only 6% of the conidia were viable at 31° C. However, temperature alone was not an important factor for the longevity of *H. oryzae* conidia *in vitro*. Relative humidity was also an influential factor for conidial survival. Conidia lost little viability for up to six months at 31° C and at 20% relative humidity. However, at 31° C and at 95% relative humidity, conidia failed to germinate after one month. At low temperatures and at all relative humidities, viability of the conidia were at the highest levels. Page et al. (90) concluded that under field conditions, the conidia would more than likely not survive the winter because they failed to germinate at 31° C at 95% relative humidity and only survived three months at 24° C. Therefore, the primary function of the conidia would seem to be connected with the spread of the fungus instead of its persistence.

Helminthosporium solani Dur. & Mont., the causative agent of silver scurf on potato (*Solanum tuberosum* L.), affects the appearance and skin color of tubers, increases water loss, and has been associated with the incidence of internal black spot (83, 15). Lennard (72) studied the environmental conditions that influence the spread of the disease on tubers placed in storage. He concluded that silver scurf did not develop at any temperature when the atmosphere was dry, however, disease severity increased with high humidity and increased temperatures. Moderate to severe damage (20% or more of surface area covered) occurred at 5° C. When the tubers were stored for five months between 6-9° C, the percentage of surface area affected was between 20 and 40%, but when stored above 9° C, 50% or more of the surface area of the tubers were affected. In 2000, Hilton et al. (56), reported that a conidial suspension of *H. solani* incubated at 15° C with 95% relative humidity for one month followed by two months at 85% relative humidity produced the greatest differences in disease severity between potato cultivars. They found that if favorable environmental conditions persisted for too long, resistance was not prevalent and no differences were observed between the susceptible and resistant cultivars.

The duration of leaf wetness plays an important role in the development of many plant pathogens. For instance, the disease incidence of *Cercospora beticola* Sacc., a warm, wet-weather disease, was found to increase on sugar beets (*Beta vulgaris* L.) with longer periods of wetness. Wallin and Loonan (121) found that the number of lesions after a 48- and 72-hr moist period was more than 30 and 80 times, respectively, than the number of lesions produced after 24 hrs. The most favorable temperature for lesion development

was 29° C, however, lesions did develop at 24°C and 27°C. Rathaiah (94) demonstrated that *C. beticola* was able to penetrate sugar beets by the interruption of leaf wetting with daily dry intervals of 1 or 6 hours' duration, of which 6 hours were more effective.

Alternaria solani (Ell. & G. Martin) Sor., the causative agent for early blight of tomato (*Lycopersicon esulentum* Mill.) is a destructive disease in the tomato industry. In 2000, Vloutoglou and Kalogerakis (120), reported that the inoculum concentration, wetness duration, and plant age were responsible for the development of early blight of tomato. At 24° C, infection of *A. solani* also depended on the duration of leaf wetness. In this experiment, leaf wetness requirements varied according to the host cultivar. For the cultivar 'Skala RZ', leaf wetness required only 4 hrs in order for disease development to occur, however, 6 hrs of leaf wetness was required for the cultivar 'Rio Rojo'.

Vloutoglou and Kalogerakis (120), concluded that these findings were similar to Bashi & Rotem (10) who demonstrated that tomatoes infected with *A. solani* would require leaf wetness periods of 4 and 8 hrs at 25 and 15° C, respectively. It is important to note that in this experiment, leaf wetness up to 24 hrs resulted in an increase leaf surface area affected by the pathogen as well as increased defoliation. After 24 hrs, if the leaf wetness persisted, disease development leveled off. This could be due to a lack of available sites for infection. Susceptibility to this pathogen was observed at all growth stages, but as the plants matured, susceptibility increased.

Didymella bryoniae (Auersw.) Rehm is the causative agent for gummy stem blight of cucumber *Cucumis sativus* L. Army and Rowe (4) stated that infections caused by this pathogen were mostly influenced by the duration of surface wetness. Svedelius and

Unestam (112) found that continuous 100% relative humidity was necessary for subsequent lesion expansion and that free water on the cucumbers leaf surface was necessary for initial infection. In 1985, VanSteekelenburg (118) concluded that at 65% relative humidity, leaves were rarely infected, but were infected at 95% relative humidity and was more severe when leaf surfaces were continually wet. Free water that remained on the cucumber leaves for one hour was sufficient for infection but continual leaf wetness was required for lesion expansion.

From the previous studies, temperature and relative humidity surrounding the infected sites may be important in understanding how *Corynespora* leaf spot develops in *S. ionantha* tissue. If *C. cassicola* sporulation was affected by temperature and relative humidity, disease incidence and severity would be high in all irrigation treatments used in this study. As the plants were watered, excess water evaporated and increased the humidity around that plant. With the mist treatment, water was dispersed not only to the plants, but into the air surrounding the plants. This would increase the relative humidity around the foliage, in addition to the evaporating moisture trapped by the dense canopy of the crown. Since all irrigation treatments were located in a small greenhouse, relative humidity could have been high and of equal mean values for all treatments. This could have been due to the relative humidity being similar for all irrigation treatments or due to the relative humidity of one treatment (mist) having a profound influence on the relative humidity in other treatments that were in close proximity to each other.

The duration of leaf wetness could have had an effect on the development of *Corynespora* leaf spot on *S. ionantha*. Because water is applied only to the root zone, the drip and ebb & flow treatments should not have been effected by this duration of leaf wetness, however, with the mist treatment the water is applied directly to the foliage and remains there until it is absorbed by the plant itself or it is evaporated. However, due to the length of the mist cycles, the foliage would have remained wet for longer periods. Even with the partitions in place, the cooling fans could have distributed water to other treatments on either side of the mist treatment areas or even across the walkway to the other treatments. Even if the water concentration was not enough for the duration of leaf wetness, it is possible that the mist droplets that had reached outside of the mist treatment areas could have been carrying conidia to the foliage of plants in other treatments

In order to better understand how this pathogen is spread, it is suggested that the three irrigation treatments be tested separate from one another, at least separate the drip and ebb & flow treatments from the mist to test if the disease incidence will decrease. If the mist is not present with the other treatments, it is thought that the relative humidity will not be as much of a factor for the other two treatments, as well as it would reduce the possibility that spores are loosened and carried by other mist droplets or air currents to new infection sites.

CHAPTER III

EFFECT OF FUNGICIDES ON *CORYNESPORA CASSIICOLA* SPORULATION

Introduction

Benomyl (benzimidazole fungicide), captan (chloralkylthio fungicide), and sulfur were once commonly used in *S. ionantha* production to manage fungal diseases (110).

Benomyl and captan have been used to control powdery mildews and *Botrytis* sp.; benomyl has also been used to control *Rhizoctonia* sp. Sulfur, which has fungicidal properties, is a major element that is vital for the growth and development of *S. ionantha*. This macronutrient not only plays an important role in the synthesis of proteins, but is also responsible for boosting the defense mechanisms of *S. ionantha* (89). A fourth fungicide that has been applied to *S. ionantha* is dinocap (dinitrophenyl), which was primarily used to control powdery mildew on fruit, vegetables, nursery, and ornamental crops, but is no longer available for use (40).

Strider (110) found that benomyl was efficacious for controlling mildew at 1, 2, or 4 oz a.i./100 gal without the occurrence of phytotoxicity in forty eight cultivars of *S. ionantha*. Dinocap and sulfur also controlled powdery mildew, but were phytotoxic to the petals (110).

In recent years, other fungicides have been used to control diseases in *S. ionantha* such as thiophanate-methyl (Cleary's 3336) and chlorothalonil (Daconil). Thiophanate-methyl is a systemic fungicide that is widely used on turf, flowers, shade trees, and ornamentals to

combat diseases such as *Alternaria* leaf spot, *Rhizoctonia* stem rot, *Cyclindrocladium* cutting rot, *Fusarium* root diseases, and powdery mildew (26, 39, 82). This broad-spectrum fungicide is a benzimidazole compound that is unstable in nature, but quickly hydrolyzes to carbendazim (MBC), which is considered to be a primary toxic agent (42). Thiophanate-methyl has been used to control *C. cassiicola* in *S. ionantha*, but recently has been ineffective in slowing fungal growth *in vitro* (Mark Windham, personal communication). Thiophanate-methyl has been effective in controlling *C. cassiicola* on such plants as *Ligustrum sinense* Lour., *Ficus benjamina* L. (Moraceae), and *Lycopersicon esculentum* L. in addition to chlorothalonil, mancozeb, and benomyl (25, 67). However, benomyl was not effective in controlling *C. cassiicola* on *F. benjamina*. The ineffectiveness of benomyl to control *C. cassiicola* on *F. benjamina* may be a result of pathogen resistance to the fungicide. If *C. cassiicola* is demonstrating signs of resistance to the benomyl fungicide, it would make sense that *C. cassiicola* is resistant to thiophanate-methyl since the two fungicides are within the same class. Cross resistance of fungicides has been observed with the benzimidazole family (45, 31). Resistance to thiophanate-methyl has also been observed for *Cercospora kikuchii* (Matsumoto and Tomoyasu) and *Cercospora beticola* Sacc. (59, 124).

Chlorothalonil is a foliar protectant fungicide and is applied to ornamentals, turf, vegetables, and fruit to control leaf spots, blights, powdery mildews, and *Phytophthora* dieback diseases (17). The mode of action for chlorothalonil involves the interaction with glutathione molecules within the fungal cells (29). This combination inhibits the cells' available glutathione, therefore preventing the glutathione-dependent enzymes

from functioning. In 2005, Hagan et al. (52) concluded that chlorothalonil provided better control of rose diseases at 2-week spray intervals than at 4-week spray intervals. When the fungicide was sprayed to the canopy of roses, the canopy of sprayed plants were larger than unsprayed foliage that developed symptoms of foliar diseases. When chlorothalonil was applied to roses resistant to blackspot, little growth increase was observed. Phytotoxicity observed on rose leaves included irregular 'burnt' or brown spots on the adaxial surface, bronzing or chlorosis of leaves, and premature defoliation (52).

Propiconazole (Banner MAXX®) is a sterol-inhibiting fungicide that is systemic and is registered by the EPA as a fungicide for field nurseries, ornamentals, agricultural crop, and turf. Propiconazole has been used to control diseases caused by *Fusarium* sp., *Colletotrichum* sp., and powdery mildews. Dickens (35) found that propiconazole reduced the spread of white rust (*Puccinia horiana* Henn.) on chrysanthemum (*Chrysanthemum indicum* L.). Propiconazole reduced disease severity before and after symptom development and killed telia once sporulation began. Plants treated with propiconazole were stunted.

The objective of this study was to determine if the foliar application of fungicides would reduce the sporulation and growth of *C. cassicola* in *S. ionantha* tissue. The hypothesis was that fungicidal sprays would reduce the ability of *C. cassicola* to sporulate and grow in infected tissue beyond the normal two week spray interval.

Methods and Materials

Fungicides used in this study were propiconazole (Banner MAXX®, Syngenta Crop Protection, Inc., Greensboro, NC) 0.29 mL/100 mL (0.04% a.i.), thiophanate-methyl (Cleary's 3336, W. A. Cleary, Dayton, NJ) 0.1 mL/100 mL (0.04% a.i.), and chlorothalonil (Daconil, The ORTHO Group, Columbus, OH) 0.06 mL/100 mL (0.02% a.i.). Each fungicide solution was placed in respective spray bottles and applied at a height of 25 cm above the foliage. Plants were sprayed to run off. The fourth treatment was a water control, where mist irrigation was applied to the plant. Each treatment within a replication consisted of ten severely diseased *S. ionantha* (cultivar 'Everfloris') for a total of forty plants per replication. Plants were placed in a complete randomized block design and arranged in ten rows of four. Each row was composed of one plant from each treatment. Plants were watered with overhead mist irrigation using micronet vibro-mist nozzles, violet color, and orifice size 0.032 (30 psi: 9.59 gal/hr) (Sonne-Gro, Inc., Knoxville, TN). The mist irrigation came on daily at 8-10 a.m., 11:30-1:30 p.m., and 3-5 p.m. (Batrow Inc., Short Beach, CT). During the cycles, the mist cycled on two minutes and off for ten minutes. The greenhouse temperature was maintained at 27°C. Each week for eight weeks, one leaf from each of the forty plants was collected. The leaves were labeled and placed in a Ziploc sandwich bag (Glad Products Co., Oakland, CA). Leaves were surface disinfested in 1000 mL of an aqueous solution of 0.05% sodium hypochlorite (Clorox Company, Oakland, CA) and 12 drops of Tween 20 (Fisher Chemical, Fair Lawn, NJ). Leaves of each treatment were disinfested separately. After thirty seconds, the disinfested leaves were rinsed four times in water for five seconds

each. The leaves were placed in moist chambers consisting of filter paper (qualitative: P8; porosity: coarse; flow rate: fast) (Fisher Scientific, Pittsburgh, PA) inside of a 100 x 15 mm Petri dish (Fisher Scientific, Suwanee, GA) that had been moistened. Petri dishes were wrapped with parafilm "M" (Pechiney Plastic Packaging Company, Menasha, WI). Chambers were incubated for seventy-two hours under 40 watt T-12 Sylvania cool white plus fluorescent light bulbs (OSRAM Sylvania, Danvers, MA) at 22°C.

After incubation, leaves were examined through the Petri dish for sporulation using an Olympus SZH10 stereo microscope (Olympus America Inc, Center Valley, PA). Sporulation in a lesion appeared as long black hyphae in a starburst formation in the center of the lesion. After microscopic examination, one lesion was excised from each leaf. If sporulation was observed, the lesion with sporulation was selected for culturing. If no sporulation was observed, a lesion that best represented the symptoms of *Corynespora* leaf spot lesions were chosen. Lesions were excised from the leaves, using sterile surgical blade scalpels No. 11 (Feather Safety Razor Co., LTD Medical Division, Osaka, Japan) and disinfested as described above, rinsed three times in water, and placed in Petri plates on 20 mL of Difco™ potato dextrose agar (PDA) (Becton, Dickinson & Co., Sparks, MD) that contained 30 mg each of chlortetracycline hydrochloride and streptomycin sulfate (SIGMA Chemical Company, St. Louis, MO). Forty plates were incubated as previously described for five to six days.

Statistical Analysis

The data were analyzed using the Cochran-Mantel-Haenszel (CMH) test, which assumes a common odds ratio and a null hypothesis that states X and Y are conditionally

independent (101). This test was used to calculate the percent of leaves with sporulation and percent of isolate development of *C. cassicola*.

Results

No fungicide treatment prevented sporulation of *C. cassicola* or stopped the growth of the fungus from excised lesions placed on media. (Figure 3-1 & Figure 3-2).

Therefore, the hypothesis that the use of fungicides would reduce the ability of *C. cassicola* to sporulate and grow in infected tissue beyond the normal two week spray interval must be rejected and conclude that fungicidal spray intervals can not be lengthened beyond the recommended two week spray interval.

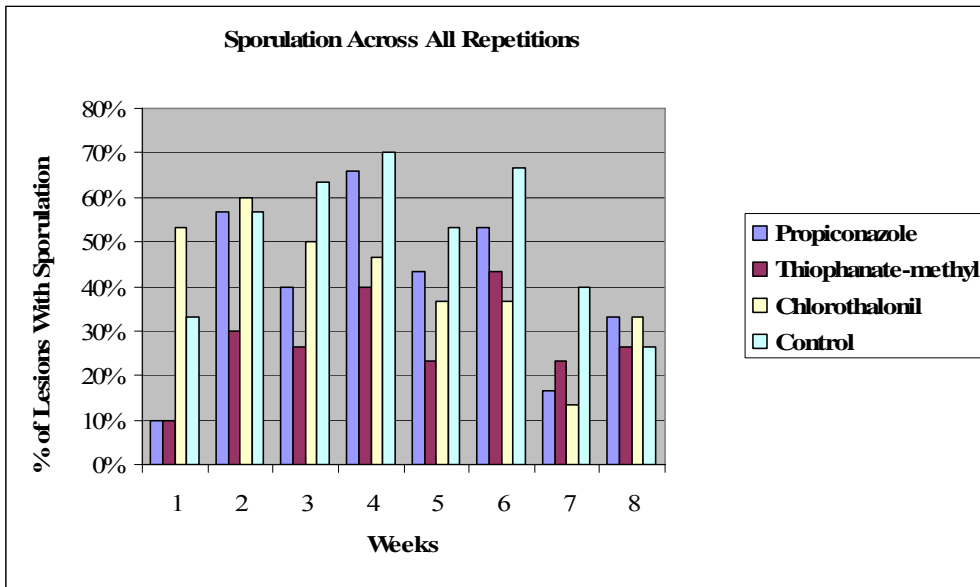


Figure 3-1. Percentage of leaves with sporulation of *C. cassicola* that had been sprayed with propiconazole, thiophanate-methyl, chlorothalonil, or water (control) one week before data collection.

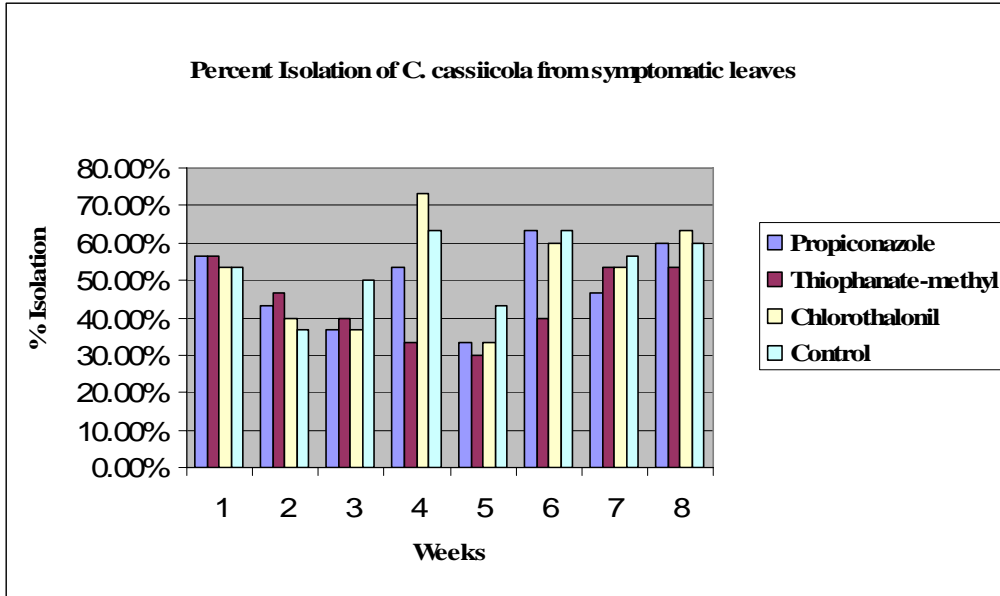


Figure 3-2. Percent isolation of *C. cassiicola* from symptomatic leaves isolated after they had been sprayed with propiconazole, thiophanate-methyl, chlorothalonil, or water (control). Data collection began one week after the leaves were sprayed.

Propiconazole and thiophanate-methyl were the only treatments that reduced sporulation one week after the chemicals were applied to the foliage. By the second week, the reduction in sporulation ceased. The CMH test indicated that sporulation differed weekly for the propiconazole and chlorothalonil treatments and the control. This was based on the number of observations versus the expected number for the presence of sporulation in the propiconazole treatment which alternated from high levels of sporulation to low levels of sporulation from week to week. Sporulation in the chlorothalonil treatment was high compared to the other three treatments in the first four weeks. Sporulation during week 7 dropped significantly and week eight remained at the expected numbers of the CMH test. The control treatment was relatively high for sporulation in weeks one

through four, the expected sporulation remained as expected for week five, and increased significantly by week 6. However, the observed number dropped well below the expected number in weeks 7 and 8. Thiophanate-methyl was the only treatment where sporulation remained constant on a weekly basis. Lesions on the leaves of the thiophanate-methyl treatment sporulated consistently 28% of the time throughout the three repetitions (Figure 3-1).

Propiconazole and thiophanate-methyl were constant over weeks for the ability to suppress isolation of *C. cassiicola* from treated leaves. In the chlorothalonil treatment, the number of observations versus the expected number for the presence of fungal growth increased in all weeks except for week 7.

Discussion

No fungicide, used in this study, was effective in reducing sporulation or fungal growth of *C. cassiicola* in *S. ionantha*. Isolates of *C. cassiicola* obtained from *S. ionantha* tissue have demonstrated resistance to thiophanate-methyl (Mark Windham, personal communication). Fungicide resistance has lead African violet growers to increase rates of the fungicide and/or reduce intervals between fungicide applications.

Resistance to benzimidazole fungicides (thiophanate-methyl) have reportedly been due to point mutations at the β -tubulin gene, which result in an altered amino acid sequences at the benzimidazole-binding site (77). Numerous studies have shown changes in the β -tubulin gene at codons 6, 50, 167, 198, 200, and 240 that confers field isolate resistance to many phytopathogenic fungi to benzimidazole fungicides (69, 44, 127, 1). In 1971,

thiophanate-methyl was used to control purple seed stain in soybeans (*Cercospora kikuchii* [Matsumoto and Tomoyasu] Gardner) (59). By the late 1980s, isolates of *C. kikuchii* were reported to be resistant to thiophanate-methyl. When usage of thiophanate-methyl was stopped, the frequency of resistant strains declined steadily. However, when farmers began using thiophanate-methyl again, the frequency of resistant strains increased rapidly (85). Similar results were found for *Helminthosporium solani* Durieu & Mont., *Botrytis cinerea* Pers., and *Monilinia fructicola* Wint. (79, 127, 76).

Although minimal, some control was observed with thiophanate-methyl. The isolation technique used in this study was selective for resistant isolates of *C. cassiicola*. Dovas et al. (38) reported that under selection pressure of various field conditions, survival of both *Cercospora beticola* Sacc. benomyl-sensitive and benomyl-resistant isolates were of equal fitness and present in sugar beet (*Beta vulgaris* L.) fields in northern Greece. According to Gisi et al. (46), isolates with higher fitness attributes generally have a shorter incubation and sporulation time, or the larger lesion size and sporulation capacity increases in frequency in disease epidemics after a certain time of development. As to whether or not these resistant isolates are capable of surviving without the presence of thiophanate-methyl, is not known. In some incidences, if the fungicide applications cease, the frequencies of resistant isolates in the pathogen populations will also cease (77). However, Ruppel et al. (99) reported that resistant benomyl isolates of *C. beticola* were capable of survival without the presence of the benomyl fungicide.

Since resistance to thiophanate-methyl has been observed in *C. cassiicola* infected on *S. ionantha*, neither this fungicide nor others within the same class of fungicide should be

used for disease control. Cunha and Rizzo (31) reported that 182 of 238 isolates of *H. solani* were resistant to thiophanate-methyl and benomyl in potato fields where only thiophanate-methyl had been used. Ruppel (98), also reported cross-resistance to the same benzimidazole fungicides in an attempt to control *C. beticola* on sugar beet.

Propiconazole was also ineffective in controlling *C. cassiicola* in symptomatic tissue of *S. ionantha*. Sporulation and isolation development was consistently high for all weeks in the combined analysis of the three repetitions. Propiconazole is a demethylation inhibitor (DMI). This group of fungicides is responsible for inhibiting the demethylation at the 14- α carbon of 24-methylenedihydrolansterol, which is a precursor of ergosterol in fungi (16). However, mutations have been reported at the 14- α -demethylase (CYP51) gene. A single mutation in the CYP51 gene of the fungus *Uncinula necator* [Schw.] Burr., causal agent of powdery mildew of grape, results in an amino acid change from tyrosine (Y) to phenylalanine (F) at codon 136 (Y136F) which is found in all triadimenol-resistant isolates (33). The same mutation was found for the fungus *Erysiphe graminis* DC (34). In laboratory-induced resistant isolates of *Penicillium italicum* Wehmer. and *Ustilago maydis* DC (Cda.), several modifications of other amino acids of the CYP51 genes were shown to be associated with DMI resistance (64, 22, respectively). Schnabel and Bryson (104) also reported that propiconazole resistant isolates of *M. fructicola* were more difficult to control than propiconazole sensitive isolates from peach orchards in South Carolina and Georgia. It is possible that isolates of *C. cassiicola* obtained in this study have similar resistance mechanisms.

Another possible explanation for the lack of effectiveness of propiconazole may have been due to the low rate of propiconazole used in this study. A lower rate was necessary because phytotoxicity has been reported on *S. ionantha* sprayed with propiconazole. Clarkson et al. (27) reported that reduced dose applications of propiconazole were capable of containing the spread of leek rust caused by *Puccinia allii* Rud. on leeks (*Allium porrum* L.). However, with the appearance of new rust pustules, the reduced dosage of propiconazole was ineffective. Although the reduced rate was ineffective, propiconazole may have been more effective, if it had been applied before symptoms had developed. At the lower rate, propiconazole was ineffective as a curative fungicide. It might be more effective as a preventative fungicide.

Chlorothalonil, a protective fungicide, is effective in controlling many leaf spots, powdery mildews, and foliar blights (17). However, if the pathogen is in the host before the application of chlorothalonil, the fungicide is not as effective as it would have been if it was applied before infection. In this study, *S. ionantha* plants were infected and symptomatic for *Corynespora* leaf spot before the fungicide was applied. Therefore, it is not surprising that the chlorothalonil treatment was not effective. Also, chlorothalonil may not have been effective because all treatments were placed under mist irrigation which could have washed the fungicide from the leaves of *S. ionantha*. Both Johnson et al. (61) and Schenck (102) reported that when applications of chlorothalonil were applied after symptoms of gummy stem blight *Didymella bryoniae* (Auersw.) appeared on watermelon *Citrullus lanatus* (Thunb.) Matsum & Nakai, the fungicide was ineffective in controlling the disease when compared to plants sprayed before symptoms

occurred. *Cercospora beticola* was effectively controlled on sugarbeet when chlorothalonil was applied in protective treatments (83-87% disease control), however, when applied after symptoms developed, disease control decreased to 45-76% (2).

Because of the failure of the three fungicides used in this study, strobilurin fungicides should be tested for effectiveness of control because they use different mechanisms to inhibit fungal growth and sporulation. They are used to control a broad spectrum of plant pathogens (128). According to Gisi (47), the mode of action for strobilurin fungicides involves inhibiting the mitochondrial respiration by binding to the Qo site (the outer, quinone oxidizing pocket) of the cytochrome bc_1 enzyme complex (complex III), therefore blocking the electron transfer in the respiration pathway and leading to energy deficiency due to a lack of ATP², and are known as Qo inhibitors (QoIs). Strobilurins are also known to produce longer retention of green leaf tissue, as well as significant yield enhancements (50). Pernezny et al. (92), reported azoxystrobin, a strobilurin fungicide, in conjunction with mancozeb and fumoxate provided excellent control of *C. cassicola* on tomato (*Lycopersicon esculentum* Mill.). Compared to the untreated control, the diseased severity values of the fungicide treatment only reached 10-15% in addition to doubling the marketable yield. Because resistance has been reported with these fungicides, it is important to note that no more than two sequential applications of this fungicide group should be made and applications should be alternated with another broad-spectrum fungicide.

Another suggestion involves using a mixture or alternating applications of fungicides (30). Culbreath et al. (30) discovered that to control early (*Cercospora arachidicola*

S.) and late (*Cercosporidium personatum* Berk. & M. A. Curtis) leaf spot of peanut (*Arachis hypogaea* L.), a mixture or alternate applications of benomyl and chlorothalonil were suggested, since benomyl alone was not able to control the spread of leaf spot in the field. If applied before symptoms occur, chlorothalonil could work in conjunction with a curative/systemic fungicide. With this method, the leaves and petioles of the African violet would double the protection on both the outside and inside of the plant organ. In addition to doubling the protection, the risk of producing resistant isolates would be lowered because the pathogen would have to combat two fungicides at the same time or if the fungicides were alternated, then the pathogen would not have time to adapt to either one.

Although chemical controls are only one aspect of controlling the spread of a pathogens' population, it is still very important and effective. The economic loss that *Corynespora* leaf spot has caused to the *S. ionantha* industry, is not known. However, if chemical compounds are not identified that are effective in controlling the disease, economic losses may sky rocket within the next few years in *S. ionantha* production facilities.

CHAPTER IV

SUSCEPTIBILITY OF *SAINTPAULIA IONANTHA* LEAVES TO *CORYNESPORA CASSIICOLA* AS BY LEAF AGE INFLUENCED

Introduction

Age of the host can also affect susceptibility. Different interactions between the host and the pathogen can be affected by the surface area of plant organs such as: chemicals released by both the host and the pathogen, a conducive environment for infection development, and various developmental stages and age of the plant organs. This type of resistance can determine the severity of disease incidence. Wang and Lin (122) found that susceptibility of peanuts (*Arachis hypogaea* subsp. *fastigiata*) to rust (*Puccinia arachidis* Speg.) changed as the plants aged. Older leaves became more resistance to rust. Viljanen-Rollinson et al. (119) found that the germination of *Erysiphe pisi* Syd. conidia increased more on young leaflets of peas (*Pisum sativum* L.) than on the older leaflets. Carver and Adai (23) also discovered that the germination of *E. graminis* f.sp. *avenae* conidia were more prominent on the younger leaves of oats (*Avena sativa* L.) than on the older leaves. Douglas et al. (37) found an increase of *E. graminis* f.sp. *avenae* conidial germination on the leaves of adult oat plants compared to the leaves of seedlings. Studies on barley (*Hordeum vulgare* L.) by Ayres & Woolacott (6) and Russell et al. (100) shared the same outcome.

Chase (25) reported that *Corynespora* leaf spot was more severe during propagation of zebra plants and that high humidity and leaf wetness contributed to disease development.

On soybeans, Boosalis and Hamilton (14), concluded that as plants aged, lesions of *Corynespora* leaf spot increased in size and girdled the tap roots and adjacent stem tissue. Fajola and Alasoadura (41) found similar results with *Corynespora* leaf spot on tobacco. Older leaves had higher disease severity than did younger leaves.

Many studies have found a relationship between plants' organ age and disease susceptibility including studies with *Corynespora* leaf spot. Therefore, the objective of this study was to determine if leaf age influenced the susceptibility of *S. ionantha* to *Corynespora* leaf spot. Susceptibilities of the foliage to this pathogen may be important in propagation. The hypothesis is that leaf age affects the susceptibility of *S. ionantha* to *Corynespora* leaf spot.

Methods and Materials

For this study, leaves of different ages (juvenile, mature, and senescing) of the cultivar 'Michelle' were collected to observe the development of lesions caused by *Corynespora* leaf spot. Juvenile leaves were chosen from the second node from the top of the plant; mature leaves were chosen at the sixth node from the top of the plant; and senescing leaves were chosen from the second node from the bottom of the plant. The side of the plant from which a leaf was chosen for inoculation was randomly decided by a coin toss. Ten plants were used per replication and the experiment was repeated three times.

In both the inoculated treatment and the uninfected controls, leaves were wounded with a quilter's pin (plastic head extra large steel size 28-1 3/4" (44 mm)) (Prym-Dritz Corporation, Spartanburg, SC). Each leaf was carefully wounded fifteen times

throughout the adaxial surface so as not to puncture through to the abaxial surface. In the inoculation treatment, mycelium of *C. cassiicola* was applied directly to the injured area. After wounding, both treatments were randomly arranged in five rows of four. After the initial setup, plants were observed for lesions every third day. As lesions appeared and were at least 1 mm in diameter, the lesions were measured with a Mitutoyo Absolute Digimatic Calibrator (Mitutoyo Canada Inc., Toronto, Ontario). Measurements involved the width and the length of the lesions. Lesion area was calculated using the formula for the area of an ellipse ($A=\pi ab$, i. e. $A=3.1416 \cdot L \cdot W$). Three lesions were measured per leaf. If lesions coalesced, lesions were measured as separate entities. All plants were watered with mist irrigation using micronet vibro-mist nozzles, violet color, and orifice size 0.032 (30 psi: 9.59 gal/hr) (Sonne-Gro, Inc., Knoxville, TN). Mist irrigation came on three cycles a day between 8-10 a.m., 11:30-1:30 p.m., and 3-5 p.m. (Batrow Inc., Short Beach, CT). During each cycle, the mist came on for two minutes and off for ten minutes. The greenhouse temperature was maintained at 27°C.

Statistical Analysis

This experiment was analyzed as a complete randomized block using the SAS program (101). The Least Significant Differences (LSD), at a criterion alpha level of 0.05, calculated the differences between the mean lesion area.

Results

Repetition 1. After one week, lesions were observed on juvenile leaves. Lesions were observed on the senescing leaves six days later. Lesions were not observed on mature

leaves until the fourteenth day after they were observed on the juvenile leaves. The number of lesions present on all leaf ages increased throughout the six weeks of observation. On the forty-second day, the number of lesions present on the juvenile, mature and senescing leaves were 7, 8, and 5, respectively.

Repetition 2. Ten days after inoculation, lesions were observed on all three leaf ages. The number of lesions continued to increase on all three leaf ages throughout week 6. However, it is important to note that the mature leaf had the highest number of lesions throughout the experiment.

Repetition 3. After one week, lesions were observed on both the juvenile and senescing leaves ten days after inoculation. Lesions on mature leaves occurred three days after lesions appeared on the juvenile and senescing leaves. At the end of week 6, the juvenile leaves had the highest total number of lesions with six.

Significant differences between the mean lesion area of the leaf age and by week were observed when tested at a criterion alpha level of 0.05. When the total number of lesions were considered as a composite sample, the highest number of lesions occurred on the mature leaves, followed by the juvenile leaves (Figure 4-1).

When the lesion area was calculated with the Least Significant Differences (LSD) test, the number of lesions on the mature leaves and on the juvenile leaves were significantly different. Lesions on the mature leaves had the smallest lesion area at 2.76 mm and lesions on the juvenile leaves had the highest lesion area of the three leaf ages at 3.58 mm (Table 4-1).

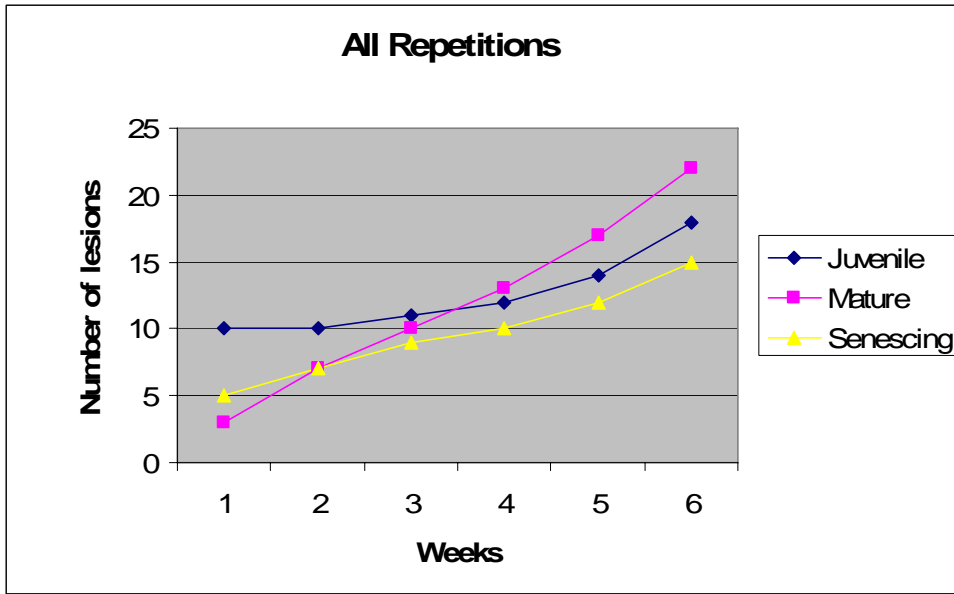


Figure 4-1. Total number of lesions on the leaves in all three repetitions.

Leaf Stage	Mean Lesion Area (mm ²)
Juvenile	3.58 a
Mature	2.76 b
Senescing	2.89 ab

Table 4-1. Effect of *Saintpaulia ionantha* leaf age on lesion size (area) resulting from infection by *C. cassiicola*.

^{ab} Means followed by the same letter do not differ according to LSD (p=0.05).

Because the mature leaves are more resistant to *C. cassicola* than the juvenile and senescing leaves, the hypothesis that leaf age influences the susceptibility of *S. ionantha* to *Corynespora* leaf spot can be accepted.

Discussion

For many plants, resistances to plant pathogens develop with increasing age. In some circumstances, after the plant has matured and continues to age, resistance begins to decrease (63, 75, 81, 91, 117). In this experiment, juvenile leaves, which were chosen from the second node from the top of the plant, were the most susceptible to *Corynespora* leaf spot, while the mature leaves, which were chosen six nodes from the top of the plant, were the most resistant. The senescing leaves, chosen from the second node from the bottom of the plant, were as susceptible to the fungus as the juvenile leaves.

Several factors can influence the susceptibility of the juvenile leaves. Jhooty and McKeen (60) concluded that the thickness of the cuticle was positively correlated with resistance in wild strawberries, *Fragaria ovalis* Wils. and *Fragaria chiloensis* Duchesne, to powdery mildew *Sphaerotheca macularis* (Wallr. ex. Fr.) Cooke. For *F. ovalis*, cuticles of young leaves were thin on both surfaces and leaves were highly susceptible to infection. The adaxial surface of the *F. chiloensis* leaves had thicker cuticles and was resistant to *S. macularis*, while the abaxial surface had a thinner cuticle and was more susceptible. Wetzstein and Sparks (125) found that as pecan leaves (*Carya illinensis* (Wang) K. Koch) matured in age, resistance to pecan scab (*Cladosporium caryigenum* (Ell. et Lang) Gottwald) increased. The older leaves had thicker cuticles than did the

younger, more susceptible leaves. Cuticle thickness was not measured in this experiment, but it could have played a role in providing resistance to mature leaves since they are thicker and harder to tear. In future experiments, the role of cuticle thickness should be investigated.

Another factor that could have played a role in the susceptibility of the juvenile leaves to *Corynespora* leaf spot was the presence of glandular trichomes. If the glandular trichomes of *S. ionantha* secreted a resinous material, spores could have been captured in the adhesive substance and not released. However, with the mature leaves, the glandular trichomes could have had a chemotropic effect and chemically inhibited spore germination and growth of *C. cassicola* (125). An example of this chemotropic effect, is the secretion of malic by glandular trichomes of *Cicer arietinum* L. (48). The abundance of trichomes could also effect disease development. An increased number of trichomes, could provide a conducive environment for spore development due to the trapping of humidity near the leaf's surface by the trichomes (125). Turechek and Stevenson (116) studied the development of pecan scab on the two pecan cultivars 'Wichita' and 'Sumner' in response to leaf wetness and leaf age. From their results they concluded that infection for the cultivar 'Wichita' decreased as the temperature increased (22 to 25° C), however, as the leaves matured and periods of leaf wetness increased (no less than 12 hrs), so did infection. With increasing leaf age, the cultivar 'Sumner' had less infection frequencies, smaller lesion size, and lower conidia production.

Another entry site for plant pathogens, is foliar trichomes. Layne (71) reported that foliar trichomes of tomato (*Lycopersicon esculentum* Mill.) produced a conducive

environment for the entry of *Corynebacterium michiganense* (E. F. Smith) Jensen, the causal agent of bacterial canker on tomato. Results concluded that the adaxial surface of young tomato leaves were more susceptible to infection than older leaves. The resistance demonstrated by the older leaves was believed to be the result of “fewer active, infectible sites per unit area suitable for infection by the pathogen” (71). Also, higher infection frequency was found on leaves with long, septate hairs with bulbous bases as opposed to short hairs or glandular hairs. The long appressed glandular trichomes possessed by *S. ionantha*, may explain the resistance demonstrated by the mature leaves. However, the increase of glandular trichomes on the juvenile leaves could have aided in the entrapment of spores, which seemed to have more per unit area compared to the mature and senescing leaves.

The mature leaves and to some extent the senescing leaves, may have released fungitoxic substances that inhibited the growth and sporulation of *C. cassicola*. This may have resulted because as the leaf ages, the leaf exudates changes (49). Gottwald (49) states that once the leaves mature and reach their full size, cuticle formation catches up with the expansion, and phenolic substances increase in the palisade parenchyma layers of the leaf blade (125). The inhibition of pathogen growth and reproduction due to the increase of phenolic substances has been reported to affect *Cercospora beticola* on sugar beet (*Beta vulgaris* L.), *Ventruria* on pear and apple leaves, as well as *Botrytis cinerea* Pers. on *Vitis* and *Cyclamen* (18, 53, 68, 103). Conversely, *C. cassicola* itself may release a toxin that may break down the defenses of the host defense mechanisms, especially the juvenile *S. ionantha* leaves.

The production of toxins by *C. cassiicola* has been reported to influence the susceptibility of tomato. Onerirosan et al. (87) reported that various isolates of *C. cassiicola* that were highly pathogenic to tomato produced a toxin that affected only susceptible cultivars and were ineffective against resistant cultivars. They predicted that the size of the lesion found on tomato may be a result of the amount of toxin produced by isolates and that virulence was correlated with toxin production. Large spreading lesions on the foliage and stem were associated with highly virulent isolates that produced relatively large quantities of toxin. Weakly virulent isolates produced pinpoint-sized lesions and produced low levels of toxin. The varying lesion sizes found on the tomato leaves due to the amount of toxin production, could be one explanation as to why the juvenile leaves of *S. ionantha* produced lesions greater in size (mean lesion area of 3.58 mm) than on the mature leaves (mean lesion area 2.76 mm). Onerirosan et al. (87) suggested that the increased knowledge of the tomato-specific toxin would be beneficial in the propagation of developing tomato resistance cultivars to *C. cassiicola*. The same would hold true for the propagation of *S. ionantha*. Similar uses have been found in the toxin detection of *Helminthosporium victoriae* F. Meehan & Murphy on oats and *H. sacchari* (B. de Haan) Butl. on sugar cane (126, 108).

Nutrient input can also influence the survivability of various fungal pathogens. Hill et al. (55) reported that the younger leaves of *Luzula sylvatica* (Huds.) Gaud. were found to have much higher apoplast pH than older leaves and that the apoplast pH and NH_4^+ concentration increased during leaf expansion before declining prior to senescing. The authors also reported a higher concentration of bulk foliar tissue pH, NH_4^+ , and N levels

in younger leaves than older leaves. If the chemical make up of *S. ionantha* leaves is similar to *L. sylvatica*, then it may explain why the mature leaves are resistant to *Corynespora* leaf spot and the juvenile leaves are more susceptible. The susceptibility demonstrated by the senescing leaves may be a result of the declining apoplast pH and NH_4^+ .

In order to control the spread of *Corynespora* leaf spot in commercial greenhouses, propagators should focus on using the mature *S. ionantha* leaves as the mother leaves when propagating in order to reduce the inoculum available to infect the plantlets. By reducing initial inoculum levels, the development of the epidemic could be delayed and possibly maximum levels of disease incidence and/or severity reduced. However, this attempt to reduce inoculum pressure may be lost if propagation and the growing of plugs and mature plants are conducted in the same greenhouse. Air currents, due to fans, will facilitate the movement of conidia from infection sites to susceptible leaves of plantlets still attached to the mother leaves. Propagation should occur in facilities separated from the areas where plugs and mature plants are grown.

SUMMARY

Cultural control methods were evaluated to determine their effect on development and spread of *Corynespora cassicola* (Berk. & Curt) Wei on African violet (*Saintpaulia ionantha* Wendl.). Three irrigation treatments, drip, mist, and ebb & flow, were ineffective in reducing the spread of *Corynespora* leaf spot on *S. ionantha*. Increased humidity, variation in temperature, and duration of leaf wetness may have contributed to increased symptom development. Since all irrigation methods failed to reduce disease incidence, recommendations to growers concerning irrigation method can not be made. No fungicide treatment was found to reduce sporulation or growth within symptomatic tissues. Thiophanate-methyl has been effective in controlling *C. cassicola* on *S. ionantha* in the past, but was ineffective in this study. Its failure could be due to resistance developing in response to continual use and increased applications of the fungicide. At lower rates, propiconazole was ineffective despite being a curative and systemic fungicide. Chlorothalonil was ineffective because it was not applied before symptoms developed. In the age-susceptibility experiment, juvenile and senescing leaves were more susceptible to *Corynespora* leaf spot than the mature leaves. Senescing leaves should not be used in propagation. This will reduce initial inoculum providing that propagation is separated from areas where plugs and mature plants are located.

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