RESISTANCE TO CERCOSPORA LEAF SPOT AND POLYPLOID INDUCTION TO IMPROVE *LAGERSTROEMIA*

by

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(Under the Direction of John Ruter)

ABSTRACT

Crape myrtles (*Lagerstroemia spp.*) are an economically important crop in the southeastern United States cultivated for their showy flowers, bark, and attractive foliage. The goals of this research aim to evaluate crape myrtles for Cercospora leaf spot resistance and improve crape myrtles through polyploid induction. Resistance to Cercospora leaf spot exists among crape myrtle cultivars descended from a cross between *L. indica* and *L. fauriei*. Cultivars most resistant to Cercospora leaf spot in Blairsville, GA, are 'Apalachee', 'Muskogee', 'Natchez', and 'Miami'. Cultivars least resistant to Cercospora leaf spot are 'Ozark Spring', 'Victor', 'Dynamite', and 'Pink Velour'. Resistance to Cercospora leaf spot exists in *Lagerstroemia subcostata* hybrids selections observed in Watkinsville, GA. Selections of dark-foliaged crape myrtles with improved resistance over commercial cultivars were identified. Fungicides were tested *in vitro* to control the causal agent of Cercospora leaf spot, *Pseudocercospora lythracearum*. The fungicides: thiophanate methyl (3336 WP), propiconazole (Banner Maxx II®), azoxystrobin (Heritage®), mancozeb (Dithane® 75DF), chlorothalonil (Daconil Ultrex®) effectively stopped fungal growth. Fludioxonil (Medallion 50WP) was not effective in reducing fungal growth. A method was developed for inducing polyploid crape myrtles using the chemical oryzalin. Survival of seedlings across three treatment periods was 46%, with a tetraploid conversion rate of 5.4%. Tetraploid crape myrtles showed modified leaf and stomata morphology.

INDEX WORDS: *Lagerstroemia*, crape myrtle, oryzalin, mutagenesis, polyploid, tetraploid, plant breeding, flow cytometry, AUDPC, plant disease, *Cercospora*, *Pseudocercospora*

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CHAPTER 1

INTRODUCTION

Crape myrtle (*Lagerstroemia spp.*) is a tree commonly planted in the southeastern United States for its showy summer flowers and vibrant fall foliage. Originally introduced to America in the 1700's from Asia, crape myrtle is widely cultivated and planted throughout the southeastern United States (Wang et al. 2016). In 2019 over three million crape myrtles were sold, accounting for a market value of over 69.9 million dollars (USDA Horticultural Census 2019). The southeastern region of the United States produces 75% of crape myrtles (National Agriculture Statistics 2020). Over 200 crape myrtle cultivars exist, with half available to nurseries for production (Wang et al. 2011).

Over 50 crape myrtle species have been reported (Cabrera 2004), but fewer than ten are cultivated for ornamental use (Parajuli 2023). The most common species for use in the landscape are *Lagerstroemia indica* and *Lagerstroemia fauriei* (Wang et al. 2011). *Lagerstroemia indica* is a large shrub ranging from 3.0 m to 9.0 m in height and 4.5 to 7.5 m in the canopy (Dirr 2002). *Lagerstroemia indica* produces 15.0 to 20.0 cm flower panicles that are showy with various colors (Dirr 2002). *Lagerstroemia fauriei* is a tree that can grow from 10.0 to 15.0 m in height and 7.5 to 10.0 m in the canopy (Creech 1985). *Lagerstroemia fauriei* produces flowers in small panicles which bloom only once per season (Wang et al. 2011). *Lagerstroemia indica* and *L. fauriei* were crossed at the USDA National Arboretum to improve resistance to powdery mildew starting in the 1960s and thru the 1980s (Egolf 1986; Egolf 1987a; Egolf 1987b; Egolf 1990a, Egolf 1990b; Einert and Watts 1973). The resulting cultivars make up some of the most popular selections in production today.

Historically, one of the main benefits of planting crape myrtle in the landscape is its relative lack of pest and disease issues (Chappell et al. 2012). However, with the introduction of the crape myrtle bark scale from Asia to Texas in 2004, crape myrtle is no longer a low-maintenance plant (Gu et al. 2014). Crape myrtle is mainly affected by two diseases, powdery mildew (*Erysiphe australiana*) and Cercospora leaf spot (*Pseudocercospora lythracearum*) (Hagan 2010). Although neither disease is fatal, they can negatively impact the beauty of crape myrtles and the value of the plants in a nursery (Hagan et al. 1998). Research has focused on breeding powdery mildew resistance (Pounders et al. 2013). Some resistance to powdery mildew has been imparted through *Lagerstroemia indica* × *Lagerstroemia fauriei* hybridization (Hagan et al. 2002; Chappell et al. 2012). Less work has been done with breeding for Cercospora leaf spot (*Pseudocercospora lythracearum*) resistance compared to powdery mildew.

Several studies of Cercospora leaf spot impacts on various crape myrtle species and cultivars have been performed (Hagan 2001; Baysal-Gurel et al. 2017; Parajuli et al. 2023), but there is still disagreement about how some popular cultivars react to Cercospora leaf spot. Take, for example, the cultivar 'Acoma'. Selected in 1972 and released in 1986 by the USDA National Arboretum, 'Acoma' is a semidwarf, bushy crape myrtle with pure white florets and resistance to powdery mildew. Hagan (2001) and Hagan et al. (1998) claim 'Acoma' is susceptible to Cercospora leaf spot, while several observations in Louisiana (Holcomb et al. 2006; Holcomb 2001; Holcomb 2003; Holcomb 2001; Holcomb 2005; Holcomb et al. 2005; Holcomb et al. 2007) claim 'Acoma' is resistant. While climate could be the reason for these discrepancies, the Hagan studies occurred in Alabama, and the Holcomb studies in Louisiana, two states near each other with a similar humid, subtropical climate. Hagan (2001) observed that rainy weather, heavy dews, and warm, cloudy weather could accelerate disease development. Parajuli et al. (2023)

observed that fluctuating rainfall levels can increase Cercospora leaf spot disease severity. Additionally, Parajuli et al. (2023) demonstrated that the same cultivars planted in different plots can show different levels of disease resistance to Cercospora leaf spot due to various factors, including the age and size of the plants.

Another reason for these differences in resistance could be rater bias. Rater bias is the inherent bias introduced when different people rate a plant for disease severity. Rater bias can increase the likelihood of type II errors (incorrectly rejecting the null hypothesis) (Chiang et al. 2016). Thurn et al. (2019) compiled a list of known studies involving Cercospora leaf spot resistance on crape myrtles. The list contains 63 cultivars and four species of crape myrtle. Of the 63 cultivars, the studies disagreed on the Cercospora leaf spot resistance of 34 cultivars. Notably absent from these lists are crape myrtles with dark foliage. Dark-foliage crape myrtles have been gaining popularity since their introduction in 2009 (Pounders et al. 2013; J. Berry Nursery 2023).

Little is known about the disease cycle and biology of *P. lythracearum*. Cercospora leaf spot is characterized by brown angular lesions on the leaves and becomes apparent in August or September, depending on the USDA zone and cultivar (Hagan et al. 1998). On a susceptible plant, lesions spread through the canopy, turning leaves yellow and red before defoliating. However, defoliation can occur rapidly early in the season, leaving some selections bare in mid-September. Repeated fungicide applications from early summer through fall have successfully managed Cercospora leaf spot (Hagan and Arkidge 2013; Hagan and Akridge 2006; Baysal-Gurel 2017).

P. lythracearum was first identified by Heald and Wolf (1911). It was named *Cercospora lythracearum* until it was reclassified as *Pseudocecrospora lythracearum* by Liu and Guo

(1992). *Pseudocercospora* species are an anamorph, or asexual state, of *Mycosphaerella* (Park et al. 2017). The sexual *Mycosphaerella* stage of *P. lythracearum* has not been observed, and it is unknown if it exists. *Pseudocercospora* species are significant plant pathogens (Park et al. 2017; (Beckman and Payne 1982; Kim et al. 2011; Weiland and Koch 2004; Secor et al. 2010). *Pseudocercospora* species can be challenging to identify from among species using only morphological characteristics, so host specificity and multilocus sequence data are often used (Ávila et al. 2005; Crous et al. 2015). *P. lythracearum* is distributed mainly around tropics, sub-tropic, and warmer temperate areas (Kim and Shin 1999; Silva and Pereira 2008). Most *Pseudocercospora* species are host-specific (Crous et al. 2013; Crous et al. 2015). Host specificity of *Pseudocercospora* species is thought to occur at three gene loci, ITS, EF-1a, and ACT (Crous et al. 2013). The host specificity of *P. lythracearum* is unknown, but it has only been observed on plants in the genus *Lagerstroemia*.

The primary treatment for control of *P. lythracearum* is fungicides (Hagan 2006). Previous studies of fungicide treatment on Cercospora leaf spot in crape myrtle have been performed (Hagan and Akridge 2006; Hagan and Akridge 2013; Baysal-Gurel 2017). Fungicides effective for the treatment of Cercospora leaf spot are Elite™ (tebuconazole), Heritage® (azoxystrobin), Eagle 20EW® **(**myclobutanil), Cleary's 3336 (thiophanate methyl), Isofetamid 400SC (isofetamid), Instrata™(29.9% chlorothalonil, 1.2% fludioxonil, 4.7% propiconazole), Mankocide DF (30% copper hydroxide 15% mancozeb), and Mural 45 WG (30% azoxystrobin, 15% benzovindiflupyr).

However, due to the repetitive application of the same fungicides for many years, it is unknown if *P. lythracearum* is developing resistance to commonly applied fungicides.

There are no published reports of an *in vitro* fungicide assessment on *P. lythracearum*. However, i*n vitro* fungicide studies can show which fungicide is most helpful in slowing growth, inhibiting sporulation, spore germination, and inhibiting pathogenicity (Iacomi-Vasilescu et al. 2014). The amount of sensitivity a fungicide can cause is measured as the EC_{50} (Iacomi-Vasilescu et al. 2014). The EC_{50} is the concentration of fungicide that reduces mycelial growth by 50%. The EC_{50} is calculated by the regression of the radial fungal growth value against the $\log_{10}$ value of the fungicide concentration.

The process through which *Pseudocercospora lythracearum* reinfects crape myrtle annually is unknown. However, it is suspected that *P. lythracearum* overwinters in the fallen leaf litter below the crape myrtle or on the dormant leaf buds. The other most damaging disease of crape myrtle, powdery mildew, is known to overwinter in the buds of crape myrtle (Shi and Mmbaga 2006). Most pathogens related to *P. lythracearum* are known to overwinter in the fallen leaf litter below the plant (Verma and Sharma 1999; Payne and Waldron 1983; Cruz and Dorrance 2009).

The location of the overwintering of *P. Lythracearum* is critical for control recommendations given to growers. Knowing when and where the disease is active will guide future recommendations for managing this disease. It is unknown at what time of year the infection of crape myrtle by *P. lythracearum* occurs or how long after infection sporulation occurs. The efficacy of fungicide applications could be improved if infection and sporulation of *P. lythracearum* were known.

Several species of *Psuedocercospora* are known to enter their hosts through stomatal openings (Babu et al. 2009; Beckman and Payne 1982). Stomatal abundance significantly impacts *Psuedocercospora* disease severity (Akinsanmi et al. 2012). Akinsanmi et al. (2012)

showed that *Psuedocercospora* infection and the number of stomata on *Macadamia integrifolia* fruit were related by a significant positive linear relationship. The study showed that stomatal abundance could be used to select cultivars with *Psuedocercospora* resistance in a breeding context. Other morphological factors related to *Cercospora* disease severity are leaf size (Cook 1981) and trichome density (Du et al. 2009). Stomata and trichomes defend against disease by creating physical barriers to infection (Akinsanmi et al. 2012, Du et al. 2009). Therefore, the variation in crape myrtle leaf morphology may contribute to Cercospora leaf spot resistance.

Polyploidy is the state of an organism having more than two complete sets of chromosomes. Polyploidy exists naturally in plants and has been a significant driver of plant evolution (Soltis et al. 2010). Colchicine has been commonly used to induce polyploidy in plants since its discovery by Blakeslee et al. (1937). Colchicine is a chemical isolated from the plant *Colchicum autumnale* (Caperta et al. 2006). Colchicine works by preventing the formation of the mitotic spindle during mitosis, arresting mitosis, and thus doubling the genome of the cell to which it was applied (Caperta et al. 2006). A method for inducing tetraploidy in *Lagerstroemia indica* exists using colchicine (Zhang et al. 2010). Another chemical commonly used for generating polyploidy in plants is oryzalin (Thao et al. 2003; Väinölä 2000). Some studies characterize oryzalin as a more effective, less toxic, and a better alternative to colchicine (Ramulu et al. 1991; van Tuyl et al. 1992; Tosca et al. 1995). A method for inducing polyploidy in crape myrtle using oryzalin has not been reported. Oryzalin works similarly to colchicine, arresting mitosis by disrupting the formation of the mitotic spindle and doubling the genome of the affected plant (Morejohn et al. 1987). The manipulation of ploidy is an effective tool for improving the valuable characteristics of various crops and ornamentals, including citrus (Wu and Mooney 2002), azalea (De Schepper et al. 2004), and pomegranate (which is in the same

family as crape myrtle) (Shao et al. 2003). The impact of induced polyploidy is often quite variable (Thao et al. 2003). Adverse effects of induced polyploidy include stunted growth, irregular growth, and plant death (Thao et al. 2003). Some of the favorable aspects caused by induced polyploidy are increased resistance to disease and abiotic (drought, cold, nutrient, disease) stresses, larger flowers, leaves, and increased vigor and sterility (Ranney et al. 2006; Thao et al. 2003; Zhang et al. 2010; Ye et al. 2010; Li and Ruter 2017). Out of the over 50 *Lagerstroemia* species, ploidy of two *Lagerstroemia* species is reported: *Lagerstroemia indica* (Chen et al. 2003) and *Lagerstroemia speciosa* (Singhal and Gill 1984). The most relevant is *L. indica,* as the other species are not commonly grown by the nursery industry in temperate climates. Both species are diploid, and their 1n chromosome number is 24. Flow cytometry is widely used to measure the ploidy level of plants (DeLaat et al. 1987) and is a simple method of measuring ploidy (Meng and Finn 2002). Flow cytometry works by binding a fluorochrome to the nucleus and measuring the fluorescence (Dolezel 2005). The intensity of the fluorescence corresponds to the amount of nuclear DNA present. The ploidy can be estimated by comparing the fluorescence of the unknown sample to that of a selection with a known ploidy (Li and Ruter 2017).

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CHAPTER 2

SUSCEPTIBILITY OF *LAGERSTROEMIA* SPECIES AND CULTIVARS TO CERCOSPORA LEAF SPOT

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Abstract

Lagerstroemia (crape myrtle) is a genus of horticulturally important crops in the southeast United States. Crape myrtles are impacted by the disease Cercospora leaf spot (*Pseudocercospora lythracearum*) which causes defoliation and reduces the value of affected plants in the nursery. Crape myrtle cultivars were rated over six months from June to November for Cercospora leaf spot incidence in 2021 and 2022 in Blairsville, and Watkinsville, Georgia. Cultivars most resistant to Cercospora leaf spot are 'Apalachee', 'Muskogee', 'Natchez', and 'Miami'. Cultivars most susceptible to Cercospora leaf spot are 'Ozark Spring', 'Victor', 'Dynamite', and 'Pink Velour'. *L. indica* × *fauriei* hybrids were correlated with higher resistance to Cercospora leaf spot than *L. indica*. Likewise, *L. limii* × *indica* hybrids were correlated with high susceptibility to Cercospora leaf spot. Hybrids of dark-foliage *L. indica* and *L. subcostata* were more resistant than pure dark-foliage *L. indica* plants. Resistance to Cercospora leaf spot was not consistent among species with some populations having individuals with both very high and very low resistance. Hybridization of *L. indica* with *L. fauriei* and *L. subcostata* yield the highest likelihood of creating a crape myrtle resistant to Cercospora leaf spot.

Introduction

Lagerstroemia (crape myrtle) is a horticulturally important genus in the southeastern United States, accounting for over 69 million dollars in sales in 2019 (USDA Horticultural Census, 2019). Over 50 crape myrtle species have been reported (Cabrera 2004; Liu et al. 2013), but fewer than ten are cultivated for ornamental use (Parajuli 2023). The most common species for ornamental use are *Lagerstroemia indica* and *Lagerstroemia fauriei* (Wang et al., 2011). *Lagerstroemia indica* is a large shrub ranging from 3.0 m to 9.0 m in height and 4.5 to 7.5 m in

the canopy (Dirr 2002). *Lagerstroemia indica* produces 15.0 to 20.0 cm flower panicles that are showy with various colors (Dirr 2002). *Lagerstroemia fauriei* is a tree that can grow from 10.0 to 15.0 m in height and 7.5 to 10.0 m in the canopy (Creech 1985). *Lagerstroemia fauriei* produces flowers in small panicles which bloom only once per season (Wang et al. 2011). In the 1960s and thru the 1980s, crosses between *L. indica* and *L. fauriei* were made at the USDA National Arboretum to improve resistance to powdery mildew (Egolf 1986; Egolf 1987a; Egolf 1987b; Egolf 1990a; Egolf 1990b; Einert and Watts 1973). The resulting cultivars make up some of the most popular selections in production today. Hybridization between *L. indica* and *L. fauriei* imparts many valuable traits, including powdery mildew resistance and exfoliating bronze bark (Pounders et al. 2007). Some cultivars from *L. indica* and *L. fauriei* hybridization have shown resistance to Cercospora leaf spot (Hagan et al. 1998). The species *L. limii* has shown resistance to Cercospora leaf spot (Hagen et al. 1998; Parajuli 2023).

Crape myrtles are planted for their large, long-lasting inflorescence, exfoliating bark, and few pest and maintenance problems. One of these pest problems is Cercospora leaf spot caused by *Pseudocercospora lythracearum*. *Pseudocercospora lythracearum* was first identified by Heald and Wolf (1911). It was named *Cercospora lythracearum* until 1992 when it was reclassified as *Pseudocecrospora lythracearum* by Liu and Guo (Liu and Guo 1992). *Pseudocercospora* species are an anamorph, or asexual state, of *Mycosphaerella* (Park et al. 2017). The sexual *Mycosphaerella* stage of *P. lythracearum* has not been observed, and it is unknown if it exists. Although Cercospora leaf spot does not cause plant mortality, it can negatively impact the beauty of crape myrtles and the value of the plants in a nursery (Hagan et al. 1998). Cercospora leaf spot can be controlled using bi-monthly fungicide applications; resistant cultivars are the preferred control method (Hagan and Arkidge 2013).

Cercospora leaf spot is characterized by brown, round to irregular lesions on the leaves and becomes apparent in August or September, depending on the USDA zone and cultivar (Hagan et al. 1998). During warm wet conditions, leaf spots increase from August to October (Chappell et al. 2012). Weather plays a prominent role in Cercospora leaf spot development, with rainy weather, heavy dews, and warm, cloudy weather accelerating disease development (Hagan 2001). On a susceptible plant, lesions spread through the canopy, turning leaves yellow and red before defoliating (Chappell et al. 2012).

Several studies of Cercospora leaf spot impacts on various crape myrtle species and cultivars have been performed (Hagan 2001; Baysal-Gurel et al. 2017; Parajuli et al. 2023; Chappell et al. 2012). However, there is still disagreement about how some popular cultivars react to Cercospora leaf spot. Cercospra leaf spot has been reported on *Lagerstroemia indica, L. fauriei, L. limii, and L. subcostata* (Parajuli et al. 2023; Chappell et al. 2012; Baysal-Gurel et al. 2017). Interspecific hybrids can be made among some of these species (Pooler 2003) and is a focus of breeding programs to create new traits in crape myrtle cultivars (Pounders et al. 2007). Additionally, no public data exists on the susceptibility of dark-foliage crape myrtle cultivars to Cercospora leaf spot. Dark-foliage crape myrtles introduced in 2009 have become popular with consumers (Pounders et al. 2013). This study aimed to evaluate *Lagerstroemia* species and hybrids for their reaction to Cercospora leaf spot and to determine their resistance in the Piedmont and Blue Ridge regions of Georgia.

Materials and Methods

Study Sites

A plot of 41 commercially available *Lagerstroemia* cultivars in Blairsville, GA (34.8761° N, 83.9584°W) at the Georgia Mountain Research and Education Center and two plots of *Lagerstroemia* selections at the University of Georgia Horticulture Farm in Watkinsville, GA (33.8629° N, 83.4088° W) were observed from June to November 2021 and 2022. In addition, two or three replications of each cultivar were planted in Blairsville.

At the Blue Ridge mountain site in Blairsville, GA (549 m elevation, USDA hardiness zone 6b (USDA 2012)), the plot comprised four rows of trees, 10 to 15 years of age, spaced 4.6 m apart. Cultivars were randomized within the rows.

The Watkinsville area in the Piedmont region was comprised of a breeding program of *Lagerstroemia* (*indica* × *fauriei*) × *subcostata*, *L. limii*, *L. indica*, *L. indica* × *subcostata*, *L. indica* × *limii*, *L. limii* × *indica*, and *L.* ((*indica* × *fauriei*) × *subcostata*) × *limii*)) plants. Plants were planted between 2010 and 2019 at the University of Georgia Horticulture Farm in Watkinsville, Georgia (220 m elevation, USDA hardiness zone 8a (USDA 2012)). Selections of *Lagerstroemia* (*indica* × *fauriei*) × *subcostata* were replicated once, while all others had no replication. The *L. subcostata* breeding lines were from a seed source in Taiwan, and the *L. limii* selections were received as seed from South Korea.

Plant Evaluations

Crape myrtle plants were observed bimonthly for the severity of Cercospora leaf spot and defoliation due to Cercospora leaf spot in Blairsville from 7 June 2021 to 11 November 2021 and from 12 July 2022 to 21 October 2022 and in Watkinsville from 1 July 2021 to 18 November 2021 and 16 June 2022 to 27 October 2022.

A disease rating scale of 0-9 was created based on the Horsfall-Barratt scale (Horsfall and Barratt 1945) to rate the amount of disease observed on the crape myrtles. The ratings were a quality scale corresponding to the percentage of leaves with spots and defoliated leaves, such that: 0=0% of leaves affected, 0 = 0%, 1 =1%-5%, 2 = 5%-10%, 3 = 10%-15%, 4 = 15%-20%, 5 = 20-25%, 6 = 25%-40%, 7 = 40%-60%, 8 = 60%-80%, 9 = 80%-100% (Horsfall and Barratt 1945). Cultivars were given a rating of low, medium, or high resistance to Cercospora leaf spot based on Area Under The Disease Progress Curve. High resistance was defined as less than 150 AUDPC in 2021, and 40 AUDPC in 2022, moderate resistance between 150 and 350 AUDPC in 2021 and 40 and 150 AUDPC in 2022, and low resistance was defined as above 350 AUDPC in 2021 and 150 AUPDC in 2022.

Data were analyzed using a one-way analysis of variance and t-test in the statistical programming language R (R Core Team 2022). The plugin 'epifitter' (Alves and Del Ponte, 2021) was used to calculate the area under the disease progress curves (AUDPC, $A_k = \sum_{i=1}^{N_i-1} \frac{(y_i + y_{i+1})}{2}(t_{i+1} - t_i)$). Tukey's honestly significant differences (HSD) test was used for mean comparison (alpha = 0.05). Weather data was collected from the website www.weather.uga.edu.

Results

Cercospora leaf spots appeared naturally in late June in Watkinsville and late July in Blairsville. The disease steadily increased from initial spot development to peak at different times depending on the cultivar or seedling selection. Crape myrtle accessions highly susceptible to Cercospora leaf spot had a leaf spot peak in late August to September in Watkinsville and

mid-September in Blairsville before defoliating shortly after. Highly resistant individuals retained most of their leaves until the first freeze each year, after which they defoliated.

Blairsville

Many cultivars showed differing levels of susceptibility to Cercospora leaf spot (Table 2.1). There was less disease caused by Cercospora leaf spot in 2022 compared to 2021 (Table 2.1). More disease was observed on cultivars of *L. indica* parentage compared to cultivars of *L. indica* × *fauriei* (Table 3.2). The AUDPC was 57.3 % lower in the *L. indica* × *fauriei* cultivars in 2021 and 73.7% lower in 2022 compared to *L. indica.* Cultivars were differentially affected by disease each year. In a low disease year (2022), cultivars with a low resistance to Cercopora leaf spot had a similar amount of infection as in a high disease year (2021). The cultivars with high and moderate disease resistance had less disease in 2022 compared to 2021.

Watkinsville

Cercospora leaf spot differentially affected *Lagerstroemia* species in 2021 and 2022 (Table 3.3). Similar levels of disease were observed in both years for all species and hybrid crosses except *L. limii* × *indica* and *L. (indica* × *fauriei)* × *subcostata,* both exhibiting more disease in 2021 even though observations started one week earlier in 2022. Few of the species had AUDPC values significantly different from other species. The observed difference could be due to the high variance among the species groups. Dark-foliage *L. indica* cultivars crossed with *L. subcostata* showed less disease in 2021 and 2022 when compared to the dark-foliage *L. indica* (Table 3.4).

Discussion

In this two-year evaluation, 42 cultivars in Blairsville, GA, and 13 groups of species and hybrids in Watkinsville, GA, were evaluated for Cercospora leaf spot susceptibility. Cercospora leaf spot susceptibility was quantified by calculating the area under the disease progress curve, a measure of disease severity over time. *Lagerstroemia indica* × *fauriei* cultivars had a significantly lower AUDPC than *L. indica* cultivars. A similar correlation was observed by Parajuli et al. (2023). In addition to the current study, two other studies have assessed the relationship between *L. indica* × *fauriei* hybridization and Cercospora leaf spot. Parajuli et al. (2023) found that *L. indica* × *fauriei* hybrids generally had a lower AUDPC in *L. indica* cultivars. Still, there was wide variation among the susceptibility of *L. indica* × *fauriei* cultivars. Our study supports this finding, although we observed less variation within *L. indica* × *fauriei* cultivars. Parajuli et al. (2023) observed that *L. indica* × *fauriei* 'Acoma' was among the most susceptible cultivars, while in our study, only moderate susceptibility was observed. Our study observed no *L. indica* × *fauriei* cultivars with high susceptibility, only low and moderate. Additionally, our study observed no *L. indica* cultivars with high resistance to Cercospora leaf spot. Our research determined *L. indica* 'Dynamite' to be the most susceptible cultivar evaluated, confirming the same observation from Parajuli et al. (2023). Parajuli et al. (2023) also observed that pure *L. fauriei* cultivars were resistant to Cercospora leaf spot and that the trait was consistent among all observed plants. Hagan et al. (1998) also observed that *L. fauriei* showed resistance to Cercospora leaf spot. Our study did not include pure *L. fauriei* selections. Hagan et al. (1998) observed no correlation between *L. indica* × *fauriei* cultivars and resistance, finding that *L. indica* × *fauriei* cultivars showed similar levels of disease when compared to *L. indica* cultivars. Our results disagree with the conclusion of Hagan et al. (1998) that *L. indica* × *fauriei*

cultivars are not more resistant to Cercospora leaf spot than *L. indica*. The difference in results between our study and Parajuli et al. (2023) and Hagan et al. (1998) could be due to evaluation methods and location. While Hagan et al. (1998) evaluated each cultivar once per year in late August or early September, our study and Parajuli et al. (2023) evaluated cultivars throughout the entire progression of disease development and used AUDPC to determine a cultivar's susceptibility.

Additionally, the location could impact the amount of Cercospora leaf spot observed due to differing levels of rainfall and humidity between locations. In addition to Cercospora leaf spot, crape myrtles are also affected by the fungal disease powdery mildew, caused by *Erysiphe australiana* (McAlpine) Braun and Takamatsu. Previous literature describes the high resistance of *L. fauriei* and *L. indica* × *fauriei* to powdery mildew (Hagan et al. 1998; Egolf 1986; Chappell et al. 2012). However, based on the findings from our study and Parajuli et al. (2023), it does not appear that *L. indica* × *fauriei* hybridization imparts as much resistance to Cercospora leaf spot as powdery mildew. This finding is also supported by Hagan et al. (1998).

There are some trade-offs associated with cross-breeding *L. indica* by *L. fauriei.* The main disadvantage is reduced flower size, inflorescence size, and less vibrant flower color, as *L. fauriei* has small petals with pale colors. The valuable traits associated with crossing *L. indica* × *L. fauriei* are bronze exfoliating bark and resistance to powdery mildew and Cercospora leaf spot. The most susceptible *L. indica* plants still have horticultural value because the flowers of many cultivars are larger and more vibrant than those of the *L. indica* × *fauriei* cultivars during the flowering months before Cercospora leaf spot is severe. Further improvements can be made to flower size and color by continued interspecific hybridization among other *Lagerstroemia* species.

Less disease was observed in 2022 compared to 2021. The observed difference could be due to differences in weather. Weather plays a significant role in Cercospora leaf spot development, with rainy weather, heavy dews, and warm, cloudy weather accelerating disease development (Hagan 2001). During warm, wet conditions, leaf spots increase from August to October (Chappell et al. 2012). In August 2021, in Blairsville, GA, there was 34.0 cm of rainfall compared to 8.9 cm in August 2022. In October 2021, there was 10.4 cm of precipitation compared to 2.0 cm in October 2022. The increased amount and days of rainfall in 2021 could be why more disease was observed. Parajuli et al. (2023) also observed that Cercospora leaf spot disease could vary yearly based on rainfall during the growing season.

Thirteen groups of *Lagerstroemia* species and hybrids were evaluated in Watkinsville, GA, for Cercospora leaf spot susceptibility in 2021 and 2022. There were few significant differences between these groups, with two notable standouts, *L. limii* × *indica* in 2021 being very susceptible and *L. (indica* × *fauriei)* × *subcostata* being very resistant in 2022. The lack of significant differences was likely due to the wide variation observed in each group. For example, the *L. indica* × *subcostata* group contained CANR-1 with an AUDPC of 693, a plant with severe disease completely defoliated by September, and CANR-7 with an AUDPC of 112, a plant that retained most of its leaves until the first frost each year. Variation was seen among almost every group except for *L. indica, L. limii* × *indica,* and *L. indica* × *limii.* These three groups had consistently high susceptibility among all individuals. Parajuli et al. (2023) found few significant differences between different species, finding that *L. indica* was significantly more susceptible than all other evaluated hybrids and species, including *L. subcostata* and *L. limii,* and *L. indica* × *fauriei* × *limii.* Our study was unable to confirm these relationships. Parajuli et al. (2023) observed resistance in their *L. limii* population. Resistance to Cercospora leaf spot was not seen

in our *L. limii* population, suggesting that resistance may not be consistent across an entire species.

Two dark-foliage cultivars ('Ebony Embers' and 'Ebony Flame') susceptible to Cercospora leaf spot were crossed in 2018 with *L. subcostata* selections and planted in Watkinsville, GA. These F_1 individuals were selected based on powdery mildew resistance and dark foliage. The *L. indica* × *subcostata* selections from this cross had significantly more resistance to Cercospora leaf spot than both 'Ebony Embers' and 'Ebony Flame'. Based on this finding and the discovery that *L.* (*indica* × *fauriei*) × *subcostata* hybrids were resistant to Cercospora leaf spot in 2022 support the idea that hybridization with *L. subcostata* could impart resistance in crape myrtle cultivars. This idea is further supported by Parajuli et al. (2023), who observed resistance to Cercospora leaf spot in *L. subcostata* selections, and by Rinehart et al. (2015) and Wang et al. (2022), who observed a genetic similarity between *L. fauriei* and *L. subcostata.*

No crape myrtle tested in these evaluations was 100% resistant to Cercospora leaf spot, with all selections showing varying levels of susceptibility. Differences in susceptibility exist between *Lagerstroemia* species and within species, with considerable variation in susceptibility observed. Results from this study may guide breeders in selecting species and landscapers in selecting cultivars with resistance to Cercospora leaf spot. Previous studies have been done in the northern and southern United States, but this is the first study of its kind in the Piedmont and Blue Ridge mountain regions.

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Tables

Table 2.1. Disease resistance rankings of 43 commercial crape myrtle cultivars to Cercospora leaf spot (*Psuedocercospora lythracearum*) using area under the disease progress curves (AUDPC). Studies were conducted in Blairsville and Watkinsville, GA.

Cultivar	Parentage	Resistance	2021 AUDPC	2022 AUDPC
'Byer's Red'	*L. indica*	Moderate[y]	361	84
'Byer's White'	*L. indica*	Low[x]	465	154
'Carolina Beauty'	*L. indica*	Moderate	265	47
'Catawba'	*L. indica*	Moderate	357	65
'Centennial'	*L. indica*	Moderate	282	136
'Centennial Spirit'	*L. indica*	Low	455	346
'Dynamite'	*L. indica*	Low	560	438
'Ebony Embers'[w]	*L. indica*	Low	707	574
'Ebony Flame'[w]	*L. indica*	Low	707	539
'Hardy Lavender'	*L. indica*	Moderate	241	158
'Hope'	*L. indica*	Moderate	267	130
'Ozark Spring'	*L. indica*	Low	546	416
'Pink Velour'	*L. indica*	Low	564	439
'Potomac'	*L. indica*	Moderate	286	70
'Powhatan'	*L. indica*	Moderate	334	140
'Raspberry Sundae'	*L. indica*	Low	415	264

‘Red Rocket’	*L. indica*	Moderate	292	102
‘Regal Red’	*L. indica*	Moderate	386	112
‘Seminole’	*L. indica*	Moderate	331	58
‘Velma’s Royal Delight’	*L. indica*	Moderate	275	158
‘Victor’	*L. indica*	Low	567	341
‘William Toovey’	*L. indica*	Moderate	316	119
‘Acoma’	*L. indica* × *fauriei*	Moderate	269	46
‘Apalachee’	*L. indica* × *fauriei*	High[z]	47	0
‘Biloxi’	*L. indica* × *fauriei*	Moderate	272	49
‘Choctaw’	*L. indica* × *fauriei*	High	106	10
‘Comanche’	*L. indica* × *fauriei*	Moderate	126	130
‘Hopi’	*L. indica* × *fauriei*	Moderate	412	144
‘Lipan’	*L. indica* × *fauriei*	High	80	32

‘Miami’	*L. indica* × *fauriei*	High	140	10
‘Muskogee’	*L. indica* × *fauriei*	High	78	16
‘Natchez’	*L. indica* × *fauriei*	High	92	21
‘Osage’	*L. indica* × *fauriei*	High	97	28
‘Pecos’	*L. indica* × *fauriei*	Moderate	260	98
‘Pocomoke’	*L. indica* × *fauriei*	Moderate	222	100
‘Sioux’	*L. indica* × *fauriei*	High	70	38
‘Tonto’	*L. indica* × *fauriei*	High	126	24
‘Tuscarora’	*L. indica* × *fauriei*	Moderate	176	49
‘Tuskegee’	*L. indica* × *fauriei*	Moderate	183	49
‘Wichita’	*L. indica* × *fauriei*	Moderate	205	77

	L. indica ×	Moderate	172	49
‘Yuma’	*fauriei*			
	L. indica ×	Moderate	129	35
‘Zuni’	*fauriei*			

[z] High Resistance = less than 150 AUDPC in 2021 and 40 AUDPC in 2022.

[y] Moderate Resistance = between 150 and 350 AUDPC in 2021 and 40 and 150 AUDPC in 2022.

[x] Low Resistance = above 350 AUDPC in 2021 and 150 AUPDC in 2022.

[w] Observed at the University of Georgia Horticulture Farm in Watkinsville, GA.

Table 2.2. Average AUDPC of *L. indica* (n=22) and *L. indica* × *fauriei* (n=20) cultivars in 2021 and 2022 in Blairsville, GA (USDA Zone 6b).

	Average AUDPC ±SE			
Parentage	**2021**	**Range**	**2022**	**Range**
L. indica	408±30[z] a[x]	241-567	222±36 a	47-574
L. indica × *fauriei*	163±20[y] b	47-412	50±9 b	0-144
Mean of all plants	291	47-567	140	0-574

[z] AUDPC over 350 in 2021 and 150 in 2022 is highly susceptible

[y] AUDPC under 150 in 2021 and 40 in 2022 is highly resistant

[x] Numbers followed by the sample letter are significantly different from each other at $P<0.05$ using a t-test.

Table 2.3 Area Under The Disease Progress Curve for *Lagerstroemia* species and hybrids infected with Cercospora leaf spot (*Pseudocercospora lythracearum*) in Watkinsville, GA (USDA zone 8a).

Parentage	Number of plants	2021 AUPDC[z]	2022 AUDPC
L. indica	4	707±23 bc[y]	632±50 abc
L. indica × *subcostata*	50	441±33 bc	357±32 bc
L. indica × *subcostata* (F_2)	13	448±32 bc	449±31 abc
L. indica × *subcostata* (F_3)	3	189±83 bc	189±83 cd
L. indica × *limii*	19	598±28 b	598±30 a
L .(*indica* × *fauriei*) × *subcostata*	20	339±49 c	116±20 d
L. ((*indica* × *fauriei*) × (*subcostata*)) × *limii*	11	444±53 bc	456±54 abc
L. limii	5	505±108 bc	519±115 abc
L. limii × *indica*	13	1260±151 a	603±45 a
L. limii × (open pollinated)	3	616±77 bc	613±111 ab

L. (*limii* × open pollination) × *indica*	5	581±55 bc	503±104 abc
L. (*limii* × open pollination) × ((*indica* × *fauriei*) × *subcostata*))	17	332±29 c	380±33 bc

[z] Mean AUDPC of all plants in group

[y] ANOVA used to compare means

Table 2.4. Average AUDPC for dark-foliage *L. indica* cultivars and their crosses by *L. subcostata* in Watkinsville, GA in 2021 and 2022.

Selection	**2021 Average AUDPC**	**2022 Average AUDPC**
Dark-Foliage *L. indica*	707±19 a[z]	556±33 a
Dark-Foliage *L. indica* × *subcostata*	441±33 b	358±32 b

[z] Numbers followed by the sample letter are significantly different from each other at $P<0.05$ using a t-test.

Table 2.5. Average monthly minimum temperature, maximum temperature, rainfall, and days of rainfall at the University of Georgia Mountain Research and Extension Station (Blairsville, GA) in 2021 and 2022

	Maximum Temperature (C°)		Minimum Temperature (C°)		Rainfall (cm)		Days of Rainfall	
Month	2021	2022	2021	2022	2021	2022	2021	2022
June	27.7	29.6	15.5	15.8	10.4	3.8	15	9
July	29.2	30.8	17.4	18.7	11.7	18.5	15	14
August	29.2	28.7	17.9	17.9	34.0	8.9	12	15
September	25.9	25.9	12.9	12.3	5.3	8.4	11	11
October	21.8	20.4	10.0	3.9	10.4	2.0	12	9

Weather data retrieved from Georgia Weather – Automated Environmental Monitoring Network (weather.uga.edu)

Table 2.6. Average monthly minimum temperature, maximum temperature, rainfall, and days of rainfall at the University of Georgia Horticulture Research Farm (Watkinsville, GA) in 2021 and 2022.

	Maximum Temperature (C°)		Minimum Temperature (C°)		Rainfall (cm)		Days of Rainfall	
Month	2021	2022	2021	2022	2021	2022	2021	2022
June	29.2	32.0	18.9	19.9	11.2	3.8	15	9
July	30.3	31.6	20.7	21.5	13.0	18.5	13	14
August	30.7	29.9	21.0	20.8	12.7	8.9	14	15
September	27.4	27.6	16.6	16.8	6.9	8.4	11	11
October	23.2	22.1	12.8	8.7	22.1	2.0	11	9

Weather data retrieved from Georgia Weather – Automated Environmental Monitoring Network (weather.uga.edu)

CHAPTER 3

EFFICACY OF COMMERCIAL FUNGICIDES APPLIED *in vitro* FOR CONTROL OF *PSEUDOCERCOSPORA LYTHRACEARUM*

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Abstract

Crape myrtles are impacted by Cercospora leaf spot, a disease caused by the pathogen *Pseudocercospora lythracearum* (Liu and Guo) that triggers leaf spots and defoliation which decreases the value in a nursery. The most effective control of Cercospora leaf spot in the nursery is a bi-monthly fungicide application. Media amended with commonly used fungicides for treating Cercospora leaf spots were inoculated with three *P. lythracearum* isolates *in vitro*. The fungicides thiophanate-methyl (3336 WP), propiconazole (Banner Maxx II®), azoxystrobin (Heritage®), mancozeb (Dithane® 75DF), and chlorothalonil (Daconil Ultrex®) were effective at stopping conidia germination and growth at labeled rates. The fungicide fludioxonil (Medallion 50WP) was ineffective at stopping conidia germination and radial fungal growth.

Introduction

Crape myrtles (*Lagerstroemia sp.*) are an economically important crop in the southeastern United States (USDA Horticultural Census, 2019). Crape myrtles are planted for their large, showy flower panicles and exfoliating bark (Pounders et al. 2010). Crape myrtles are produced in nurseries, from small liners to trees in containers or in a field. *Pseudocercospora lythracearum* (Liu and Guo) is a fungal pathogen that causes Cecrospora leaf spot on crape myrtles (Chappell et al. 2012). In the southeastern United States, Cercospora leaf spot is the dominant disease of crape myrtles (Hagan and Arkidge 2013). Cercospora leaf spot is not fatal but causes defoliation and decreases the value of a crape myrtle crop (Hagan et al. 1998; Chappell et al. 2012). Cercospora leaf spot is characterized by brown round and irregular lesions on the leaves and becomes apparent in August or September, depending on the USDA zone and cultivar (Hagan et al. 1998). On a susceptible plant, lesions spread through the canopy, from

lower leaves to higher, turning leaves yellow and red before defoliating (Parajuli et al. 2023). Defoliation can occur rapidly from August to October (Hagan 2001).

Previous studies (Hagan and Arkidge 2006; Hagan and Arkidge 2013; Baysal-Gurel 2017) have used fungicides to treat Cercospora leaf spots in a simulated nursery setting. Fungicides effective for the treatment of Cercospora leaf spot are Elite™ (tebuconazole), Heritage® (azoxystrobin), Eagle 20EW® (myclobutanil), Cleary's 3336 (thiophanate methyl), Isofetamid 400SC (isofetamid), Instrata™ (29.9% chlorothalonil, 1.2% fludioxonil, 4.7% propiconazole), Mankocide DF (30% copper hydroxide 15% mancozeb), and Mural 45 WG (30% azoxystrobin, 15% benzovindiflupyr).

It is not known if *P. lythracearum* is developing fungicide insensitivity. Fungicide insensitivity has been shown to build in fungal pathogens when fungicides are sprayed on an area for many years (Lucas et al. 2015; Russell 1995). Fungicide resistance is a concern in ornamental nursery systems, with several organisms developing resistance to fungicides (Bika et al. 2021; Daughtrey and Benson 2005). *Pseudocercospora fijiensis*, a banana pathogen, has shown resistance to propiconazole, chlorothalonil, and mancozeb, common fungicides used to control *P. lythracearum* (Aguirre 2016). Despite the lack of recorded control failures, fungicide resistance is a concern in *P. lythracearum* due to the repeated spraying of fungicides in nurseries. In addition, the interaction between these fungicides and *P. lythracearum in vitro* is unknown. For these reasons, this study seeks to evaluate commonly used fungicides *in vitro* for their effect *P. lythracearum* growth and to determine if fungicide insensitivity is developing.

Materials and Methods

Fungal Isolates

One isolate of *Pseudocercospora lythracearum* was obtained from the University of Georgia Horticulture Research Farm (Watkinsville, GA, (33.8629° N, 83.4088° W), USDA zone 8a (USDA 2012)) and two isolates from the University of Georgia Mountain Research and Extension Center (Blairsville, GA, (34.8761° N, 83.9584° W), USDA zone 6b (USDA 2012)). The isolates were cultured on V8-juice agar (15g Difco Bacto agar (BD, Franklin Lakes, New Jersey), 900 mL deionized water, 100 mL clarified V8 juice (Campbell Soup Company, Camden, New Jersey) and 1g $CaCO_2$) in petri dishes. To culture *P. lythracearum*, a sterile hypodermic needle was used to remove stroma from the *Lagerstroemia* leaf tissue. The needle was used to drag the stroma lightly across the surface of 100 mL V8-juice agar. Colonies that grew from the dragged stroma were isolated in pure culture on V8-juice 100 mL agar and were allowed to grow for two months in an incubator at 25°C with a 12-hour inflorescent light cycle to produce enough fungal tissue for the sporulation induction procedure. A square 5 mm agar piece was excised from the edge of a 2-month-old *P. lythracearum* culture to create inoculum. The tissue was placed in a 1.5 mL tube filled with 1 mL of deionized water, where it was macerated with a scalpel and broken into small pieces. The tissue solution was pipetted onto a plate of V8-juice 100 mL agar and spread evenly across the plate with a glass stir rod. The cultures were placed under twelve-hours of light at 25°C. After three weeks, the cultures sporulated. Cultures were allowed to mature for an additional week before spores were harvested.

Deionized water was poured onto a *P. lythracearum* culture that had sporulated. A paintbrush was used to disturb and separate spores from the colonies. The spore solution was poured into a beaker. Ten µL of spore solution were pipetted into both sides of a hemocytometer.

The spore suspension was counted at 100x magnification using a hemocytometer. The spore suspension was diluted to 20,000 conidia/mL in the first trial and 10,000 conidia/mL in the second. The concentration of conidia was reduced in the second trial due to difficulties in counting large numbers of germinated conidia. Finally, 100 mL of spore solution was added to four replications of each fungicide-amended medium.

Media Preparation

Six fungicides were selected based on the 2022 Georgia Pest Management Handbook (2022) recommendations to determine which effectively controlled *P. lythracearum*. These six fungicides were chosen for comparison to Hagan and Arkidge (2006) and Hagan and Arkidge (2013) to identify a potential shift in fungicide sensitivity. The fungicides selected were chlorothalonil (Daconil Ultrex, Syngenta AG, Basel, Switzerland), azoxystrobin (Heritage, Syngenta AG, Basel, Switzerland), fludioxonil (Medallion 50WP, Syngenta AG, Basel, Switzerland), thiophanate methyl (Cleary's 3336, Nufarm, Melbourne, Australia), mancozeb (Dithane 75DF, Corteva Agriscience, Indianapolis, Indiana), and propiconazole (Banner Maxx, Syngenta AG, Basel, Switzerland). Fungicide media was prepared with 7.5 grams of Bacto agar, 50 mL of clarified V8-juice, 1 g of calcium carbonate, 450 mL of deionized water, and either one or two times the recommended rate of the fungicide (if the rate was given in a range, the lower rate of the range was used). The rates used were as follows: 0.54 mL of Cleary's 3336, 0.39 ml of Banner Maxx, 0.078 g of Heritage, 0.037 g of Medallion 50WP, 0.59 g of Dithane 75DF, 0.83 g of Daconil Ultrex.

The media solution was autoclaved, then fungicide was added after the media cooled to 55 degrees F. The number of colonies were counted on each petri dish to determine the number of germinated spores.

Experimental Design

There were thirteen treatments, six recommended-rate fungicides, six double-the-recommended-rate fungicides, and one control. Each group had four replicates, and the experiment was conducted twice.

Three isolates were collected from research farms, one from the University of Georgia Horticulture Farm in Watkinsville, Georgia, and two from the Georgia Mountain Research and Education Center in Blairsville, Georgia. The isolates were named "CercB," "CercGr1", and "CercGr2".

After adding the spore solution to the plates, they were put into a growth chamber with twelve hours of light at 25˚C. The treated plates were observed after three days, and the number of colonies was recorded. Statistics were performed in R Studio (R Core Team 2022). A Tukey's HSD test was used for mean separation. Data were arcsine transformed before statistical analysis due to differential colony-forming units applied to petri dishes between the two trials. Data was transformed back before presentation in table 3.2.

Results

Colony growth was observed on non-amended control plates for all three isolates. Colony germination data were arcsine transformed before statistical analysis due to varying amounts of initial inoculum added to each plate. Colony growth ranged from 271 to 1970 colonies for non-amended plates. All fungicides inhibited colony formation except the fungicide fludioxonil (Table 2.2). The recommended rate of fludioxonil significantly reduced growth by 28.8-31.5% in the 'CercB' isolate and 63.4% in the second trial for the 'CercGr1' isolate. Colony growth was not significantly reduced by fludioxonil in all other isolates. Doubling the rate of fludioxonil significantly reduced colony growth compared to the recommended rate in the first trial of

'CercGr1'. Colony growth was entirely inhibited by the recommended rates of thiophanate methyl (3336 WP), propiconazole (Banner Maxx II®), azoxystrobin (Heritage®), mancozeb (Dithane® 75DF), and chlorothalonil (Daconil Ultrex®).

Discussion

Protective fungicide applications are commonly used for treating Cercospora leaf spot on crape myrtles in nurseries. Treating a given disease with an effective fungicide is essential to prevent wasting resources on applying an ineffective fungicide. Unlike herbicides and pesticides, which are often applied after a weed or pest is present, fungicides must be applied before the presence of inoculum to prevent infection. Fungicides break down over time due to UV radiation and weather exposure, so they must be applied regularly to protect a crop. Coverage of the entire susceptible area of the crop with a fungicide is necessary to prevent infection unless the fungicide is systemic. 184,339 kg of fungicides were used on woody ornamentals in nurseries (National Agricultural Statistics Service 2011). Of the fungicides used in our study, 50,439 kg of chlorothalonil, 41,004 kg of mancozeb, 25,673 kg of thiophanate methyl, and 1,542 kg of azoxystrobin were used in 2009.

Previous studies (Hagan and Arkidge 2006, Hagan and Arkidge 2013) have documented that fludioxonil is ineffective at controlling Cercospora leaf spot. Fludioxonil insensitivity has been reported in *Psuedocercospora liquidambaricola,* suggesting that the genus *Psuedocercospora* could be insensitive to fludioxonil (Ekemn and Williams-Woodward 2019). Fludioxonil hyperactivates the high osmolarity glycerol signaling pathway through group III hybrid histidine kinases (Bersching and Jacob 2021). Loss of function mutations in the group III histidine kinases imparts resistance in fungi to fludioxonil (Bersching and Jacob 2021). Fludioxonil is not effective for the control of Cercospora leaf spot on its own but is effective

when mixed with chlorothalonil and propiconazole (Hagan and Arkidge 2013). Therefore, a fludioxonil management plan would not be suitable for managing Cercospora leaf spot.

Our study confirms the findings of Hagan and Arkidge (2013) that azoxystrobin and thiophanate methyl are effective at controlling the growth of *Psuedocercospora lythracearum*. Hagan and Arkidge (2013) observed that fludioxonil, propiconazole, and chlorothalonil were ineffective at treating Cercospora leaf spot. Our study finds that *in vitro,* only fludioxonil is ineffective at controlling *Psuedocercospora lythracearum,* while propiconazole and chlorothalonil are effective. No fungicides used by Hagan and Arkidge (2013) were 100% effective at preventing Cercospora leaf spot when applied bi-monthly from July to September.

The efficacy of these fungicides in a nursery setting could be different since the isolates in our study were not taken from nursery populations. The research plots from which these isolates were collected have never been sprayed with fungicides, which could explain the differences in control between our study and Hagan and Arkidge (2013) since they observed a crape myrtle population that had been sprayed with fungicides in the past. Isolates must be collected from an area where fungicides are regularly sprayed to determine if insensitivity exists in *Psuedocercospora lythracearum* populations. Additionally, more isolates of *Psuedocercospora lythracearum* should be collected to increase the possibility of finding potential fungicide insensitivity.

Chlorothalonil and mancozeb were both effective at preventing *Psuedocercospora lythracearum* colony growth. The risk of *Psuedocercospora lythracearum* developing insensitivity to chlorothalonil and mancozeb is unlikely due to their multisite modes of action (FRAC groups M5 and M3, respectively). Resistance to thiophanate methyl, azoxystrobin, propiconazole, and fludioxonil has been documented across wide varieties of ascomycetes,

including cercosporid fungi (Hu et al. 2015; Kienath and Zitter 1998; Imazaki et al. 2006; Canas-Gutierrez 2009; Chen et al. 2013; Wang et al. 2021). Tank mixing of a single-site mode of action fungicide is recommended to avoid the creation of fungicide-insensitive strains (Damicone and Smith 2009). Tank mixing can increase the efficacy of fungicides for treating Cercospora leaf spot (Hagan and Arkidge 2013). Fludioxonil tank mixed with chlorothalonil and propiconazole effectively controlled Cercospora leaf spot, the addition of the two more effective fungicides. Fludioxonil is included in this mixture to attack target fungi with multiple modes of action and prevent resistance.

Cercospora leaf spot can be controlled with fungicide applications; however, care should be taken to avoid the development of fungicide insensitivity. Future studies should test *Psuedocercospora lythracearum* isolates obtained from an area historically sprayed with fungicides to determine if fungicide insensitivity exists within the population of this organism.

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Tables

Table 3.1. Fungicides and rates used to test *in vitro* fungicide efficacy against *Pseudocercospora lythracearum*

Trade Name	Active Ingredient	FRAC Code[1]	Labeled Rate[2] (per 100 gal.)	Amended Rate (per 500mL)	Twice Amended Rate (per 500mL)
3336 WP	Thiophanate-methyl	1	14 fl oz	0.54 mL	1.09 mL
Banner Maxx II®	Propiconazole	3	10 fl.oz	0.39 ml	0.78 ml
Heritage®	Azoxystrobin	11	2 oz	0.078 g	0.15 g
Medallion® WDG	Fludioxonil	12	1 oz	0.037 g	0.074 g
Dithane® 75DF	Mancozeb	M3	1 lb	0.59 g	1.19 g
Daconil Ultrex®	Chlorothalonil	M5	1.4 lb	0.83 g	1.66 g

[1] Numerical classification of fungicide mode of action groups based upon the Fungicide Resistance Action Committee (FRAC) designation.

[2] The lower labeled rate for Cercospora leaf spot control was used when a concentration range was provided on the product label.

Table 3.2. Percent control of three *P. lythracearum* grown on V8-100 growth media amended with the fungicides Fludioxonil, Thiophanate Methyl, Azoxystrobin, Chlorothalonil, Mancozeb, and Propiconazole.

		Trial 1			Trial 2		
Fungicide	Active Ingredient	'CercB'	'CercGr1'	'CercGr2'	'CercB'	'CercGr1'	'CercGr2'
Control	N/A	0%	0% b	0% ab	0% ab	0% b	0% b
Medallion	Fludioxonil	33%	20% bc	45% def	38% d	45% fg	N/A
Medallion Double Rate	Fludioxonil	N/A	55% ef	62% de	33% d	41% fg	55% ef
3336 WP	Thiophanate Methyl[y]	0 h	0 h	0 h	0 h	0 h	0 h
Heritage	Azoxystrobin	0 h	0 h	0 h	0 h	0 h	0 h
Daconil	Chlorothalonil	0 h	0 h	0 h	0 h	0 h	0 h
Dithane	Mancozeb	0 h	0 h	0 h	0 h	0 h	0 h
Banner Maxx®	Propiconazole	0 h	0 h	0 h	0 h	0 h	0 h

[z] Numbers followed by the sample letter are not significantly different from each other at $P<0.05$ using Tukey's HSD means comparison test. Data was arcsine transformed prior to Tukey's HSD.

[y] Doubled rate of remaining fungicides are not presented due to lack of colony growth.

CHAPTER 4

PLOIDY CHANGES OF *LAGERSTROEMIA SPP*. INDUCED BY ORYZALIN

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Abstract

Ploidy induction is a standard tool for improving ornamental plants. A reliable method for inducing tetraploids in crape myrtles (*Lagerstroemia sp.*) exists but is time-consuming and uses colchicine, a chemical that is dangerous and difficult to handle. The chemical oryzalin has been used to induce tetraploids in other crops but never with crape myrtle. Therefore, a protocol was developed for the induction of tetraploidy in crape myrtles using oryzalin, resulting in a 5.9% tetraploid induction rate. Tetraploid plants had longer and broader leaves in two sample groups and longer stomata in all three sample groups. Tetraploid induction with oryzalin is safer and faster than induction with the chemical colchicine. In addition to tetraploids, triploid, hexaploid, and mixaploid plants were observed. Sterility was observed in some mixaploid plants.

Introduction

Crape myrtle (*Lagerstroemia spp.*) is a tree commonly planted in the southeastern United States for its showy summer flowers and vibrant fall foliage. Originally introduced to America in the 1700's from Asia, crape myrtle is widely cultivated and planted throughout the southeastern United States (Wang et al. 2016). In 2019 over three million crape myrtles were sold, accounting for a market value of over $69.9 million (USDA Horticultural Census 2019). 75% of crape myrtles are produced in the southeast (National Agriculture Statistics 2020). Over 200 crape myrtle cultivars exist, with half available to nurseries for production (Wang et al. 2011). Highly bred and selected, crape myrtles have many uses in the landscape, from trees to miniature potted plants. Despite breeding efforts thus far, opportunities exist to improve crape myrtle traits further. For example, some crape myrtles suffer from low vigor and small flowers. Others suffer from low resistance to diseases (powdery mildew [*Erysiphe lagerstroemiae* (Braun and

Takamatsu)] and Cercospora leaf spot [*P. lythracearum* (Liu and Guo)]) and low resistance to insect herbivory (crape myrtle bark scale). Polyploidization could improve these traits (Thao et al. 2003, Zhang et al. 2010, Ye et al. 2010).

Induction of polyploidy is a common technique for improving ornamental plants (Thao et al. 2003, Zhang et al. 2010). There are multiple ways to induce polyploidy in plants, the two most common being treatment with colchicine or oryzalin (Blakeslee et al. in 1937, Thao et al., 2003). Colchicine and oryzalin work by preventing the formation of the mitotic spindle during mitosis, thus arresting mitosis and doubling the genome of the cell (Caperta et al. 2006; Morejohn et al. 1987). Polyploid induction is a tool used to improve various ornamental factors in horticultural crops, including more vigorous growth, larger flowers, and higher levels of disease resistance (Hilu 1993; Shao et al. 2003). Unfortunately, colchicine has a low affinity for plant tubulins and a high affinity for animal tubulins, making it highly toxic to humans (Morejohn et al. 1987). Oryzalin specifically binds to plant tubulins, allowing for the use of lower concentrations for tetraploid induction, and the active ingredient is safer for humans to handle (Lehrer et al. 2008).

Crape myrtle is an invasive species in the southeastern United States (Reichard 1994). Crape myrtles quickly reseed themselves, creating thousands of seeds each season (Pounders et al. 2006). Induction of triploidy in an invasive landscape plant can reduce the fertility and spread of invasive plants that spread by seed (Trueblood et al. 2010; Czarnecki et al. 2014). Fertility is reduced in male and female structures, and seeds are often unviable or not created in triploid plants (Trueblood et al. 2010; Czarnecki et al. 2014). Induction of triploidy is possible by crossing a diploid plant with a tetraploid plant (Wang et al. 2016; Navarro et al. 2015).

Ploidy level of plants is commonly determined by flow cytometry or chromosome counts (Zhang et al. 2010, Li and Ruter 2017, Thao et al. 2003). It is possible to confirm ploidy level by observing stomata characteristics, such as length, width, density, and area (Murti et al. 2012, Dwivedi et al. 1986, Yang et al. 2006, Carvalho et al. 2005). Confirming ploidy by stomata is quicker and less expensive than flow cytometry and chromosome counting, although it is less accurate (Zhang et al. 2010).

Multiple treatment protocols exist for crape myrtle polyploidization using colchicine (Ye et al. 2010, Zhang et al. 2010). However, oryzalin is more effective at tetraploid induction and less toxic than colchicine (Ramulu et al. 1991, van Tuyl et al. 1992, Tosca et al. 1995). Therefore, this study aimed to create a polyploidization protocol for crape myrtle using oryzalin.

Materials and Methods

Seed Collection and Germination

Seeds were collected from the University of Georgia Horticulture Research Farm in Watkinsville, GA, in Oct 2021 before dehiscence. Seeds were placed in a greenhouse on sheets of paper to mature and dry. After drying, seeds were placed in a refrigerator in moist sand for 30 days for cold stratification at 4.4˚C. Seedlings germinated in the sand in a mist chamber with the mist spraying for 5 sec. every 30 min. Seedlings were removed from the sand two weeks after germination before the emergence of the first true leaves and washed for 30 seconds in preparation for treatment with oryzalin.

Treatment

Twenty-seven treatment beakers were filled with a 60 mL solution of 100 µL of Surflan A.S. (40.4% oryzalin solution) (United Phosphorous Limited, Mumbai, India) and 59.9 mL of

deionized water for a solution of 2.3 mM oryzalin. Beakers containing 15 seedings each were treated for four, six, and eight hours. The beakers were placed on a rotating shaker at 120 revolutions per minute. Treated seedlings were removed from the oryzalin solution and rinsed for 30 seconds three times with running deionized water. After rinsing, seedlings were potted in 3:1 perlite: PRO-MIX high porosity substrate with biofungicide and mycorrhizae (Premier Tech Horticulture, Quakertown, PA) in 200 cell × 4.5 cm deep flats (T.O. Plastics, Clearwater, MN) and placed in a greenhouse at the University of Georgia Horticulture Research Farm in Watkinsville, GA (33.886045, -83.420179) under 30% shade cloth and a humidity dome. After one month, treated seedlings sprouted true leaves, and root systems had established. They were moved to 280 mL square deep vacuum pots (HC Companies, Twinsburg, OH). Seedlings were fertilized weekly with 200 mg/L Jack's 20–10–20 Peat Lite (20N–4.9P–16.6K) (JR Peters Inc., Allentown, PA) and placed in a greenhouse without shade. The day/night greenhouse conditions were 25/20˚C and 40%/30% relative humidity.

Seedlings grew to a height of 15 cm before tissue was harvested for flow cytometry.

Experimental Design

Orzyalin (40.4% Surflan A.S) was applied to attempt to double the genome of three *Lagerstroemia* selections. Each *Lagerstroemia* selection was treated for four, six, and eight hours in the oryzalin solution for nine treatment groups. Each treatment group was replicated three times. A total of 405 seedlings were treated. The selections treated were "Lag-2019-4" (*L. subcostata* × *limii*), "Lag-2016-5" (*L. limii*), and "R3P7W" (*L. indica* × *subcostata*). Selections were made based on perceived ornamental value.

Ploidy Analysis

Harvested tissue was analyzed for ploidy level with a Beckman Coulter cytoFLEX flow cytometer (Beckman Coulter, Inc., Brea, CA) at the University of Georgia Cytometry Shared Resource Laboratory. First, young tissue was harvested and chopped in a dish with a razor blade for ten seconds. Next, Cystain UV Precise P nuclei extraction buffer (0.5 mL) was applied to chopped tissue and allowed to sit for 5 min. After 5 min, the liquid in the dish was pipetted into a 5 mL test tube through a 100 nm filter. Next, 1 mL of Cystain UV Precise P staining buffer (4′,6-diamidino-2-phenylindole or DAPI) was added to the liquid in the test tube and mixed using a pipette. Finally, 300 µL of the solution was pipetted into a 96-well plate and taken to a Beckman Coulter cytoFLEX flow cytometer to be analyzed for ploidy level.

Leaf Morphological and Anatomical Characteristics Analysis

Leaf length, width, stomatal density, stomatal length, and stomatal width per $1mm^2$ were measured to evaluate potential differences between diploid and tetraploid plants. Five months after the seeds were germinated, five of the most recently matured leaves were measured from each tetraploid to determine leaf length and width. A representative sample of three diploid offspring was used as the control. The same protocol was used to measure the leaf characteristics of the diploid plants as tetraploid plants. Stomata were counted using a Dino-Eye Eyepiece Camera from Dino-Lite Digital Microscopes (New Taipei City, Taiwan) attached to an Olympus (Tokyo, Japan) bx51 microscope. Stomata in five $1mm^2$ areas were counted on the abaxial side of five mature leaves. Stomata were measured using the measuring tool in the DinoXcope application from Dino-Lite Digital Microscopes (Los Angeles, CA). Twenty-five stomata were measured on the abaxial surface of five mature leaves. ANOVA and t-tests were performed in R Studio (R Core Team 2022).

Results

Tetraploid induction

The most effective treatment for inducing tetraploids with oryzalin was the 8-hour treatment (Table 2.1). The percent of plants converted to tetraploids in the 4, 6, and 8-hour treatments were 1.4%, 0%, and 5.9%, respectively. The percent of plants that survived for each treatment group were 44%, 45.15%, and 47.4%. The percent of mixoploids observed for each group was 14.8%, 27.3%, and 13.3%. Less than half of this study's plants treated with oryzalin survived all three treatments.

Determination of Tetraploids

Tetraploids were determined using a histogram from the Cytexpert (Beckman-Coulter Inc., Brea, California) computer program. Because the three mother plants used were different species and open-pollinated, diploid histograms peaked in slightly different areas for each of the three mother plants. Tetraploid plants had histogram peaks at about double the channel of the diploid peaks when analyzed with a flow cytometer. Mixoploids were determined by the existence of two peaks or more peaks. Mixoploids observed were 2x-4x, 2x-4x-8x, and 2x-4x-6x. The diploids peaked between 8,000 and 9,000 median fluorescence, and the tetraploids peaked between 15,000 to 17,000 median fluorescence intensity (Figure 4.1).

Leaf Morphological and Anatomical Characters

Leaf length and width differed from the tetraploids, hexaploids, and triploids to the diploids among the three seedling groups (Table 2.2). Leaf length and width increased for the "Lag2016-5" and "Lag2019-4" seedlings groups. For the "R3P7" group, leaf length and width decreased.

There was no difference in stomatal density per 1 mm^2 between diploids and polyploids (Table 2.2). However, the stomatal length significantly increased in tetraploids compared to diploids in the "Lag2016-5" and "Lag2019-4" groups. No significant difference exists for stomatal length in "R3P7"; however, the P value (0.0529) was only slightly higher than the alpha value (0.05).

Discussion

Oryzalin successfully induces polyploidy by stopping mitosis in the anaphase stage of mitosis (Thao et al. 2003). However, the application damages the treated plants. In agriculture, oryzalin is used as an herbicide. The percent of plants that survived was about the same for all three-time treatments, so it does not appear that exposing a plant to a low level of oryzalin for 8 hours is more damaging than exposing it for 4 hours. However, there was a higher tetraploid conversion rate in the 8-hour treatment group compared to the four and 6-hour treatment groups. In addition, for all time treatments, the percent of mixoploids induced was vastly higher than the percent of tetraploids generated. The 6-hour treatment produced more mixoploids than the 4 and 8-hour treatments.

Increasing the time a plant is in the oryzalin solution increases the likelihood of a tetraploid conversion. However, based on the data in this study, increasing the amount of time a seedling is exposed to oryzalin does not decrease seedling survival. Therefore, prolonged oryzalin exposure could increase tetraploid conversion without decreasing seedling survival.

The survival rate was about the same between the three-time treatment groups. It is unknown when treatment time will begin to affect the survival rate, but it should be explored. A future study should test a 12- and 24-hour treatment to explore the trade-off between lower

survival and tetraploid conversion rates. The concentration of oryzalin could also be increased, although oryzalin is only slightly soluble in water (2.5 mg/L), so it could be challenging to dissolve more than the 2.3mM concentration used in our study. Additionally, a surfactant or penetrant could be added to the oryzalin solution to increase contact between oryzalin and plant tissue. The rate of mixoploid conversion was higher in the 6-hour treatment group. Higher mixoploid conversion is likely due to a lack of time to fully convert the meristematic tissue to tetraploid cells, leaving some cells transformed and some diploid. Since oryzalin acts on mitosis to cause tetraploidy, if a cell did not undergo mitosis while being treated, it could not have had its genome duplicated (Morejohn et al. 1987).

Previous research has created a method for tetraploid induction of *Lagerstroemia* using colchicine. In that study, Zhang et al. (2010) developed a technique with a 60% tetraploid conversion rate with a similar survival rate and observed a higher conversion rate than was found in this study. The previous study used embryo rescue to propagate the tetraploid cells into full plants. Embryo rescue is time-consuming and resource-intensive, so fewer seedlings were treated by Zhang et al. (2010) 45 compared to the 405 in this study. As a result, 17 tetraploid seedlings were created by Zhang et al. (2010), more than the ten generated in this study.

Changes in leaf anatomy often correlate with ploidy levels in plants because of increased cell size (Dwivedi et al. 1986). Therefore, anatomical changes (e.g. stomatal length, width, area, and frequency) can be used to evaluate ploidy level (Murti et al. 2012, Dwivedi et al. 1986, Yang et al. 2006, Carvalho et al. 2005). Zhang et al. (2010) found that leaf index, stomata length, width, and frequency effectively evaluated ploidy in crape myrtles. However, our study finds that only stomatal length effectively assesses ploidy in crape myrtles. Differences may be due to variations in crape myrtle species used for tetraploid induction.

The induction of tetraploids in crape myrtle using the chemical oryzalin presents a new method for breeding crape myrtles. Future work should investigate changes in flower morphology between diploids and tetraploids and the hybridization of diploid and tetraploid plants.

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Tables and Figures

Table 4.1. Ploidy of crape myrtle hybrid seedlings after treatment in a 2.3mM oryzalin solution for 4, 6, and 8 hr.

Ploidy After Treatment	**Time in Oryzalin Solution[z]**		
	4 Hours	6 Hours	8 Hours
Diploid	36[y] (60.0%)[x]	31 (50.8%)	38 (58.5%)
Tetraploid	2 (3.3%)	0 (0.0%)	8 (12.3%)
Mixoploid	20 (33.3%)	28 (45.9%)	18 (27.7%)
Triploid	3 (3.3%)	1 (1.6%)	0 (0.0%)
Hexaploid	0 (0.0%)	1 (1.6%)	0 (0.0%)
Number Survived	60 (44.0%)	61 (45.2%)	64 (47.4%)
Total Seedlings Treated[w]	135	135	135

[z] At 2.3mM oryzalin solution (100 µL of Surflan A.S. (40.4% oryzalin solution) and 59.9 mL of deionized water).

[y] Number of seedlings at respective ploidy

[x] Percent of surviving seedlings at respective ploidy

[w] Total seedlings tested is combined seedlings from three crape myrtle selections of 15 seedlings replicated three times

Table 4.2. Comparison of leaf anatomical characteristics between diploids and tetraploids among the three *Lagerstroemia* groups

	Lag2019-4[x]		Lag2016-5[w]		R3P7[v]	
Leaf Characteristics	Diploid	Tetraploid	Diploid	Tetraploid	Diploid	Tetraploid
Leaf Length (cm)	4.6 a[z]	5.6 b	4.3 a	6.9 b	3.9 a	2.5 b
Leaf Width (cm)	2.7 a	3.5 b	2.2 a	3.3 b	2.1 a	1.5 b
Stomatal Length (μm)	88.1 a	98.1 b	94.1 a	111.9 b	96.4 a	104.2 a
Stomatal Width (μm)	62.6 a	67.2 a	65.9 a	67.9 a	61.2 a	69.9 b
Stomatal Density[y]	30.3 a	31.6 a	27.6 a	28.2 a	28.7 a	26.0 a

[z] Significant differences between diploid and tetraploid offspring within each parental group using Tukey's HSD

[y] Number of stomata in $1mm^2$ of the abaxial leaf surface

[x] *L. subcostata* × *limii*

[w] *L. limii*

[v] *L. indica* × *subcostata*

Figure 4.1. Flow cytometry histogram of a diploid and tetraploid *Lagerstroemia* (Lag2016-5) after staining nuclei with DAPI.

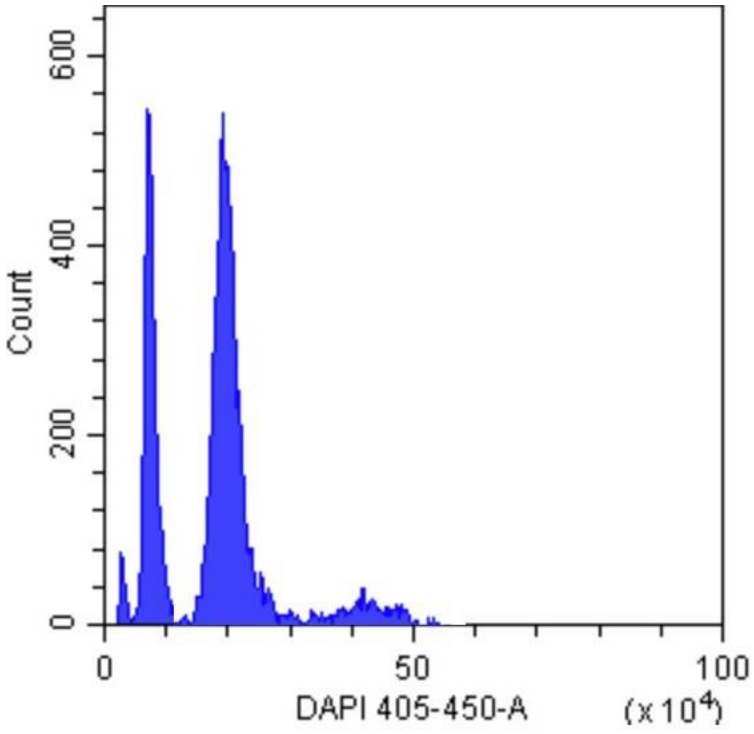

Figure 4.2. Leaves from diploid and tetraploid *Lagerstroemia limii* offspring after treatment with 2.3mM oryzalin.

Diploid "Lag2016-5"

Tetraploid "Lag2016-5"

APPENDICES

APPENDIX A

AUDPC OF ALL WATKINSVILLE BREEDING

Western Plot				
Position	**Accession No.**	**Plant ID**	**AUDPC Year 1**	**AUDPC Year 2**
R1-1		Lagerstroemia CANR-1	693	420
7		Lagerstroemia CANR-2	464	483
13		Lagerstroemia CANR-3	437	532
19		Lagerstroemia CANR-4	595	490
25		Lagerstroemia CANR-5	497	357
31		Lagerstroemia CANR-6	462	325
37		Lagerstroemia CANR-7	112	56
43		Lagerstroemia CANR-8	574	553
49		Lagerstroemia CANR-9	546	497
55		Lagerstroemia CANR-10	497	287
61		Lagerstroemia CANR-11	343	217

R3-1		Lagerstroemia CANR-12	343	210
7		Lagerstroemia CANR-13	413	259
13		Lagerstroemia CANR-14	178	154
19		Lagerstroemia CANR-15	122	84
25		Lagerstroemia CANR-16	588	332
31		Lagerstroemia CANR-17	500	504
37		Lagerstroemia CANR-18	581	497
43		Lagerstroemia CANR-19	469	427
49		Lagerstroemia CANR-20	637	560
58	Lag2019-6	Lagerstroemia CANR-21	427	399
61		Lagerstroemia CANR-22	504	525

R5-1		Lagerstroemia CANR-23	315	199
7		Lagerstroemia CANR-24	385	364
13		Lagerstroemia CANR-25	182	56
19		Lagerstroemia CANR-1 x Lag. CANR-10	504	504
22		Lagerstroemia CANR-1 x Lag. CANR-10	630	630
25		Lagerstroemia CANR-5 x Lag. CANR-10	483	483
28		Lagerstroemia CANR-6 x Lag. CANR-1	455	455
31		Lagerstroemia CANR-6 x Lag. CANR-1	490	490
34		Lagerstroemia CANR-7 x Lag. CANR-17	588	588
37		Lagerstroemia CANR-13 x Lag. CANR-7	413	413
40		Lagerstroemia CANR-16 x Lag. CANR-7	560	560

43		Lagerstroemia CANR-21 x Lag. CANR-7	245	245
46		Lagerstroemia CANR-21 x Lag. CANR-8	406	406
49		Lagerstroemia CANR-21 x Lag. CANR-8	280	280
52		Lagerstroemia CANR-21 x Lag. CANR-8	427	427
55		Lagerstroemia CANR-21 x Lag. CANR-8	357	357
58	Lag2019-5	Lagerstreomia 'Ebony Embers' x Lag2016-5	756	763
61		Lagerstreomia 'Ebony Embers' x Lag2016-5	679	693
64		Lagerstreomia 'Ebony Embers' x Lag2016-5	686	693
67		Lagerstreomia 'Ebony Embers' x Lag2016-5	630	637
R7-4		Lagerstoemia 'Ebony Flame' x Lag2016-5	525	539
7		Lagerstoemia 'Ebony Flame' x Lag2016-5	784	826

10		Lagerstoemia 'Ebony Flame' x Lag2016-5	266	280
13		Lagerstoemia 'Ebony Flame' x Lag2016-5	693	700
16		Lagerstoemia 'Ebony Flame' x Lag2016-6	490	490
19	Lag2019-2	Lagerstoemia 'Ebony Flame' x Lag2016-6	651	651
22		Lagerstoemia 'Ebony Flame' x Lag2016-6	693	728
25		Lagerstoemia 'Ebony Flame' x Lag2016-6	553	553
28		Lagerstoemia 'Ebony Flame' x Lag2016-6	483	483
31		Lagerstoemia 'Ebony Flame' x Lag2016-6	525	525
34		Lagerstoemia 'Ebony Flame' x Lag2016-6	595	637
37		Lagerstoemia 'Ebony Flame' x Lag2016-6	560	560
40		Lagerstoemia 'Ebony Flame' x Lag2016-6	714	770

43		Lagerstoemia 'Ebony Flame' x Lag2016-6	574	609
46		Lagerstoemia 'Ebony Flame' x Lag2016-6	511	511
49		Lag2015-1 x Lag2016-5	553	553
52		Lag2015-1 x Lag2016-5	322	322
55		Lag2015-9 x Lag2016-5	210	217
58		Lag2015-9 x Lag2016-6	182	196
61	Lag2019-3	Lag2015-10 x Lag2016-5	385	392
64	Lag2019-4	Lag2015-11 x Lag2016-6	343	343
67		Lag2015-12 x Lag2016-5	749	763
R9-1		Lag2015-12 x Lag2016-6	560	595
4		Lag2015-12 x Lag2016-6	567	595

7		Lag2016-1 x Lag2016-5	434	448
10		Lag2016-2 x Lag2016-5	588	602
13		Lag2016-5 x Lagerstroemia 'Ebony Embers'	420	448
16		Lag2016-5 x Lagerstroemia 'Ebony Embers'	560	791
19		Lag2016-5 x Lagerstroemia 'Ebony Embers'	700	882
22		Lag2016-5 x Lagerstroemia 'Ebony Embers'	840	749
25		Lag2016-5 x Lagerstroemia 'Ebony Embers'	980	861
28		Lag2016-6 x Lagerstroemia 'Ebony Embers'	1120	581
31		Lag2016-6 x Lagerstroemia 'Ebony Embers'	1260	483
34		Lag2016-6 x Lagerstroemia 'Ebony Embers'	1400	462
37		Lag2016-6 x Lagerstroemia 'Ebony Embers'	1540	427

40		Lag2016-6 x Lagerstroemia 'Ebony Embers'	1680	581
43		Lag2016-6 x Lagerstroemia 'Ebony Flame'	1820	497
46		Lag2016-6 x Lagerstroemia 'Ebony Flame'	1960	560
49		Lag2016-6 x Lagerstroemia 'Ebony Flame'	2100	518
52	Lag2017-2	Lag2017-2	763	798
55		Lag2017-2 x Lagerstroemia 'Ebony Embers'	413	658
58		Lag2017-2 x Lagerstroemia 'Ebony Embers'	616	777
61		Lag2017-2 x Lagerstroemia 'Ebony Embers'	644	518
64		Lag2017-2 x Lagerstroemia 'Ebony Embers'	728	378
67		Lag2017-2 x Lagerstroemia 'Ebony Embers'	504	182
R11-1		Lag2017-2 x Lag2015-1	364	287

4		Lag2017-2 x Lag2015-1	175	518
7		Lag2017-2 x Lag2015-1	273	217
10		Lag2017-2 x Lag2015-1	476	511
13		Lag2017-2 x Lag2015-1	203	273
16		Lag2017-2 x Lag2016-2	497	441
19		Lag2017-2 x Lag2015-6	252	280
22		Lag2017-2 x Lag2015-6	420	147
25		Lag2017-2 x Lag2016-3	273	420
28		Lag2017-2 x Lag2016-3	140	217
31		Lag2017-2 x Lag2016-3	406	413
34		Lag2017-2 x Lag2016-3	203	427

37		Lag2017-2 x Lag2016-3	399	357
40		Lag2017-2 x Lag2016-3	413	287
43		Lag2017-2 x Lag2016-3	336	574
46		Lag2017-2 x Lag2016-3	273	511
49		Lag2017-2 x Lag2016-5	553	588
52	Lag2019-1	Lag2016-6 - OP	504	413
55		Lag2016-5/2016-6 - OP	581	630
58		**5 x 10 (2018 CANR)**	140	140
61		**7 x 17 (2018 CANR)**	350	350
64		**10 x 21 (2018 CANR)**	77	77
67		**EMPTY**		

	Eastern Plot			
Position	**Accession No.**	**Plant ID**		
R1-2	**Lag2015-1**	L. subcostata SED 11-0016 T1 Seed from Tifton PEA PIP	168	35
3	**Lag2015-12**	L. subcostata SED 11-0016 T2 Seed from Tifton PEA PIP	553	133
4	**Lag2015-2**	L. subcostata SED 11-0015 M1 Seed from Monrovia Cairo, GA	147	49
6	**Lag2015-3**	L. subcostata SED 11-0015 M6 Seed from Monrovia Cairo, GA	77	21
7	**Lag2015-4**	L. subcostata SED 11-0015 M7 Seed from Monrovia Cairo, GA	266	49
11	**Lag2016-1**	L. subcostata FC-03 CANR SED 11-0016; Seed from Tifton PEA PIP; Burgundy Fall Color; Lt. Pink Flowers	805	301
15	**Lag2018-1**	Lagerstroemia M2, Plant 2 seedling (SED12-0152) - CANR (nice pink)	280	147
16	**Lag2015-5**	L. subcostata FC-09 CANR SED 11-0015; Seed from Monrovia Cairo, GA; Burgundy Fall color	455	203

18	**Lag2016-2**	L. subcostata 11-09 Seed treatment studies 2010; 1 day oryzalin spray; lt. pink 7/18/11; med. pink 8/13/12	476	105
19	**Lag2016-3**	L. subcostata Seed treatment studies 2010; 7 days oryzalin spray; med. pink 8/21/12;	77	21
25	**Lag2015-6**	L. subcostata Seed treatment studies 2010; 1 day oryzalin spray; medium pink 8/21/12;	483	238
R2-1	**Lag2015-10**	L. subcostata SED 11-0015 Seed from Monrovia Cairo, GA; Light pink, soft, compact 10/12/11	525	182
2	**Lag2015-9**	L. subcostata 11-08; Seed treatment studies 2010; 3 days oryzalin spray; medium pink 7/11; medium pink 8/21/12;	665	238
6	**Lag2015-11**	L. subcostata DWF 001; SED 11-0016 Seed from Tifton PEA PIP; medium pink 8/21/12	532	49
8	**Lag2016-4**	L. subcostata Seed treatment studies 2010; 7 days oryzalin spray; medium pink 8/21/12;	168	21
10	**Lag2017-1**	L. subcostata Seed treatment studies 2010; Control; medium pink 8/21/12;	189	105
11	**Lag2018-3**	Lagerstroemia M4, Plant 4 seedling (SED12-0150) - CANR (great fall color)	462	196
12	**Lag2018-2**	Lagerstroemia M8, Plant 9 seedling (SED12-0155) - CANR (nice dark pink)	329	168

16	**Lag2015-8**	L. subcostata SED 11-0015 Seed from Monrovia Cairo, GA; Light pink, soft, compact 10/12/11	35	35
17	**Lag2015-7**	L. subcostata SED 11-0015 Seed from Monrovia Cairo, GA; Light pink, soft, compact 10/12/11	91	42
R3-1		Lagerstroemia 'Cecil Pounder' (GEN10-0090) - from ABG (S. McMahan/SFA Arb., irradiated, pink flowers)	651	651
3		Lagerstroemia Ebony Embers	707	539
4		Lagerstroemia Ebony Flame	707	574
10		Lagerstroemia (David Creech)	763	763
11		L. limii SED13-0270 (Chollipo Arboretum, Korea) PD 5/16/13	217	217
12		L. limii SED13-0270 (Chollipo Arboretum, Korea) PD 5/16/13	301	301
15	**Lag2016-5**	L. limii SED13-0270 (Chollipo Arboretum, Korea) PD 5/16/13	686	742
18	**Lag2016-6**	L. limii SED13-0270 (Chollipo Arboretum, Korea) PD 5/16/13	777	791
19		L. limii SED13-0270 (Chollipo Arboretum, Korea) PD 5/16/13	546	546