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Resistance of Selected Rose Cultivars to Variants of *Marssonina rosae* in Mississippi¹

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Abstract

Seven isolates of *Marssonina rosae* (imperfect stage of *Diplocarpon rosae*) exhibited differences in colony color on potato-dextrose agar and in conidial measurements. Four of the isolates selected for pathogenicity tests differed in virulence when inoculated to 76 selected rose cultivars. The Chickasaw variant was the least virulent and the Oktibbeha variant was the most virulent. Cultivars 'Brandy', 'Choo Choo Centennial', and 'First Prize', showed resistance to all variants. 'Coral Destiny', 'Golden Masterpiece', 'Goldilocks', 'Jennifer Hart', and 'Spartan' showed resistance to three variants. Forty-two cultivars showed susceptibility to all variants tested.

Index words: *Diplocarpon rosae*, blackspot, Rose

Significance to the Nursery Industry

Blackspot, the most important disease of landscape roses world wide, is caused by *Marssonina rosae* (*Diplocarpon rosae*). Most modern rose cultivars demonstrate little or no resistance to blackspot and many cultivars that are reported to be resistant are in fact susceptible when exposed to different variants of the pathogen under varying environmental conditions. This study describes variations in conidia size, colony color, and pathogenicity, which were found in isolates of *M. rosae* from different counties in Mississippi. Four *M. rosae* isolates were selected for pathogenicity tests on 76 selected landscape rose cultivars.

Introduction

Blackspot is the most important rose disease worldwide. Previous reports (2, 4, 8) firmly documented differential pathogenicity of *Marssonina rosae* (Lib.) Lind (imperfect stage of *Diplocarpon rosae* Wolf) isolates to various species and cultivars of roses. Other reports (1, 3, 5) report different plant response to a single isolate.

Blackspot is particularly destructive in Mississippi and other regions where favorable temperature and moisture conditions for leaf infection exist for extended periods of the year. This is especially significant in Mississippi, since roses are grown at 75% of the home units (personal communication, Robert Haygood). There is some commercial rose production in the state and many rose fanciers face difficulties effecting blackspot control since it is apparently impossible to purchase modern plants with known resistance. We established a Disease Research Rose Garden (through encouragement by University Patrons) that would

¹Received for publication March 16, 1992; in revised form September 14, 1992. Published as Mississippi Agricultural and Forestry Experiment Station Journal Series No. _____.

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provide material that could be researched to, possibly, locate internal mechanisms connected to resistance factors that might be transferrable, by some mechanism, into a commercially acceptable plant that would then be resistant to blackspot. No specific information was available on *M. rosae* performance in Mississippi. Therefore, research was begun to determine the existence of *M. rosae* variants within the state with anticipation of information that might contribute to blackspot resistance and benefit growers in selecting and tending roses.

Materials and Methods

Isolation and growth. Fungal isolates were obtained from infected leaves from seven counties that represented different rose growing conditions in the state to obtain a diversity of fungal variants (Table 1). After infected leaves were washed with running tap water for 5 min, they were submerged in 70% ethanol for 3 min then in 1% NaOCl for 3 min. Pieces of leaflets were plated on yeast-malt extract agar (YMEA) and plates were maintained at room temperature [26°C (79°F)]. This procedure usually produced pure cultures of *M. rosae*.

Inoculum. Emerging fungal growth from host tissue was blended in sterile water in a micro-blender. Aliquots [1 ml (0.03 oz)] of minute hyphal fragments and conidial suspension mixture were transferred to YMEA in plates and the plates were rotated until the agar surface was covered with inoculum. Inocula for all tests were produced on YMEA containing thiamine, inositol, pyridoxine and biotin (7) and minor elements iron, manganese and zinc (2). Conidia of 7-day-old cultures were scraped from the agar and blended in water for 30 sec in a micro-blender. The suspension was diluted to 2.5×10^4 conidia/ml (8.5×10^2 conidia/oz) in sterile water. Cultures were recycled through susceptible plants every 2–3 months to maintain a high level of virulence and inoculated leaflets of individual variants also were preserved under freezer conditions (–12°C).

Morphology. Two hundred conidia from 7-day-old cultures of each of the seven isolates were measured microscopically. To determine variations in colony color, spore suspensions of equal concentrations of the four variants used in pathogenicity tests were streaked on potato-dextrose-agar (PDA). Three streaks, measuring 7 cm × 6 mm and 25 mm apart (2.76×2.36 and 1 in), were made and colony color was determined 4-wk later using the scale of Ridgway (6).

Pathogenicity tests. Four *M. rosae* isolates, Chickasaw, Hinds, Marion and Oktibbeha (named for the county of origin), were tested for pathogenicity on a number of cultivars utilizing a detached leaf technique (1, 4). Leaflets at peak growth stage were detached and placed on sterile, moist

(2% sucrose) cotton discs in culture plates (1, 4). For each test, five leaflets per cultivar were placed in each of two plates and inoculated with five leaflets in one plate treated with sterile water. Two tests were conducted on 76 cultivars that included floribunda, grandiflora, hybrid tea, large flowered climber and miniature roses. Leaflets were sprayed until wet with minute droplets of spore suspension from a model 15 Devilbiss atomizer under a transfer hood. Uninoculated leaves were sprayed with sterile water. Leaflets in plates were incubated on a laboratory bench at room temperature [22–24°C (72–75°F)] under linear banks of double 40 watt Gro-lux lamps each 120 cm (47 in) long and 62.5 cm (25 in) above the plates. Lights were on 8 hr and off 16 hr per day. Symptoms developed at 10–14 days and readings were made at 16 days. A visual rating of symptom development scored highly resistant (HR) for asymptomatic leaflets, resistant (R) for leaflets with less than 10% of the leaflet affected and susceptible (S) with more than 10% of the leaflet affected. Symptom expression on leaflets in duplicate plates were consistent under the test conditions. Two tests were performed.

Results

Isolation and growth. Standard isolation techniques, used earlier in this research, permitted overgrowth of *M. rosae* by many organisms. The sterilization technique used here produced clean cultures, usually pure, of *M. rosae* within 14 days. Since mycelial growth was usually very slow, flooding agar surfaces with blended spore suspensions considerably shortened production time of inoculum to 7 days for each test.

Morphology. Spore measurements of the seven isolates from different sources across the state were different (Table 2). The Wayne county isolate had the largest conidia and was followed in order by variants from Chickasaw, Quitman, Marion, Oktibbeha, Sharkey, and Hinds counties. Colony color varied from fucous black for Marion and Oktibbeha isolates to pale mouse gray (Chickasaw isolate) and light ochraceous salmon (Hinds isolate).

Pathogenicity. The Chickasaw isolate was the least virulent of the four tested, causing susceptible reactions on 60.3% of the cultivars. Three cultivars were not inoculated with this isolate due to insufficient plant material. The Oktibbeha isolate was the most virulent, with 89.5% of the cultivars susceptible. The Hinds isolate, 85.7%, and Marion isolate, 80%, were also highly virulent. Cultivars 'Brandy',

Table 2. Conidial dimensions of seven variants of *Marssonina rosae* from rose plants from seven Mississippi counties.

Isolate	Length ^y	Width ^y
Wayne	25.42 ^z	7.37 ^z
Chickasaw	23.71	6.03
Quitman	22.86	5.78
Sharkey	22.15	5.30
Marion	21.79	5.65
Oktibbeha	21.77	5.72
Hinds	21.18	5.33
LSD (P = 0.05)	1.96	0.16

^yMeasurements of water-mounted conidia in microns.

^zData represents means of 200 conidia.

Table 1. Sources of *Marssonina rosae* variants.

County	City	Source area
Chickasaw	Houston	interior flatwoods
Hinds	Oakley	brown loam
Marion	Columbia	lower coastal plain
Oktibbeha	Mississippi State	blackland prairie
Quitman	Lambert	upper delta
Sharkey	Rolling Fork	lower delta
Wayne	Waynesboro	lower coastal plain

Table 3. Reaction of 76 rose cultivars to inoculation with variants of *Marssonina rosae* from four Mississippi counties.

Cultivar	Isolates				Cultivar	Isolates			
	Chickasaw	Hinds	Marion	Oktibbeha		Chickasaw	Hinds	Marion	Oktibbeha
Americana	S ²	S	S	S	King's Ransom	R	S	S	S
American Pride	S	S	S	S	Kolner Karneval	S	S	S	S
Arlene Francis	S	O	S	S	Kordes Perfecta	S	S	S	S
Betty Prior	S	S	S	S	Lavender Jewell	O	S	S	S
Bing Crosby	S	S	S	S	Little Eskimo	S	S	S	S
Blaze	S	S	S	S	Mister Lincoln	S	S	S	S
Brandy	HR	HR	HR	HR	Misty Dawn	S	S	S	S
Carrousel	S	S	S	S	Montezuma	S	S	S	S
Charlotte Armstrong	S	S	S	S	My Valentine	R	S	R	S
Chattem Centennial	S	S	S	S	Nocturne	S	R	S	S
Choo Choo Centennial	HR	HR	HR	HR	Oklahoma	R	S	S	S
Coral Destiny	R	S	R	R	Olympiad	R	S	S	S
Corn Silk	S	S	S	S	Orange Honey	S	S	S	S
Crimson Glory	R	S	R	S	Oregold	S	S	S	S
Cupcake	S	S	S	S	Pascali	R	S	S	S
Diamond Jubilee	R	S	R	S	Peace	S	S	S	S
Don Juan	S	S	S	S	Pink Portrait	O	S	S	S
Double Delight	S	S	O	S	Prairie Princess	R	S	S	S
Double Joy	S	S	S	S	Razzle Dazzle	S	S	S	S
Dr. Brownell	R	S	S	R	Royal Highness	S	S	S	S
Eclipse	R	S	S	R	Saratoga	R	R	S	S
Evening Star	S	S	S	S	Shreveport	S	S	S	S
First Prize	HR	HR	HR	HR	Spartan	R	R	R	S
Floradora	S	S	S	S	Summer Sunshine	R	O	S	S
Frau Karl Druschki	R	S	R	S	Sunblest	S	O	S	S
Galaxy	O	S	R	S	Super Star Supreme	R	S	S	S
Garden Party	S	O	S	S	Sutter's Gold	S	S	S	S
Genevieve	S	O	S	S	Sweet Surrender	R	S	S	S
Golden Masterpiece	R	R	R	S	Tiffany	S	S	S	S
Goldilocks	R	O	R	R	Tribute	S	S	S	S
Helen Traubel	S	S	R	S	Tropicana	S	S	S	S
Intrigue	S	S	S	S	Uncle Joe	R	S	S	S
Ivory Tower	S	R	S	S	Viva	S	R	S	S
Jennifer Hart	R	S	R	R	Whiskey Mac	R	S	S	S
John F. Kennedy	S	S	S	S	White Knight	R	S	S	S
Joseph's Coat	S	S	S	S	White Masterpiece	S	S	S	S
Karl Herbst	R	R	S	S	White Queen	R	S	S	S
Katherine T. Marshall	R	S	R	S	Yankee Doodle	S	S	S	S

²Disease ratings: HR = highly resistant, clean leaflets; R = resistant, less than 10% of leaflet affected; S = more than 10% of leaflet affected; O = variety not inoculated with this isolate.

'Choo Choo Centennial', and 'First Prize' were highly resistant to all four isolates. 'Goldilocks' was resistant to three isolates, but was not inoculated with the Hinds isolate. Various cultivars (Table 3) showed resistance to some isolates but not to others. 'Coral Destiny', 'Golden Masterpiece', 'Jennifer Hart', and 'Spartan' showed resistance to three of the four isolates, but all were not resistant to the same isolate. Forty-two cultivars were susceptible to all four isolates tested.

There were variations in conidial measurement, colony color, and virulence of isolates of *M. rosae* from different counties in Mississippi. This shows that different variants may occur within the rather limited area of an individual state. Three cultivars showed resistance to all four isolates used. However, with variants possibly carried into the state on rose canes and the changes which may occur within variants over time within the state, a single site performance rating of cultivar susceptibility to *M. rosae* would provide limited information for resistant cultivar selection.

The pathogenicity test indicated highly variable virulence potential among isolates. However, it is possible that none of these variants used in our test are as virulent as others,

which may already occur in areas where roses are grown. If true, this would strongly emphasize the limitations of simple hybridization to obtain general blackspot resistance. Hopefully, the newly emerging technology of genetic engineering may be useful in producing lasting resistant cultivars of roses to the blackspot disease.

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Effect of Fertilizer and Irrigation on Leachate Levels of $\text{NH}_4\text{-N}$, $\text{NO}_3\text{-N}$, and P in Container Production of *Nephrolepis exaltata* 'Fluffy Ruffle'¹

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Abstract

Nitrogen leaching into surficial aquifers continues to become more of a problem in several areas of the U.S., and thus potential for regulation of foliage plant producers is increasing. A factorial experiment evaluated liquid and controlled-release fertilizer sources at three irrigation levels [100, 200, or 300 ml (3.4, 6.8, or 10.2 oz) per 15 cm (6 in) pot twice weekly] for $\text{NH}_4\text{-N}$, $\text{NO}_3\text{-N}$, and P in leachate. Samples were collected weekly for 12 weeks beginning the last week of September. Plant grade and top fresh weights were similar for all treatments, but large variations occurred in $\text{NH}_4\text{-N}$, $\text{NO}_3\text{-N}$, and P levels in leachate due to irrigation level. Increasing irrigation level above 100 ml (3.4 oz) twice weekly resulted in increases of $\text{NO}_3\text{-N}$ present in leachate, with levels as high as 126 mg/pot observed toward the end of November. $\text{NH}_4\text{-N}$ levels were affected by irrigation during the first seven weeks of the experiment but, after week 2, were lower than one mg/pot. Phosphorus levels ranged from 0.9 to 5.7 mg/pot in leachate with responses to irrigation treatment throughout the experiment.

Index words: controlled-release fertilizer, foliage plant production, liquid fertilizer, nitrate nitrogen, surficial aquifer

Species used in this study: 'Fluffy Ruffle' fern (*Nephrolepis exaltata* (L.) Schott 'Fluffy Ruffle')

Significance to the Nursery Industry

Concern over nitrogen runoff into surface aquifers and leaching into ground water may lead to regulation of current production practices employed by plant producers. Limited information is available on best management practices (BMP's) for greenhouse production to ensure leaching of N into surficial aquifers does not exceed permitted standards. This experiment provides information regarding plant quality and leachate concentrations from foliage plant production using a liquid vs. a controlled-release fertilizer and three irrigation levels. These results can be used to help formulate BMP's for the foliage plant production industry.

Introduction

Both liquid and controlled-release fertilizers can be used effectively during foliage plant production (3, 4, 5, 8, 10, 14, 23) and the amount of irrigation necessary to produce quality plant material has been studied (3, 7, 17). Other research has evaluated the area of surficial aquifer (ground

water) contamination caused by leachates from agricultural systems (1, 6, 9, 11, 12, 19, 20, 21, 22), including runoff water studies from container-grown crops (18). However, little research has been conducted regarding leachate quality from potted foliage plant production. This study was established to determine leachate levels of $\text{NH}_4\text{-N}$, $\text{NO}_3\text{-N}$, and P, and effects on plant quality when using a liquid vs. a controlled-release fertilizer and different irrigation levels during production of 'Fluffy Ruffle' fern.

Materials and Methods

Rooted liners of *Nephrolepis exaltata* 'Fluffy Ruffle' were planted into 15 cm (6 in) pots using Vergro Container Mix A without superphosphate (Verlite Company, Tampa, FL 33610) consisting of peat:vermiculite:perlite (2:1:1 by vol) with a water-holding capacity by volume of 72.8 percent and a pH range of 5.8 to 6.5. Plants were placed in a shaded glasshouse where they received $380 \mu\text{mol}\cdot\text{s}^{-1}\cdot\text{m}^{-2}$ (2000 ft-c) maximum light intensity at plant level with temperatures varying between 20–35°C (68–95°F) depending upon ambient temperature. Based on previous research (16), irrigation was scheduled twice weekly for all plants at 100, 200, or 300 ml (3.4, 6.8, or 10.2 oz) of deionized water per 15 cm (6 in) pot at each irrigation. Fertilizer treatments

¹Received for publication June 23, 1992; in revised form September 14, 1992. Published as Florida Agricultural Experiment Station, Journal Series No. R-02474.

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