Marssonina rosae Variants in Mississippi and Their Virulence on Selected Rose Cultivars

IDA WENEFRIDA, Graduate Student, and JAMES A. SPENCER, Plant Pathologist, Department of Plant Pathology and Weed Science, Mississippi State University, Mississippi State 39762

ABSTRACT

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Spore size, colony color, and pathogenicity of *Marssonina rosae*, imperfect stage of *Diplocarpon rosae*, were determined to ascertain the occurrence of fungus variants. Isolates were obtained from 10 rose cultivars in the rose disease research garden at Mississippi State University and from two unidentified cultivars in Oktibbeha and Wayne counties. Spores ranged from 22.1 to 25.3 μm in length and from 5.3 to 7.1 μm in width. Isolates were divided into four color groups: clove brown, light pinkish cinnamon, light mouse gray, and clove brown with apricot and salmon buff spot mixture. Four pathogenic variants were found among the isolates. Isolate ELT from the cultivar Electron was the most virulent and was more severe on Electron than on other cultivars. *Rosa roxburghii* was immune to all isolates.

Rose black spot, caused by *Marssonina rosae* (Lib.) Died. (imperfect stage of *Diplocarpon rosae* F. A. Wolf), is the most important disease of rose worldwide (1,2,4) except in regions of low seasonal rainfall (1,13). Fungicides and resistant cultivars are currently used as control measures (1,3,5,6).

Development of resistance to *M. rosae* is complicated by variability of the fungus and multiple gene action in rose genes governing resistance traits (14,15). *M. rosae* isolates from different rose cultivars and geographic locations vary greatly. Palmer et al (10) reported differences associated with geographic origin of the pathogen and cultivar source of conidia. Jenkins (7) identified different races of the fungus among isolates obtained from 22 locations across the United States. Although evidence suggests differences in virulence (3,7), the effects of environment, age, condition of host leaves, and inoculum density of the fungus must be considered when resistance is evaluated (9–11).

Strategies for developing resistance require determination of the nature and extent of the pathogen variation, pathogenicity, and the existence of a gene-for-gene relationship. The Disease Research Rose Garden at Mississippi State University provides germ plasm that can be screened to quantify the degree and diversity of genetic resistance to a variable fungus population and that may subsequently be transferred into horticulturally acceptable lines. Four cultivars in the original planting (obtained from few sources) showed good initial resistance to the prevailing fungus variants, but later, as the garden was enlarged with new plant additions (from diverse sources), these cultivars showed greater susceptibility. Presumably, additional fungus variants were imported with plants or developed within the garden population. Because no specific information was available on *M. rosae* variant types in Mississippi, we investigated the existence of variants in the research garden and evaluated the response of selected rose cultivars to these variants.

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MATERIALS AND METHODS

Fungus sources and isolation. Isolates of *M. rosae* were obtained from black spot-infected leaflets of 10 rose cultivars in the rose disease research garden. In addition, one isolate was obtained from each of two unidentified rose cultivars from Wayne (WAY) and Oktibbeha (OKT) counties, which are approximately 243 and 6.8 km, respectively, from the garden. We gave the isolates from the research garden names that were abbreviated versions of the names of the source cultivars: CEL (Celebrity), CCC (Choo Choo Centennial), DBS (Dainty Bess), DDL (Double Delight), ELT (Electron), GLD (Graceland), HLT (Heartlight), PLD (Proud Land), SSN (Spanish Sun), and SBT (Sunbright).

Isolations were made in July 1989. Infected leaflets were washed under running tap water for 5 min, then submerged in 70% ethyl alcohol (ETOH) for 3 min and then in 1% sodium hypochlorite for 2 min for surface decontamination. Portions of diseased leaflets were placed on yeast-malt extract agar (YMEA) containing 0.2% yeast extract, 2.0% malt extract, and 20% agar per liter of distilled water modified with thiamine (100 μg), biotin (5 μg), inositol (5 μg), pyridoxine (100 μg) (13), $Fe(NO_3)_3 \cdot 9H_2O$ (0.2 mg), $ZnSO_4 \cdot 7H_2O$ (0.2 mg), and $MnSO_4 \cdot 4H_2O$ (0.1 mg) (8) (per liter of medium). Plates were incubated on an office bench with diurnal light from a nearby (1.67 m) southern-exposure window at a temperature of 21.5 ± 2 C for fungal growth and isolation of pure cultures of *M. rosae*.

Conidial measurements. Conidia from individual 5-wk-old isolates were separately blended in a vitamin solution of thiamine, biotin, pyridoxine, and inositol (8,13) mixed with a minor-element solution of iron nitrate, zinc sulfate, and manganese sulfate (8). Aliquots (1.5 ml) of blended conidia of separate isolates were transferred to separate plates containing YMEA. Plates were rotated circularly so inoculum would cover the agar surface and were incubated under the same conditions used for isolating pure cultures. Two hundred conidia (50 from each of four plates) from 12-day-old cultures of each isolate of *M. rosae* were measured. The experiment was a randomized complete block design with four replications. Each plate was an experimental unit. Analysis of variance and mean separation were performed with Duncan's multiple range test at $P \leq 0.01$.

Colony color. Conidia from individual 2-wk-old cultures were blended in the vitamin-mineral solution, and the conidial suspensions were diluted to 2.5×10^4 conidia per milliliter. A 5-mm loop of fine chrome wire was dipped into the conidial suspension and used to make streaks (7 cm × 5.5. mm) on potato-dextrose agar in culture plates. Three streaks were spaced 1.5 cm apart in each 9-cm plate. Four plates of each isolate were randomized in a complete block design on a laboratory bench and incubated under the same conditions used for isolation of cultures and production of conidia for measurements. Colony color, determined after 3 wk of growth of cultures in four plates, was highly consistent for a given isolate. Colony colors were determined from color plates in *Color Standard and Color Nomenclature* (12).

Pathogenicity. Inoculum was prepared from conidia from 7-day-old cultures blended and diluted as indicated for the colony color test. Leaflets to be inoculated were collected from mature leaves three to five nodes from plant tops. Leaflets were washed under running water for 5 min and in a mild soap solution for 2 min to remove debris and were then

rinsed three times in distilled water. They were placed on cotton disks wetted with 2% sucrose (7) in 9-cm plates. Each plate contained three or four leaflets, depending on leaflet size. Leaflets were spray-inoculated with a conidial suspension containing 2.5×10^4 conidia per milliliter until wet.

Inoculated leaflets were maintained at 26 ± 2 C under linear banks of double-bulb, 40-W Gro-lux lamps (8 hr on, 16 hr off per day) each 120 cm long and 62.5 cm above the plates (light intensity $25.8 \pm 1.1\ \mu E \cdot m^{-2} \cdot s^{-1}$) for 21 days. Symptom data were recorded at that time. The experiment was a completely randomized design with three replications arranged in a factorial of isolates and cultivars. The symptom index was rated on a scale from 0 to 5, where 0 = no black spot symptoms, 1 = 1–15% of leaflet surface affected, 2 = 16–25%, 3 = 26–40%, 4 = 41–75%, and 5 = 76–100% of the leaflet surface covered with black spot symptoms.

Three pathogenicity tests were conducted. In the first (pathogenicity test I), each isolate was inoculated onto eight cultivars from which isolates originated: Celebrity, Dainty Bess, Double Delight, Electron, Graceland, Proud Land, Sunbright, and Spanish Sun. For pathogenicity test II, six isolates of *M. rosae* (CCC, CEL, DBS, ELT, GLD, and OKT) were selected based on differences in conidial size, colony color, and pathogenicity in the first test. Variability in virulence was assessed on rose cultivars Bienvenu (grandiflora), Tiffany (hybrid tea), and Showbiz (floribunda) and on *Rosa roxburghii,* a species rose. Selection of cultivars was based on leaflet response to infection in the rose garden. The inoculum concentration was increased to 4×10^5 conidia per milliliter. Methods, design, and data recording for tests II and III were the same as for test I. In test III, isolates CEL, ELT, GLD, and OKT were selected for further testing based on their performance in test II. Plant selection was the same used in test II.

All pathogenicity tests were completely randomized designs with three replications arranged in a factorial treatment arrangement. The factors were isolates and cultivars. All data were subjected to analysis of variance, and means were separated by a multiple range test for significance at $P \leq 0.01$.

Table 1. Mean conidial size of isolates of *Marssonina rosae*

Isolate[y]	Length[z] (μm)	Width[z] (μm)
WAY	25.3 a	6.9 ab
ELT	24.9 ab	7.1 a
CCC	24.4 abc	7.0 a
SBT	24.4 bc	7.1 a
PLD	24.1 bc	6.6 abc
DDL	23.9 c	6.9 abc
HLT	23.7 c	6.5 abc
GLD	23.6 c	6.7 abc
SSN	22.6 d	6.3 bc
DBS	22.3 d	6.2 c
CEL	22.3 d	5.3 d
OKT	22.1 d	6.1 c

[y] Isolates are named after source cultivar (ELT = Electron, CEL = Celebrity, DBS = Dainty Bess, CCC = Choo Choo Centennial, PLD = Proud Land, GLD = Graceland, DDL = Double Delight, SBT = Sunbright, SSN = Spanish Sun, HLT = Heartlight) or county (OKT = Oktibbeha Co., WY = Wayne Co.).

[z] Based on measurement of 50 conidia per culture from each of four plates of the fungus growing on potato-dextrose agar. Means within a column followed by the same letter are not significantly different according to Duncan's new multiple range test at $P \leq 0.01$.

RESULTS AND DISCUSSION

Conidial measurements. Conidial lengths (22.1–25.3 μm) and widths (5.3–7.1 μm) measured in this study were in general agreement with sizes previously described for the fungus (20–25 × 5–6 μm) (16) except that the conidial widths (6.1–7.1 μm) of all isolates except CEL (5.3) were larger than those in that description. Conidia from isolates CCC, ELT, and WAY were the largest (24.4–25.3 × 6.9–7.1 μm) (Table 1). Conidia of isolates CEL, DBS, OKT, and SSN were significantly shorter (22.1–22.6 μm) than those of the other isolates. Isolates DDL, GLD, HLT, PLD, and SBT had conidial measurements intermediate between those of the first two groups. These size differences support the hypothesis of morphological variants of the organism in the rose garden.

Colony color. Colony color varied among the 12 isolates. In streak cultures, isolates CCC, CEL, PLD, SBT, and WAY were pinkish cinnamon; isolates DBS and SSN were light mouse gray; isolates DDL, ELT, and HLT were clove brown; and isolates GLD and OKT were clove brown with apricot and salmon buff mixtures. These differences were important in determining morphological variants.

Pathogenicity. In pathogenicity test I, the highest symptom index (4.17) (Table 2), which represented 41–75% of the leaflet covered by symptoms, was expressed by isolate ELT on cultivar Electron. ELT also was the only isolate that produced a symptom index greater than 3.0 on all cultivars. The least virulent isolates on all cultivars, and their highest symptom indexes, were DDL (2.67), HLT (2.33), SBT (2.83), and SSN (2.83). There was no relationship between virulence and conidial size, as evidenced by the action of isolate WAY, with conidial size 25.3 × 6.9 μm and symptom indexes of 1.33 (the lowest of all isolates) on both Electron and Proud Land, and isolate ELT, with conidial size 24.9 × 7.1 μm and symptom indexes of 4.17 on Electron

Table 2. Mean symptom index[x] produced by isolates of *Marssonina rosae* on detached leaflets of eight rose cultivars

	Cultivar[z]							
Isolate[y]	Celebrity	Dainty Bess	Double Delight	Electron	Graceland	Proud Land	Sunbright	Spanish Sun
CCC	3.17 ab	2.50 def	2.83 abc	2.83 bc	3.00 ab	2.50 bc	2.67 a	2.83 bc
CEL	3.67 a	3.33 ab	3.00 ab	3.17 b	2.83 ab	2.83 ab	3.00 a	3.00 b
DBS	3.00 bc	2.67 def	3.17 a	2.33 cde	2.67 bc	2.67 ab	3.00 a	3.00 b
DDL	2.67 bcde	2.33 efg	2.33 bcde	1.67 fg	2.17 cd	2.00 cd	2.00 b	2.33 ab
ELT	3.67 a	3.67 a	3.33 a	4.17 a	3.33 a	3.17 a	3.33 a	3.33 a
GLD	2.50 cde	3.00 bcd	2.50 bcd	2.33 cde	2.50 bc	2.50 bc	2.33 b	2.00 b
HLT	2.00 f	2.33 efg	2.00 de	2.00 ef	1.67 d	2.00 cd	2.00 b	2.17 b
OKT	3.00 bc	3.17 abc	2.83 abc	2.17 def	2.67 bc	2.83 ab	3.00 a	2.50 ab
PLD	3.17 ab	2.83 bcde	2.17 de	2.67 bcd	2.83 ab	3.00 ab	2.00 b	2.33 b
SBT	2.83 bcd	2.00 g	1.83 e	2.00 ef	1.83 d	1.83 de	2.83 bc	2.17 d
SSN	2.33 def	2.00 g	1.83 e	1.67 fg	1.67 d	2.00 cd	2.17 b	2.83 a
WAY	2.17 ef	2.17 fg	2.33 cde	1.33 g	1.67 d	1.33 e	2.33 b	3.00 a

[x] 0 = no black spot symptoms, 1 = 1–15% of leaf surface covered by black spot symptoms, 2 = 16–25%, 3 = 26–40%, 4 = 41–75%, and 5 = 76–100% of leaf surface covered by black spot symptoms. Means within a column followed by the same letter are not significantly different according to Duncan's multiple range test at $P \leq 0.01$.

[y] Isolates are named after source cultivar (ELT = Electron, CEL = Celebrity, DBS = Dainty Bess, CCC = Choo Choo Centennial, PLD = Proud Land, GLD = Graceland, DDL = Double Delight, SBT = Sunbright, SSN = Spanish Sun, HLT = Heartlight) or county (OKT = Oktibbeha Co., WAY = Wayne Co.).

[z] Cultivars were some of those from which isolates of *M. rosae* were obtained.

Table 3. Mean symptom index[y] produced on roses by isolates of *Marssonina rosae* in pathogenicity test II

Isolate[z]	Cultivar: Bienvenu	*Rosa roxburghii*	Showbiz	Tiffany
CCC	1.83 d	0.00 a	1.00 d	2.17 b
CEL	2.83 b	0.00 a	2.00 b	2.83 a
DBS	2.17 c	0.00 a	1.50 c	1.50 c
ELT	3.83 a	0.00 a	3.17 a	2.83 a
GLD	3.17 b	0.00 a	2.67 a	2.17 b
OKT	3.00 b	0.00 a	2.83 a	2.83 a

[y] 0 = no black spot symptoms, 1 = 1–15% of leaf surface covered by black spot symptoms, 2 = 16–25%, 3 = 26–40%, 4 = 41–75%, and 5 = 76–100% of leaf surface covered by black spot symptoms. Means within a column followed by the same letter are not significantly different according to Duncan's multiple range test at $P \leq 0.01$.

[z] Isolates are named after source cultivar (CCC = Choo Choo Centennial, CEL = Celebrity, DBS = Dainty Bess, ELT = Electron, GLD = Graceland) or county (OKT = Oktibbeha Co.).

Table 4. Mean symptom index[y] produced on selected roses by isolates of *Marssonina rosae* in pathogenicity test III

Isolate[z]	Cultivar: Bienvenu	*Rosa roxburghii*	Showbiz	Tiffany
CEL	2.33 a	0.00 a	1.33 b	2.33 ab
ELT	3.00 a	0.00 a	2.00 ab	2.67 a
GLD	2.33 a	0.00 a	1.33 b	1.00 c
OKT	2.33 a	0.00 a	2.33 a	1.67 bc

[y] 0 = no black spot symptoms, 1 = 11–15% of leaf surface covered by black spot symptoms; 2 = 16–25%, 3 = 26–40%, 4 = 41–75%, and 5 = 76–100% of leaf surface covered by black spot symptoms. Means within a column followed by the same letter are not significantly different according to Duncan's multiple range test at $P \leq 0.01$.

[z] Isolates are named after source cultivar (CEL = Celebrity, ELT = Electron, GLD = Graceland) or county (OKT = Oktibbeha Co.).

and 3.17 on Proud Land.

Statistical analyses revealed significant interaction between isolates and cultivars in all tests. Therefore, differences in isolate pathogenicity depend on the cultivar.

In pathogenicity test II, isolates differed significantly in pathogenicity, which indicated different levels of cultivar susceptibility. Isolate ELT produced the highest symptom index of all isolates on Bienvenu (3.83) and Showbiz (3.17) and equaled the highest index on Tiffany (2.83) (Table 3). Isolate CCC produced the lowest symptom index of all isolates on Bienvenu (1.83) and Showbiz (1.0). Isolate DBS produced the lowest index (1.50) of all isolates on Tiffany. *R. roxburghii* showed no symptoms from any of the six isolates CCC, CEL, DBS, ELT, GLD, and OKT. Uninoculated leaflets showed no symptoms.

Isolates CEL, ELT, GLD, and OKT were selected for pathogenicity test III, and they differed significantly in pathogenicity on Showbiz and Tiffany (Table 4). Again, *R. roxburghii* showed no symptoms, indicating immunity to the variants. Isolates CEL, GLD, and OKT produced identical symptom indexes on Bienvenu. ELT was the most virulent isolate on Bienvenu and Tiffany and was the most virulent overall. The lowest symptom index (1.0) was expressed by GLD on Tiffany, and CEL and GLD expressed an index of 1.33 on Showbiz.

In summary, *M. rosae* isolates collected from the rose research garden and from roses in Oktibbeha and Wayne counties varied in conidial size, colony color, and pathogenicity. Significant variation in symptom indexes was observed among isolates. All isolates were more virulent on Bienvenu and Showbiz in test II than in test III, and isolates GLD and OKT were more virulent on Tiffany in test II than in test III. CEL and ELT differed less in virulence on Tiffany in tests II and III. In general, isolates ELT and GLD were more virulent on Bienvenu than on the other cultivars in both tests. This indicated greater susceptibility in Bienvenu. It is possible that several variants of *M. rosae* were introduced into the area on roses imported from out-of-state nurseries.

The results emphasize the difficulty of developing resistance to black spot by conventional hybridization techniques because of the many variants of the pathogen and the varying susceptibility of rose cultivars. A genetic engineering approach to the development of black spot resistance, which would attempt to transfer resistant genes from immune species roses such as *R. roxburghii* to hybrid tea roses with desirable horticultural characteristics, seems desirable.

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