Resistance of Daylily Cultivars to the Daylily Rust Pathogen, *Puccinia hemerocallidis*

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Abstract. Daylilies (*Hemerocallis* sp.) have traditionally been considered to be pest-free. However, a rust disease caused by *Puccinia hemerocallidis* Thüm. has become an increasing problem in the United States since 2000. The objective of this study was to evaluate daylily cultivars for resistance to daylily rust. From a greenhouse evaluation of 84 inoculated cultivars, 14 (17%) were classified as resistant; 13 (15%) were classified as moderately resistant; 22 (26%) were classified as moderately susceptible; and 35 (42%) were classified as fully susceptible. Several cultivars displayed very high levels of resistance and may be utilized by breeders to develop cultivars with improved resistance to daylily rust. Information about the resistance and susceptibility of cultivars also will help commercial growers and gardeners select cultivars that have the potential to dramatically reduce damaging outbreaks of rust.

Daylilies are one of the most economically important ornamental plants cultivated in the United States. Daylilies are very popular perennials because they are available in several colors, shapes, and sizes; they are able to survive with very little care in a wide range of climates; and they are drought-resistant and, before daylily rust, almost disease- and pest-free (Williams-Woodward and Buck, 2002).

Daylily rust caused by *Puccinia hemerocallidis* was introduced into the United States in 2000 (Williams-Woodward et al., 2001) and has become an increasing problem for growers. First found in Georgia, daylily rust has spread rapidly throughout the United States and has been reported as far north as Minnesota and west as Oregon and California (U.S. Dept. of Agriculture, 2002). *Puccinia hemerocallidis* is heteroecious. The uredinal/telial host is daylily and the alternate (spermatophyte/aerial) host is *Patrinia* (Valerianaceae), a genus of rhizomatous or stoloniferous herbaceous perennials, some of which are grown as ornamentals (Jelitto and Schacht, 1990). However, infection of the alternate host is not required for the fungus to infect daylily. The uredinal or repeating spores stage on daylily can re-infect the same host plant, leading to epidemics. Bright

yellow-orange to rust-brown pustules containing urediniospores are produced on upper and lower daylily leaf surfaces within 7 to 14 d after infecting plants (Williams-Woodward et al., 2001). A wide range of symptoms associated with infected hosts has been reported. These include bright yellow-orange spots to streaks, water-soaked tan spots with darker borders, larger yellow lesions, and smaller more discrete spots (Williams-Woodward and Buck, 2001).

Since the American Hemerocallis Society began acting as the official registry of daylily cultivars in 1947, more than 40,000 cultivars have been registered and published in the form of check lists (Baxter, 1999; Monroe, 1973, 1984, 1994; Monroe and Monroe, 1989; Stuntz et al., 1957), making it one of the most intensively bred crops during the past 60 years. Preliminary work at the Univ. of Georgia reported nearly 50% of the cultivars were classified as highly susceptible while only 8% were labeled least susceptible (Williams-Woodward and Buck, 2001). A more in-depth study of cultivar reactions is needed to help nurseries, daylily breeders, and gardeners avoid epidemic levels of daylily rust similar to those observed in 2001. The objective of this study was to evaluate 84 daylily cultivars, including widely available commercial cultivars, for resistance to the rust pathogen *P. hemerocallidis* in a greenhouse environment.

Materials and Methods

Plant culture. Forty-one cultivars from Walter's Garden (Zeeland, Mich.) or Sing-

ing Oakes Garden (Blythewood, S.C.) (set 1) and 43 cultivars from McCorkle's Nursery in Dearing, Ga. (set 2) were evaluated in the greenhouse. Plants were maintained in 3.8-L pots containing a commercial 9 bark :1 sand mixture (McCorkle's Nursery). Cultivars in set 1 were placed on greenhouse benches in a rust-free polyhouse until initiating the experiment when pots were transferred to a greenhouse with average night/day temperature of 22 and 26 °C. Cultivars from McCorkle's Nursery (set 2) were grown at their original site (Dearing, Ga.) until being transferred to the greenhouse. Plants were irrigated as needed and fertilized with Osmocote Plus 15%-9%-12% (15.0N-3.96P-9.96K) controlled-release fertilizer (Scotts Co., Marysville, Ohio). Insect and arthropod pests were controlled using standard practices.

Pathogen culture and inoculation. An isolate of *P. hemerocallidis* was collected from an infected daylily plant ('Pardon Me') that was received by the Univ. of Georgia disease diagnosis clinic in 2000 (Williams-Woodward et al., 2001). This isolate was maintained on daylily plants of the same cultivar in the greenhouse with average temperatures of 24 °C day/22 °C night, respectively. For each experiment, urediniospores were collected from 10 to 12-d-old pustules using a vacuum spore collector, suspended in 0.05% (v/v) Tween 20 (J.T. Baker, Phillipsburg, N.J.), and filtered through five layers of sterile cheesecloth. Spores were enumerated by hemocytometer, then diluted to 1–2 × 10⁵ spores/mL in 0.05% (v/v) Tween 20. For each trial, three plants of each cultivar were watered to saturation, sprayed with the spore suspension until runoff, sealed individually in clear plastic 25 cm × 20 cm × 60 cm bags, and stored at 24 °C in the dark. After 24 h, bags were removed and plants were transferred to the greenhouse. Foliage was not wetted during each experiment. Foliage was evaluated for rust 5× between 9 and 21 d after inoculation using a 1 to 5 rating scale. Ratings were 1 = no disease; 2 = reduced lesion number and no sporulation; 3 = reduced lesion number but not sporulation; 4 = slightly reduced lesion number and/or amount of sporulation; and 5 = fully susceptible (greatest number of lesions and/or amount of sporulation). This rating scale was based on the reaction of the *Hemerocallis* 'Pardon Me', which typically would have most lesions sporulating within 10 to 12 d (Mueller et al., unpublished). Area under disease progress curve (AUDPC) values were calculated in which: X_i = the foliar rating at the i th observation; t_i = time (days between ratings) at the i th observation; and n = total number of observations (Tooley and Grau, 1984). Plants in set 1 were cut off just above the crown after trial 1 was completed, treated with mancozeb at 1.8 g·L⁻¹ (Fore FloXL, Dow AgroSciences, Indianapolis), then transferred to a rust-free polyhouse for 40 d. These plants were then used in the second trial using the same techniques. For the plants from McCorkle's Nursery, a different set of plants was used for the second trial.

Statistical analysis. Data were subjected to analysis of variance using general linear

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Table 1. Foliar reaction of 41 commercial daylily cultivars inoculated with *Puccinia hemerocallidis* in the greenhouse (set 1).

Cultivar	Reaction ^z	Trial 1		Trial 2	
		Rating ^y	AUDPC ^x	Rating ^y	AUDPC ^x
Prairie Blue Eyes	R	1.0	9.0	1.0	9.0
Carolyn Criswell	R	1.0	10.8	1.0	9.0
Mardi Gras Parade	R	1.0	9.0	1.0	11.0
Woodside Ruby	R	1.7	10.7	1.7	12.7
Hush Little Baby	R	1.0	10.5	1.3	13.2
Follow Your Heart	R	1.3	10.2	1.3	15.7
Chicago Apache	R	2.0	18.0	1.0	9.7
Buttered Popcorn	R	1.0	12.0	1.0	16.0
Gordan Biggs	MR	2.0	12.2	2.0	18.3
Texas Sunlight	MR	2.0	17.2	1.7	14.2
Stella de Oro	MR	2.0	16.5	2.0	19.0
Joan Senior	MR	2.3	19.8	2.3	18.2
Ed Murray	MR	3.0	22.7	2.7	18.0
Nanuq	MR	2.0	20.3	2.3	21.3
Daring Deception	MR	2.0	15.3	4.0	26.8
Bertie Ferris	MR	2.0	19.8	3.0	24.2
Baja	MS	3.0	26.8	3.0	26.3
Anzac	MS	3.3	25.3	4.0	27.8
Mary's Gold	MS	2.7	27.3	3.0	26.0
Razzmatazz	MS	2.3	22.5	3.7	31.5
Tropical Reflection	MS	3.0	26.5	4.0	27.7
Advance Party	MS	4.0	28.8	3.3	25.5
Ice Carnival	MS	3.0	24.3	4.0	31.3
Midnight Rambler	MS	3.0	28.3	3.3	27.8
Cherry Cheeks	MS	3.3	31.8	3.0	24.8
Pearl Harbor	MS	2.3	31.3	3.0	26.3
My Melinda	S	4.0	37.2	4.0	29.2
Catherine Neal	S	5.0	38.2	3.7	32.5
Enchanted Rainbow	S	4.3	32.2	5.0	38.5
Siloam Plum Tree	S	5.0	38.8	4.7	36.8
Chorus Line	S	4.7	41.3	4.0	34.8
Along the Way	S	5.0	41.7	5.0	36.0
Barbara Mitchell	S	5.0	41.0	4.3	38.3
Strawberry Candy	S	5.0	42.3	5.0	37.3
Mary Todd	S	5.0	43.5	4.7	37.7
Hyperion	S	5.0	45.0	4.7	36.3
Love Those Eyes	S	5.0	42.8	5.0	41.2
Bright Sunset	S	5.0	42.5	5.0	44.7
Rocket City	S	5.0	44.3	5.0	43.8
Little Grapette	S	5.0	45.0	5.0	44.7
Christmas Carol	S	5.0	45.0	5.0	45.0
Pardon Me (control)	S	5.0	45.0	5.0	45.0
LSD _(0.05)		0.7	4.9	0.6	4.6
cv (%)		14.8	11.0	12.2	10.8

^zR = resistant; MR = moderately resistant; MS = moderately susceptible; and S = susceptible.

^yRating taken 21 d after inoculation using a 1 to 5 rating scale where: 1 = no disease; 2 = reduced lesion number and no sporulation; 3 = reduced lesion number but not sporulation; 4 = slightly reduced lesion number and/or amount of sporulation; and 5 = fully susceptible. This rating scale was based on the reaction of the cultivar 'Pardon Me', which typically would have most lesions sporulating within 10 to 12 d.

^xAUDPC = area under disease progress curve in which: X_i = the foliar rating at the i th observation; t_i = time (days between ratings) at the i th observation; and n = total number of observations.

models procedure (PROC GLM) of SAS (SAS Institute, Cary, N.C.). Trials and replications were considered random variables, while daylily cultivars were considered a fixed variable. Means were separated by Fisher's protected least significant differences (LSD) at $P \leq 0.05$. Cultivar ratings and AUDPC values from trials one and two for each set were compared using Pearson's correlation (PROC CORR) of SAS.

Results and Discussion

The daylily cultivars screened for resistance to *P. hemerocallidis* are listed in Tables 1 and 2.

Trials one and two were positively correlated ($P < 0.0001$) for cultivar rating ($r = 0.87$; $r = 0.76$) and AUDPC values ($r = 0.90$; $r = 0.80$) for set 1 and set 2, respectively. These high correlation coefficients indicate reactions to *P. hemerocallidis* were similar for both trials in each set of cultivars, despite the application of mancozeb 40 d prior to the second trial in set 1. Cultivars were separated into four groups based on their susceptibility to rust. Resistant (R) plants had no or very few lesions. If lesions did occur on the resistant plants, they were restricted by a hypersensitive reaction or did not sporulate (Fig. 1 A and B). Moderately resistant (MR) plants had very few lesions

Table 2. Reaction of 43 commercial daylily cultivars inoculated with *Puccinia hemerocallidis* in the greenhouse (set 2).

Cultivar	Reaction ^z	Trial 1		Trial 2	
		Rating ^y	AUDPC ^x	Rating ^y	AUDPC ^x
Green Flutter	R	1.0	9.0	1.0	10.0
Plum Perfect	R	1.0	10.7	1.3	10.0
Frankly Scarlet	R	1.3	11.5	1.0	10.3
Mama Cha Cha	R	2.7	11.5	2.7	11.2
Chinese Scholar	R	1.3	10.8	1.3	12.0
Charlie Pierce Memorial	R	2.0	19.2	1.3	19.8
Black Eyed Stella	MR	2.0	21.3	2.3	20.5
Nagasaki	MR	2.3	18.7	3.0	24.3
Siloam Double Classic	MR	3.0	18.8	2.3	25.8
Mini Pearl	MR	2.0	23.5	2.7	22.0
Little Business	MR	2.3	19.3	3.7	27.3
Little Sweet Sue	MS	3.7	28.7	3.7	19.5
Susie Wong	MS	3.3	27.2	3.7	23.7
Seeing Red	MS	3.3	26.3	2.7	26.2
Little Heavenly Angel	MS	3.7	26.8	3.3	26.7
Patio Parade	MS	4.0	29.0	3.3	25.0
Witches Wink	MS	2.3	21.0	4.0	34.5
Todd Monroe	MS	3.3	26.3	4.0	29.7
Amy Louise	MS	4.0	29.2	4.0	27.8
Jeune Tom	MS	3.3	27.8	4.0	29.8
Magic Obsession	MS	3.7	30.3	4.0	28.2
Grape Magic	MS	3.7	28.7	4.3	31.7
Becky Lynn	MS	3.7	28.8	4.3	33.3
Judith	S	4.7	31.0	4.7	33.5
Red Rum	S	4.7	30.3	4.3	34.3
Leebea Orange Crush	S	4.3	32.3	4.3	32.8
Cranberry Baby	S	4.0	26.7	4.7	38.8
Little Maggie	S	4.7	35.7	4.0	34.0
Little Grapette	S	4.3	34.3	4.7	38.3
Elegant Candy	S	4.7	39.2	4.7	35.8
Lavender Blush	S	5.0	41.3	4.7	34.2
Night Beacon	S	5.0	37.8	4.7	41.0
Custard Candy	S	5.0	42.0	4.7	39.8
David Kirchhoff	S	5.0	44.7	4.7	38.3
Always Afternoon	S	4.7	39.7	5.0	43.8
Apolodorus	S	5.0	43.2	4.7	40.7
Ancient Glow	S	5.0	43.8	3.7	40.7
Here Comes Sallyann	S	5.0	41.8	5.0	43.0
Tickled Pink	S	5.0	44.7	4.7	40.3
Seducer	S	5.0	44.7	5.0	41.2
Creative Art	S	5.0	45.0	5.0	43.7
Benchmark	S	5.0	45.0	5.0	44.7
Raspberry Candy	S	5.0	45.0	5.0	44.7
Pardon Me (control)	S	5.0	44.7	5.0	45.0
LSD _(0.05)		1.1	7.1	1.0	6.8
cv (%)		18.1	14.7	16.2	13.8

^{z, y, x}See footnotes for Table 1.

compared to 'Pardon Me' plants but produced some urediniospores. Moderately susceptible (MS) plants had slightly reduced lesion number or delayed sporulation compared to 'Pardon Me' plants. Susceptible plants were similar to 'Pardon Me' in number of lesions and level of sporulation (Fig. 1E). Of the 41 cultivars in set 1, eight (20%) were classified as R; eight (20%) were classified as MR; 10 (24%) were classified as MS; and 15 (36%) were classified as S (Table 1). Of the 43 cultivars in set 2, six (14%) were classified as R; five (12%) were classified as MR; 12 (28%) were classified as MS; and 20 (46%) were classified as S (Table 2). Combined, 32% of the cultivars were either R or MR. Data on flower color and size, foliage type (dormant vs. evergreen), and ploidy level were collected for each cultivar, but none of these plant traits exhibited a significant correlation with rust resistance (data not presented).

Different reactions to daylily rust have been reported previously (Williams-Woodward and

Fig. 1. (A) Small lesions that did not sporulate ('Buttered Popcorn', resistant); (B) larger lesions that did not sporulate ('Nanuq', moderately resistant); (C) very large yellow lesions that did not sporulate until 18 d after inoculation, if at all ('Ice Carnival', moderately susceptible); (D) large yellow-orange lesions that sporulated 12–14 d after inoculation ('Lavender Blush', susceptible); (E) bright yellow-orange lesions ('Pardon Me', susceptible); and (F) lesions on scape.

Buck, 2001). Symptoms on the foliage of 'Pardon Me' were described as bright yellow spots to streaks, while symptoms on 'Gordon Condon' were described as water-soaked tan spots with darker borders (Williams-Woodward and Buck, 2001). In our studies, we also observed a wide variety of symptoms. For example, 'Pardon Me' produced small bright yellow spots within 4 d and these spots turned into sporulating lesions within 8 to 12 d after inoculation (Fig. 1E). Cultivars like 'Ice Carnival', 'Mini Pearl', and 'Seeing Red' produced large, dull yellow spots that did not start sporulating until 18 d after inoculation or later (Fig. 1C). However, cultivars like 'Lavender Blush' had large yellow lesions that started sporulating as early as 12 to 14 d after inoculation (Fig. 1D). A few cultivars like 'Buttered Popcorn', 'Charlie

Pierce Memorial', 'Follow Your Heart', and 'Gordon Biggs' had very few restricted lesions produced on the lowest leaves; however, these lesions did not sporulate (Fig. 1 A and B). On some cultivars, such as 'Here Comes Sallyann' and 'Bright Sunset', lesions were formed on the scape as well as the leaves (Fig. 1F).

Currently, there are no data indicating the presence of different races of *P. hemerocallidis*. Hernandez et al. (2002) compared the internal transcribed spacer (ITS) region of *P. hemerocallidis* isolates from different geographical areas in the United States to isolates from Asia. The variation in the ITS sequences between Asia and the United States and within each geographical area indicated that the specimens are the same species. In our study, we used *P. hemerocallidis* from a single source. Resistant

and susceptible cultivars identified in our study could be used to assess a collection of *P. hemerocallidis* for genetic differences.

Fungicides such as azoxystrobin (Heritage, Syngenta Crop Protection, Greensboro, N.C.) and chlorothalonil (e.g., Daconil Ultrex, Syngenta Crop Protection) have been shown to reduce *P. hemerocallidis* urediospore germination (Buck and Williams-Woodward, 2001) and lesion development (Buck and Williams-Woodward, 2003). However, control measures, such as fungicides, have dramatically increased daylily production costs. The use of resistant cultivars would reduce production costs by eliminating, reducing, or delaying rust infection. Furthermore, cultivars with resistance provide nurseries and enthusiasts with an environmentally safe option for controlling

daylily rust and may be used by breeders to develop future cultivars.

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