A Variety of Collecephalus hemerocalli Pathogenic on Hemerocallis

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ABSTRACT

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A fungal isolate (DAR), obtained from daylily plants grown in Maryland was compared with *Collecephalus hemerocalli*. Notable differences existed between the two fungi in the amount of growth and degree of pigmentation on three culture media at six temperature levels. Differences in growth of the two fungi were obtained at both 12 and 32 C. Conidia of DAR were significantly greater in length and width than those of *C. hemerocalli*. Isolate DAR was consistently less pathogenic than *C. hemerocalli* and separation of the two was determined by responses of 10 daylily cultivars. Since these fungi differ significantly in morphology and pathogenicity, DAR is considered a distinct variety of *C. hemerocalli*. It is described as *C. hemerocalli* var. *macrosporum* n. var.

Additional key words: Hemerocallis, leaf-streak.

The occurrence of leaf-streak on daylily (*Hemerocallis* spp.) in Mississippi and a description of the causal fungus, *Collecephalus hemerocalli* J. A. Spencer, have been reported (2, 3, 4). However, the cultural characteristics of an isolate obtained from diseased plants in Maryland differed greatly from those of the Mississippi fungus (1). Mycelium of the Maryland isolate (DAR) was mostly black whereas the Mississippi fungus produced light cream to white mycelial growth. Isolate DAR proved to be pathogenic on daylily. It was considered advisable to determine whether DAR was a variant, a distinct race, or a new species.

MATERIALS AND METHODS

Initial cultures of both isolates were made from conidia from single conidiophore heads. Subsequent cultures, which provided inoculum for the various experiments, were maintained on Difco potato-dextrose agar (PDA) at 24 C. *C. hemerocalli* and DAR were grown on PDA, Sabouraud-dextrose agar (SDA), and bean pod agar (BPA) at six temperatures ranging from 12 to 32 C in 4 C increments. Each isolate was replicated seven times at each temperature, and the test was repeated four times. Each replication received a 5-mm diameter mycelial plug as inoculum. After 7 days, the radial growth was measured and an average was obtained.

Twenty-four daylily cultivars were used. Three plants of each cultivar were treated as follows: one was a control, one was inoculated with *C. hemerocalli,* and the other with DAR. The plants were maintained in 15-cm diameter clay pots in the greenhouse, fertilized every 4 months with one 6-g Agriform tablet (Agriform International Chemicals, Inc., Newark, California) per container and were watered at least twice daily.

Inoculum suspensions were produced by adding 25 ml of a 0.1% aqueous solution of Tween-20 (polyoxyethylene sorbitan monolaurate) to two plate cultures for each isolate and dislodging the spores with a flamed glass rod. Concentrations of the resultant spore suspensions were measured with a hemacytometer and adjusted to a concentration of 5×10^6 spores/ml. To enhance infection, the leaves were wounded prior to inoculation. To standardize the wounding, two pieces of stainless steel (3.8×7.7 cm) were joined with hinges and a 3-mm-thick foam pad was glued to the lower piece. Three 0.5-cm diameter holes were drilled 2 cm apart in the upper piece. Six No. E-80 insect pins were embedded in a cork, with 1 cm of the points exposed. A leaf was placed on the pad between the metal halves, and the pins were pressed through the drilled holes and into the leaf. Six leaves of each plant were wounded and each wound received one drop of inoculum. The controls were also wounded, but received a drop of the Tween-20 solution without spores. The plants were placed in a mist chamber for 65 hours, then transferred to a greenhouse bench for 18 days. The greenhouse temperature ranged from 22 to 33 C from February through August.

After 18 days, diseased leaves were collected and rated on a scale of 1 to 6. The scale numbers and associated symptoms were: 1, minimum disease, water-soaking around wounds, slight necrosis, no coalescing spots (Fig. 1-A); 2, necrotic wounds, coalescing of up to two necrotic areas (1.0-1.5 cm in diameter), less than five water-soaked streaks between wound areas (Fig. 1-B); 3, necrotic wounds, coalescing to form three large spots, more than five water-soaked streaks (Fig. 1-C); 4, necrotic wound areas coalesced to form three large spots, necrotic streaks between two wound areas (Fig. 1-D); 5, all wound areas necrotic and connected by necrotic streaks (Fig. 1-E); and 6, all wound areas necrotic, with necrotic streaks extending to leaf tip or length of leaf (Fig. 1-F).

Wet mounts for conidial and conidiophore measurements were taken from week-old cultures on PDA. The length and width of 500 conidia (×1,000) and 100 conidiophores (×450) were measured. Conidiophores were measured from the point of origin at the hypha to the tip, and included the enlarged terminal cell.

Black sclerotia-like bodies were produced on autoclaved daylily leaves placed on water agar and incubated in darkness at 25 C. These bodies appeared 5-7 days after inoculation and covered the leaf at the end of 1 month. Radial measurements (×100) of 100 bodies on leaf tissue were made in two directions. The height of 50 bodies was determined by measuring free-hand sections (×100).

RESULTS

Growth temperature.—The optimum temperature for growth of both fungi on PDA was 24 C. The Maryland isolate (DAR) produced significantly greater radial growth than did *C. hemerocalli* at all temperatures (Table 1). On SDA, DAR was distinguishable from *C. hemerocalli* by significant differences in radial growth at 12 and 32 C (Table 1). On BPA, DAR differed from *C. hemerocalli* at all temperatures (Table 1); DAR hyphae consistently produced dark pigments, whereas those of *C. hemerocalli* remained light colored (Fig. 2).

Pathogenicity.—Ten of 24 cultivars showed distinct

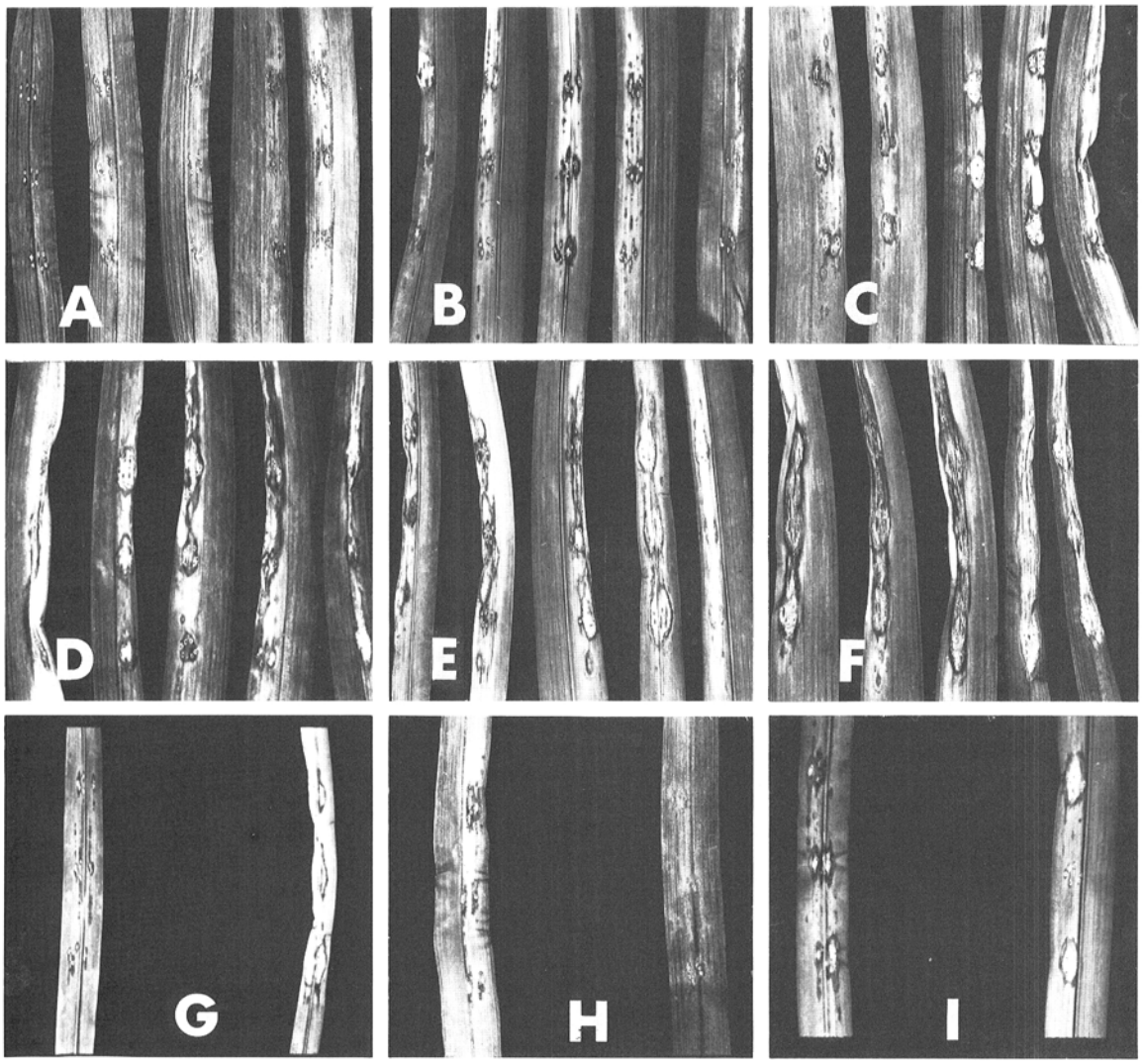

Fig. 1. A-I. Pathogenesis of *Collecephalus hemerocalli* and *C. hemerocalli* var. *macrosporum* on daylily (*Hemerocallis* sp.) **A-F)** Disease rating scales of 1-6. **G-I)** Cultivar reactions to *C. hemerocallis* (left) and *C. hemerocalli* var. *macrosporum* (DAR) (right) on cultivars **G)** Boutonniere, **H)** Iris Lady Lawrence, **I)** Little Cherub.

differential responses to the two fungi (Table 2). Seven were more susceptible to *C. hemerocalli,* but the other three were more susceptible to DAR. Differences in susceptibility and resistance were broad: the range in the disease ratings was 1.2 to 5.0 for cultivars inoculated with isolate DAR, and 1.4 to 5.4 for cultivars inoculated with *C. hemerocalli.* Within the 10 cultivars which showed differential responses, cultivars Boutonniere, Iris Lady Lawrence, and Little Cherub were selected to clearly separate these fungi. Boutonniere was relatively resistant (rating 1.4) to *C. hemerocalli* but susceptible (rating 3.7) to DAR (Fig. 1-G), Iris Lady Lawrence was susceptible (rating 2.9) to *C. hemerocalli* but resistant (rating 1.2) to DAR (Fig. 1-H). Little Cherub was susceptible (rating 3.1) to *C. hemerocalli* but resistant (rating 1.7) to isolate

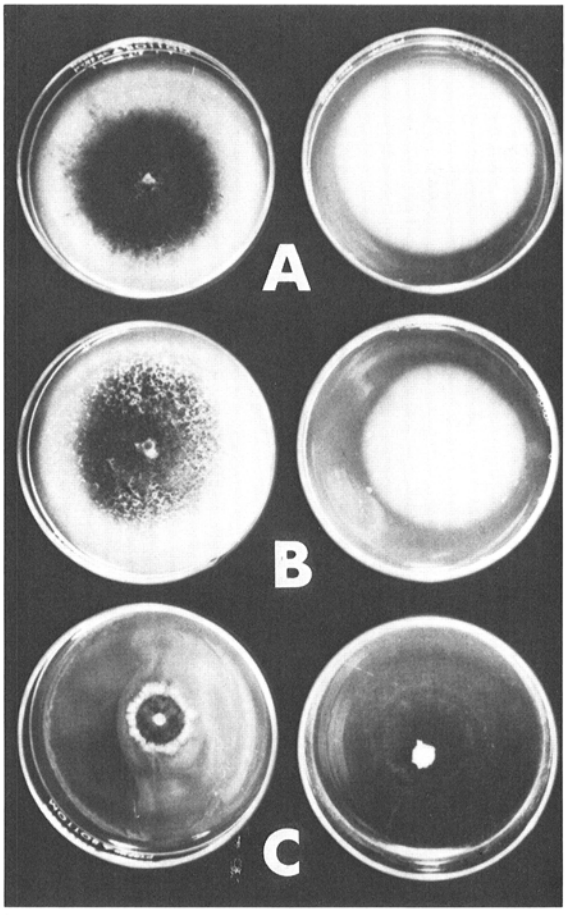

Fig. 2-(A to C). Radial growth of fungus isolate DAR (left) and *Collecephalus hemerocalli* (right) on potato-dextrose agar at **A)** 24 C, **B)** 28 C, and **C)** 32 C.

TABLE 2. Disease scale ratings[y] for *Collecephalus hemerocalli* and isolate DAR on 24 daylily (*Hemerocallis* sp.) cultivars

Hemerocallis sp. cultivar	Disease rating[y]	
	C. hemerocalli	Isolate DAR
Little Cherub	3.1 a[z]	1.7 b
Whirley Bird	3.6 a	2.2 b
Queen of Gonzales	2.2 a	1.9 a
Dutchess of Windsor	2.3 a	2.8 a
Gannymede	2.9 a	2.1 a
Helene	3.1 a	2.5 a
Picture	4.6 a	3.4 b
Granada	2.9 a	2.1 b
Babette	3.4 a	3.1 b
Morning	5.4 a	4.4 b
Queen of Dallas	3.5 a	2.9 a
Dancing High	4.0 b	5.0 a
Purple Waters	3.0 a	2.2 b
Gold Cargo	2.7 a	3.1 a
Armada	3.4 a	3.9 a
Iris Lady Lawrence	2.9 a	1.2 b
Salmon Rose	5.0 a	4.8 a
Princess	2.4 a	2.6 a
Boutonniere	1.4 b	3.7 a
Vulcan	3.4 b	3.7 a
The Sultan	3.2 a	3.3 a
Gay Troubadour	3.9 a	3.0 a
Plum Mist	4.3 a	3.8 a
Purple Sage	4.2 a	3.7 a

[y]1 = minimum disease, water-soaked wounds, slight necrosis; 2 = necrotic wounds, coalescing of up to two necrotic areas; 3 = necrotic wounds, coalescing to form three large spots, five or more water-soaked streaks; 4 = wounds coalesced to form three large spots, necrotic streaks between two wound areas; 5 = wounds necrotic and connected by necrotic streaks; 6 = wounds necrotic with necrotic streaks extending to leaf tip or length of leaf.

[z]Duncan's multiple range test, $P = 0.05$. Treatments within each row having the same letter are not significantly different.

TABLE 1. Radial growth (mm) of *Collecephalus hemerocalli* and fungal isolate DAR from daylily incubated for 7 days on potato-dextrose agar (PDA), Sabouraud-dextrose agar (DSA), and bean pod agar (BPA)

Medium	Fungus	Radial colony growth (mm)					
		12 C	16 C	20 C	24 C	28 C	32 C
PDA	*C. hemerocalli*	22.3 b[z]	37.7 b	52.6 b	61.0 b	40.5 b	9.8 b
	isolate DAR	31.8 a	46.7 a	68.4 a	82.4 a	78.1 a	27.6 a
SDA	*C. hemerocalli*	24.3 a	29.9 a	50.3 a	68.8 a	69.4 a	8.9 b
	isolate DAR	18.4 b	30.7 a	49.6 a	64.3 a	68.4 a	19.6 a
BPA	*C. hemerocalli*	15.8 b	22.3 b	38.4 b	48.7 b	47.2 b	9.8 b
	isolate DAR	23.9 a	32.4 a	47.7 a	57.4 a	57.3 a	23.9 a

[z]Duncan's multiple range test, $P = 0.05$. Comparisons within each medium having the same letter are not significantly different.

DAR (Fig. 1-I).

Morphology.—Conidia of isolate DAR were significantly longer and wider (average dimensions 9.4 × 3.2 μm) than those of *C. hemerocalli* (average dimensions 5.8 × 2.3 μm). Measurements of conidiophores of the two fungi were not significantly different. The average diameter (379 μm) of the sclerotia-like bodies of *C. hemerocalli* was significantly greater than that (306 μm) of DAR, but there was no difference in measurements of height.

A second isolate (DAR-2) from Maryland later was compared with the original isolate (DAR). Close similarities were observed in pathogenicity tests on selected differential hosts, in appearance in growth-temperature studies, and measurements of conidia of DAR-2 agreed with those of DAR.

DISCUSSION

The primary objective in this study was to determine whether two species, races, or varieties of the fungus were present. Evidence was obtained for separating the two fungi based on significant differences in growth on different media and at a range of temperatures as well as on differences in general appearance. Separation by plant responses can be made with the cultivars Boutonniere, Iris Lady Lawrence, and Little Cherub. Inoculation of Boutonniere with DAR produced a disease rating mean of 3.7 which showed susceptibility. On the other hand, inoculations of the same cultivar with *C. hemerocalli* produced a rating mean of 1.4 which showed resistance to *C. hemerocalli*. With Iris Lady Lawrence and Little Cherub the reverse disease reactions were obtained. Morphologically, these fungi were separated by significant differences in length and width of conidia and in diameter of the sclerotia-like bodies. Because of differences in pathogenicity, cultural characteristics, and morphology DAR is described as a variety of *C. hemerocalli* as follows:

Collecephalus hemerocalli var. *macrosporum* n. var. Mycelium 2.6 - 10.6 μm (medium 5.2 μm) μm diameter, niger tinctum; conidia 5-20 × 2-6 μm (medium 9.4 × 3.2 μm), leniter tincta, in mass a cremea, glabra, unicellularia suballantoidea vel cylindrica, in capitulis oedocephaloideis 29.3 - 112.5 × 4.3-6.5 μm (medium 66.3 × 5.3 μm) constitutis enata. Sclerotialia 100 - 470 μm (medium 306 μm) latis × 110-190 μm (medium 166 μm) altis.

Holotype.—Dried culture, DAR, isolated from diseased leaves of *Hemerocallis* sp. from Glenn Dale, Maryland, USA; 1 June 1970, has been deposited as Culture No. 71856 in the National Fungus Collections, Beltsville, Maryland.

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