

**Leaf-Streak of Daylily:
Infection, Disease Development, and Pathological Histology**

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ABSTRACT

Infection of daylily plants was obtained when spore suspensions of *Collecephalus hemerocalli* were applied to wounded leaves and to nonwounded leaves, where the fungus gained entrance through stomates and by direct penetration of the leaf surface. At 23 to 28 C with adequate moisture, disease development was extensive and the fungus grew throughout affected leaves.

Sporulation was heavy under favorable conditions and dissemination of inoculum was promoted naturally by splashing water and leaf movement. The fungus persisted through unfavorable environmental conditions as thickened, pigmented mycelium or as hard, black, sclerotialike bodies within dead leaves.

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The occurrence of leaf-streak on daylily (*Hemerocallis* spp.) and a description of the causal fungus, *Collecephalus hemerocalli* have been reported (2, 3). No attempt was made at that time to present detailed information on the infection process, disease development, and pathological histology of the disease. This paper reports subsequent investigations in these areas.

MATERIALS AND METHODS.—Plants growing out of doors were maintained in 3.8-liter nursery cans, heeled-in with sawdust, and fertilized every 4 months with one 6 g Agriform tablet (Agriform International Chemicals, Inc., Newark, California) per container. During prolonged dry periods, the plants were irrigated by a sprinkler system. Four plants of the varieties 'Plum Mist', 'Salmon Rose', 'Dancing High', 'Vulcan', and 'Boutonniere' were maintained outdoors, and three plants of each were maintained indoors for test purposes. Greenhouse plants were maintained in 15-cm clay pots, fertilized as above, and, depending upon moisture conditions, watered at least twice each day.

Inoculation of plants in the greenhouse was accomplished by wounding the leaves with the points (0.5 mm exposed) of six straight pins placed in a cork and then spraying the leaves with a spore suspension. Inoculated plants were moved to a mist chamber for 48 hr then transferred to a greenhouse bench for observation.

Fungus cultures for all experiments were maintained on potato-dextrose agar (PDA) at 24 C. Inoculum was prepared by adding sterile distilled water to one 7-day-old culture on PDA in a dish, dislodging the spores with a flamed glass rod, and bringing the volume up to 200 ml.

To study the methods of natural penetration, small moist chambers were made using plastic petri dishes. Openings (ca. 2.54-cm wide) were cut on opposite sides of the dishes so that a leaf could be sandwiched between the upper and lower halves. Moistened filter paper was placed on the inside flat surface of the upper and lower plate halves and moist

cotton was placed around the leaf at the openings to maintain a high humidity. Circles (ca. 0.5-cm diam) were drawn with India ink on the leaves of a potted plant, one drop (from a 30-ml bottle dropper) of inoculum was placed within the circle, and the plate halves were secured with a clamp attached to a ring stand.

After 30-hr incubation in the dishes, the treated sections of leaves were removed, killed in Formalin-acetic acid-alcohol, dehydrated by using the tertiary butyl alcohol series (1) and embedded in Tissuemat, 52.5 C (Fisher Scientific Co., Pittsburgh, Pa.). Tissue sections were cut 10- to 15- μ thick and stained with safranin-fast green. Samples from plants inoculated in the greenhouse were obtained after symptoms developed and subsequent treatment of this material was the same as that listed above.

RESULTS.—*Infection process.*—In the greenhouse, preliminary attempts to obtain infection by spraying spore suspensions on nonwounded leaves failed. When the leaves were wounded prior to spraying, infection occurred. Secondary infection, however, was noted in many instances on nonwounded leaves of the same plant within 4 weeks after the plants were removed from the mist chamber. Generally, initial symptoms of water-soaked tissue around the wounds were readily visible at the time plants were removed from the mist chamber. Further development of symptoms increased rapidly as the lesions enlarged, turned tan to brown, and frequently coalesced and elongated to form necrotic streaks that often progressed to the leaf tip (Fig. 1).

Sections of 15 intact, nonwounded, inoculated leaves showed only two instances of direct penetration by a spore germ tube (Fig. 2). No evident specialized penetration structures were noted. Many spores were observed on the cuticle surface and these appeared to be budding. Spores also were observed within stomatal depressions. It was difficult to determine whether some were germinating by germ tube or simply producing bud cells. In some instances (Fig. 3), however, apparently a few spores

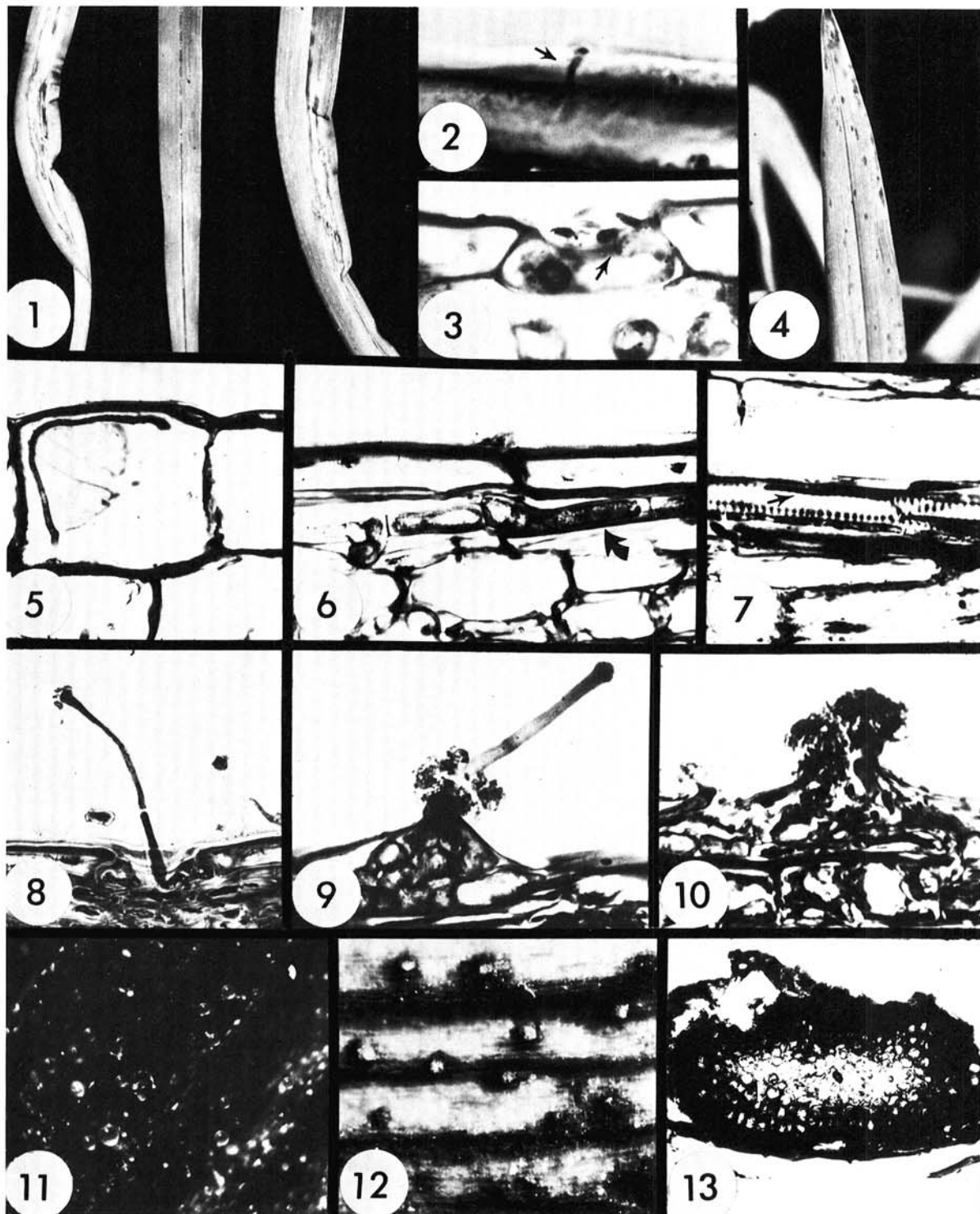


Fig. 1-13. 1) Leaf-streak symptoms produced by *Collecephalus hemerocalli* on daylily 10 days after wounding leaves and inoculating with a spore suspension. 2) Spore germ tube (arrow) penetrating leaf directly. 3) Spore germ tube (arrow) growing into stomatal opening. 4) Water-soaked type symptoms on young leaf. 5) Mycelium following inner contour of epidermal cell. 6) Enlarged mycelium (arrow) in mesophyll cells. 7) Darkly stained mycelium (arrow) within xylem vessel element. 8) Conidiophore growing out of stomate. 9) Conidiophore produced from massed mycelium has emerged through ruptured epidermis. 10) Multiconidiophore production from mycelial mass; small conidiophores produce some conidia prior to rupture of epidermis. 11) Portion of diseased leaf showing conidiophores with terminal slime droplet in which conidia are held. 12) Black bodies with apex exposed above epidermis show a white, crusty spore mass. 13) Sectioned sclerotialike body showing rounded internal cells that are surrounded by a dark layer of cells.

were producing germ tubes which were beginning to grow into the stomatal openings.

Disease development and pathological histology.—Leaves of plants in outdoor plots showed initial symptoms of the disease (Fig. 4) soon after they emerged in late February. Warm temperatures and high relative humidity appeared to be essential for infection and rapid and extensive development of the disease. The mycelium was easily observed in epidermal and mesophyll cells (Fig. 5, 6) and later was found in xylem vessel elements (Fig. 7). As the cells became degraded, and in the presence of sufficiently high humidity, the mycelium aggregated and enlarged in the epidermal cells. Conidiophores were initiated from this massed mycelium and grew out through stomatal openings or burst through the epidermis (Fig. 8, 9, 10). One to six conidiophores emerged from each site. Some conidiophores extended above the leaf surface and produced numerous conidia in slim droplets (Fig. 11). Other conidiophores began to produce conidia before they were fully emerged and the spores were liberated as the conidiophores burst through the epidermis. Conidiophores were observed on both leaf surfaces. Spread of the disease was accomplished through transfer of conidia by splashing rain, sprinkler irrigation, and rubbing together of wind-blown leaves. Under favorable conditions, the disease often developed on some cultivars to the extent that up to 80% of the leaves were affected to varying degrees. During drought conditions, spread of the disease was suppressed until sufficient moisture again was available.

As the older leaves entered senescence or as they were killed by the disease, black sclerotialike bodies appeared subepidermally (Fig. 12). These structures were small and easily overlooked during dry conditions. Sectioned bodies revealed a mass of somewhat rounded cells in the interior (Fig. 13). The body wall was composed of a layer of two to four dark cells. No evidence of spore initiation or production was observed in these sections. Occasionally, crusty spore extrusions were observed on the emerged surface of the black bodies, but these could not be identified and their origin (method of

production) is unknown. These bodies, along with thickened, pigmented mycelium within dead leaves served to preserve the fungus during times of moisture stress and overwinter. Dead leaves collected from October to February and placed in moist chambers produced conidiophores with conidia from which the fungus could be isolated throughout the winter. Conidiophores often were observed on the sclerotialike bodies.

DISCUSSION.—The investigation showed that infection and progressive disease development were, as with many other organisms, dependent on an adequate supply of moisture. During rainy periods or with sprinkler irrigation, the amount of infection was increased and development of symptoms was enhanced. This observation suggests that wider spacing of plants and elimination of overhead irrigation will aid in controlling the disease by permitting plant parts to dry out quicker after rains as well as reduce humidity around the plants by increasing air movements. There was limited evidence of direct penetration of leaves by the fungus which could rule out requirements for natural wounding of leaves. After the fungus had entered the host, there were apparently no barriers to its spread since mycelium was found throughout the leaf. Production of the sclerotialike bodies possibly is triggered by stress on the organism in the dead leaves or by reduced light intensity brought about by the shading effect of overhanging leaves. Spores apparently released from these bodies are believed to be produced only under suitable moisture and temperature conditions, and, once they are initiated, the maturation process occurs rapidly.

LITERATURE CITED

1. JOHANSEN, D. A. 1940. Plant microtechnique. McGraw-Hill Book Co., Inc. New York. 523 p.
2. SPENCER, J. A. 1968. A new leaf-streak disease of *Hemerocallis* spp. Plant Dis. Repr. 52:751-753.
3. SPENCER, J. A. 1972. *Collecephalus hemerocalli*, the cause of daylily leaf-streak: morphology, taxonomy, and cultural characteristics. *Hemerocallis J.* 26:12-16.