A Zonal Leaf Spot of Waterhyacinth Caused by Cephalosporium Zonatum[1]

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INTRODUCTION

In August of 1971 the author found a zonal leaf spot occurring on waterhyacinth (*Eichornia crassipes* (Mart.) Solms.) at the Spring Bayou Area of central Louisiana and shortly thereafter at Newman's Lake in north-central Florida. Isolations from both areas produced an identical fungus, subsequently identified as a *Cephalosporium* sp. The only other fungus reported on waterhyacinth in the United States is *Fusarium roseum* (6).

The first report of a zonal leaf spot of waterhyacinth was by Padwick from India in 1946 (5). He attributed the cause to a new fungus, naming it *Cephalosporium eichhorniae* Padwick. Tims and Olive (9) reported a zonal leaf spot of fig (*Ficus carica* var. Celeste) in southern Louiciana 2 years later and, apparently unaware of Padwick's description, described the causal agent as a new fungus, *C. fici* Tims & Olive. Zonal leaf spots attributed to *C. eichhorniae* have been reported on coffee (*Coffea arabica*), ramie (*Boehmeria nivea*), and *Croton dispar* in Sierra Leone (2, 3). Other workers have attributed zonal leaf spots on coffee (*C. arabica, C. liberica, C. robusta*) in India (4) and Costa Rica (8) and on ramie and *Luffa* sp. in Nigeria (1) to *C. zonatum* Sawada. This latter fungus is considered by the Commonwealth Mycological Institute to be identical to both *C. eichhorniae* and *C. fici* and to be the preferred name (4, and personal communication). Some Indian workers disagree and consider at least *C. eichhorniae* and *C. fici* to be distinct species (7). It is the purpose of this paper to further describe the fungus, here to be considered as *C. zonatum*, and to further explore its supposed synonomy with *C. eichhorniae* and *C. fici*.

SYMPTOMS

The disease is first evident as small sunken lesions on both leaf surfaces and the petiole. Infected areas gradually enlarge and coalesce, becoming distinctly zonate with light brown bands alternating with narrower dark brown bands (Fig. 1, A). Lesions are oval to irregular in shape with the surfaces often covered with a weft of cottony mycelium. Under humid conditions, the hyphae spread out several millimeters in advance of the lesion, producing phialides and spore heads over both lesion surfaces. The lesions continue to enlarge until the leaf dies or conditions ensue unfavorable for disease development.

ISOLATION AND MAINTENANCE

Cephalosporium zonatum can be easily isolated from infected tissues by streaking conidia onto Petri plates containing water agar and transferring after germination. Infected tissues on which the fungus is not sporulating can be cut into small pieces, surface sterilized for 5 to 7 min in a 1% sodium hypochlorite solution, and transferred to plates of potato dextrose agar (PDA). After several days, small white colonies will grow out of the tissues and hyphal tips can be transferred to other plates. Cultures used in this study were maintained at room temperature on plates containing PDYA (PDA plus 0.5% yeast extract) and were transferred at 2-month intervals.

MORPHOLOGY AND CULTURE CHARACTERISTICS

Cephalosporium zonatum produces a well developed aerial mycelium, hyaline in color. Slender, tapering phialides arise directly from the vegetative hyphae or from funiculose strands of hyphae. The phialides are borne singly or, less frequently, are branched (Fig. 1, B and C). Oval, uni-cellular conidia are produced singly from the apex of the phialides and collect in mucilaginous heads. Conidia average 4.3 by 7 μ (3-5.2 by 4.5-9 μ) while conidial heads may reach 50 μ in diameter.

At room temperature, *C. zonatum* grows slowly even on rich media and usually does not cover the entire surface of the plate. The periphery of the colony is slightly irregular in outline and sporulation occurs after linear growth ceases. Sporulation can be induced by exposing cultures to 12 hr of fluorescent light daily for about 7 days. On PDA, growth is cottony with abundant aerial mycelia, the center of the colony being raised to 5 mm. Zonations are not evident unless growth occurs under strong light. The colony is white above, becoming slightly tan with age and sporulation, but with no pink coloration. It is dark yellow-brown below with conspicuous foldings. Phialides are produced on funiculose hyphae. On CzapekDox agar, growth is cottony with thick aerial mycelia. Cultures are off-white to buff above, with no pink coloration, and cream to dark brown below with marked foldings. Drops of exudate often occur toward the center of the colony. On corn meal agar, growth is sparse with evident zonations and little aerial mycelia. Small aerial tufts, 1 to 2 mm diameter, occur on some cultures in an annular pattern. Sporulation occurs early, without exposure to light, but is sparse. Colonies are grey-white.

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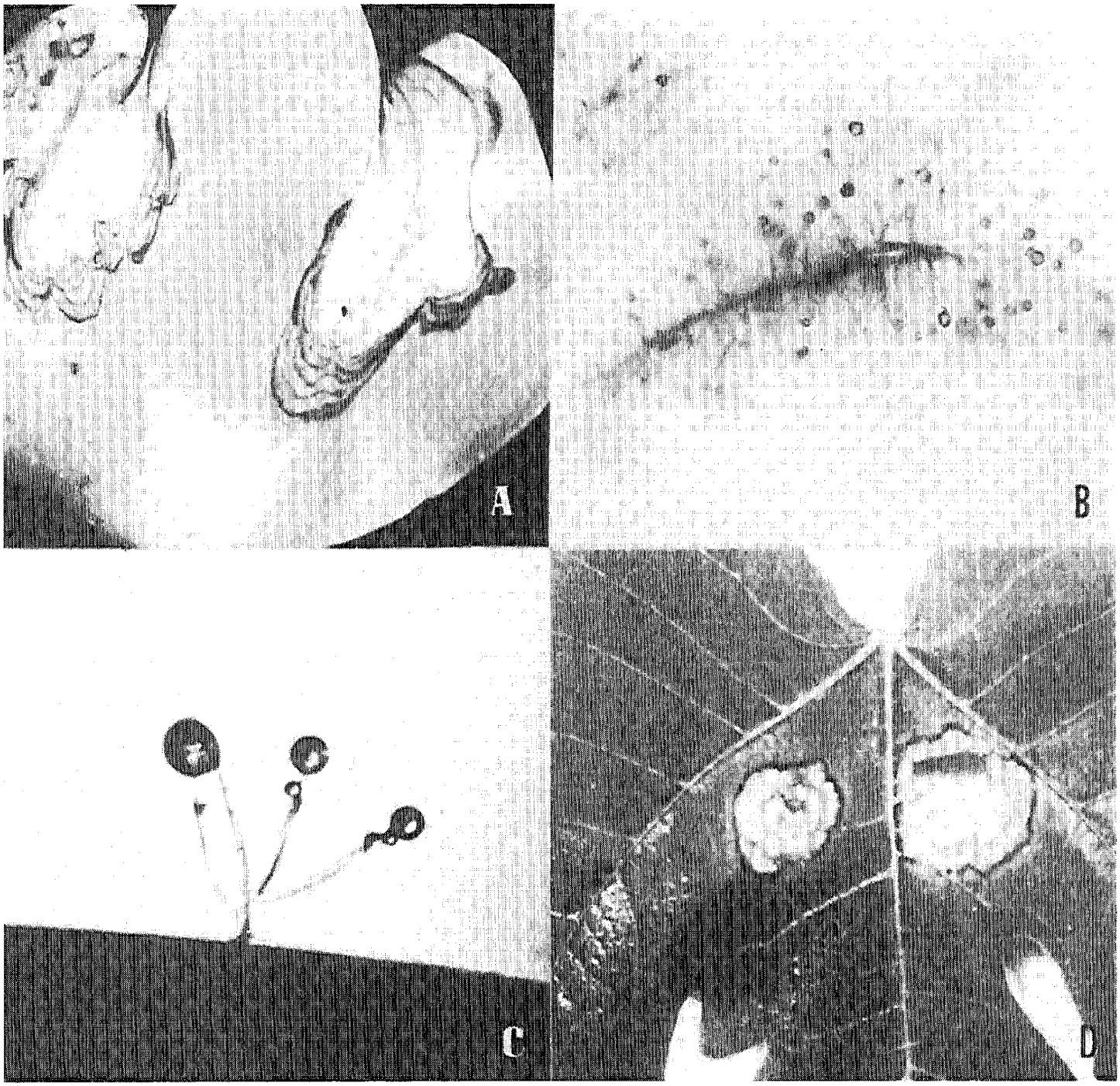

Figure 1. Symptoms and hyphal characteristics of *Cephalosporium zonatum*. A, waterhyacinth leaf after artificial inoculation; B, funiculose hyphae bearing phialides and conidial heads on potato-dextrose-yeast agar; C, branched phialide with conidial heads; D, fig leaf after artificial inoculation.

PATHOGENICITY

Conidia of *C. zonatum* were collected from 4-week-old cultures which had been exposed to 12 hr of fluorescent light daily for 7 days. Conidia were suspended in a spreader-sticker and sprayed onto both surfaces of waterhyacinth leaves. The plants were grown in 15 cm clay pots lined with plastic bags and, after inoculation, were covered with plastic bags and incubated at 25 C and 12 hr of fluorescent light daily for 14 days. The plastic bags were removed at 2, 4, and 14 day intervals. After 5 days, small oval lesions were visible on the leaves of all the plants, but after 14 days those leaves which had been under plastic for the full 14 days had much larger lesions. In addition, sporulation was evident on these leaves while those leaves under plastic for 2 and 4 days had no surface mycelium. Thus, high humidity is a requirement for growth and sporulation of the fungus on the host.

To determine the growth optimum of the fungus in culture and on the host, two methods were used. For the

former, equal portions of the fungus were placed on Petri plates containing PDYA and incubated for 28 days at 15, 20, 25, 30, and 35 C. Colony diameters were then measured. For studying growth on the host, agar blocks 5 mm sq were cut from 3-week-old cultures and placed on the upper surface of waterhyacinth leaves. Plants were covered with plastic bags and incubated at 20, 25, and 30 C and 12 hr of fluorescent light daily for 14 days. After incubation, lesion diameters were measured. The data (Figure 2) show that growth in culture correlates closely with growth on the host, the optimum for both being 25 C.

HOST RANGE

Cephalosporium zonatum is currently being screened as a potential agent for biological control of the waterhyacinth and it is, therefore, necessary to know its capacity for infecting a wide range of plants. To determine this, plants representing 12 families were grown in 10 cm clay pots, 2 to 3 plants per pot, and were inoculated 3 to 6 weeks after germination. Exceptions were *E. crassipes*, *F. carica* var. Celeste, and *Pontedaria lanceolata* which were vigorously growing when obtained and had leaves from a week to a few months old. Four leaves on each plant were inoculated by placing an agar block 5 mm sq on the upper surfaces. Blocks were cut from 3-week-old cultures grown on PDYA and control plants received agar blocks without mycelia cut from the same plate. Each pot of plants was covered with a plastic bag and incubated at 25 C and 12 hr of fluorescent light daily for 7 days. To further substantiate the pathogenicity of *C. zonatum* to fig, leaves of this plant were sprayed with a conidial suspension from 4-week-old cultures. The leaves were covered with plastic bags and incubated as above. Results from this study indicate that *C. zonatum* can infect a wide range of plants, cotton being the notable exception (Table 1.) The pathogenicity of the fungus to members of the Cucurbitaceae, Euphorbiaceae, and Moraceae (Fig. 1, D) agrees well with previous reports of its pathogenicity to *Luffa* sp. (1) Cucurbitaceae), *Croton dispar* (3) (Euphorbiaceae), and ramie (3) (Urticaceae, close relatives of the Moraceae). Yet, despite this wide host range, reports of its occurrance on these hosts in North America are not known, except for fig. Thus, on these grounds the fungus need not necessarily be excluded from consideration as a possible biocontrol agent of waterhyacinth.

TABLE 1. REACTION OF VARIOUS PLANTS TO *C. zonatum*.

Family and binomial of test plant	Reaction[a]
Amaryllidaceae	
Allium Cepa L.	**
Chenopodiaceae	
Beta vulgaris L.	**
Chenopodium album L.	**
Cruciferae	
Raphanus sativus L.	**
Cucurbitaceae	
Citrullus vulgaris Schrad.	**
Cucurbita pepo Mill.	**
Euphorbiaceae	
Ricinus communis L.	**
Gramineae	
Zea mays L.	**
Haloragaceae	
Myriophyllum brasiliense Cambess.	*
Leguminosae	
Phaseolus vulgaris L.	**
Vigna unguiculata (L) Walp.	**
Malvaceae	
Gossypium hirsutum L.	--
Moraceae	
Ficus carica L. var. Celeste	**[b]
Pontederiaceae	
Eichhornia crassipes (Mart) Solms.	**
Pontederia lanceolata Nutt.	**
Solanaceae	
Lycopersicon esculentum Mill.	**
Nicotiana tabacum L.	**

[a]** = susceptible, * = moderately susceptible, -- = resistant.
[b]Also susceptible when inoculated with a conidial suspension.

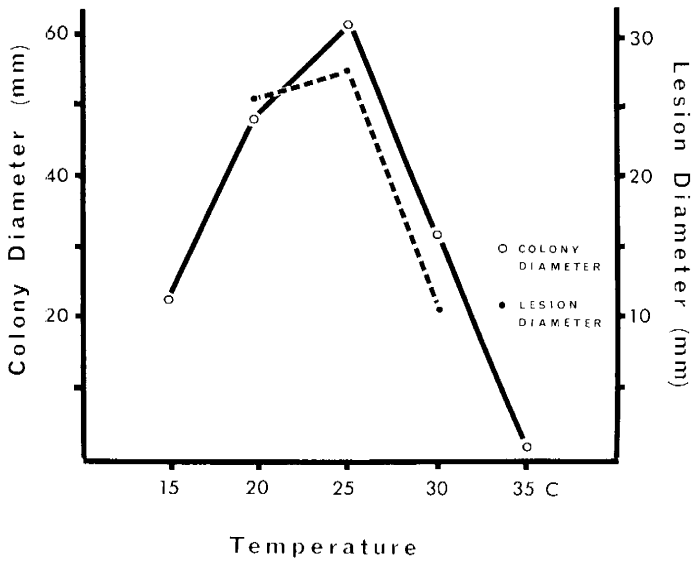

Figure 2. Comparative growth rates of *C. zonatum* on potato-dextrose-yeast agar and waterhyacinth leaves.

DISCUSSION

The decision to consider the fungus as *C. zonatum* arises from several factors. Sakapure and Thirumalachar's (7) key to Indian Cephalosporia distinguishes between *C. fici* and *C. eichhorniae* on the basis of three characters: *C. fici* produces a pink colony color on PDA and Czapek-Dox agar, while *C. eichhorniae* is white; *C. fici* has funiculose hyphae, while *C. eichhorniae* does not; and *C. fici* has phialides with swollen bases, while *C. eichhorniae* has phialides without swollen bases. This last character is not mentioned in Tims and Olive's original description of *C. fici* (9) and the Indian key does not mention *C. zonatum* though Nag Raj and George (4) report that it occurs in India. In contrast, the author's isolates do not produce a pink colony color on these media, nor do the phialides have swollen bases, but the phialides are borne on funiculose hyphae and the cultures are pathogenic to both waterhyacinth and fig var. Celeste. In addition, plants reported as susceptible to *C. eichhorniae* and *C. zonatum* are either the same species, as with *C. arabica* (2, 4, 8), or have close relatives which are susceptible to the author's isolates. Thus the data suggest that these three

fungi are not distinct species, but are synonyms of *C. zonatum*. Attempts to locate Sawada's original description of *C. zonatum* have not as yet been successful, but a comparison of those for *C. fici* (9) and *C. eichhorniae* (5) reveals that they are nearly identical. Additional work is underway to test the pathogenicity of the Louisiana and Florida isolates on coffee and to obtain cultures of *C. zonatum* from Costa Rica for comparison.

The possibility of using *C. zonatum* as a biological control agent of waterhyacinth does not seem favorable at the present time. Studies indicate that the fungus is restricted to the leaves and petioles of the plant and that even on these its progress is not rapid. It does not seem capable of killing that plant nor of seriously hindering its prolific growth. In those areas where the disease was first noted it did not appear to be causing significant damage as many of the plants were uninfected. The possibility exists, however, that applications of the fungus at high concentrations could cause significant damage and this area is now being explored.

ACKNOWLEDGEMENTS

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Pathogenicity of Fungi and Bacteria from India to Hydrilla and Waterhyacinth[1]

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INTRODUCTION

Surveys were made in southern India from November 1971 to January 1972 in a search for plant pathogens as possible biocontrol agents of hydrilla (*Hydrilla verticillata* Royle) and other aquatic plant problems in Florida. Hydrilla has existed much longer in India than in Florida and, being a cosmopolitan plant of warm temperate to tropical regions (7), occurs extensively throughout India. For this reason, India was considered to be one of the places where chances are good for finding suitable biocontrol agents of hydrilla. While on this tour, pathogens of waterhyacinth (*Eichornia crassipes* (Mart.) Solms.) were also collected. Several promising pathogens were isolated in India on this tour from diseased plants and were brought to this country in strict compliance with quarantine regulations as specified by the U. S. Department of Agriculture and Florida Department of Agriculture and Consumer Services. About 100 cultures of fungi and bacteria were obtained and studied using the quarantine facilities available at the Plant Pathology Department of the University of Florida.

MATERIALS AND METHODS

Isolation of pathogens and culturing. Plant pathogens were isolated from specimens of hydrilla and waterhyacinth that had lesions, rots, and browning. Specimens of some diseased plants were cut into small pieces, surface-sterilized with 0.1% aqueous solution of mercuric chloride for 1.0 min and rinsed thoroughly with sterile water. These pieces were then plated on potato dextrose agar (PDA) or nutrient agar (NA) in petri plates and incubated at 25 C. Any emergent fungi and bacteria were isolated. Other diseased specimens were incubated in moist chambers to allow the slow-growing parasites to

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