Isolation and Effect of Temperature on Spore Germination, Radial Growth, and Pathogenicity of Curvularia senegalensis

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ABSTRACT

Curvularia senegalensis was isolated from sugarcane seeds, greenhouse bench sand, sugarcane field soil, and air, and cultured on potato-dextrose agar (PDA) amended with various fungicides and streptomycin; and from leaf lesions on sugarcane and johnsongrass seedlings on PDA amended with streptomycin. The cardinal temperatures for spore germination in 1% sucrose solution were 10, 20 to 35, and 40 C; for radial growth of the fungus in culture, 10, 30, and 35 C; and for pathogenicity on sugarcane and johnsongrass seedlings, 15, 25 to 30, and 35 C, respectively.

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Additional key words: selective media.

Curvularia senegalensis (Speg.) Subram. has been isolated from paint work and soil (3) and reported as a pathogen on sugarcane seedlings (1) and other plants (2). Byther & Steiner (1) isolated the fungus from naturally infected florets and sugarcane seeds on a cane leaf agar amended with benomyl, nonionic surfactant-NPX, and streptomycin. I was unable, on their medium, to recover the fungus from sugarcane seeds in Louisiana because of the rapid and abundant growth of mucoraceous fungi. The preparation of the cane leaf agar (4) is time consuming and mature field grown cane leaf is not available during winter in Louisiana. Potato-dextrose agar (PDA), however, is readily available. This note reports the development of a selective medium based on PDA to isolate *C. senegalensis* from seeds, bench sand, soil and air, and a study of the effect of temperature on its spore germination, radial growth rate, and pathogenicity.

The selective medium contained 39 g PDA, 3 g agar, 0.1 g benomyl, 0.1 g maneb, 0.15 g PCNB, 1 g Tergitol-NPX (an alkylphenylhydroxypolyoxyethylene), 100 μg/ml streptomycin, in 1 liter of distilled water. *C. senegalensis* was recovered by agitating 5 g seeds in 400 ml sterile distilled water containing four drops of Tween 80 for 5 to 10 min. The suspension was passed through sterile cheesecloth and a borosilicate funnel (0.45-μ pore size). The residue on the filter pad was washed off with 15 ml of sterile distilled water, and 0.2 ml of the wash was mixed with 20 ml of warm (48 C) medium in a petri plate. The fungus was recovered from sand and soil by a serial dilution plate method; from air by exposing the cool medium in petri plates for 2 to 24 hr; and from leaf lesions by a routine plating technique.

C. senegalensis was readily recovered from sugarcane seeds; from greenhouse bench sand, and from air; from sugarcane field soil and air on the selective medium; and from lesions on leaves of sugarcane and johnsongrass seedlings on PDA amended with 100 μg/ml streptomycin. *C. senegalensis* in bench sand and air may also be important sources of inoculum which causes leaf spotting of sugarcane seedlings in the greenhouse.

Colonies of other sugarcane leaf pathogens, such as *Cochliobolus lunatus* Nelson & Haasis (*Curvularia* state) (2), and *Helminthosporium sacchari* (B. de Haan) Butl. (2), were also recovered on the selective medium from sugarcane field air. This selective medium, thus, can be used to collect spores of such leaf pathogens from the field air and to determine the species frequency and seasonal fluctuations of such pathogens in the field.

Germination of spores was studied three times each in tap water and 1% sucrose solution at 5, 10, 15, 20, 25, 30, 35, 40, and 45 C. Conidia from 2- to 3-week-old PDA cultures at 25 C were suspended in the appropriate solutions stored overnight at each of the nine temperatures. The spore suspensions were filtered through cheesecloth and standardized by dilution. Germination was observed microscopically in petri plates to which five well-separated drops of the spore suspension had been added. To determine the latent period of spore germination, the spores were fixed and stained with lactophenol cotton blue at 30-min intervals for 4 hr. To determine the cardinal temperatures for spore germination, the spores were fixed and stained in the same way after incubation for 6 hr at 15 C and higher; for 26 hr at 10 C; and for 120 hr at 5 C.

The latent period in spore germination varied with the age of spores and temperatures, but not with the different solutions used. At 25 C, the germination of spores from 3-month-old PDA cultures started in 2 to 3 hr and from 2- to 3-week-old cultures in 0.5 to 1 hr in tap water and 1% sucrose solution. At 30 and 35 C, the time required for spores to germinate in any of the two solutions was similar to that at 25 C, but at 10 C, the time was more than 16 hr. The percentage of spores that germinated in 1% sucrose solution was greater than that in tap water at the same incubation temperature. The cardinal temperatures for spore germination in the two solutions were 10, 20 to 35 (in tap water, 25 to 30 C), and 40 C. No spores were observed to germinate in any of the two solutions at 5 C within 120 hr.

The radial growth rate of colonies was measured daily for 6 days on PDA, oatmeal agar, and 2% water agar at 5 C intervals from 5 to 45 C. The average daily rate of growth in mm (colony radius) in two

experiments for the nine temperatures on PDA was as follows: no growth at 5, 40, and 45 C; 0.5 at 10 C; 3.7 at 15 C; 5.8 at 20 C; 10.3 at 25 C; 13.3 at 30 C; and 1.7 at 35 C. There was a significant difference (5% level) in the daily rate of growth at 15, 20, 25, and 30 C. The average daily rate of growth on oatmeal agar and water agar was similar to that of PDA at all temperatures.

The effect of temperature (15 to 45 C, at 5 C intervals) on pathogenicity of *C. senegalensis* was determined by incubating seedlings of sugarcane and johnsongrass for 4 days in plastic bags with added water to maintain the high humidity. The leaves of inoculated seedlings were sprayed with a spore suspension before incubating, and those of control seedlings with sterilized distilled water. In repeated experiments, *C. senegalensis* caused many lesions on inoculated leaves of both species at 25 and 30 C, fewer at 20 C, few at 15 and 35 C, and none at 10 and 40 C; no lesions were observed on the controls. The fungus was re-isolated from the lesions. The results suggest that the temperature range at which infection is likely to occur limits the pathogenicity of *C. senegalensis* and permits its control by maintaining the greenhouse temperature above 35 C.

LITERATURE CITED

1. BYTHER, R. S., & G. W. STEINER. 1972. Four sugarcane seedling diseases in Hawaii: Causal agents, control, and selective medium for isolation. Phytopathology 62:120-124.
2. COMMONWEALTH MYCOLOGICAL INSTITUTE. 1968. Plant host, pathogen index to Review of Applied Mycology, Volumes 1-40 (1922-1961). Published by the Institute, Kew, England. 820 p.
3. ELLIS, M. B. 1966. Dematiaceous Hyphomycetes. VII. Curvularia, Brachysporium, etc. Mycol. Papers No. 106, Commonw. Mycol. Inst., Kew, England. 57 p.
4. WISMER, C. A., & H. KOIKE. 1967. Testing sugarcane varieties against eye spot, brown spot, red rot, and leaf scald diseases in Hawaii. Proc. Int. Soc. Sugar Cane Technol. 12:1144-1153.