Effect of Moisture on Conidiophore Morphology of Cristulariella pyramidalis

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ABSTRACT

Cristulariella pyramidalis incubated at 21 C on wet lesions of pecan leaves formed single-headed conidiophores only. Conidiophores were branched 35% of the time when leaf lesions were incubated at 96% relative humidity (RH) and 21 C. Hyphal-tip cultures from germinating branched conidiophores produced an average of 60% branched fruiting structures on pecan leaves at 96% RH. However, only single-headed conidiophores formed when the same leaves were wetted thoroughly and incubated in a saturated atmosphere.

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Cristulariella pyramidalis Waterman & Marshall attacks plants in a large number of diverse genera. Fruiting structures or conidiophores (pyramidal heads, *sensu* Waterman and Marshall) are produced dorsoventrally on leaf lesions. Waterman and Marshall (5) reported that nontypical heads were occasionally found in which the central hypha was pinnately branched with each lateral branch forming a pyramidal head. Occurrence of conidiophores with single pyramidal heads from Alabama and some with two or more pyramidal heads from Georgia has been reported (2). The effect of temp and moisture levels on lesion size and conidiophore numbers has been demonstrated (3); 13% as many conidiophores developed at 96% relative humidity (RH) as on wet leaves. An understanding of fructification morphology, and the conditions under which changes may appear, facilitates species determinations and prevents proliferation of taxa based on phenotypic variation. These investigations were conducted to define some moisture conditions that influence conidiophore morphology, and to determine if a strain could be isolated that would reproduce branched conidiophores under all moisture conditions.

MATERIALS AND METHODS.—Leaves of potted pecan seedlings were inoculated with agar disks of *C. pyramidalis* as described earlier (2). Inoculated plants were sprayed with water, covered with a pre-wetted polyethylene bag, and placed in a tray of water in a constant-temp incubator. After 24 h of incubation, the polyethylene bags and agar-disk inocula were removed, and free water was allowed to evaporate from the leaves. Subsequently, the plants were placed in a Vapor-Temp Controlled Relative Humidity Chamber (Blue M Electric Co., Blue Island, Ill.) at 96 ± 2% RH and 21 C. After 48 to 96 h incubation, branched or multi-headed conidiophores were picked off the leaves with the aid of a microscope. These conidiophores were used to inoculate healthy pecan leaves which were incubated as above for 72 h. Plants were uncovered again, water dried from the leaves, and reincubated at 96 ± 2% RH for 48 h at 21 C. Following incubation, leaves were examined for fruiting structures and number and types of conidiophores recorded.

Branched conidiophores were transferred to potato-dextrose agar from the third generation developed on pecan leaves. Four hyphal-tip cultures were made from

hyphae originating from each of five germinating conidiophores. Subsequently, agar disks cut from them were used as inoculum.

The influence of high RH versus free water on conidiophore morphology was evaluated further on plants infected with isolates from branched conidiophores. Infected pecan plants were either sprayed to run-off, bagged, and incubated 48 h; or leaves were cut

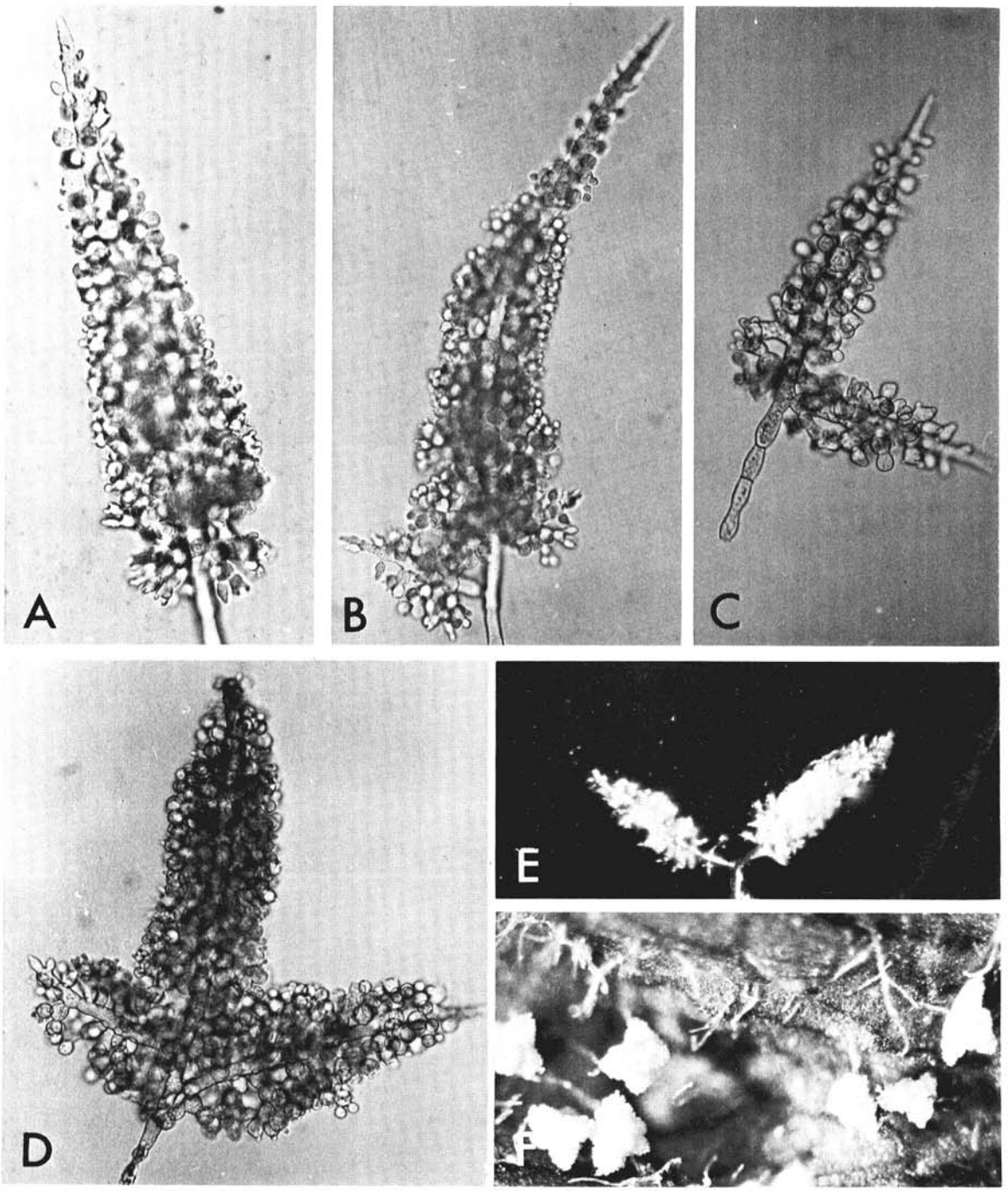

Fig. 1-(A to F). Morphological features induced in conidiophores of *Cristulariella pyramidalis* by different relative humidities: **A)** Single-headed conidiophore developed in saturated atmosphere; **B)** Partly developed branch on conidiophore; **C)** Early stage in synchronously developing heads; **D)** Mature branched conidiophore; **E)** Branched stipe type of conidiophore; **F)** Conidiophore with three or more heads. Conidiophore in Fig. B-F formed in relative humidities of 96 ± 2%.

from them and placed in a petri dish containing a water-saturated filter paper, the leaves wetted, and the assemblage wrapped in polyethylene and incubated 48 h.

RESULTS.—A total of 1,339 conidiophores developed on 14 plants at 96 ± 2% RH; 35% of the conidiophores were branched. When branched conidiophores were used as inoculum, 61% of the conidiophores that developed in the third generation on pecan leaves were branched.

The 20 hyphal-tip cultures obtained from third generation branched conidiophores produced 331 conidiophores in the fourth generation, 56% of which were branched. Pecan leaves from these tests, after incubation in petri dishes, produced an average of 268 single-headed conidiophores per cm^2 . The test was repeated using hyphal-tip cultures which had yielded the highest number of branched conidiophores. From these leaves, 120 conidiophores yielded 60% branched fruiting structures. Subsequent tests yielded similar results.

Only single-headed conidiophores developed on plants with wet leaves incubated 72 h at 21 C in polyethylene bags (Fig. 1-A). Early stages in development of branches from the primary stipe with more advanced development in the main or central head under high RH conditions are shown in Fig. 1-B. Synchronous development of heads in both immature and mature conidiophores is shown in Figs. 1-C and 1-D, respectively. Another type of stipe branching is illustrated in Fig. 1-E. Compact heads with three or more branches were found among some conidiophores (Fig. 1-F). Other conidiophores were found that were irregularly or imperfectly developed at 96 ± 2% RH and 21 C.

In two experiments, single-headed conidiophores formed in the humidity chamber when leaves remained dry and RH was 100%.

DISCUSSION.—Attempts to isolate a strain of *C. pyramidalis* that would produce branched conidiophores under both high RH conditions and water-saturated or wet-leaf conditions failed in these tests. Inoculum from branched conidiophores growing in pecan leaves did not produce like progeny on wet leaves. In other experiments, multi-headed conidiophores developed at high RH on lesions caused by single-headed conidiophores incubated on wet leaves. Branching was suppressed when conidiophores formed on leaves in the presence of free water.

Single-headed conidiophores developed abundantly when leaves were wet or when RH was 100%. Absence of water on leaves restricted the number of conidiophores that developed (3), but relative humidity of 96 ± 2% promoted development of three or more branches by slightly over 60% of the fruiting structures. Thus, whether *C. pyramidalis* develops single- or multi-headed conidiophores is dependent on prevailing moisture conditions.

Hopp (1) found that sporophore morphology of *Ganoderma applanatum* could be altered by culturing the fungus under certain RH conditions. He found normal fruiting bodies formed only when aeration was good and supply of water to the mycelium was adequate. Under such conditions, normal sporophores were produced at 73% RH, but abnormal ones grew at 53 or 100% RH. Plunkett (4) showed that normal expansion of the pileus of *Collybia velutipes* and *Polyporus brumalis* was prevented by poor aeration. In experiments with *C. pyramidalis*, only single-headed conidiophores developed when leaves were wet or the atmosphere was saturated in the growth environment. When pecan leaves were allowed to dry and RH decreased to 96 ± 2% in the circulating air of the humidity chamber, branching development occurred.

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