Survival of *Fusarium moniliforme* Hyphae and Conidia in Grain Sorghum Stalks

S. K. MANZO, Former Graduate Student, and L. E. CLAFLIN, Associate Professor, Department of Plant Pathology, Kansas State University, Manhattan 66506

ABSTRACT

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Fusarium moniliforme conidia and vegetative hyphae from colonized sterile grain sorghum (*Sorghum bicolor*) stalk sections stored in canning jars above and below the ground in Kansas overwintered (1979 and 1980) without loss of viability or pathogenicity. No specialized structures such as thick hyphae, chlamydospores, or sclerotia were observed in 1979 although chlamydospore-like cells were observed on some hyphae in 1980. No loss in viability of *F. moniliforme* conidia or hyphae was detected when stored at −16 C for 6 mo. This indicates that low winter temperatures per se have no lethal effects on *F. moniliforme* and that specialized structures may not be essential for overwintering of *F. moniliforme*.

Fusarium moniliforme Sheld., described in 1904, occurs in both tropical and temperate regions of the world as a pathogen of numerous crops (2). Chlamydospore production has not been reported (2). The fungus has been isolated from overwintered host tissues (5), but survival mechanisms for overwintering in the temperate regions are unknown.

Nyvall and Kommedahl (4,5) observed *F. moniliforme* hyphae threefold to fourfold thicker than normal hyphae in stalks of corn (*Zea mays* L.) that had been inoculated or naturally infested with the organism and stored at 1 C for 4 mo. They concluded that thickened hyphae were survival structures of *F. moniliforme* after noting that the fungus was isolated more frequently when tissues contained thickened hyphae. They did not observe thickened hyphae in stalks that were stored at 25 C.

The objectives of this study were to ascertain the overwintering survival mechanisms and the capacity of *F. moniliforme* conidia and nonthickened hyphae to survive in sorghum (*Sorghum bicolor* (L.) Moench) stalks.

MATERIALS AND METHODS

A mixture of five *F. moniliforme* isolates obtained from corn seeds, cornstalks, sorghum seeds, sorghum stalks, and from field soil were used in this study. Ten split pieces (15 cm long) of healthy sorghum stalks were placed in each of 15 glass quart canning jars and sterilized by autoclaving (1979) or by using propylene oxide (1980). A concentrated conidial suspension of *F. moniliforme* was added aseptically to each jar and shaken thoroughly to distribute the inoculum over the stalks. For the 1979 studies, the mouth of each jar was covered with three layers of regular filter paper before the lid band was tightened. During the 1980 tests, holes (1–1.5 cm in diameter) were bored in the lids and the holes plugged with cotton. Inoculated stalk sections in the jars were incubated for 3 wk at room temperature (23–28 C).

After incubation, a piece of sorghum stalk colonized by *F. moniliforme* was removed randomly from each of five jars for initial observations and tests. Samples of sporulating mycelia from each piece of stalk were removed with tweezers and placed on several drops of distilled water on sterile microscope slides to dislodge mature conidia. The remaining mycelial mat then was transferred to a sterile petri plate containing distilled water. Mycelial mats were teased apart with dissecting needles in several changes of distilled water until all hyphae were completely free of conidia. Mycelia were then observed microscopically to determine morphological characteristics, including the presence of chlamydospore-like structures. Fifty mycelial units from each stalk were plated on potato-dextrose agar amended (PDA-A) with the following antibiotics: streptomycin sulphate (0.1 g/L), chlortetracycline hydrochloride (0.05 g/L), and chloramphenicol (0.05 g/L). Plates were checked regularly to determine hyphal viability and production of contaminant-free colonies.

The conidial suspension on each microscope slide was observed (×400) without staining or heating for morphological structures and to ensure that hyphae were not present, then washed into a beaker with distilled water. The process of removing mycelia from stalks or dislodging the conidia and observing by microscope was repeated several times to ensure that adequate conidia or mycelia were present. The five conidial suspensions were mixed, thoroughly stirred, then counted with a hemacytometer. After ascertaining the conidial concentration, serial dilutions of the suspension were made. One milliliter of the diluted suspension was placed in each of 10 petri plates containing PDA-A. *F. moniliforme* colonies from these plates were counted and compared with expected colony numbers using a chi-square test for determining conidial viability.

In mid-December, the canning jars containing colonized sorghum stalks were packed (five into each of two cartons). One carton was buried in the field soil to the depth of the lids and the other carton of jars was placed above the ground in an unprotected location. To determine the effects of extended cold temperature on the viability of conidia and hyphae, two of the five canning jars kept in the laboratory that contained colonized stalk tissue showing profuse, sporulating growth of *F. moniliforme* were placed in a freezer at −16 C for 6 mo. The remaining three jars sampled for initial observations and tests were kept in the laboratory at room temperature (23–28 C).

The jars were collected in late March and 50 samples of mycelia/conidia were removed with a needle from each jar and placed on PDA-A plates, which were incubated at room temperature and examined after 2 days. After confirming that *F. moniliforme* had survived the winter, stalk tissue from several jars incubated under various conditions (described previously) were sampled. Mycelia and conidia were separated and examined for morphological characteristics. To determine hyphal viability, several hundred hyphal units (free of thickened structures) from each test were plated on PDA-A. Conidial viability was measured in the manner described previously.

Parasitic root colonization ability of *F. moniliforme* was determined by incorporating tissue from the overwintered (aboveground and belowground) colonized stalk pieces in jars into five clay pots (15 cm diameter) that contained auto-

Present address of first author: Plant Pathology Section, Institute for Agricultural Research, Ahmadu Bello University, Samaru-Zaria, Nigeria.

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claved soil. *S. bicolor* cv. Pink Kafir was sown in the pots, and after 28 days, the plants were harvested. Roots were washed and dipped in 5% (v/v) aqueous sodium hypochlorite solution for 2 min. Root sections (1–1.5 cm) were placed on Komada's selective medium (3). After 7 days, *F. moniliforme* colonies were counted. Controls consisted of roots from sorghum plants grown in autoclaved soil.

RESULTS

Before testing, viability of *F. moniliforme* conidia and hyphae was nearly 100%. No thickened hyphae or swollen structures were observed. Compared with initial tests, conidia and hyphae obtained at the end of testing (late March) in 1979, regardless of treatment, appeared normal with no loss in hyphal viability. A chi-square test on conidial viability showed no significant ($P = 0.05$) differences between expected and observed numbers of *F. moniliforme* colonies. This indicates that temperatures as low as −30 C do not significantly reduce conidial viability. Similar observations for both hyphae and conidia were made on the *F. moniliforme* cultures maintained at −16 C for 6 mo.

Results of the 1980 test confirmed the previous year's viability tests for the respective fungal propagules. Some hyphae showed swollen, chlamydospore-like cells with thin, single-layered cell walls. The diameter of such cells was twice that of normal hyphal cells. One hundred units of mycelia free of chlamydospore-like cells and 45 units of mycelia with chlamydospore-like cells were plated on PDA-A. All germinated and sporulated.

On PDA-A, mycelia from overwintered stalk tissue tended to be more dense and slightly slower to sporulate than mycelia from stock cultures.

Limited loss in infectivity (parasitic colonization) occurred in the sorghum seedling bioassay using overwintered fungal infested stalks, because an average of 80 and 20% of roots from the treated and control seedlings, respectively, yielded *F. moniliforme* when plated on Komada's medium.

DISCUSSION

Under Kansas winter temperatures, conidia and vegetative hyphae of *F. moniliforme* overwintered with little or no loss in viability. In 1979, temperatures lower than −30 C were not uncommon in January and February. Chlamydospore-like cells were detected in 1980 but not in 1979, when winter temperatures were lower than in 1980. This indicates that special structures are not essential for winter survival of *F. moniliforme*. Storage of *F. moniliforme* for 6 mo at −16 C did not result in any loss of conidial or hyphal viability and the exceedingly low temperatures did not induce formation of special structures. However, chlamydospore-like cells were observed in *F. moniliforme* hyphae when the cultures were grown on PDA and Komada's media at 23–28 C. Such swollen cells were more abundant when *F. moniliforme* was grown in petri plates contaminated with a bacterium, tentatively identified as *Bacillus mucoides*. This indicates that swollen cells observed in some hyphae (in the 1980 tests) may not have been formed in response to low temperatures. Others (4–6) have reported that low temperatures and/or dry conditions adversely affect viability of *F. moniliforme* and induce thickened hyphae.

Banihashemi and deZeeuw (1) observed that *F. oxysporum* f. sp. *melonis* populations were affected only to a small extent by low winter temperatures but the population declined sharply in the spring with the advent of milder weather. Similar observations on *F. moniliforme* were noted in sorghum "rhizosphere" soil (S. K. Manzo and L. E. Claflin, *unpublished*).

Low temperatures retard microbial activities and delay residue decay, whereas moderate temperatures during spring and summer encourage microbial activities and hasten decay of crop residue. This results in the decline or disappearance of *F. moniliforme* from such tissues (S. K. Manzo and L. E. Claflin, *unpublished*; 6).

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