STUDIES ON THE ECOLOGY OF FUSARIUM MONILIFORME SHELD. UNDER SORGHUM CULTURE

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EFFECTS 0F WINTER ON SURVIVAL 0? FUSARIUM MONILIFORME HYPHAE AND CONIDIA

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ABSTRACT

Fusarium moniliforme Sheld. conidia and vegetative hyphae were capable of overwintering (1979 and 1980) without any loss of viability or pathogenicity in grain sorghum (Sorghum bicolor (L.) Moench.) stalks under Kansas conditions. No structures such as thick hyphae, chlamydospores or sclerotia were observed in 1979 although chlamydospore-like cells were observed on some hyphae in 1980* Additionally, no loss in viability of conidia or hyphae was observed when P. moniliforme was stored at -16C for six months. This suggests that low winter temperatures have no lethal effects on F. moniliforme and also shows that special overwintering structures are not essential for overwintering of F. moniliforme.

Fusarium moniliforme Sheld., described in 1904, occurs in both tropical and temperate regions of the world as a a pathogen or numerous crops (2). Chlamydospore production has not been reported (2) although the fungus can be isolated from overwintered host tissues (7, 10). The survival mechanisms for overwintering in the temperate regions are unknown for F. moniliforme.

Myvall and Kommedahl (9, 10) observed hyphae three to four-fold thicker than normal F. moniliforme hyphae from

corn (Zea mays L.) stalks that had been inoculated or naturally infested with the organism and stored at 1 C for four months. It was concluded that the thickened hyphae were survival structures of F. moniliforme as the fungus was isolated at a higher frequency from tissues containing thickened hyphae. The thickened hyphae were not observed in stalks that were stored at 25 C.

The objective of this study was to ascertain the overwintering survival mechanisms and the ability of F. moniliforme conidia and nonthickened hyphae to survive under natural conditions in sorghum stalks.

MATERIALS AND METHODS

A mixture of five F. moniliforme isolates were utilized in this study. The isolates were obtained from corn seeds, corn stalks, sorghum seeds, sorghum stalks and from field soil. Ten split pieces (15 cm long) of nondecayed 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 aseptically added to each jar and thoroughly shaken to distribute the inoculum over the stalks. For the 1979 winter 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 were plugged with cotton. The

inoculated stalks in the jars were incubated for three weeks at room temperature (23-28 C).

Prior to packaging these jars, five jars were randomly selected and a piece of sorghum stalk containing the growing and sporulating F. moniliforme was randomly removed from each jar. 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 the loosely attached conidia. The mycelial mat was then transferred to a sterile petri plate containing distilled water. The mycelial mats were teased apart with dissecting needles in several changes of distilled water until all hyphae were completely free of conidia. The mycelia were then observed microscopically for determining morphological characteristics including the presence of chlamydospore-like structures. Fifty mycelial units from each stalk were plated on PDA-A. Plates were regularly checked to determine viability and colony identification. Identification of F. moniliforme was confirmed by the presence of microconidia in chains.

The conidial suspension on each microscope slide was observed (400 X), without staining or heating for morphological structures and to ensure that hyphae were not present and then washed into a beaker with distilled water. The process of removing mycelia from stalks or dislodging the conidia and microscope observations was repeated several times to ensure that adequate conidia or mycelia numbers were

present. The five conidial suspensions were mixed, thoroughly stirred, and then counted with a haemocytometer. 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 potato dextrose agar amended (PDA-A) with the following antibiotics (Sigma Chemical Co., St. Louis, MO 63178): Streptomycin sulphate (0.1 gm/liter), Chlorotetracycline hydrochloride (0.05 gm/liter) and Chloramphenicol (0.05 gm/liter). F. moniliforme colonies from these plates were counted and compared with expected colony numbers using a Chi-square test for determining conidia viability.

In mid December the canning jars containing inoculated sorghum stalks were packed (five into each of two cartons). One carton was buried in the field with the lids of the jars exposed. The second carton was placed outside of the laboratory building in an unprotected location. The remaining jars were maintained in the laboratory at room temperature (23-28 C).

Jars were collected in late March and fifty small colonies of mycelia/conidia from each jar were removed with a needle and placed on PDA-A. The plates were incubated at room temperature and examined after two days. After confirming that F. moniliforme had survived the winter, several jars incubated under various conditions (described above) were sampled; mycelia and conidia were separated and examined for morphological characteristics. Two or three hundred small

hyphae units (free of thickened structures) per test condition were plated on PDA-A to measure viability. The conidia viability was measured in same manner as previously described.

To determine the effects of extended cold temperature on the viability of conidia and hyphae, two canning jars containing stalks infested with F. moniliforme were placed in a freezer at -16°C for six months.

Parasitic (root colonization) ability of F. moniliforme was determined by incorporating tissue from the overwintered stalk pieces, retrieved from jars exposed to winter conditions, into five clay pots (15 cm diam) containing autoclaved soil. Sorghum ("Pink Kafir") was sown in the pots and after 28 days the plants were harvested. Roots were washed and dipped in 0.05% aqueous sodium hypochlorite solution for two minutes. Root sections (1 - 1.5 cm) were placed on Komada selective medium (5) and examined after seven days for F. moniliforme colonies. Controls consisted of roots from sorghum plants grown in autoclaved soil.

RESULTS

Prior to testing, viability of F. moniliforme conidia and hyphae was nearly 100%. No thickened hyphae or swollen structures were observed. The conidia and hyphae obtained at the termination of testing (late March) in 1979 appeared normal and there was 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 which suggests that low temperatures did not reduce conidial viability. Similar observations for both hyphae and conidia were made on the F. moniliforme cultures maintained at -16°C for six months.

Result of the 1980 test confirmed the previous year's viability tests for the respective fungal propagules. However, limited number of hyphae produced thin, single layered cell walls with a diameter 2-fold greater than normal hyphae. 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.

In both 1979 and 1980 tests, overwintered mycelia plated on PDA, tended to produce denser hyphae with slightly delayed sporulation when compared to mycelia from stock cultures.

No loss in pathogenicity (parasitic colonization) was evident in the sorghum seedling bioassay using overwintered fungal infested stalks, as 80 and 20% of roots from the treated and control seedlings, respectively yielded F. moniliforme when they were plated on Komada medium.

DISCUSSION

Lockwood (6) suggested that fungi are most capable of surviving adverse conditions when structures such as chlamydospores, sclerotia and oospores are produced. However, special structures of Cephalosporium gramineum and Gaeumannomyces graminis are unknown and yet these fungal organisms are

known to survive adverse climatic periods (3, 4).

Under Kansas conditions, conidia and normal vegetative hyphae of F. moniliforme overwinter with limited or no loss in viability. In 1979, temperatures exceeding -30 C were not uncommon in January and February. Chlamydospore-like cells were detected in 1980 but not in 1979, even though the winter of 1979 was severe than that of 1980. This indicates that special structures are not essential for F. moniliforme survival at very low temperatures. Also storage of F. moniliforme for 6 months at -16 C did not result in any loss of conidial or hyphal viability and neither did the exceedingly low temperatures induce formation of special structures. However, chlamydospore-like cells were observed in F. moniliforme hyphae when the cultures were grown on PDA and Komada medium 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 (L. Claflin - personal communication). This suggests that swollen cells observed in some hyphae (in the 1980 tests) may not have been formed in response to low temperatures. Others (9, 10, 11) have reported that low temperatures and/or dry conditions are abiotic factors that adversely affect F. moniliforme viability and induce thickened hyphae.

Results of this study show that F. moniliforme does not need specialized structures to survive low temperatures.

Hanihashemi and De Zeeuw (1) observed that F. oxysporum f. sp. melonis populations were affected only to a

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POPULATION DYNAMICS OF FUSARIUM MONILIFORMS IN GRAIN SORGHUM FIELD SOIL

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ABSTRACT

Fusarium moniliforme Sheld. population levels were determined at monthly intervals over a two-year period from samples collected from a field with a previous history of fusarium root and stalk rots. Populations from soil shaken from sorghum roots ("rhizosphere" soil) was consistently higher than soil populations obtained from between sorghum rows (furrow soil). In both categories of soil,the population *of* F. moniliforme increased as the sorghum plants grew and matured. The population remained relatively unchanged or declined only slightly during the winter season but declined very rapidly during the spring season. The population varied from 2 propagules per gram soil (oven dry equivalent) at the end of May and 158 propagules/g soil in September for furrow soil, and between 12 propagules/g soil in May and 1,046 propagules/g soil in September and October for "rhizosphere" soil. The decline in population in the soil during the spring was considered to be a result of the progressive decay of roots, especially the cortical layers.

Evidence provided shows that there are seasonal variations in the populations of F. moniliforme in field soil. When fiold soil was infested with conidia and mycelia of

F. moniliforme, the population decline to zero in 10 and 14 weeks under greenhouse and field conditions, respectively. F. moniliforme failed to colonize pieces of autoclaved sorghum stalks incorporated in infested field soil. These results showed that F. moniliforme is a soil invader (root-inhabitant) and not a soil inhabitant, and it has a poor competitive saprophytic ability.

Fusarium moniliforme Sheld. incites seed rots, seedling blights, root and stalk rots of numerous cereal crops throughout the world (6). Numbers and species of Fusarium, found in cultivated and noncultivated soil, have been documented (8, 11, 12, 15, 19, 20, 26, 29, 30, 32) but in most reports, F. moniliforme was not mentioned or consisted of only a small portion (1-4%) of the total Fusarium spp. isolated. Some Fusarium spp. populations have been monitored in soils either naturally or artificially infested, in the absence of host plants, for determining their ability to survive in the soil (2, 3, 4, 13, 18, 21, 22, 23, 24, 27, 28, 31). Longevity of Fusarium spp. is quite variable as some species rapidly declined to non-detectable levels after 8 or more months following soil infestation and/or incubation. The importance of soil microorganisms, soil moisture and temperature on Fusarium spp. survival in the soil is documented in other reports (2, 4, 7, 13, 23, 25). Spring-like climatic factors were attributable to the sharp decline of F. oxysporum f. sp. melonis and this decline coincided with the progressive

decomposition of infested plant residue (2). F. moniliforme has been considered a soil-inhabiting fungus, capable of competing with other soil organisms (5).

Minimal research has been reported on the ecology and population dynamics of F. moniliforme in the soil under field conditions. Continuous monitoring of F. moniliforme populations in the field on a monthly basis to determine seasonal variations has not been reported. A thorough knowledge of the ecology of soil-borne plant pathogens is considered essential for biological and cultural control of such pathogens (1,10).

The purpose of this investigation was to study the population dynamics of F. moniliforme under field conditions on a year-round basis, and determine whether or not F. moniliforme is a soil inhabitant.

MATERIALS AND METHOD

F. moniliforme population studies were conducted on a farm near Manhattan, Kansas that had been planted to grain sorghum (Sorghum bicolor (L) Moench) for the previous 8 years. The soil was classified as a Chase silty clay loam, an Aquic Argiudolls, with a pH of 6.4. The effective calcium carbonate level exceeded 1123 Kg available phosphorus per hectare and an exchangeable Potassium rate of 561.6 Kg/ha. No herbicides were added and all tillage operations were performed in accordance with normal sorghum production. The grain sorghum was planted the first week of June and the final plant population

was 120,708 plants/ha with rows 76.4 cm apart and 11 cm between plants in the row. Fusarium root and stalk rot was observed each year during the growing seasons.

At monthly intervals, 35 soil samples were removed from between the rows of the field in a randomized design. Cores 15 cm in depth by 1.8 cm diameter were obtained at each collection. The soil cores were bulked and thoroughly mixed in plastic bags and referred to as furrow soil (FS).

Twenty-five randomly selected grain sorghum plants were dislodged and soil adhering to the roots was gently removed and placed in plastic bags. This collection was referred to as "rhizosphere soil" (RS).

Soil moisture were determined from two subsamples of each collection by the oven-dry method. The remaining soil was maintained (overnight) at 5 C until processing. Three 25-g (dry weight equivalent) subsamples were removed from each bag of FS and RS and placed in 250-ml graduate cylinders. Sterile distilled water was added (1:10 w/v) for a total of 250 ml. The suspension was transferred to 1-liter flask and thoroughly mixed by shaking and with a magnetic stirrer for 20 minutes. From each stock suspension, 10 ml were pipetted into a 250 ml Erlenmeyer flask and mixed with 90 ml of sterile distilled water (1:10 v/v) and stirred for 10 minutes. The 1:10 (total 1:100 w/v) dilution of stock suspension was adequate for both FS and RS samples. While stirring, 1 ml of the suspension was added to each of 15-20 petri plates containing Komada (14) fusarium-selective medium and distributed

on the agar surface with a sterile glass rod. The plates were maintained at 23-28 C for 7-10 days. Komada medium was preferred over other selective media as F. moniliforme colonies were easily distinguished from other Fusarium spp. Suspect or doubtful F. moniliforme colonies were confirmed in situ by the presence of microconidia in chains. The colonies observed on each plate from the three replicates of FS and RS samples were counted and recorded.

F. moniliforme populations in artificially infested natural field soils:

Six plots (2. x. 5) were established on July 1, 1979 in the same field that was previously described. All debris from the previous season was removed and soil was mounded around the plot edges to form a levee. Plots were maintained free of vegetation. Equal volumes (2 liters) of a conidial/mycelial suspension containing five F. moniliforme isolates were sprayed and immediately incorporated into the soil on each of three, randomly selected, plots. The control consisted of spraying sterile water on the three remaining plots.

Thirty-five soil-cores were collected from each plot soon after the inoculum was incorporated. Soil moisture levels, soil dilutions and the procedure for determing F. moniliforme populations were conducted as previously described. For the first two sampling dates, soil dilutions of 1:1,000 and 1:10,000 (w/v) were used for the infested soil and thereafter dilutions consisted of 1:100 and 1:1,000 (w/v). Soil samples were collected at 2-week intervals until

F. moniliforme populations were undetectable and, thereafter, sampling occurred at 2-month intervals.

F. moniliforme populations in field soil under greenhouse conditions:

Nonsterile soil from the previously described field was collected and placed in six wooden flats (60 x 45 x 10 cm). Six hundred grams (wet wt.) of autoclaved sorghum stalks (none exceeding 2 cm long) were incorporated into the soil of each flat. An equal (500 ml) volume of a mycelial/conidial suspension of F. moniliforme was added to each of three, randomly selected flats and thoroughly incorporated. The remaining three flats served as control. The flats were watered as needed to maintain a moisture content of 25-35% (w/v).

Twenty soil cores were obtained from each flat with a number 8 cork borer, and the cores from each flat were bulked separately. Soil moisture levels, soil dilutions and F. moniliforme colony counts were identical to previous procedures. Soil cores were removed and tested 0, 4, 8, 10 and 12 weeks after the soil flats were prepared. Stalk samples from the flats were collected at 4 and 8 weeks after incorporation. The stalks (100 per flat) were washed with distilled water, sliced lengthwise and one-half of each piece placed on Komada medium. The number of stalk sections, from each flat, infested with F. moniliforme was recorded.

RESULTS

F. moniliforme populations in natural field soil :

F. moniliforme populations were much greater in RS soil as the maximum number of propagules was 1,050/g soil, whereas the maximum number detected in FS soil was 150/g soil (Fig. 1). The fungal population increased over 10-fold (RS soil) from the period of June to September. The population remained relatively static until the advent of warmer temperatures in the spring, then decreased to almost non-detectable levels in May. Lower populations were observed in 1980 than in 1979. The 1980 winter was relatively mild with abundant moisture to hasten decay. Cortical tissue sloughing off was commonly observed at crop maturity.

F. moniliforme populations in artificially infested natural field soil:

F.moniliforme propagules in artificially infested soils devoid of vegetation declined to non-detectable levels 14 weeks after the test was initiated (Fig. 2). The soil remained free of propagules during the remainder of the test (6 months).

F. moniliforme populations in soil under greenhouse conditions:

F. moniliforme populations in artificially infested soil flats averaged 6,500 propagules/g soil but declined to nondetectable levels within 10 weeks thereafter. Populations in the naturally infested soil averaged 125 propagules/g soil initially and also declined to nondetectable levels within 10 weeks (Fig. 3).

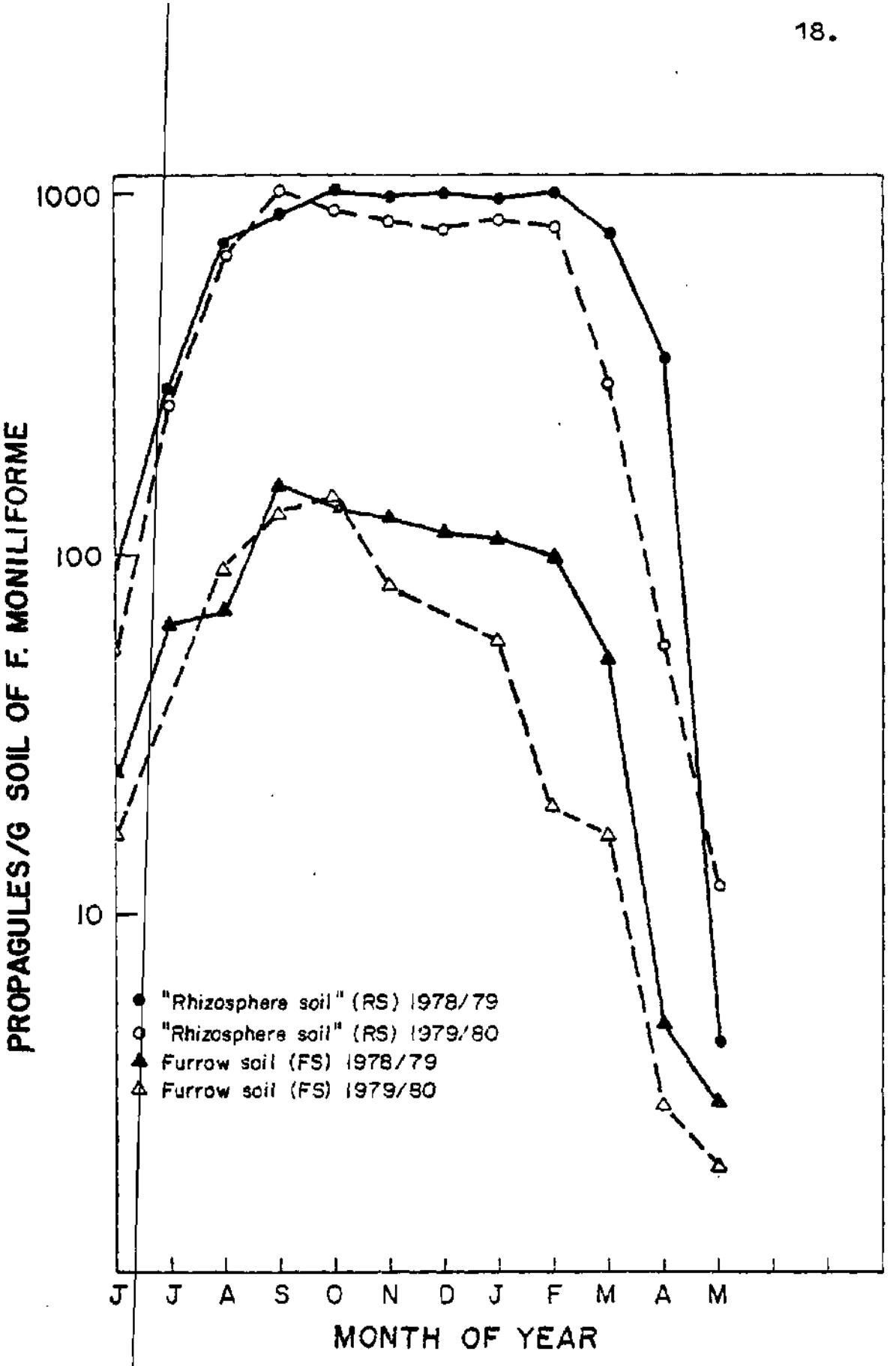


Fig. 1 Monthly numbers of Fusarium moniliforme propagules/g soil from a sorghum field near Manhattan, Kansas. Each point represents the average number from 3 subsamples.

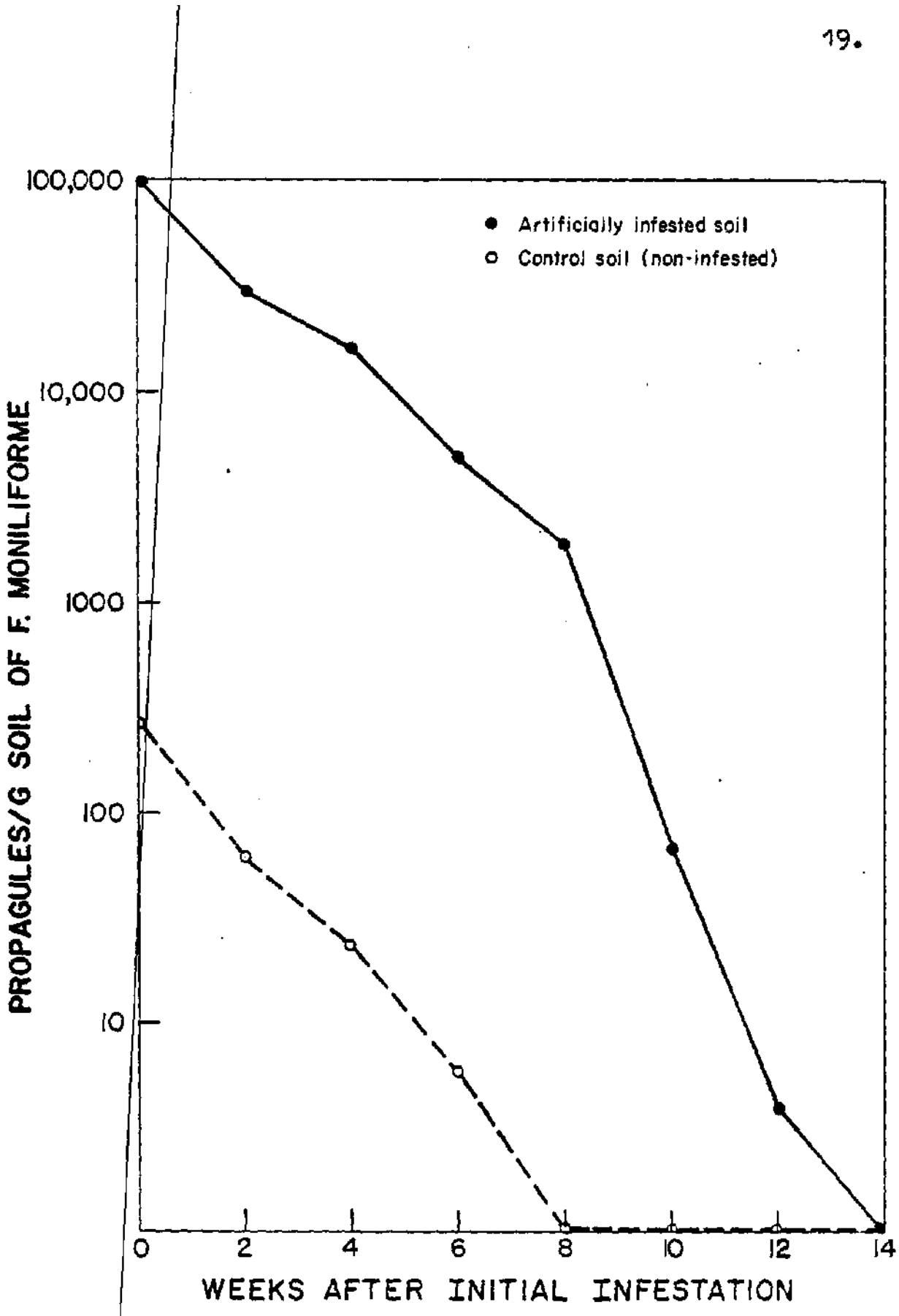


Fig. 2 Decline in numbers of Fusarium moniliforme propagules after initial infestation of field soil under field conditions. Each point represents the average of 3 replicate plots with 3 subsamples per plot.

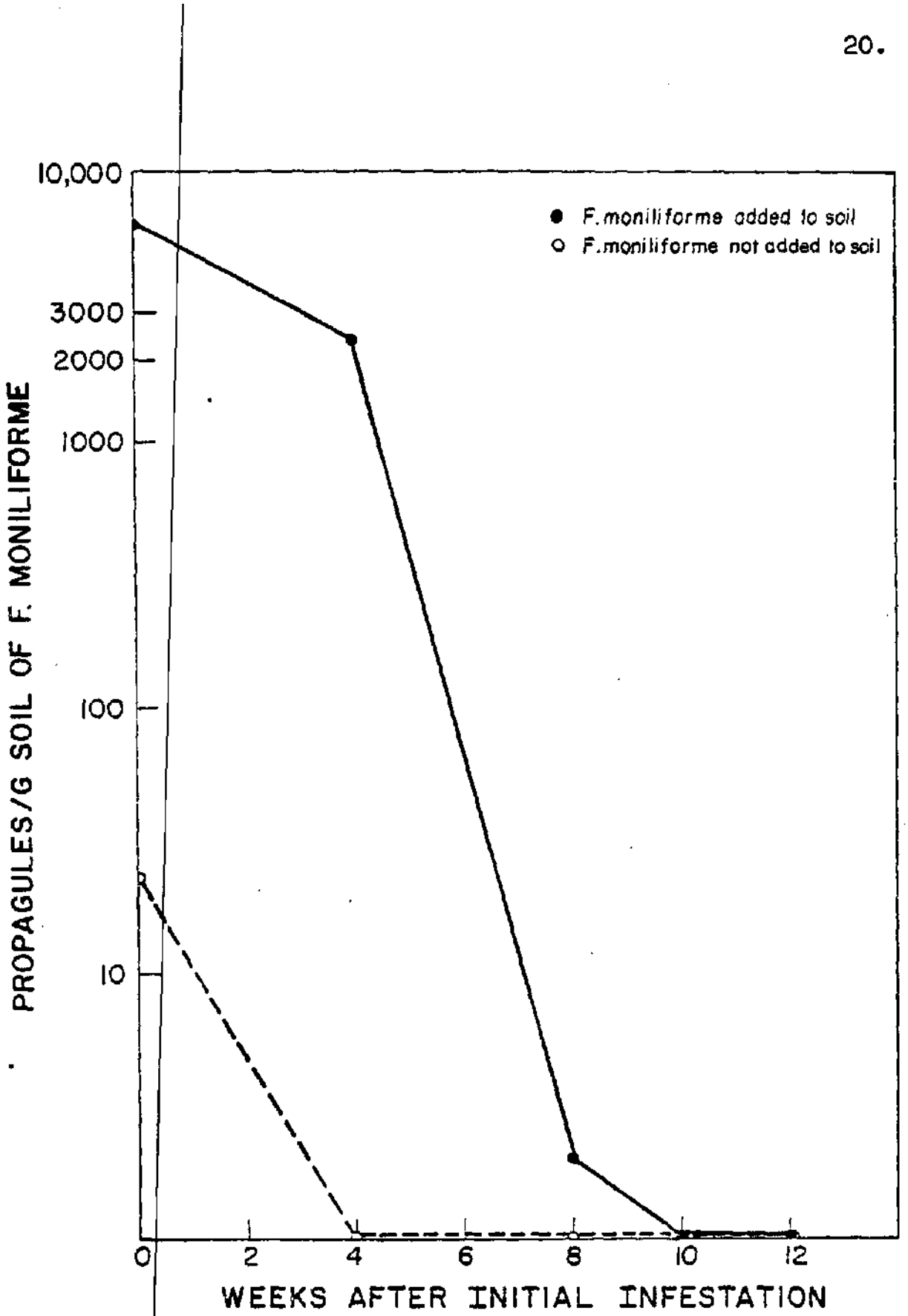


Fig. 3 Decline in numbers of Fusarium moniliforme propagules after initial infestation of field soil under greenhouse conditions. Each point represents the average of 3 replicate plots with 3 subsamples per plot.

No stalks from the naturally infested soil (control) yielded F. moniliforme. From the artificially infested soil, F. moniliforme was isolated from only 2 stalks (300 stalks tested) 4 weeks after the test was initiated. After 8 weeks, however, no positive isolations were observed. At the onset of the investigation, an average of 23 F. moniliforme propagules/g control soil was recorded.

DISCUSSION

F. moniliforme populations varied considerably between seasons. RS soil contained many more propagules th FS soil. The F. moniliforme population peak in September a October in field experiments can be correlated with physic gical crop maturity, and this was accompanied by a sloughi off and decay of the cortical root tissue. Population decl during the winter was very limited as the low winter tempe rature may have suppressed microbial antagonists. Manzo an Claflin (16) have demonstrated that low temperature does n directly affect conidia or hyphae viability. The decline i F. moniliforme populations during the spring season coinci with an increase in microbial activities and decay or root tissues; especially cortical tissues which harbor large numbers of F.moniliforme. A significant correlation betwe decomposition of crop debris and decline in F. moniliforme populations within the crop tissues has been observed (17

Garrett (9) defined a soil-inhabiting fungus as o with ability to survive indefinitely as a soil saprophyte a root inhabitant (soil invader) as one with an expanding parasitic phase on the living host plant and a declining saprophytic phase after death of the host. Based on our da presented in Fig. 1, 2 and 3, F. moniliforme fits the defi tion of a root inhabitant. We conclude, therefore, that F. moniliforme is a root inhabitant and not a soil inhabitan Conceivably, those colonies isolated from the various soil

tests (RS and FS soils) were not from saprophytic clones in the soil mass but from undecomposed host tissues, especially the root cortical tissues. Since our data shows that F. moniliforme is not a soil inhabitant, the most logical source of the fungus in the soil was from the root cortex tissues which sloughed into and were mixed with the test soils. Additionally, this would also explain the significant difference between FS and RS populations as more cortical tissue would be present in RS samples. Limited differences in populations of FS and RS were detected in late spring as most of the cortical tissue at that time was decomposed.

Grassy weeds are known host plants for various Fusarium spp. (26, 27). In the sorghum field, up to 450 F. moniliforme propagules/g soil were obtained by gently removing soil from the roots of grasses. Grassy weeds (primarily Setaria spp. and Digitaria sanguinalis (L.) Scop.) were observed in the furrows of the sorghum field during the boot stage of sorghum growth. This would also partially explain the increase in F. moniliforme populations in FS soil as the growing season progressed. Based on our data, the number of F. moniliforme propagules isolated from field soil would depend on the extent to which undecomposed crop residue (especially root cortical tissue) is mixed with the soil.

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SIGNIFICANCE OF GRAIN SORGHUM RESIDUE IN PARASITISM AND SURVIVAL OF FUSARIUM MONILIFORME SHELD.

by

Siman K. Manzo

ABSTRACT

Field and greenhouse studies showed that Fusarium moniliforme Sheld. aggressively invaded and colonized grain sorghum plants in the early stages of development and colonization continued to increase until the plants were physiologically mature. Colonization of stalk tissues did not always result in disease symptoms. Overwintered stalks were found to be a major reservoir of F. moniliforme (2×10^5 propagules/g stalk). F. moniliforme population in stalks varied according to the extent of stalk decay as shown by a negative correlation. F. moniliforme was found to have a high competitive parasitic ability which is essential for its survival.

Fusarium moniliforme Sheld. has been regarded a soil-borne pathogen (1, 2) and a soil invader (22) but not a soil inhabitant. F. moniliforme is commonly isolated from living and dead host tissues (13, 17, 18, 24, 27) indicating that crop residue is an important source of F. moniliforme inoculum in the field. Knowledge of F. moniliforme populations in crop residue is useful in determining the proper tillage operations for decreasing the incidence of Fusarium stalk and root rots.

Root pathogens that are neither soil inhabitants nor

good competitive saprophytes depend on early parasitic invasion of hosts for survival and, as a result, such fungi are the early colonizers and "possessors" of host tissue (4, 5, 6, 8). Some investigators (3, 12, 14, 15, 20, 21, 23, 26) consider F. moniliforme a secondary parasite, having the capacity to invade only mature, senescent or predisposed plants. Others (13, 28), assert that F. moniliforme invades corn (Zea mays L.) stalks in the early stages of growth which generally results in systemic infections (13).

The purpose of this investigation was to: 1) ascertain the growth stage of sorghum plants when F. moniliforme infections are most likely to occur, and 2) demonstrate the importance of stalks as a principal reservoir for overwintering of F. moniliforme inoculum under field conditions.

MATERIALS AND METHODS

Time of infection of sorghum stalks by F. moniliforme:

The research plots were established and maintained during the 1978 and 1979 growing seasons at the Rocky Ford Farm near Manhattan, Kansas. Two sorghum (Sorghum bicolor (L.) Moench) hybrids, SC5-99 (tolerant) and Wheatland X 2536 (susceptible) were chosen because of their previously tested genetic response to F. moniliforme. The plots were planted between June 1 and 4 in rows 76.4 cm apart with plants spaced 11 cm apart in the rows. Herbicides were not applied to the test area.

Twenty-five plants were randomly selected from each

hybrid on a monthly basis beginning in June and ending in October. Leaves, leaf sheaths and roots were removed from the stalk. The stalks were cleansed of foreign debris by brushing and washing in several changes of distilled water and then split lengthwise and stalk rot symptoms noted. Three sections (10 X 3 X 2 mm) were removed from 4 areas on each half of the stalk. Those areas tested included the crown, first and second internodes, third and four internodes and the remaining upper portions of the stalk including the peduncle. Samples from each area included cortical and pith tissue and a portion of the node. The tissue was placed on medium (16) and incubated for 10 days at room temp (23-28 C). F. moniliforme was confirmed by the presence of microconidia in chains.

Sorghum stalks as a reservoir of F. moniliforme inoculum:

Overwintered sorghum stalks from the 1977 growing season were collected from the Rocky Ford Farm in May, 1978. The stalks were classified into 5 categories (I - V) based on the extent of decay, viz., 0 to 10% (I); 11 to 25% (II); 26 to 50% (III); 51 to 75% (IV); and 76 to 95% (V). The stalks were cut into small pieces (3 to 5 cm) and bulked separately according to the category. The moisture content of each category was ascertained with 2 subsamples by the oven dry procedure. For each category, three - 10g (dry wt. equiv.) subsamples were placed in a Waring blender containing 200 ml sterile distilled water and ground for two minutes

followed by a cooling period of 25 minutes and then ground for an additional two minutes. The suspension was filtered through a single layer of cheesecloth. An additional 30 ml sterile distilled water was used for rinsing the blender and cheesecloth. The filtrate was collected in a graduated cylinder and distilled water added to bring the total volume to 250 ml. Serial dilutions of 10^{-1} , 10^{-2}, 10^{-3}, and 10^{-4} were made from each stock suspension. One ml of each dilution was pipetted into each of 10 petri plates containing Komada medium and distributed on the agar surface with a sterile glass rod. The plates were incubated at 23-28 C for 10 days and then observed for F. moniliforme colonies.

To further demonstrate the relationship between stalk decay and F. moniliforme populations, the following was carried out:

In November (1978), stalks exhibiting F. moniliforme stalk rot symptoms were collected, cut into sections (5 cm), thoroughly mixed and divided into 7 equal portions. Six of the portions were utilized in the moisture experiments while the remaining portion was used to determine the moisture content and the initial number of F. moniliforme propagules in the stalk samples. Each of the 6 stalk portions was thoroughly mixed with nonsterile soil from the Rocky Ford Farm and placed in clay (30 cm diam.) pots. Three of the pots were kept moist by watering as needed to maintain a 20 - 40% w/w ratio whereas the other 3 pots were maintained dry. The pots were maintained under greenhouse conditions

(22-28 C).

Three subsamples (10 g dry wt. equiv.) were collected from the pots every four weeks. The samples were ground, filtrate collected and dilutions prepared as previously described.

One operation in the cultural practices of grain sorghum production in the field involves incorporating the stalks and other debris into the soil. The tillage operation is normally conducted in the fall after harvesting or in the spring prior to planting. To test the hypothesis that incorporation of stalks into the soil will increase stalk and root rots the following tests were established and consisted for 10 pots/treatment with F. moniliforme in naturally - or artificially - infested sorghum stalks. These stalks were cut in 2 cm sections and 150 g added per pot as follows:

I. Naturally infested stalk sections incorporated in autoclaved soil;

II. Artificially infested stalk sections incorporated in autoclaved soil;

III. Naturally infested stalk sections incorporated in nonautoclaved soil;

IV. Artificially infested stalk sections incorporated in nonautoclaved soil;

V. F. moniliforme conidia/mycelia (without stalk tissue) incorporated (75,000 propagules/cc soil) in nonautoclaved soil;

VI. F. moniliforme conidia/mycelia (without stalk

tissue) incorporated (75,000 propagules/cc soil) in nonautoclaved soil;

VII. No inoculum was added to the nonautoclaved soil;

VIII. No inoculum was added to the autoclaved soil and pots (control).

Nine surface sterilized (0.5% NaOCl for 2 min) sorghum ("Pink Kafir") seeds were planted in each pot. The control pots were placed in an adjacent room of the greenhouse to avoid contamination. After emergence, the seedlings were thinned to 6/pot, grown for 6 weeks (ca. 27 C), harvested, washed and labelled as to the treatment. The roots were placed in 0.5% NaOCl for 2 min and rinsed in several changes of sterile distilled water. From each seedling, 6 sections of roots (1 - 1.5 cm lengths) were removed from ca. 2.5 cm distance below the crown and placed on Komada medium and incubated at 25 - 28 C for 10 days. Duncan's Multiple Range Test was used to analyze between treatments.

RESULTS

Time of infection of sorghum stalks by F. moniliforme :

F. moniliforme was detected in crown and first and second internode tissues of both hybrids approximately one month after planting (Fig. 1 and 2). The frequency of F. moniliforme isolations increased during the growing season but was most pronounced during the reproductive growth stage (August).

At the conclusion of the growing season, over 80%

of the tissues from the crown and first and second internodes of the susceptible hybrid yielded F. moniliforme colonies. The frequency was slightly less in the tolerant hybrid (Fig. 2).

F. moniliforme was commonly isolated from stalk tissue devoid of rot symptoms. Throughout the 2-year investigation, stalk rot symptoms were only observed in the peduncle area of the tolerant hybrid. Stalk rot symptoms were observed (ca. 20-30%) in the susceptible hybrid in 1978 and, in 1979, symptoms were not observed except in a limited number of peduncles although no difficulty was encountered in isolating F. moniliforme from the tissues. In general, stalk rot symptoms were not observed prior to the hard dough stage and those plants with symptomatic brace roots also exhibited stalk rots whereas plants with normal symptomless brace roots rarely showed stalk rot symptoms.

Sorghum stalks as a reservoir of F. moniliforme:

In excess of 1×10^5 F. moniliforme propagules overwintered in those sorghum stalks with minimal decay (Fig. 3) whereas the number of propagules declined to less than 100 in stalks exceeding 75% decayed tissue. Throughout the testing, the stalks with increasing percentages of decay also resulted in a lesser number of propagules recovered and showed a strong negative correlation ($r^2 = 0.957$).

Moisture is an important component in reducing the number of F. moniliforme propagules in stalk tissue. A 10-fold

decrease was observed in F. moniliforme infested stalks incorporated in moist soil as compared to those in dry soil (Fig. 4) where the propagule numbers remained static throughout the testing program. Moisture hastens decomposition, and hence provide a more suitable habitat for other microorganisms to compete with F. moniliforme.

In our tests to confirm the hypothesis that incorporation of stalks in the soil provides inoculum, high (82-97) percentages of "Pink Kafir root" pieces were infected with F. moniliforme in those tests involving naturally or artificially infested stalks and F. moniliforme propagules incorporated in sterile and nonsterile soil (Table 1). A higher incidence of root infection was observed in plants grown in autoclaved soil with inoculum added (although lesser necrosis occurred). Approximately 30% of the roots were infected from those plants grown in nonsterile soil without inoculum added. At the onset of testing, the nonsterile soil contained 18 propagules/g of soil. Although precautions were taken, 5% of the roots were infected with F. moniliforme in those plants grown in autoclaved pots and soil without added inoculum. This may be attributable to the seed source (Manzo, unpublished data) or outside contamination.

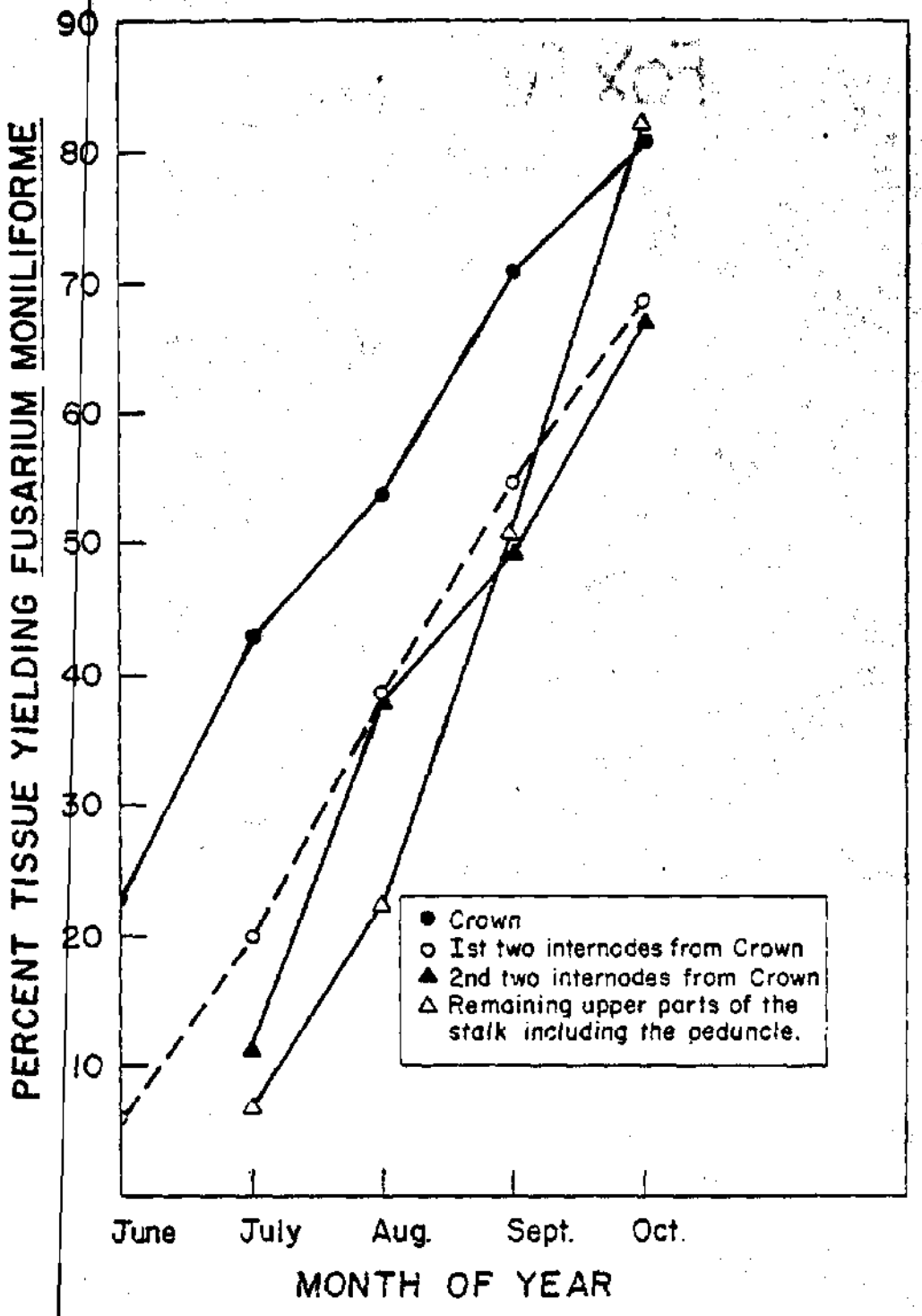


Fig. 1 Fusarium moniliforme infection of susceptible hybrid grain sorghum (Wheatland X 2536) stalks at various stages of plant growth under field

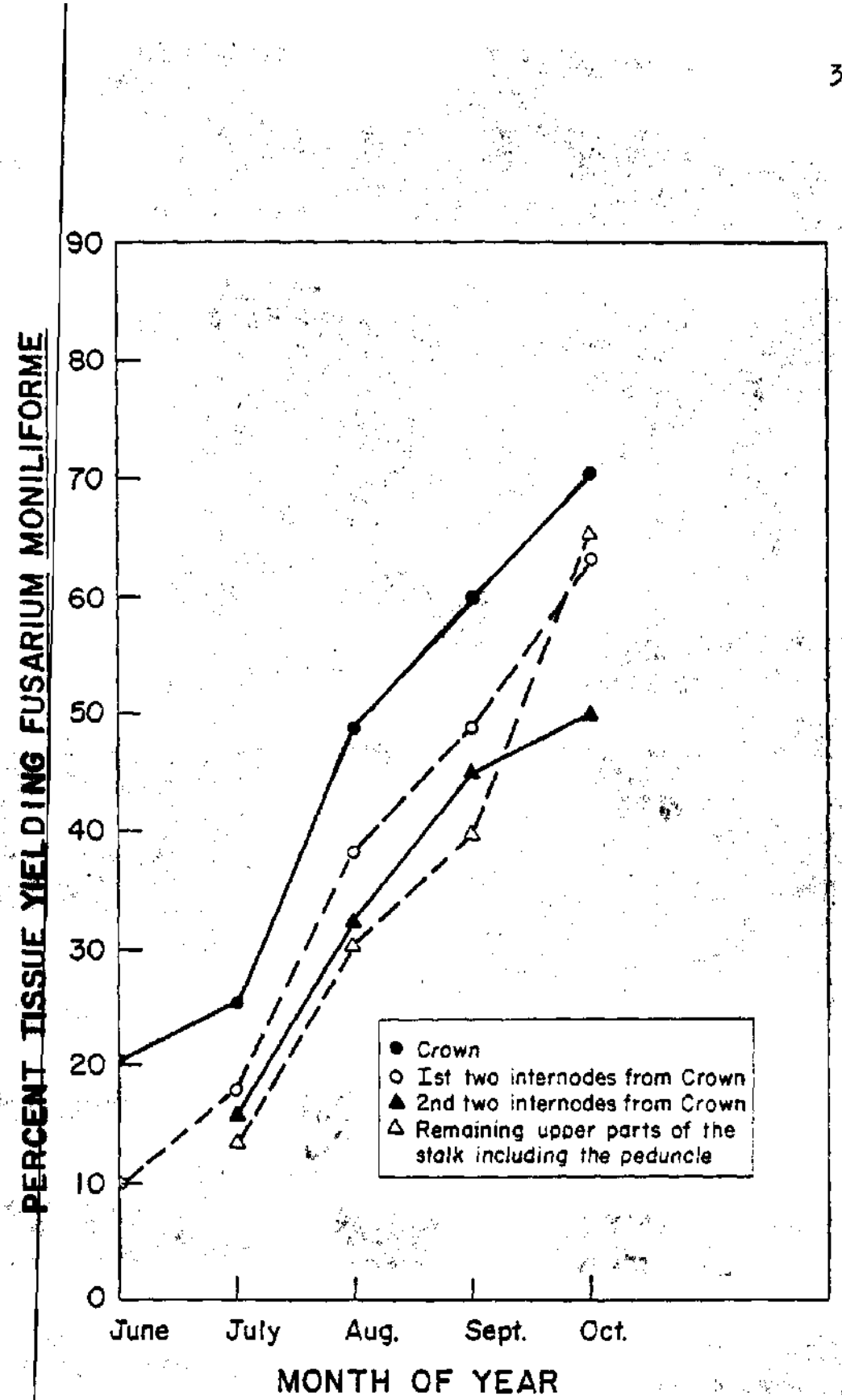


Fig. 2 Fusarium moniliforme infection of tolerant hybrid grain sorghum (SC5-99) stalks at various stages of plant growth under field conditions. Each point represents the average of 2-season observations.

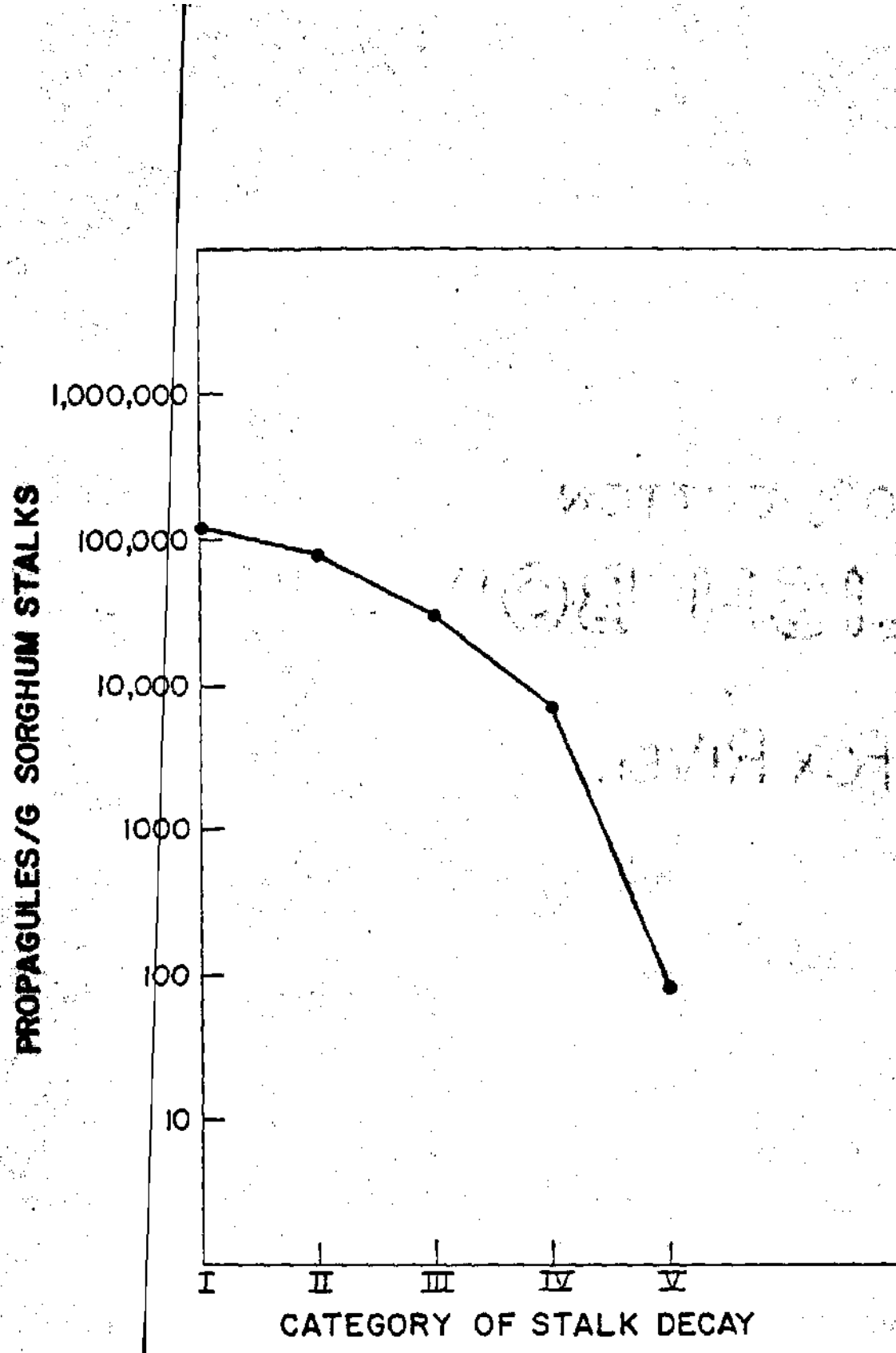


Fig. 3 Number of Fusarium moniliforme propagules in naturally infested sorghum stalks at various stages of decay. Each point represents the average of 3 subsamples.

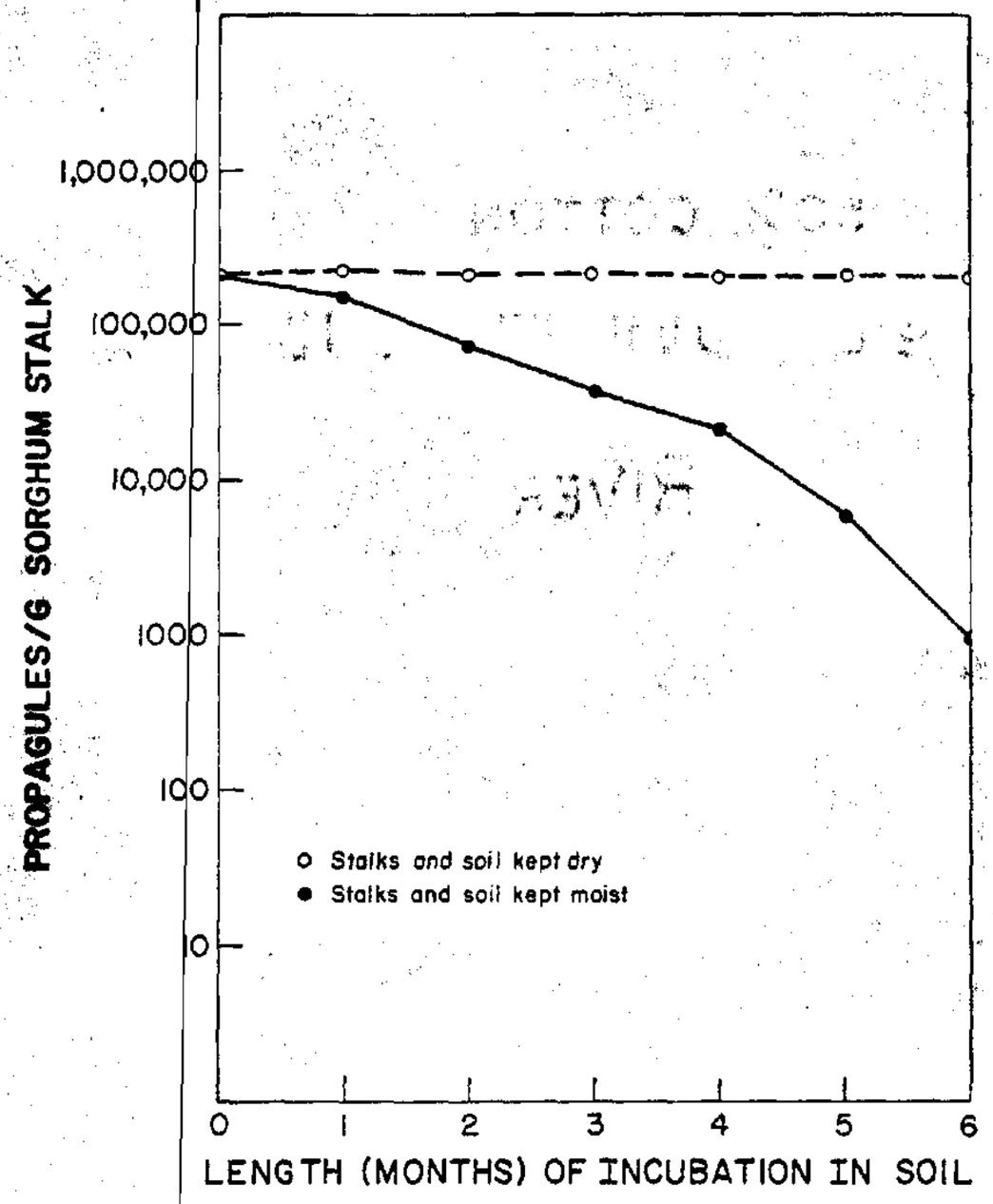


Fig. 4 Effect of moisture on Fusarium moniliforme populations in naturally infested sorghum stalks. Each point represents the average of 3 subsamples per sampling date.

Table 1 Effects of incorporating *fusarium moniliforme* infested sorghum stalks into soil at planting time on root infection

Identification	Treatment: Inoculum Source	Soil: Autoclaved	Soil: Not Autoclaved	%(Mean) Roots Infected
1.	Naturally infested stalks	X		90.8 b[1]
2.	Artificially infested stalks	X		96.7 a
3.	Naturally infested stalks		X	82.0 c
4.	Artificially infested stalks		X	84.7 c
5.	Conidia/mycelia suspension	X		91.4 b
6.	Conidia/mycelia suspension		X	82.0 c
7.	Natural field soil		X	29.5 d
8.	Natural field soil	X		5.0 e

[1]% (mean)shown against each treatment number represents the average of 10 replicates. Means followed by a common letter are not significantly different (p = 0.05).

DISCUSSION

Results of this study demonstrate that F. moniliforme is an aggressive parasite and is capable of infecting actively growing sorghum plants. Since F. moniliforme is not a soil inhabitant (22), the capability to colonize host tissue early in the season assures that the organism will survive similar to Cephalosporium gramineum (4,5,6,19) and F. roseum "culmorum" (8). F. moniliforme can be disseminated on sorghum seed (S. Manzo-unpublished) although preliminary testing reveals that most of the inoculum is destroyed by common chemical seed treatments.

Stalk tissue is a major overwintering reservoir for F. moniliforme as 2 X 10^5 propagules/g of tissue were recovered from overwintered stalks prior to planting. Stalk decomposition is important as a 20-fold decrease in propagule numbers was observed in highly decomposed (75-95%) in comparison to stalks with limited decay (0-10%). Other reports (7,9,10,11,25), have emphasized the importance of leaving the stalks on the soil surface as the root and stalk rot incidence was less than when stalks were incorporated in the soil. Tillage operations disseminate the inoculum throughout the soil profile which enhances the probability of roots coming in contact with stalk debris. If tillage operations are needed for seed bed preparation prior to planting, tillage practices to enhance decay such as shredding the stalks and plowing early in the fall while soil temperatures are warm would be beneficial. Incorporating stalk debris in the spring prior to planting

would seemingly increase root infection. If fall tillage operations are not recommended due to water and soil conservation practices, no till or minimum tillage should be exercised at the time of planting to avoid incorporating infested stalks in soil. Burning or removal of stalk residue would be beneficial in minimizing root infection.

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SEED TRANSMISSION OF FUSARIUM MONILIFORME IN GRAIN SORGHUM

S.K. MANZO

ABSTRACT

Fusarium moniliforme was detected in seeds from 27 of 28 untreated grain sorghum hybrids from 6 different sources. *F. moniliforme* propagules were more commonly detected as a surface contaminant, ranging from 0 to 56%. In contrast, internally-borne inoculum ranged from 0 to 24%.

MATERIALS AND METHOD

Thirty-four grain sorghum (*Sorghum bicolor* (L.) Moench) hybrids from 6 companies were utilized in this study. With the exclusion of one source (company E), the seeds were not treated with chemical protectants. For each hybrid tested, 100 seeds were randomly selected and one-half of the seeds (nonsurface disinfested) were sliced longitudinally with a scalpel followed by a 2-min soak in 0.5% NaOCl and rinsed in several changes of sterile, distilled water. The seeds were split longitudinally and placed on Komada medium. The plates were incubated at 27 C for 7 days.

RESULTS AND DISCUSSION

The percentages of *F. moniliforme* ranged from 0 to 56% and 0 to 24% in nonsurface disinfested and surface disinfested seeds, respectively (Table 1). Most of the

F. moniliforme inoculum was on the periphery of the seed coat; however, in several tests, a significant number of propagules were detected in surface disinfested seeds indicating that the inoculum source was internal. This would be an important inoculum source in fields where a crop sequence program is practiced with a non-host crop utilized in the rotation.

The efficacy of seed treatments in disinfesting the seeds of externally-borne inoculum is exemplified by the low (2%) infestation in 2 of the 6 hybrids treated with a seed protectant (Table 1). The damping-off and seedling blight stages of infection are normally not a problem in Kansas unless prolonged periods of cool temperatures and excessive rainfall persist. With the advent of seed protectants, replanting due to seedling blights is a rarity. The cause of surface-borne F. moniliforme infested seeds may be attributable to the harvesting operation.

Table 1 Percentages of nonsurface and surface disinfested grain sorghum seeds infested with F.moniliforme.

Seed Source	F. moniliforme infested seed (%)	
	Nonsurface disinfested	Surface Disinfested
Company "A"[X]		
Hybrid # 1	44	10
" # 2	42	14
" # 3	52	16
" # 4	54	24
" # 5	34	12
" # 6	42	8
Company "B"		
Hybrid # 1	56	10
" # 2	54	20
" # 3	54	18
" # 4	44	14
Company "C"		
Hybrid # 1	52	22
" # 2	10	0
" # 3	50	14
" # 4	28	4
" # 5	12	2
" # 6	46	12
Company "D"		
Hybrid # 1	30	10
" # 2	22	4
" # 3	28	8
" # 4	18	6
" # 5	0	0
" # 6	42	12
" # 7	6	20
" # 8	4	0
" # 9	8	2
" # 10	6	4

contd..

Table 1 (contd.)

Seed Source	*F. moniliforme* infested seed (%)	
	Nonsurface disinfested	Surface Disinfested
Company "E"[Y]		
Hybrid # 1	0	0
" # 2	0	0
" # 3	0	0
" # 4	2	2
" # 5	2	0
" # 6	0	0
Pink Kafir	4	2

[X] At the request of the cooperating hybrid seed companies, the names and numbers of the hybrids are to remain unidentified.

[Y] Hybrids from this source were treated with a seed protectant.

EFFECT OF HERBICIDES ON *FUSARIUM MONILIFORME* SHELD POPULATIONS

S.K. MANZO

ABSTRACT

Atrazine, Cyanazine, Terbutryn, Propazine and Propachlor were evaluated for their efficacy in reducing *Fusarium moniliforme* populations. Propachlor totally suppressed *F. moniliforme* growth at 20 fold increase over the normal recommended applications. Herbicides applied at the recommended rates were ineffective in reducing *F. moniliforme* stalk and roots rots.

Herbicides are commonly used for controlling weeds in grain sorghum fields. The role of herbicides in controlling diseases of other commodities is well documented (1, 2, 3,4). The potential effect of herbicides in reducing *F. moniliforme* populations is reported herein.

MATERIALS AND METHODS

Atrazine (Aatrex 80 WP), Cyanazine (Bladex 80 WP), Terbutryn (Igran 80 WP), Propazine (Milogard 80 WP) and Propachlor (Ramrod 65 WP) are labelled herbicides for weed control in grain sorghum production. Each herbicide was added at concentrations of 15 and 30 mg (formulated) for each gram of potato dextrose agar (PDA) after the autoclaved medium had cooled to 45°C. After 24 hours, a small (5 mm^2) section of an *F. moniliforme* culture was placed in the center

of each petri plate containing the amended PDA and incubated at room temperature (22 - 27°C) for 7 days.

Initial tests showed that Propachlor was the most effective of the herbicides tested. As a result, Propachlor concentrations of 1.75, 3.5, 7.0, 35, 70 and 350 mg per 100 g PDA were tested and these rates were equal to ½X, 1X, 2X, 10X, 20X, and 100X of the recommended (4 lb/A) rate, respectively.

RESULTS

F. moniliforme mycelial growth was inhibited from 30 to 50% at the 15 and 30 mg/g PDA rate and only microconidia were observed in the treatments involving herbicides with the exception of Propachlor. Neither microconidia nor macroconidia was observed and nearly total inhibition of mycelial growth was observed in the medium containing Propachlor at 15 and 30 mg/g PDA. The mycelial growth was completely inhibited at 60 mg Propachlor/g PDA. However, hypae growth occurred after small sections of the mycelial mat were transferred to nonamended PDA. No detectable effects on F. moniliforme were observed at the 1.75, 3.5 and 7.0 mg Propachlor/g PDA.

DISCUSSION

Of the five herbicides tested, only Propachlor had a total suppressive effect on F. moniliforme at 60 mg/g PDA. The 60 mg/g PDA rate is 20 times the recommended field rate

(4 lbs/A) of Propachlor. This would be totally unrealistic in terms of cost per acre for F. *moniliforme* control and additionally, this rate would most likely be lethal to sorghum plants. The success of herbicides in controlling soilborne diseases of other crops is most likely due to differences in chemical composition as well as cultural factors such as band applications.

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APPENDIX

Tables

Table 1. Numbers of Fusarium moniliforme propagules/g soil obtained monthly from a sorghum field near Manhattan, Kansas

Month	Mean numbers of F. moniliforme propagules/g soil			
	1978/79		1979/80	
	Furrow Soil	"Rhizosphere" Soil	Furrow Soil	"Rhizosphere" Soil
June	25[1]	94	17	53
July	64	292	43	258
August	70	747	93	670
September	158	867	132	1015
October	135	1046	147	898
November	128	970	82	842
December	117	1029	67	818
January	112	980	58	860
February	99	1012	20	838
March	52	783	17	303
April	5	365	3	57
May	3	45	2	12

[1] Numbers in columns represent the mean of 3 subsamples.

Table 2. Decline in numbers of *Fusarium moniliforme* population in artificially infested field soil maintained under field conditions

Weeks after Infestation	Mean Number of Propagules Isolated	
	Infested Soil	Control Soil
0	104,106[1]	278
2	32,122	62
4	16,711	24
6	4,962	6
8	1,954	1
10	68	0
12	5	0
14	0	0
22	0	0
30	0	0
38	0	0

[1]Numbers represent the mean of 3 replicate plots with 3 subsamples per plot.

Table 3. Decline in Fusarium moniliforme population in artificially infested field soil maintained under greenhouse conditions

Weeks After Infestation	Mean Numbers of Propagules Isolated	
	Infested Soil	Control Soil
0	65,000 [1]	23
4	2,371	1
8	2	0
10	0	0
12	0	0

[1] Numbers represent the average of 3 replicate plots with 3 subsamples per plot.

Table 4. *Fusarium moniliforme* infection of susceptible hybrid grain sorghum (Wheatland X 2536) stalks at various stages of plant growth under field conditions[1]

	% *F. moniliforme* isolated from:			
	Crown	1st two internodes from crown	2nd two internodes from crown	Remaining upper parts of stalk
June 1978	21	4	-	-
July 1978	28	15	10	3
August 1978	53	33	32	18
September 1978	77	60	47	52
October 1978	88	80	65	77
June 1979	25	8	-	-
July 1979	57	25	13	10
August 1979	55	43	45	27
September 1979	65	50	52	50
October 1979	73	57	70	90

[1]The sorghum hybrid was planted first week of June and isolations made at the end of each month.

Table 5. *Fusarium moniliforme* infection of tolerant hybrid grain sorghum (SC5-99) stalks at various stages of plant growth under field conditions[1]

Date	% F. moniliforme isolated from:			
	Crown	1st two internodes from crown	2nd two internodes from crown	Remaining upper parts of stalk
June 1978	13	8	-	-
July 1978	23	13	12	7
August 1978	45	40	28	25
September 1978	63	52	47	42
October 1978	73	65	57	63
June 1979	28	12	-	-
July 1979	37	23	20	20
August 1979	53	37	37	40
September 1979	57	47	43	38
October 1979	68	62	43	68

[1]The sorghum hybrid was planted first week of June and isolations made at the end of each calendar month.

Table 6. Number of Fusarium moniliforme propagules/g of sorghum stalks at various stages of decay

% of stalk decay	Mean number of propagules/g stalk[1]
0 - 10	137,083[2]
11 - 25	83,542
26 - 50	31,167
51 - 75	7,542
76 - 95	83

[1]Propagules were obtained by grinding stalks in sterile distilled water with commercial waring blender for 4 minutes, filtering through a single layer of cheesecloth and plating 1 ml of the diluted filtrate into each of 10 petri plates containing Komada medium.

[2]Numbers represent an average of 3 replications.

Table 7. Effect of moisture on longevity of Fusarium moniliforme propagules in naturally infested stalks incorporated in dry and moist soil at 27 C

Incubation period (months)	Mean F.moniliforme propagules/g stalks[1]	
	Dry Soil	Moist Soil
0	226,333[2]	226,333
1	231,380	151,195
2	229,392	72,127
3	220,062	38,083
4	201,565	21,132
5	216,148	6,407
6	204,562	1,017

[1]Propagules were obtained by grinding stalks in sterile distilled water with commercial Waring blender for 4 minutes, filtering through a single layer of cheesecloth and plating 1 ml of the diluted filtrate into each of 10 petri plates containing Komada medium.

[2]Numbers represent at average of 3 replications.

Table 8. Isolation frequency of *Fusarium moniliforme* from sorghum seedling roots subjected to various sources and levels of *F. moniliforme* inoculum under green house conditions[1]

Replication No.	Treatment I	II	III	IV	V	VI	VII	VIII
1	86.1	94.4	80.6	83.3	91.7	83.3	27.8	0.0
2	97.2	100.0	80.6	88.9	88.9	77.8	30.6	16.7
3	86.1	97.2	94.4	80.6	91.7	69.4	33.3	5.6
4	91.7	94.4	77.8	83.3	94.4	86.1	22.2	0.0
5	91.7	100.0	88.9	80.6	91.7	80.6	25.0	8.3
6	97.2	97.2	86.1	83.3	91.7	72.2	30.6	0.0
7	91.7	97.2	75.0	83.3	94.4	80.6	38.9	5.6
8	91.7	100.0	77.8	88.9	88.9	86.1	30.6	11.1
9	88.9	94.4	80.6	91.7	97.2	91.7	25.0	2.8
10	86.1	91.7	77.8	83.3	83.3	91.7	30.6	0.0
Mean	90.8b[2/]	96.7a	82.0c	84.7c	91.4b	82.0c	29.5d	5.0e

[1] Sorghum seedlings were exposed for 6 weeks to 8 (I-VIII) types of treatments in clay pots under greenhouse conditions. Treatments received were:

I. Naturally infested stalk sections incorporation in autoclaved soil and pots;

II. Artificially infested stalk sections incorporated in autoclaved soil and pots;

III. Naturally infested stalk sections incorporated in non-sterile soil and pots;

IV. Artificially infested stalk sections incorporated in non-sterile soil and pots;

Table 8 contd...

V. F. moniliforme conidia/mycelia (without stalk tissue) were incorporated (75,000 propagules/cc soil) in autoclaved soil and pots;

VI. F. moniliforme conidia/mycelia (without stalk tissues) were incorporated (75,000 propagules/cc soil) in non-sterile soil and pots;

VII. Soil and pots were not autoclaved and stalks and mycelia/conidia were not added. An infestation of 18 F. moniliforme propagules/g soil came naturally.

VIII. Soil and pots autoclaved and no stalks or conidia/mycelia incorporated (control).

2/ means followed by same letter are not significantly (p = 0.05) different.

Table 9. Analysis of variance for Fusarium moniliforme infection of sorghum seedling roots under green house conditions

Source	D.F.	Sum of Squares	Mean Square	F
Treatment	7	79712	11387	453**
Replication	72	1807	25	
Total	79	81520		

**Significant at 1% level.

STUDIES ON THE ECOLOGY OF FUSARIUM MONILIFORME SHELD. UNDER SORGHUM CULTURE

by

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ABSTRACT

Fusarium moniliforme populations were determined at monthly intervals over a two-year period by the dilution plate technique. Populations were consistently higher in rhizosphere soil than in soil obtained between grain sorghum (*Sorghum bicolor* (L.) Moench) rows (furrow soil). Populations remained stable or declined only slightly during the winter but declined rapidly during the spring. The mean number of propagules per gram of soil (oven dry equivalent) varied from 2 at the end of May to 147 in October in furrow soil, and from 12 in May to 1,046 in October in rhizosphere soil. When field soil was artificially infested with *F. moniliforme* conidia and mycelia, the population declined to zero in 10 and 14 weeks under greenhouse and field conditions, respectively. *F. moniliforme* failed to colonize pieces of autoclaved sorghum stalks incorporated in infested field soil, indicating that it is a soil invader (root inhabitant) and not a soil inhabitant.

F. moniliforme conidia and mycelia overwintered (1978-79 and 1979-80) in grain sorghum stalks without loss of viability or pathogenicity. No thickened hyphae, chlamydospores or sclerotia were observed in 1979 although chlamydospore-like cells were observed on some hyphae in 1980. No loss in viability of conidia or hyphae was observed when *F. moniliforme* was stored at -16°C for 6 months.

In both field and greenhouse studies, *F. moniliforme* aggressively invaded and colonized seedling grain sorghum

plants and colonization continued until the plants were physiologically mature. Colonization of stalk tissues did not always result in disease symptoms. Overwintered stalks with minimal decay were the major reservoir of F. moniliforme (2×10^5 propagules/g stalk). Populations in stalks were negatively ($r^2 = 0.957$) correlated with stalk decay.

F. moniliforme was detected in 27 of 28 untreated grain sorghum hybrid seed lots from 6 different sources. The propagules were more commonly detected as a surface contaminant than internally, as the percentages of nonsurface disinfested seeds (100 seeds tested/lot) affected ranged from 0 to 56% while surface disinfested seeds ranged from 0 to 24%.

The herbicides Atrazine, Cyanazine, Terbutryn, Propazine and Propachlor did not reduce F. moniliforme populations when applied at the field rate. Propachlor, applied at 20 times the recommended rate, totally suppressed F. moniliforme growth in culture.