CULTURAL STUDIES TO DETERMINE RESISTANCE TO *FUSARIUM MONILIFORME* STALK AND ROOT ROT DISEASE OF SORGHUM

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PREFACE

Much has been said and doubtless much more remains to be said about world food requirements in the immediate future.

According to Nobel Peace Prize winner Dr. Norman E. Borlaug during a special seminar at Oklahoma State University: "Producing enough food and distributing it evenly among the people of the world will be two big challenges facing mankind in the next few years."

I believe the elimination of world hunger depends mostly upon the training of developing countries in proper agricultural techniques and the use of food aid only in case of emergency. There is always a real sense of accomplishment, satisfaction, dignity and nobility in serving to the home dinner guest, fruit and vegetables grown in the backyard rather than in opening an imported, pasteurized can. Ice cream always tastes especially good when it is topped off with fresh, home grown strawberries. Accordingly, working on more agricultural techniques and distributing them via training among the people of the world is a definite long term solution. For the centuries to come, we expect agricultural production to keep pace with the demand.

The production of sufficient quantities of food for the world population requires not only a better application of new agricultural techniques of production, but also an efficient adaptation and utilization of the current research results in different subjects of agriculture and related sciences.

One of the important objectives for better application of new agricultural techniques is to reduce losses due to plant diseases and pests.

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I am alone, however, solely responsible for any errors of fact or judgement that appear in this study.

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CHAPTER I

INTRODUCTION

Sorghum has been grown in Africa since before the birth of Christ. It was introduced into the United States of America during the middle 1800's (23). In recent years sorghum has known a tremendous increase in popularity, especially in the United States which is now the world's largest producer. Under mechanized agriculture, yields approach an average of 1.3 tons per acre or 3 tons per hectare (23).

Sorghum is utilized mostly as forage, silage, or grain for livestock and poultry feed and to a limited extent for industrial processes where it can be substituted for corn. In Africa, sorghum is used as food and feed or can be fermented to make beer.

Since rainfall is light where most sorghum plants are grown, yields are dependent upon moisture. However, sorghum as a dryland summer crop is well equipped with its extensive root system for survival in semi-arid climates. Any disease that may weaken this important root system seriously threatens the environmental adaptability of this crop. Unfortunately basal stalk and root rot diseases are common

problems of cereals, and attack sorghum (14). These complex diseases have been investigated extensively, and several different species of fungi and bacteria have been found living in various associations within these complexes. Among these numerous microorganisms, many Fusarium species are involved (27). One of the most prevalent Fusarium species found as a pathogen is Fusarium moniliforme Sheldon (53). This pathogen is of worldwide significance, and is most destructive under hot and dry weather conditions. The same fungal pathogen is predominant in the sorghum stalk and root rot disease complex (16). The fungus has been reported to be air-borne (39), seed-borne (1, 33), and soil-borne (4). It is at least a facultative parasite (4), if not a facultative saprophyte, which overwinters on old sorghum stalks and roots of the previous seasons (37). The fungus survives in soil for many years as mycelium in plant debris (38). It is an extremely variable organism with the capacity to mutate and generate new strains that may be even more destructive than the parent strains. The survival of this stalk rot fungus is enhanced by its prolific power of reproduction and the ability to grow under a wide range of conditions. `Fusarium moniliforme, as a wound or weak parasite, enters the plant via roots (16). Infection takes place at different stages during germination and/or seedling growth (19). Systemic infection can affect plants late in the season, near maturity and may reduce

yield from 10 to 20 percent (15). The fungus does not appear able to damage healthy, vigorous cell tissue. Plants must be predisposed (12). The pathogen produces a toxin that inhibits root growth (19). In addition, this toxin is responsible for neurological disorders and paralysis of animals fed infected plants (36).

CHAPTER II

LITERATURE REVIEW

1. Taxonomy of Fusarium moniliforme

The capacity of rapid mutation in Fusarium species has made their identification confusing. Fusarium moniliforme, a conidial state of Gibberella fujikuroi (Sawada) Wollenw., belongs to the form-class Deuteromycetes, form-order Moniliales (5, 47). The species "moniliforme" refers to the microconidia occurring in chains, which is an important taxonomic feature of the species (5).

2. Fungal Culture

In culture the fungus has hyaline to slightly greyish septate mycelia bearing hyaline conidiophores, sometimes in groups of 2 to 4. Chains of microconidia are produced only during the first few days of hyphal growth (16, 21). These chains are borne on aerial conidiophores and may produce 20 to 60 conidia per chain. Microconidia are ovoid in shape. In situ, under the low power of the microscope, microconidia can readily be observed (18). They measure 5-12 x 1.5-2.5 mμ. They occasionally become uniseptate (5). Besides the production of microconidia in chains, F. moniliforme may

eventually form irregular clumps of microconidia at the apex of the conidiophores, when collapse of the conidial chains occurs. This structure is called a false head.

Macroconidial formation is rare in many strains. When macroconidia are present, they develop from conidiophores formed as lateral branches on the hyphae. The conidiophore may consist of a single basal cell bearing 2 to 3 apical phialides. Macroconidia are inequilaterally fusiod, thin-walled with an elongated, often sharply curved apical cell and pedicellate basal cell. They are 3-4 (5-7) septate and measure 25-36 x 2.5-3.5 mμ. Swollen stromatic initial cells are frequently found which can be confused with macroconidia. Sporodochia and occasionally pionnotes develop in some strains (5).

Perithecia usually occur only on dead plant material. This sexual state is produced when correct mating strains are introduced into the culture. Perithecia measure 250-350 mμ by 220-300 mμ and possess a rough outer wall. They are superficial, dark-blue and develop along the line of contact of two mating strains. Asci are ellipsoid to clavate with 4-8 uniseriate to biseriate ascospores. Ascospores are hyaline, ellipsoid, often one septate and occasionally three septate. They measure 14-18 x 4.5-6 mμ (5). Single germinating ascospores produce microconidia in chains and three septate macroconidia typical of F. moniliforme. Both conidial and sexual states have a capacity for rapid

mutation. The presence of sectors in culture is an indication of mutation.

In culture, the fungal mycelium is generally pale peach to violet with a white powdery appearance over the surface, due to formation of microconidia. When grown on potato dextrose agar (PDA), the fungus has a tiger stripe appearance. Identification is easier in pure culture started from single conidia or hyphal tips. Growth and sporulation of F. moniliforme are highly dependent on cultural conditions, particularly the medium. Rich medium may delay formation of sporodochia or pionnotes. The color of the fungus and the shape of conidia change with respect to the medium.

3. Dispersion and Survival

Fusarium moniliforme is commonly seed-borne (1), but the infection of seed is mostly due to air-borne propagules (8). The fungus may survive in stalk fragments in or on the soil, and can grow saprophytically in stalk residue. The study of air dispersal of F. moniliforme has shown that this species makes up to 30 percent of Fusarium propagules trapped in the air of corn fields (39). Most propagules of F. moniliforme were trapped in August to September, when corn was maturing. The F. moniliforme colony counts, on corn leaves from seven samples, averaged 886 colonies per 20 cm^2 of leaf surface (39). Therefore about 83 percent of the

Fusarium spp. population consisted of F. moniliforme. In addition, F. moniliforme was isolated mainly from distal ends of corn silks (85 percent) and only occasionally from their proximal ends (24). The average population of Fusarium spp. from wind blown dust was estimated at 1500 propagules per gram of soil based on the dilution plate method, and F. moniliforme made up 56 percent of propagules (39). It was estimated that this windblown soil traveled 300 to 400 km from a distant source. The importance of rain splash dispersal of F. moniliforme was investigated during the growing seasons, and propagules in splashing rain water or in air reflected similar trends (39). The populations of F. moniliforme varied from 3 to 50 x 10^4 propagules per ml of water trapped between the leaf sheath and the stalk. Splashing rain can disperse propagules from plant to plant or wash them from leaves into sheaths. Rain splash water collected over a cornfield carried from 4 to 40 propagules of F. moniliforme per ml of water (39).

Although F. moniliforme may not be abundant in crop residues at the end of the growing season (37), it may be present in sufficient amounts or may multiply rapidly enough during the growing season on crop residues, leaf surfaces, or in rain water trapped in leaf sheaths, to build up its inoculum potential. From one or more of these sources, wind and rain could be important vectors. If propagules can be found in soil carried by wind, it seems possible that wind

might blow fragments of Fusarium infected crop residues in soil, from one field to another, where conventional tillage is common (39). Some inoculum is probably carried by insects such as stalk and ear borers, root worms and mites which are present in crop residues lying on the ground from previous seasons. Other insect pests of cereal crops may play an equally important role in the disease complex as vectors (19), to facilitate entry of fungi into the plant by their feeding activities. Fusarium moniliforme (25-39%) was isolated both externally and internally from larvae, pupae and newly emerged picnic beetles (54).

Fusarium moniliforme survived in sorghum stalks as a saprophyte in residues buried 30 cm deep at 5-35% soil moisture content and at 5-10 C soil temperature. It was found in the tissues that decomposed the least adjacent to the stalk epidermis and vascular tissues in the nodes. There is low survival in roots and small host fragments (37). The survival structures of the fungus are the individual thickened hyphae. Roots of healthy plants become infected when they grow through stalks previously infected with F. moniliforme (38). The fungus is not a soil inhabitant as it has been shown that F. moniliforme will not colonize sterile fragments buried in field soil (37, 38). It has not been found in soil apart from host tissues in several years of sampling soil (37). Therefore, it is considered to be a soil invader and not a soil inhabitant,

because of its poor competitive saprophytic ability. Conversely, Bolkan et al (4) found that a few varieties or strains (var. Subglutinans) have a competitive saprophytic ability and may colonize dead plant material. Therefore, these strains of F. moniliforme can prolong their survival by colonizing residues of plants that are not hosts.

Conidia of F. moniliforme are short-lived in soil in the absence of host tissues. They survived relatively briefly, 6 to 13 weeks depending on soil moisture and incubation temperature. Free conidia survived significantly longer in air-dried soil than in moist soil adjusted to 10, 25, or 35% moisture content; and they survived better at soil temperatures of 4 and 18 C than at 25 and 30 C. Differences in survival time, in dry and moist soil, has been explained by the more rapid or increased germination at high moisture, thus reducing the population of viable conidia (4, 41).

In general, the fungus survives mainly by colonizing host plant tissues and less frequently as free conidia in soil (42). The fungus may also survive in infected seed (1, 29), and a resultant stalk infection may originate by planting this infected seed. Experiments have been performed to ascertain the prevalence of F. moniliforme in kernels and stalks of corn in Minnesota and to determine the relationship of kernel infection to stalk rot development. Results showed no appreciable differences between the

percentage of infected crown roots on plants established from seed lots, differing substantially in percentage of infected kernels (29). Thus air-borne or soil-borne inoculum is probably more important epidemiologically than is seed-borne inoculum (25).

4. Predisposition of Sorghum to Stalk Rot

The interactions of host , pathogen and environment are important in the stalk rot syndrome. Predisposition is associated with carbohydrate shortage in root tissue, which is caused by a combination of a reduction in photosynthesis and intraplant competition for carbohydrate by the developing kernels of grain. This photosynthetic stress-translocation balance (Ps-TB) concept of predisposition of sorghum stalk rots, proposed by Dodd (10), explains the incidence of carbohydrate production and translocation in stalk rots. The consequence of this carbohydrate shortage is that root tissue has a weakened cellular defense system, allowing invasion and degeneration by soil microorganisms. Roots are infected, the plant wilts, and stalk rot develops (11). Photosynthesis is an important factor in this carbohydrate shortage. In fact, photosynthesis represents the primary means by which solar energy is converted to a usable form of energy for our biosphere. The light energy is first trapped and then converted to chemical energy in

the form of glucose. This occurs in very small subcellular organelles called chloroplasts. Thus any light reduction in the environment may be one of the main factors predisposing sorghum to stalk rot. Higher plant densities not only reduce light but also reduce the water and minerals available to each plant.

It is important to notice that all fungal pathogens associated with root rots are ubiquitous, and most appear to be unable to damage healthy, vigorous cell tissue. In other words, as long as cells remain vigorous, most host genotypes have genetic components for resistance to potential pathogens (12). Host - environment interactions are the eminent factors of predisposition (43, 44).

In the host, cellular senescence or cell death of pith tissue precedes stalk rot. Living tissue resists pathogens but dead tissue does not. Apparently, synthesis of cellular resistance substances decreases with senescence (2). The ability of several fungi to rot pith tissue correlates positively with the percentage of cell death in the tissue (12).

Leaf removal, like light reduction, lowers total sugars in the stalk and increases stalk rot development. Under normal cultural conditions, the sugar content is higher in the lower stalks of resistant hybrids than in stalks of susceptible hybrids. Higher stalk sugar content increases resistance to stalk rot (12). Root and lower stalk tissues

are decayed by several microorganisms as the tissues lose their metabolically dependent defense system because of an increase in cellular senescence caused by carbohydrate deficiency. This loss of metabolic defense is due to the combination of many factors such as insufficient photosynthesis, moisture and nutrient availability (20). High nitrogen and low potassium often are associated with an increased incidence of stalk rot. The photosynthesis rate is often lower in plants deficient in potassium.

In general, light intensity, the amount of leaf surface, moisture and mineral availablity are directly related to photosynthesis which consequently produces carbohydrates, an important factor in stalk rot resistance. The unavailability of water, an overall carrier of all plant nutrients, is an important cause of decrease in leaf area and a main predisposing factor to plant infection (34).

5. Infection

The ecology of the sorghum stalk rot penetration phase of F. moniliforme is not very clear. The higher frequency of stalk rot fungi in crowns and lower nodes and internodes is further evidence of stalk invasion by the pathogen through the roots (54). This indicates that the infection probably is initiated in the soil. Many environmental factors are known to influence the development of stalk rot. Temperature and moisture are very important in the

penetration phase. Successful penetration of the pathogen occurs in high-moisture soils close to saturation (7, 50) when temperature is above 25 C. The incidence of root and stalk rots increased in greenhouse grown corn plants when soil moisture was maintained between saturation and the normal moisture capacity (50). This condition is favorable for the pathogen. Cool, wet weather enhances fungal growth and increases the chances of penetration. Penetration may be direct or through wounds or internodal cracks. The European cornborer and root worm have been reported to carry F. moniliforme conidia, and may inoculate plants when feeding (30, 54). Conidia frequently lodge between the leaf sheath and the stalk, an area providing ideal conditions for conidial germination.

The fungus enters the plant as the growing hyphae from germinated conidia or from the dormant hyphae of the previous season. The latter situation occurs when emerging sorghum roots grow through an old infected stalk buried in the ground. Humidity and moisture are necessary for conidial germination or annulment of dormancy and fungistasis of old hyphae. Usually the plant is infected during or immediately after seed germination. The earlier the infection, the greater the loss caused by the pathogen, as susceptibility to infection decreases with plant age (52). Fusarium moniliforme is primarily a surface contaminant occurring in cracks, natural openings and insect

punctures in the seed coats. The fungus may enter the cotyledonary plate region when the stem bud breaks through the pericarp or when the plumule breaks through the apex of the coleoptile. Direct penetration of young tender tissue may occur when primary radicles or emerging adventitious roots break out (30).

The real infection phase begins after penetration. Many researchers have suggested that pectolytic enzymes are most important during the early stage of infection, resulting in the occlusion of the xylem vessel elements with pectic substances as an early sign of infection. The enzymes would affect tissues in advance of the hyphae. This suggestion led many workers to conclude that F. moniliforme was a facultative parasite and even a secondary invader of roots (49). The growth of mycelia may produce conidia, acids, enzymes and/or toxins that plug the vascular system (31).

The fungus and its toxin can reduce primary root length, number of lateral roots, and epicotyl length (19). The toxin induces the same symptoms caused by the fungus (45). Some workers have isolated this toxin from culture and it was deleterious to animals and to shoots of cereals and tobacco, but they did not determine whether this toxin was produced in plants after infection or whether the toxin facilitated penetration or infection. The fungus also produces gibberellins which are formed in roots and

translocated to shoots (28). Again it is not known whether these substances aid in penetration and infection or have some other function.

In sweet corn seed, experiments have shown that hyphae were mainly intercellular and eventually penetrated throughout the tissues (30). The growth upward through the vascular system is faster than through the parenchyma tissue. In addition to the local infections, plants can become systemically infected (15). A combination of both systemic and local infection probably occurs. Infection is severe when cool, moist conditions are followed by hot, dry weather.

6. Symptoms and Host Range

After penetration and infection, soil moisture is no longer a factor for further growth of the pathogen. Lodging of sorghum often develops when wet weather, early in the season, is followed by subsequent prolonged hot and dry conditions.

The first indications of disease are the appearance of small orange areas on the rootlets of the seminal root system. The cortical tissue of the roots and stalks are discolored. Later in the season adventitious roots are similarly affected. These lesions enlarge and may have various sizes and colors, ultimately affecting the main roots. Coloration changes to brown, then black, in advanced

stages of decay. Vascular tissue begins to decompose and rot. These variations make the stalk rot syndrome complex.

Inside the stalk the pith disintegrates and turns pink, red and finally black. The stalk may break over (lodge) near the soil level late in the season, or the plant may be stunted in a dwarf type which results in a yield reduction.

Stalk breakage occurs mostly in late infection. The initial cause involves low sugar production caused by plant stress. Drought can curtail a plant's ability to produce enough sugar to supply the food needed, to sustain plant vigor and to fill the kernels. In recent years the incidence of stalk rot caused by F. moniliforme has greatly increased in the U. S. from 10 percent to 20 percent. In some areas (southern Minnesota) stalk infection of corn averaged 60 to 100 percent (26).

Fusarium moniliforme is wide spread in both humid and sub-humid temperate zones and extends into sub-tropical and tropical zones throughout the world. It is reported from at least 32 plant families and is a major parasite of several Gramineae mostly cereal crops such as sorghum, maize, sugarcane, and rice. In rice, losses due to F. moniliforme have been reported up to 70 percent in early maturing cultivars (28). It is also an important pathogen of a very wide range of hosts, in which it may cause diseases such as seedling blight, pre- and post-emergence damping off, basal stem, crown or foot rot, root rot, lodging, stunting, and

seed rot (2).

In order to understand the root rot better, a proposed plan of study was developed to study cultural techniques which will permit a more rapid and accurate assessment of sorghum lines for resistance to F. moniliforme. In addition, the effect of water stress on the complex will be evaluated using these cultural techniques.

CHAPTER III

MATERIALS AND METHODS

1. Pathogen Isolation

Several surveys of stalk rot in fields of sorghum and corn were made to four counties in the state of Oklahoma. These counties were: Payne, Muskogee, Okmulgee and Texas. In addition to samples collected in these surveys, many other samples of sorghum stalk rot were received by the Plant Disease Diagnostic Laboratory from sorghum growers in Oklahoma. All these samples, about 45, provided a good opportunity for making several isolations of Fusarium moniliforme from diseased stalks of sorghum.

Infected stalks were collected, labeled, and split into two parts with a sterile knife for isolation. Small pieces of stalk pith tissue were selected from the reddish purple subcrown area or from the root tissue. The most obvious tissues to harbor the pathogen were the parenchyma and the sclerenchyma.

Although all crown pith tissues were reddish to dark brown internally, a large number of crown pith pieces were sterile, not infected with F. moniliforme. This suggested that in the infection process toxin production may be

involved (31).

The tissue pieces selected were surface sterilized by stirring them for 45 seconds in 10% Chlorox solution and then rinsing them in sterile distilled water. The tissue samples were finally drained on sterile paper towels and placed on potato dextrose agar with streptomycin sulfate (300 ppm) added (PDSA) in plastic petri dishes.

Surface sterilization, inoculation and transfer were done in the isolation chamber. The petri dishes were then labeled and incubated at 25 C with fluorescent light supplemented with near ultra-violet radiation (360 nm) in a growth chamber.

After two or three days, cultures were checked regularly under a dissecting microscope and any suspect growth was examined in detail with a compound microscope. Probable F. moniliforme growth was transferred to new petri dishes of PDA for further identification. After several transfers, the cultures were grown in water agar (1%) (WA) for hyphal tips and single conidial purification. Each isolate was stored in slant tubes of potato dextrose agar (PDA) and placed in the refrigerator (4-5 C) (17).

The agar used throughout the project was Bacto-agar produced by Difco Laboratories, Detroit, MI 48201, and all statistical analyses were made using Student's t-test method.

2. Similitude of Isolates

Three disks or plugs, 7mm in diameter, of each isolate were cut out of the advancing margin of seven-day-old cultures with a sterile cork borer and placed on the center of a petri dish containing PDA. The plug was placed upside down to prevent possible conidial dispersal that could induce an unwanted surface inoculation of the medium.

In order to avoid the condensation of water on the medium it was necessary to reverse the petri dishes when placing them in the incubator. Morphology and growth rates of isolates were compared and recorded.

3. Growth Rate of F. moniliforme

Fusarium moniliforme was grown at different temperatures: 21, 27, 32 and 38 C on PDA with four replications to determine the optimum temperature for fungal growth. A sufficient number of 7mm disks covered with mycelia were carefully cut out of four day-old colonies and transferred to the center of the petri dishes. One plug was used for each petri dish. The isolate used was from the Dixon farm plot near Baldhill, Okmulgee County. All petri dishes were wrapped in aluminum foil to eliminate possible variations of light intensity.

The possible changes in the environment due to multiple readings were avoided by making just one single reading at the end of the seven day period of the experiment.

The influence of light and darkness was tested with the Muskogee isolate using four replications on PDA. Eight petri dishes of PDA were inoculated with 7mm plugs of the fungal culture and four were randomly chosen and wrapped in aluminum foil. All were kept in the incubator with 12 hr light cycle of fluorescent (3,000 lux), incandescent and near ultra-violet radiations at 25 C. One single reading was done at the end of the week period.

4. Test of Infection

Six hundred seeds of sorghum cultivar Pioneer 8451 were surface sterilized (8 min) in 10% Chlorox solution and planted in PDA, five seeds per petri dish. The disease free seedlings selected after germination five days later were tested in vitro with F. moniliforme by using aluminum baking pans 7 X 11 X 21 cm fitted with doubled soft wire hangers, and paper towel to support developing seedlings (46). The system was autoclaved for 20 minutes at 121 C, 1.05 kg/cm^2 before planting the seedlings. Ten rows of five seedlings were planted per pan with four replications. The young plants were infected the same day. At infection, seedling radicles were about 3 to 4 cm long.

Seedlings were infected by placing a disc or a plug of fungal growth (7mm diam) on the hypocotyl epidermis, between the cotyledon and the growing tip of the embryonic root. The water used for the experiment was dionized distilled

autoclaved water, 100 ml per pan. This water was replenished daily to keep the paper towel continuously wet. The experiment was conducted in a sterilized growth chamber of 104 X 61 X 76 cm. Observations were made daily and the number of blighted seedlings was recorded weekly until the seedlings were two months old.

In one experiment drought stress was simulated by allowing pans of inoculated and control plants to dry out for 12 hours, one week after infection.

Three hundred germinated seeds of sorghum, Pioneer 8451, from the same seed lot were infected with F. moniliforme in the growth chamber at 25 C. The substratum used was a 1% WA in plastic weighing dishes, 7.3 X 7.3 X 2.5 cm, or in small aluminum pans, 9.5 X 6 X3.5 cm. Each dish contained 100 ml of medium and all seeds were surface sterilized for 8 min with 10% Chlorox solution. Five germinating seeds of sorghum were planted in each plastic weighing dish and 8 to 10 seeds in each aluminum pan.

The height of seedlings was recorded two weeks later.

5. Screening for Resistance

The following 20 sorghum cultivars were tested for resistance to F. moniliforme infection at 25 C using the aluminum pan system:

1. R OKY62
2. B OKY54

3. B OK11
4. Redlan
5. Dwarf Redlan
6. B OK8
7. Wheatland
8. R WD3 X Weskan-3-2-2-1
9. TX2536
10. Martin
11. B WDY18
12. R OKY15
13. R OKY8
14. R OKY78
15. B OKY55
16. B CK60
17. Dwarf Milo CI 332 VN 5001
18. R OKY34
19. R OKY47
20. TX428

All these varieties were furnished by the Agronomy Department of Oklahoma State University.

The seeds were surface sterilized for 8 minutes in 10% Chlorox solution, rinsed in sterile distilled water, and germinated. Twenty germinating seeds of each variety were planted in two aluminum pans. A suspension of F. moniliforme was prepared by blending the conidial contents of one petri dish of a rapidly growing colony with 100 ml of

sterile distilled water. The counts of conidia with an hemocytometer type counting chamber gave 3.2 X 10^3 conidia per ml. One drop of inoculum was deposited in a hole made in the seed bed before setting the seed into it.

Inoculation was also accomplished by soaking the seeds in the inoculum suspension for 3 minutes before embedding them in the WA medium for germination.

The aluminum pans were put in rectangular plastic buckets in a randomized block design. Inoculated plants and control plants were separated in different buckets to avoid undue contamination. Each bucket held 10 aluminum pans. A total of four buckets was used for one replication of 400 seedlings. In order to maintain a high relative humidity in the seedling environment, 100 ml of distilled autoclaved water was poured in each bucket. The buckets were then wrapped with a transparent plastic film and placed into a walk-in growth chamber where the environmental conditions were maintained at 25 C, 80-90% relative humidity. After two weeks, the height of plants was measured. This experiment was replicated four times.

6. Effect of Water Stress on Plants

This experiment was conducted in a growth chamber of 300 X 300 X 150 cm with a twelve-hour light cycle of fluorescent and incandescent lamps.

Seeds of sorghum cultivar Pioneer 8451 from the same

seed lot, were germinated and used for the water stress test to determine the effect of water deficits on infected sorghum seedlings.

Seedlings selected after germination were infected with F. moniliforme and tested at different levels of water stress. The stress was produced by adding polyethylene glycol (PEG) 6000 to the agar medium. The amount of PEG 6000 was determined by using the equation derived by Michel and Kaufmann (35) to obtain the desired osmotic potential of the solution:

$$s = -(1.18 \times 10\text{-}2)C - (1.18 \times 10\text{-}4)C2 + (2.67 \times 10\text{-}4)CT + (8.39 \times 10\text{-}7)C2T$$

s = desired water potential,

T = temperature in degrees C,

C = concentration, PEG 6000 g/kg H_2O.

A consistent medium able to hold sorghum seeds on the surface was selected from the different concentrations of WA adjusted with PEG 6000 to -2.8; -3.8; -4.8; -5.8; -6.8; and -7.8 bars. The water potential of WA was calculated on the basis of -1.4 bar for 2% WA (51).

One concentration of WA (4%) was chosen for its good consistency and because it mixed well with the desired concentration of PEG 6000. This concentration of WA was used throughout the entire experiment. The PEG 6000 used was produced by SIGMA Chemical Company, St. Louis, Missouri.

Six media were prepared with different percentages of PEG 6000 in 4% WA according to Table I.

Polyethylene glycol 6000 was added to autoclaved WA when the latter cooled to below 60 C.

Ten seeds were planted into each medium. Four pans of each medium were prepared, two pans were inoculated and two were not. Each aluminum pan contained 100 ml of medium. These aluminum pans were placed in buckets using a randomized block design. The buckets were wrapped with a transparent plastic film to maintain the moisture level.

The experiment was replicated four times at 25 C in the walk-in growth chamber where the environmental conditions were maintained at high relative humidity (80-90%).

The height of seedlings was recorded two weeks later.

7. Effect of Water Stress on the Pathogen

The same concentration of WA (4%) was prepared and adjusted with PEG 6000 at different osmotic potentials to test the growth of F. moniliforme at different level of water deficiency (Table I). Each medium was poured in six plastic petri dishes, and one plug (7mm diam) of the advancing margin of seven-day old colony was placed on the center of each petri dish. The inoculation was carefully done to avoid undue dissemination of conidia.

The petri dishes were incubated at 25 C with 12 hr

TABLE I

SYNTHETIC WATER AGAR MEDIUM ADJUSTED WITH POLYETHYLENE GLYCOL (PEG) 6000

Medium no.	Agar Concentration	PEG 6000 g/kg H2O*	Water Potential
0	4%	0.0	-2.8
1	4%	78.5	-3.8
2	4%	119.6	-4.8
3	4%	151.4	-5.8
4	4%	178.3	-6.8
5	4%	202.1	-7.8
6	4%	223.7	-8.8

* PEG concentration determined by using Michel and Kaufman equation (35)

fluorescent light supplemented with near ultra-violet radiation (360 nm) in a growth chamber.

Colonies were checked daily and colony diameters were measured when colonies in the most growth conducive treatments were near the edge of the plastic petri dish. Counts of conidia produced at each moisture level was determined by using a hemocytometer.

CHAPTER IV

RESULTS

1. Pathogen Isolation

Fusarium moniliforme was isolated from sorghum stalks selected from fields of four counties in the State of Oklahoma. These county locations represented the major sorghum producing areas of northwestern and northeastern Oklahoma. Collections were made from both irrigated and dry land sorghum fields. Many isolates were from the crown and roots of sorghum stalks left on the field after harvest. There was little evidence of extensive external injury or symptoms until the stalk was split into two parts. Any reddish purple pith was always suspected as evidence for this pathogen. From 45 samples collected, five isolates of F. moniliforme were purified.

2. Similitude of Isolates

The different isolates were morphologically and culturally indistinguishable from each other when grown on the same medium. Colony growth and the growth rate in millimeters per hour were not statistically different (Table II). The typical striped appearance of the cultures was

TABLE II

GROWTH COMPARISON OF 5 *FUSARIUM MONILIFORME* ISOLATES ON POTATO DEXTROSE AGAR AT 25 C

Number	Fungal Isolates	Colony Diameter (mm)*				Means
1	Dixon (Okmulgee)	78	74	77	82	77.7
2	Muskogee I	75	81	78	76	77.5
3	Muskogee II	78	69	72	70	72.2
4	Texas (Panhandle)	73	79	80	76	77.0
5	New Panhandle	68	74	79	77	74.5

*Seven day-old colonies

easily observed. Most cultures formed microconidia in chains during the first four days after inoculation onto PDA. Later, only false conidial heads were observed. Macroconidia were seldom observed. Some colonies produced sectors of different colors.

3. Growth Rate of F. moniliforme

The growth of F. moniliforme at different temperatures (Table III) showed that the optimum temperature for growth ranged from 25 C to 27 C (Figure 1), and the average growth calculated within this range was 0.30 mm per hour on PDA (Figure 2). The growth and color characteristics were subject to change when the fungus was grown on different media. Apparently, growth increased from 21 C, culminated around 27 C and then began to decrease rapidly (Figures 1 and 2).

Statistical analysis of the effect of light on F. moniliforme development indicated no significant difference between colonies grown in darkness and those grown in light (Table IV). Continuous dark had no adverse effect and did not inhibit the growth of the fungus to any great extent.

4. Test of Infection

During testing for seedling infection using the aluminum pan technique, the number of blighted sorghum seedlings counted every week was very low. This number

TABLE III

GROWTH RATE (mm) OF *FUSARIUM MONILIFORME* COLONIES* AS AFFECTED BY TEMPERATURE ON POTATO DEXTROSE AGAR

Temperature (^{o}C)	Colony Diameter (mm)				Means
21	36	66	52	42	49.0
27	72	76	78	76	75.5
32	53	42	45	44	46.0

*Seven day-old colonies of Dixon isolate

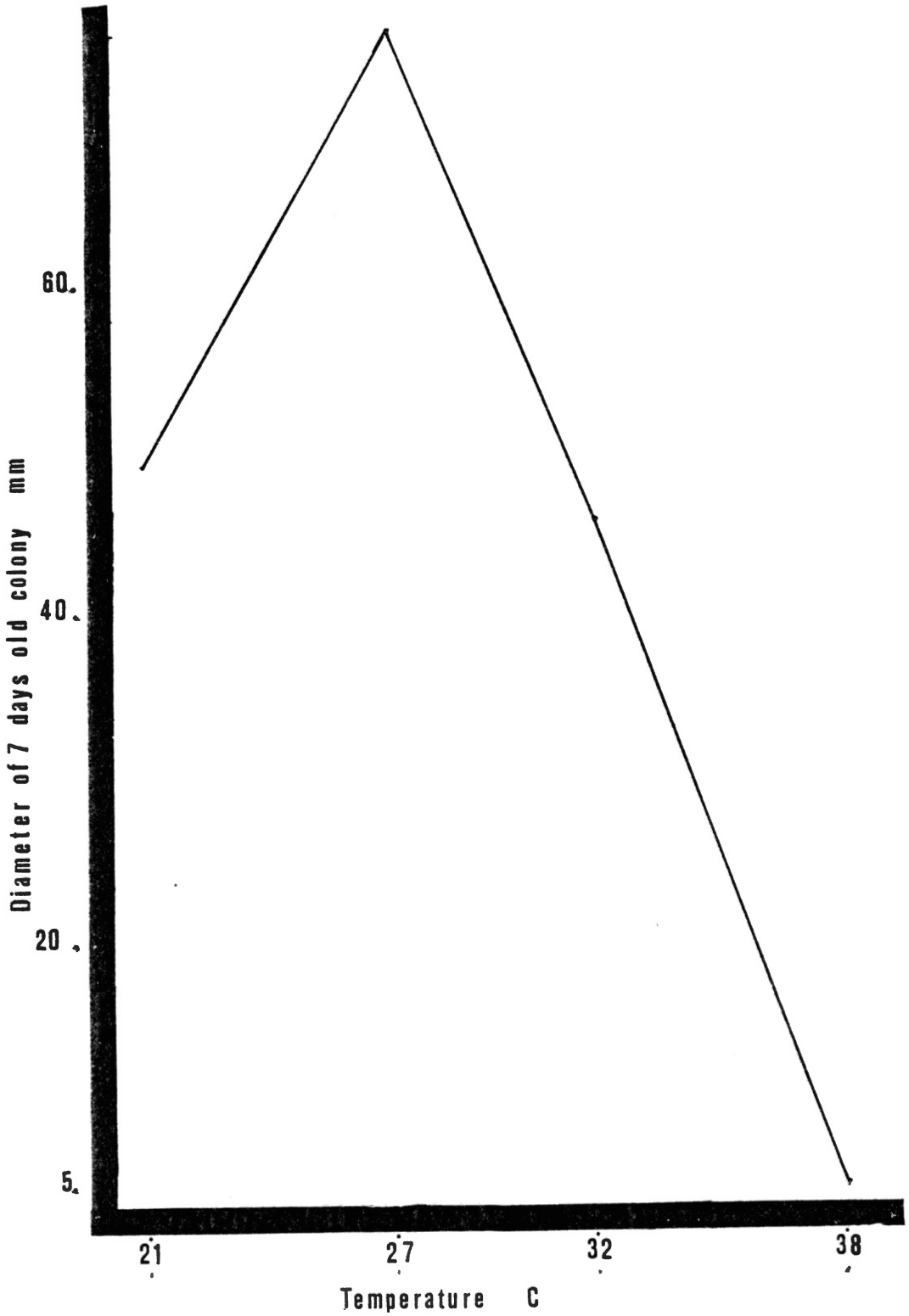

Figure 1. The Effect of Temperature on the Growth Rate of Fusarium moniliforme 7 Days After Inoculation Onto Potato Dextrose Agar

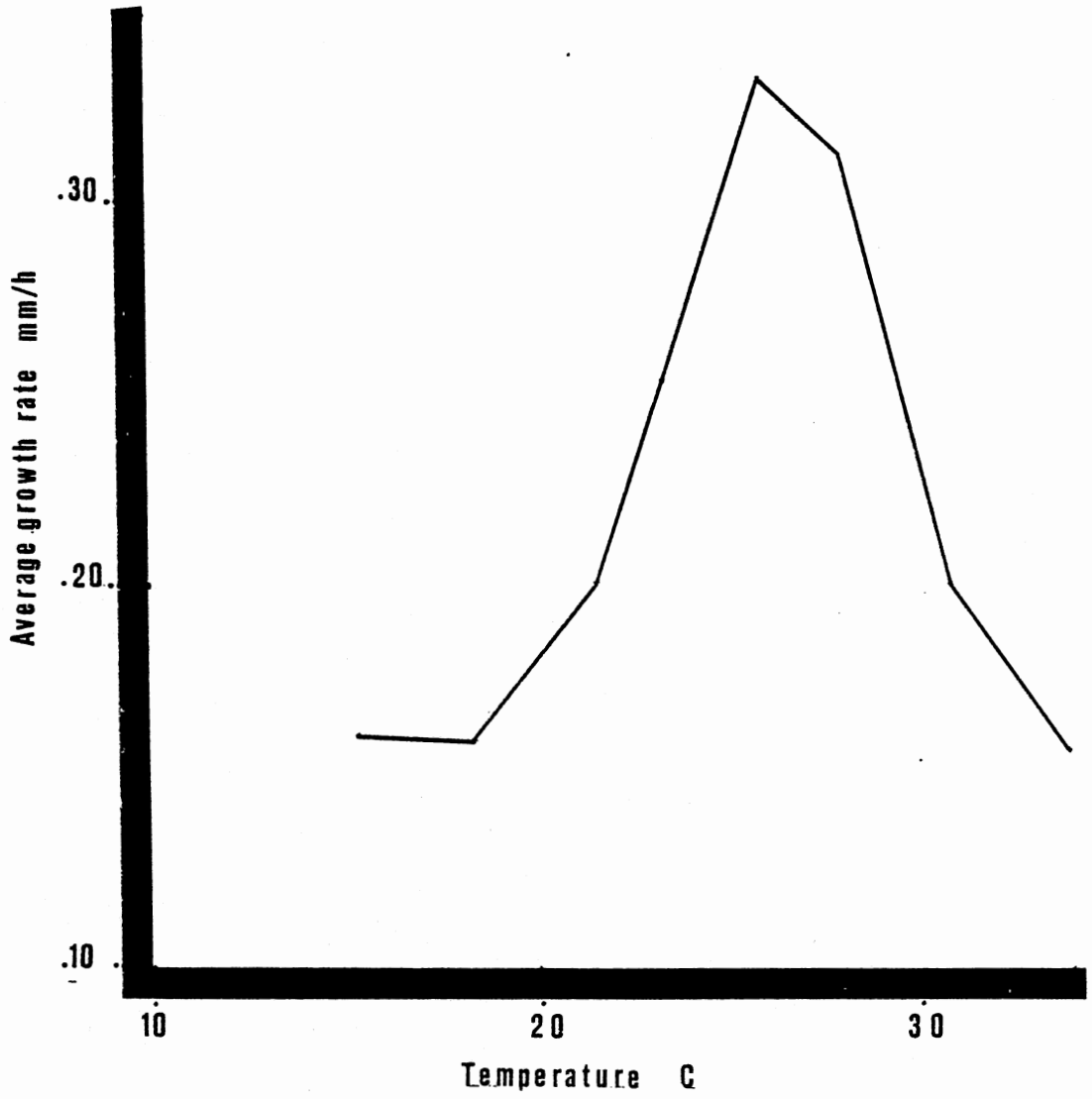

Figure 2. Effect of Temperature on the Growth Rate of Fusarium moniliforme Grown on Potato Dextrose Agar

TABLE IV

LIGHT EFFECT ON DIAMETER GROWTH (mm) OF *FUSARIUM MONILIFORME* COLONIES* GROWN ON POTATO DEXTROSE AGAR AT 25 C

	Colony Diameter (mm)				Average
Light	64	64	65	65	64.5
Dark	67	65	63	60	63.7

*Seven day-old colonies of Muskogee isolate

increased slightly when seedlings reached the three leaf-stage (Table V). Sometimes blight symptoms were not clearly expressed, but the fungus was readily reisolated from the infected plant within the root tissue of one-month-old seedlings. The number of reisolations seemed to decrease 45 days after inoculation. The first indication of infection was the appearance of the small orange areas on the rootlets of the seminal root system. Later, lateral and secondary roots were similarly affected. These lesions enlarged and ultimately affected the main roots. Coloration changed to brown, then to black in advanced stages of rot. The fresh and dry weight of stem and root (Table VI) showed a great deal of variation. However, the difference between infected and noninfected sorghum seedlings was not significant. The difference between stressed and nonstressed plant was not statistically significant, either ($P = 0.05$). However, it was possible to see a slight difference in seedling height after 12 hours of stress.

Using the water agar technique the result of the height measurement 14 days after inoculation and the number of blighted seedlings gave a highly significant response ($P = 0.01$) of the infected plants compared to the noninfected controls (Table VII). The observation of germinating sorghum seeds in sterile deionized water showed that during the first two days, sorghum seeds soaked up an appreciable amount of water and increased in size before the

TABLE V

FREQUENCE OF BLIGHTED SORGHUM SEEDLINGS AFTER *FUSARIUM MONILIFORME* INFECTION USING ALUMINUM PAN TECHNIQUE

Weeks	1st	2nd	3rd	4th	5th	6th	7th	8th	9th
Average Blighted Seedlings	1.0	0.4	2.3	3.1	4.2	4.6	3.2	1.0	0.0

TABLE VI

STEM AND ROOT WEIGHT (GRAMS) AS AFFECTED BY STRESS AND/OR *FUSARIUM MONILIFORME* INFECTION USING ALUMINUM PANS TECHNIQUE

Plant Material	Control Plants	Infected Plants	Stressed Plants	Infected and Stressed Plants
Fresh Stems	9.8	8.3	10.5	9.0
Fresh Roots	28.1	19.5	23.0	18.4
Dry Stems	2.15	2.05	2.40	2.32
Dry Roots	1.70	1.40	1.75	1.40

TABLE VII

THE EFFECT OF FUSARIUM MONILIFORME ON THE HEIGHT (cm) OF SORGHUM PLANTS GROWN ON 1% WATER AGAR AT 25 C

Trail No	Control Plants			Mean	Infected Plants			Mean
1	6.14	10.32	12.63	9.69	1.04	0.47	0.15	0.55**
2	5.22	9.65	14.75	9.87	0.04	1.43	4.56	2.01**
3	4.64	10.95	12.30	9.29	0.40	1.03	1.85	1.09**
4	5.20	11.07	13.90	10.05	2.52	4.52	3.27	3.43**
5	4.12	10.25	14.52	9.63	0.92	0.00	1.21	0.71**
6	4.74	12.25	12.71	9.90	2.50	2.76	1.62	2.29**
7	5.50	12.25	12.18	9.97	3.40	0.11	3.18	2.23**
8	6.08	7.82	15.17	9.69	0.26	1.46	3.11	1.61**
9	5.58	10.43	11.47	9.16	0.20	2.11	2.97	1.76**
10	5.16	11.47	12.37	9.66	0.00	1.98	0.00	0.66**

**Highly significant (P = 0.01)

seeds broke open when the radicle emerged.

5. Screening for Resistance

The screening for resistance of 20 selected sorghum cultivars was based primarily on height and the number of survivals. The height measurement of each plant surviving at the end of the experiment was recorded by measuring the distance from the seed level to the end of the longest leaf. A comparison of the mean height for the inoculated and noninoculated plants is presented in Table VIII.

In general, reduction in height was evident in all the infected plants. On an individual basis, quantification of seedling infection was not easy to determine.

The fungus was easily reisolated from seedling roots. The number of infected seedlings surviving in the pans versus noninoculated control plants was recorded (Table IX). Statistical analysis of the heights and the amount of seedling survival did not show any significant difference between control and infected plants. The hypothesized difference (D) chosen between resistant and susceptible cultivars was 50% of the control value. In other words, any cultivar with an average seedling survival or height less or equal to 50% of the control was considered susceptible to the pathogen. The cultivars B OKII (no. 3), Martin (no. 10) and R OKY78 (no. 14) seemed, however, to have a certain resistance to the pathogen in comparison to the other

TABLE VIII

SCREENING FOR RESISTANCE: HEIGHT (CM) OF DIFFERENT SORGHUM CULTIVARS AS AFFECTED BY *FUSARIUM MONILIFORME* INFECTION ON 1% WATER AGAR AT 25 C

Cultivars Number	Control Plants			Means	Infected Plants			Means
1	7.05	10.24	8.10	8.46	0.00	0.45	0.00	0.15
2	8.36	7.34	9.70	8.46	0.00	0.00	0.30	0.10
3	11.71	10.48	7.46	9.88	4.03	7.31	3.23	4.85
4	3.62	5.51	6.22	5.11	0.00	0.00	0.57	0.19
5	3.27	3.64	3.71	3.54	0.00	0.22	1.00	0.40
6	5.71	5.83	4.70	5.41	0.78	3.56	2.97	2.43
7	8.66	8.11	12.18	9.65	0.46	0.30	0.39	0.38
8	7.78	10.09	6.33	8.06	1.31	8.33	1.57	3.73
9	1.36	3.68	3.66	2.90	0.00	0.00	0.00	0.00
10	9.43	11.01	10.43	10.29	2.59	3.76	5.92	4.09
11	10.12	12.17	10.83	11.04	0.00	0.00	0.31	0.10
12	10.72	14.77	9.02	11.50	0.00	4.11	1.87	1.99
13	8.65	6.16	5.42	6.74	4.03	0.00	0.00	1.34
14	10.51	13.37	9.85	11.24	3.68	6.67	4.92	5.09
15	5.26	9.52	6.07	6.95	0.00	0.32	1.07	0.46
16	3.40	6.45	3.65	4.50	0.84	1.07	0.83	0.91
17	8.45	5.64	9.27	7.78	0.22	0.45	1.78	0.81
18	3.38	4.09	3.60	3.69	0.99	0.69	1.01	0.89
19	5.34	11.28	6.48	7.70	1.06	2.34	0.31	1.23
20	5.20	3.04	3.23	3.82	0.29	1.17	0.20	0.85

TABLE IX

SCREEENING FOR RESISTANCE TO *FUSARIUM MONILIFORME* OF SELECTED SORGHUM VARIETIES

Varieties		Survival in Treatment/Control		
1.	R OKY62	0/6	1/8	0/9
2.	B OKY54	0/10	0/7	2/8
3.	B OK11	6/10	7/10	4/9
4.	Redian	0/5	0/6	1/8
5.	Dwarf Redlan	0/4	3/5	2/6
6.	B OK8	1/7	4/6	5/7
7.	Wheatland	3/9	1/9	2/9
8.	R WD3 X Weskan	3/8	7/10	3/9
9.	Texas 2536	0/4	0/5	0/5
10.	Martin	4/10	4/10	7/10
11.	B WDY18	0/9	0/10	2/10
12.	R OKY15	0/9	6/10	4/9
13.	R OKY8	6/9	0/6	0/8
14.	R OKY78	5/10	5/10	6/10
15.	B OKY55	0/10	1/9	1/6
16.	B CK60	1/5	1/6	1/7
17.	Dwarf Milo	1/9	1/7	2/9
18.	R OKY34	1/5	2/8	3/5
19.	R OKY47	3/8	5/10	2/9
20.	Texas 428	1/7	3/6	2/4

cultivars. In general, there was no seedling survival greater than 56% of the control and no mean height greater than 55 mm. The comparison of mean height in infected and noninfected plants of the eight top cultivars is presented in Figure 3.

6. Effect of Water Stress on Plants

Stress predisposition, conditions unfavorable for rapid, uniform, vigorous seed germination, and seedling growth, was tested using PEG 6000. The seedlings grew slowly and were susceptible to infection and damage by F. moniliforme, which led to an uneven seedling stand and low vigor. Polyethylene glycol 6000 is a polymer regarded as chemically inert and nontoxic. The chemical induced stress (48) in germinating seeds and growing seedlings. This predisposition was an important factor in the infection process. The height measurement of infected seedlings grown at different PEG water potentials showed that the medium water potential had an important effect on the vigor and height of infected seedlings (Table X). Statistical analysis of the difference between infected stressed plants and control was highly significant ($P = 0.01$) at the water potentials -3.8 bars, -4.8 bars, -5.8 bars, -6.8 bars, and -7.8 bars. This difference was not significant at water potential -2.8 bars (Figures 4 and 5).

Root length measurement made from the cotyledon

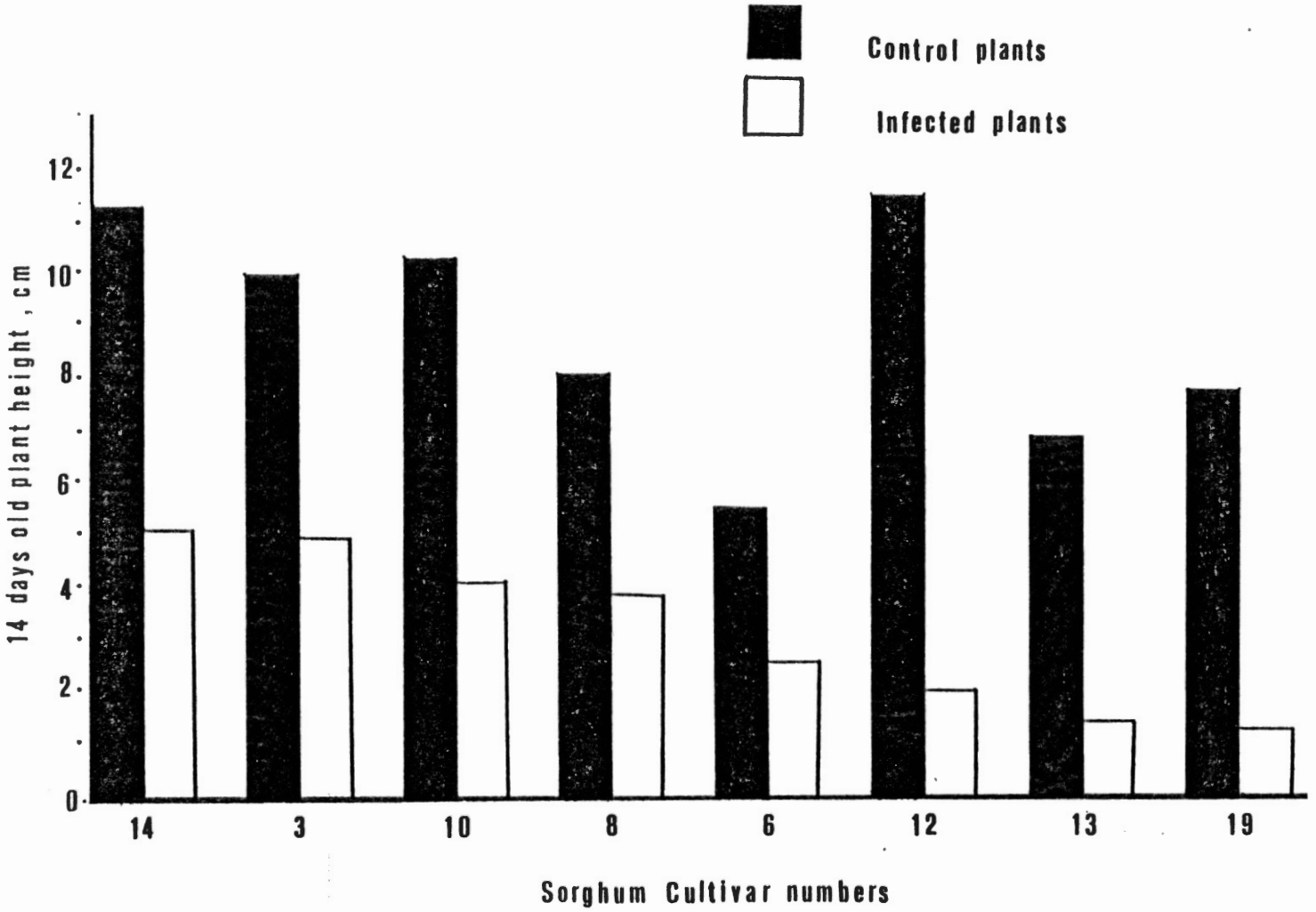

Figure 3. Comparison of Height Reductions in Eight Cultivars of Sorghum Infected With Fusarium moniliforme

TABLE X

SORGHUM SEEDLING HEIGHT (cm) AS AFFECTED BY *FUSARIUM MONILIFORME* AND POLYETHYLENE GLYCOL 6000 WATER POTENTIAL

Water Potential	Control Plants				Means	Infected Plants				Means
-2.8	13.33	12.71	11.45	12.40	12.47	6.79	14.72	14.37	11.89	11.94
-3.8	14.00	12.40	11.20	11.03	12.15	4.59	3.74	10.82	8.16	6.82**
-4.8	10.79	11.78	11.68	10.59	11.21	0.49	8.36	4.94	6.75	5.13**
-5.8	9.03	8.90	10.69	8.98	9.40	3.28	3.67	3.39	4.39	3.68**
-6.8	8.38	5.81	6.41	8.57	7.29	3.22	3.27	1.33	4.34	3.04**
-7.8	5.99	7.14	7.39	5.52	6.51	1.85	2.13	1.16	0.97	1.50**

**Highly significant (P = 0.01)

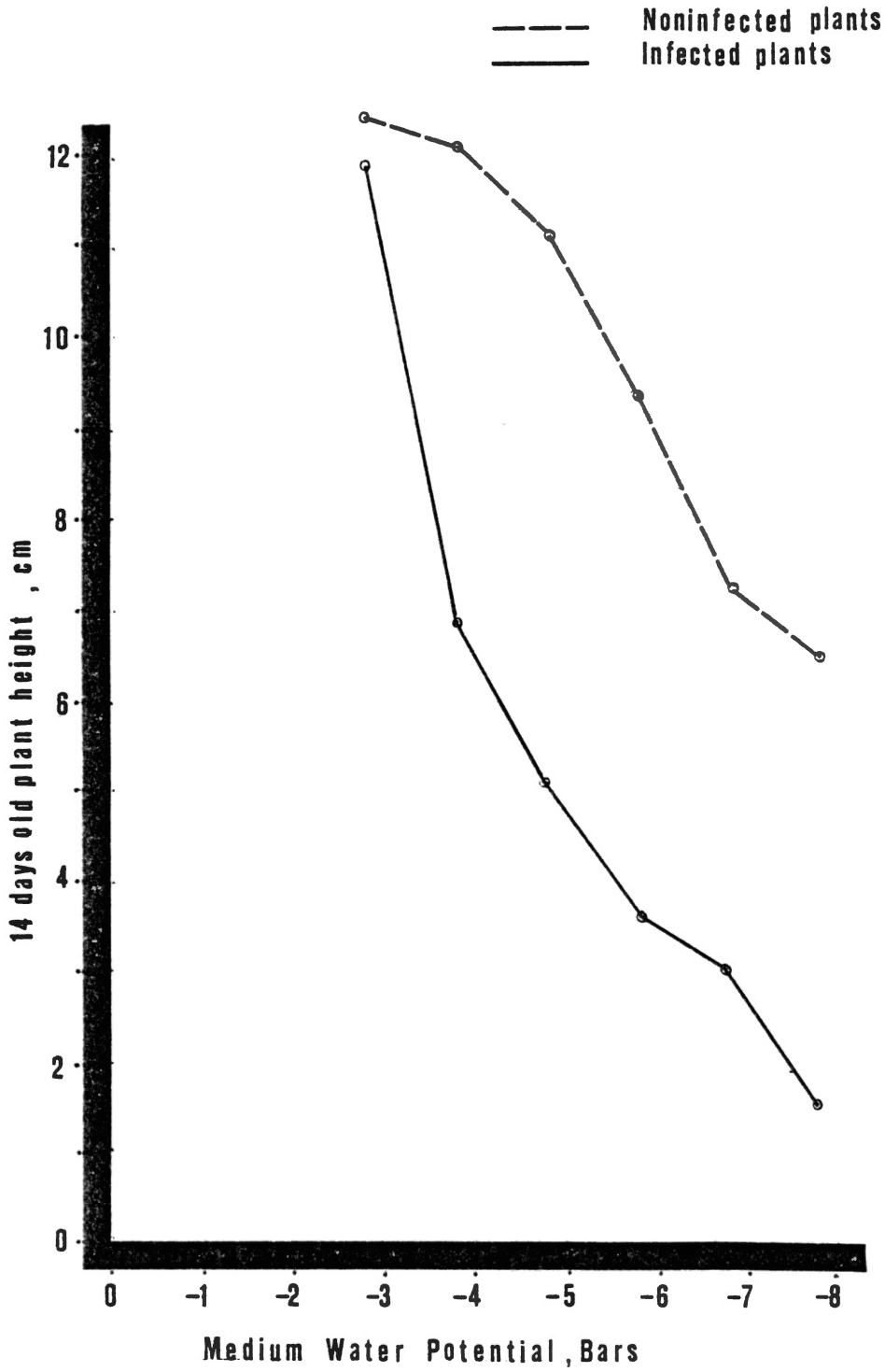

Figure 4. Effects of Water Stress on Height of Sorghum Seedlings Infected With Fusarium moniliforme

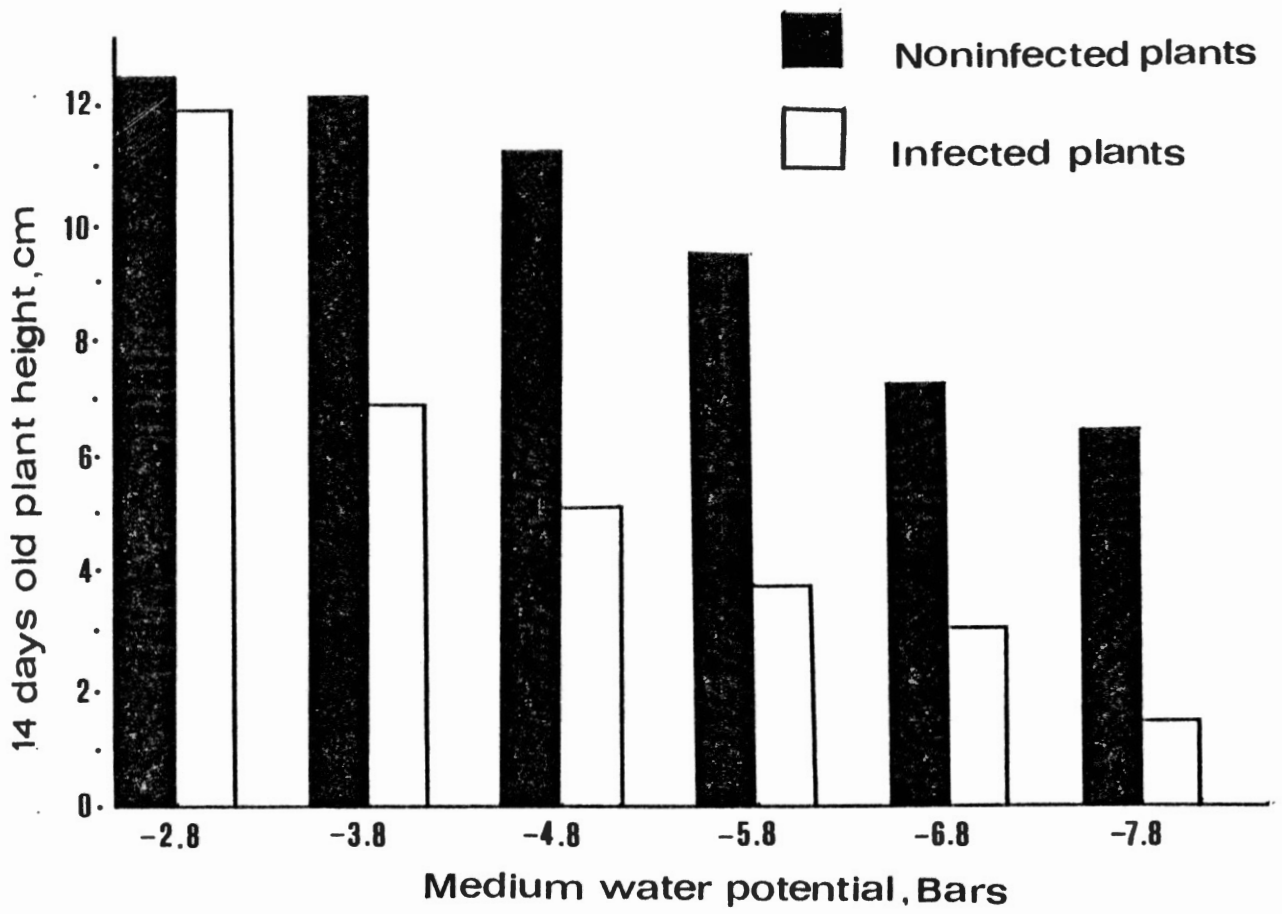

Figure 5. The Effect of Medium Water Potential on Sorghum Seedlings Infected With Fusarium moniliforme

attachment to the tip of the longest growing root showed a highly significant difference between inoculated seedlings and controls at water potentials -3.8 bars to -7.8 bars. At -2.8 bars this difference was significant for P = 0.05 (Table XI).

7. Effect of Water Stress on the Pathogen

The growth of F. moniliforme at different water potentials is recorded in Table XII. The parameters measured were: growth of the colony diameter, and the average number of conidia per petri dish. The result of this experiment showed a highly significant increase of growth and conidial production in WA (-2.8 bars) when compared to other water potentials. In WA, mycelia were thicker than in PEG solutions and the fungus tended to produce false conidial heads rather than chains. In agar PEG solutions (-3.8 bars to -8.8 bars), F. moniliforme produced numerous chains of conidia scattered over the medium surface.

The result of conidial counts per petri dish (Table XII) at different water potentials showed a highly significant difference between WA (-2.8 bars) and all PEG solutions (-3.8 bars to -8.8 bars) with more conidia being produced on WA (-2.8 bars).

TABLE XI

ROOT LENGTH (cm) OF SORGHUM SEEDLINGS AS AFFECTED BY *FUSARIUM MONILIFORME* AND MEDIUM WATER POTENTIAL

Water Potential	Control Plants				Means	Infected Plants				Means
-2.8	10.27	9.39	10.13	8.68	9.62	7.72	3.93	9.13	7.43	7.05*
-3.8	12.49	16.52	12.56	13.64	13.80	3.20	5.17	9.73	10.31	7.10**
-4.8	14.56	12.90	14.88	15.66	14.50	7.29	1.18	4.33	8.56	5.34**
-5.8	12.64	12.82	13.11	12.92	12.87	4.72	4.69	6.00	4.05	4.86**
-6.8	11.09	10.42	10.00	6.67	9.54	1.39	3.97	3.62	5.71	3.67**
-7.8	8.78	7.21	9.43	8.71	8.53	2.01	3.43	1.92	3.42	2.69**

*Significant (P = 0.05)
**Highly significant (P = 0.01)

TABLE XII

GROWTH RATE (mm) OF FUSARIUM MONILIFORME* AND NUMBER OF CONIDIA PER PETRI DISH AS AFFECTED BY MEDIUM WATER POTENTIAL

Medium Water Potential	Diameter of Fungal Colonies	Number of Conidia/dish
-2.8	75.83**	77.9×10^6**
-3.8	57.83	35.5×10^6
-4.8	60.16	45.3×10^6
-5.8	63.60	36.7×10^6
-6.8	67.00	41.9×10^6
-7.8	61.60	50.3×10^6
-8.8	59.60	34.9×10^6

*Nine day-old culture
**Highly significant compared to others

CHAPTER V

DISCUSSION

Fusarium moniliforme has been isolated from sorghum stalks (Figure 6). The long term survival of the fungus on plant debris in soil may be explained by its ability to colonize host tissue in which the fungal mycelia overwinter after harvest. The pathogen may also be disseminated by cereal stem borers, their parasites and other arthropods.

In culture, production of microconidia in chains seemed to be the early stage of conidiogenesis and false heads probably the later stage. However, it appeared that water availability may favor production of false conidial heads and water deficiency possibly induced conidial chains (21). These phenomena were evident in the experiments with water stress. At lower water potentials (less water availability) the fungus grew slowly and produced more conidial chains. False conidial heads were seldom observed. Conversely, at higher water potentials close to zero, false conidial heads replaced conidial chains. This stage may be more advanced that conidial chains.

Chains of conidia were raised above the surface of the medium while false heads formed an agglomeration of conidia

Figure 6. Fusarium moniliforme Grown on PDA. Mutant (left) and Original Strain (right).

close to the medium surface. Conidial chains may be then considered as an efficient dispersal mechanism, via air movement, to insure species conservation in drought condition. Temperature was shown to greatly influence the growth of F. moniliforme. The experiment showed that F. moniliforme grew best at temperatures between 25 C - 27 C. According to Texas county records of climatological observations on soil temperatures in 1981 (Department of Agronomy Oklahoma State University), these temperatures corresponded to those that occurred naturally in late spring (April - June) and in the fall (August - September). These periods roughly correlated with the sorghum growing season of that area. According to Cook (9) the optimum temperature of growth may increase when water becomes more available. Cook found that the optimum temperature for growth of Fusarium roseum was inversely related to water potential (9). In other words, higher water potentials lower the optimum temperature for growth. Therefore, even in the hottest summer months F. moniliforme may reach its optimum growth rate if there is not enough moisture.

In testing virulence and pathogenicity of F. moniliforme, two techniques were used: the in vitro aluminum pan technique used by Singleton and Ziv (46) and the water agar technique used in the Horticulture Department and adapted for this investigation by Dr. K. E. Conway (Plant Pathology Department). The first technique did not

give satisfactory results in this study (Figures 7 and 8). Many drawbacks were encountered such as; mechanical injuries to roots and seedlings, consistent spacing of the rows of seedlings could not be maintained because the wires were free to move along the pan edges, and some seeds dried out and others fell down into the water due to wire distortion. In general, the regularity of seed depth and spacing could not be controlled adequately with this technique. Early work describing rooting characteristics of seedlings showed that sorghum roots grew to a depth of 180 cm and laterally about 90 cm (3). When grown in rows, sorghum roots overlapped between adjacent plants and rows. One of the reasons for the great variation observed among dry and fresh weights of sorghum roots in this study was attributed to the difficulty in separating interwoven roots of sorghum seedlings.

Other disadvantages encountered were; slow growth of the fungus on the paper towel substrate and slower infection of the plants (Figure 7), number of seedling death was low compared to other techniques, and inoculation was possible only after complete emergence of seedlings (Figure 7) reducing the effect of the pathogen (Figure 8). Germination of sorghum seeds prior to planting may be an important practice in reducing stalk rot incidence, because in later infections the pathogen may be systemic and mildly virulent to nonembryonic sorghum tissue.

Figure 7. Aluminum Pan Technique: Inoculation of Sorghum Seedlings with Fusarium moniliforme.

Figure 8. Aluminum Pan Technique: Effect of Water Stress on Sorghum Seedlings.

The second method had many advantages (Figure 9). It was possible to follow the development of infection in the root system through the transparent agar medium (Figures 10 and 11). Mechanical injuries were avoided and observations of roots with the binocular microscope were facilitated. The fungus actively grew on agar and this increased seedling infections. Two different containers were used for experimentation. Plastic weighing dishes were used first (Figure 10) and were finally replaced by small aluminum pans that could be autoclaved. The fungus was primarily an external seed contaminant and entered the plant passively through wounds. The opening made in the seed coat by the hydrostatic pressure during germination of the seed (32) appeared to provide a means for fungal penetration. The early infection that coincided with this fresh wound in the seed coat had an important effect on seedling development. This was evident in the experiment by a reduction in stand counts, vigor and height of seedlings. In late infection, seeds may be already shriveled and the wound contracted.

Another important factor examined was the amount of inoculum used per seedling. Quantitative inoculation required an inoculum of fixed dosage applied on the host under standard conditions as close as possible to field conditions. The inoculum used in this study was determined to be 3.2×10^3 conidia per ml. According to Ooka and Kommedahl (40) in their air dispersal study in corn fields,

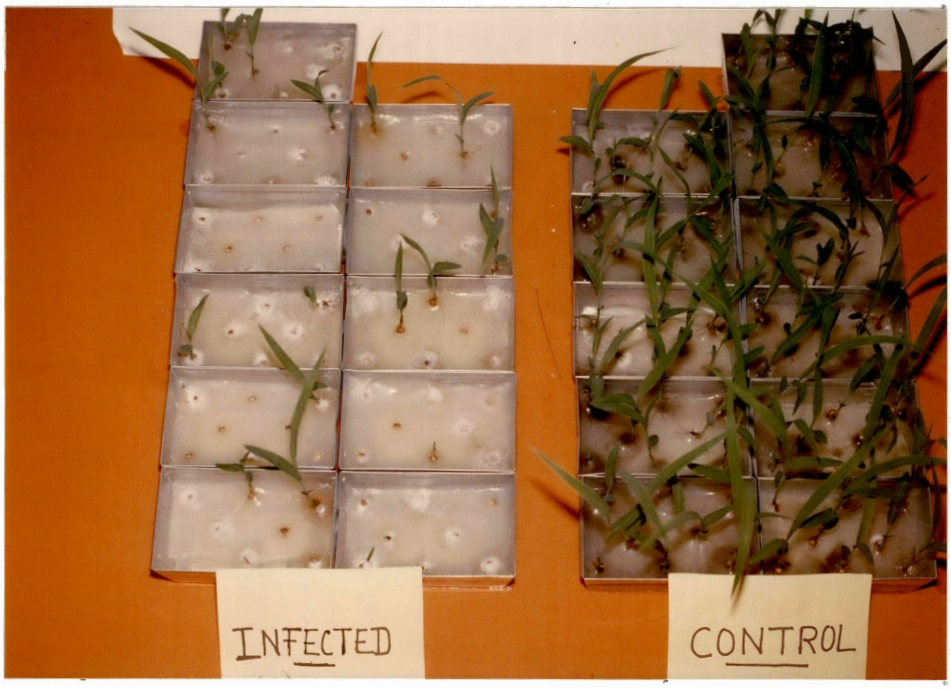

Figure 9. Small Aluminum Pan - Water Agar Technique: The Effect of Inoculation of Fusarium moniliforme Prior to Seed Germination.

Figure 10. Infected Sorghum Plant (left) and Noninoculated Control Plant Growing on Water Agar in Plastic Weighing Dishes.

Figure 11. Result of *Fusarium moniliforme* Infection of Sorghum Seed Using Water Agar- Plastic Weighing Dish Technique: Infection Clearly Observed Through Agar.

there were between 50 to 3.2 X 10^3 conidia of F. moniliforme per leaf and an average of 886 colonies per 20 cm^2 of leaf surface. In the same study, rain dispersal resulted in populations of F. moniliforme from 3 to 50 X 10^4 propagules per ml of water trapped between the leaf sheath and stalk. Apparently the amount of inoculum used in this study was well below the field level as only one drop of inoculum suspension was used per seed.

Criteria used to determine resistance in selected sorghum cultivars to F. moniliforme indicated that there was little resistance in these cultivars. However, many cultivars showing low levels of resistance in this analysis may be resistant in the field experiment, the resistance desired being the field expression. In this connection the cultivars B OK11 (no. 3), Martin (no. 10) and R OKY78 (no. 14) may need further investigation in field testing or breeding programs. Continuous attention to assay method, whether in the field or laboratory, should be placed on the temperature and the inoculum density. If the temperature and/or the inoculum level is too high, all seedlings in a population would succumb entirely, even though they may show a good field resistance (6).

Another complicating problem with Fusarium spp. is the variability in regard to their selective pathogenicity or adaptability to the host (Figure 6).

The host-pathogen-environment interaction is an

important concept to explain the incidence of root and stalk rot. Every microorganism has an optimum and minimum water potentials for growth. In nature, water potential has less effect on the growth of root pathogens than on the host plant, since most root pathogens can grow at water potentials well below the minimum required for growth of most higher plants. The permanent wilting point for most plants occurs at -15 bars, but most fungi can survive well below this point (51). In general, fungi with the highest water requirements for growth cause severe diseases in wet soil, and those with lowest water requirements cause severe diseases in dry soil. However, many Fusarium spp. are capable of growth in dry soil, but cause the most severe diseases in wet soil where they produce maximum growth. Stress induced by water deficiency primarily affects the plant, and is the most important predisposing factor for infection (Figure 12).

The important variation in growth habit observed when F. moniliforme was grown on PEG-Agar solutions did not suggest any relation between lack of vegetative growth and conidiogenesis. These observations seemed to support the findings proving that conidial production and mycelial growth occur simultaneously (22). There was no specific stage of mycelial growth related to conidiogenesis. However, it appeared that conidial production was heaviest when false heads were produced during maximal vegetative

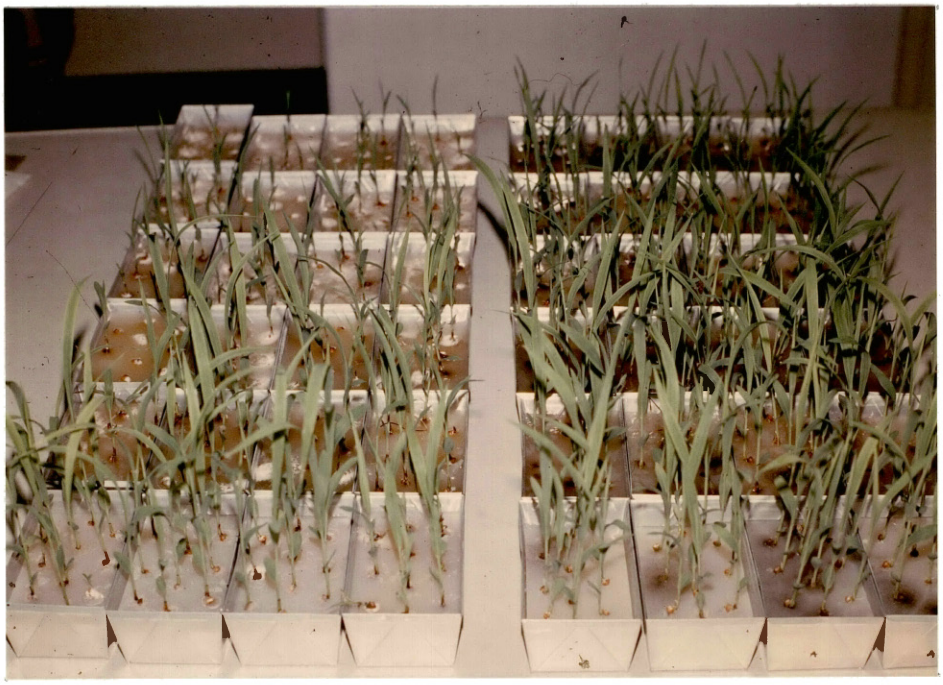

Figure 12. Effect of Water Potential and Infection of Fusarium moniliforme on Sorghum Seedlings: Infected Plants (left) and Noninoculated (right).

growth. On WA, mycelia were more profuse than in PEG-Agar and covered the entire agar surface in nine days, with abundant false heads of conidia. At lower water potentials (less water availability), mycelial development was sparse but with profuse chains of conidia. Thus, the greater virulence of F. moniliforme under lower water potentials could be attributed to an inhibitory effect on the growth response of the seedlings rather than on an enhanced rate of fungal metabolism.

CHAPTER VI

SUMMARY AND CONCLUSIONS

These studies of F. moniliforme, isolated from sorghum stalks of naturally infected fields in Oklahoma, were an attempt to determine what factors influenced fungal activities in relation to the infection of growing sorghum seedlings. The cultural experiments, such as growth at different temperatures and on different media, infection of seedlings, screening for resistance of 20 selected sorghum cultivars and the effect of water stress induced by PEG 6000 on host, pathogen and on their interaction, were initiated to elucidate factors associated with resistance of sorghum to F. moniliforme stalk and root rot disease.

The pertinent results obtained and observations made throughout this two-year investigation may be summarized as follows:

1. The fungus survives in old sorghum stalk evidently as mycelium in pith tissue. The long term survival of the pathogen in plant debris may be related to this saprophytic ability to survive continuously in host debris. Further investigation may be needed to find out whether or not the resting mycelium can colonize other plant debris in order to

lengthen its survival in soil organic matter. this may support the primary importance of soil debris as a source of inoculum.

2. The optimum temperature for growth ranged from 25 to 27 C and the average growth rate within this range was 0.3 mm per hour. These temperatures are likely obtained in late spring and fall during the growing seasons. This optimum temperature may vary with water potential in soil.

3. Fusarium moniliforme was highly virulent to sorghum seedlings of Pioneer 8451 cultivar grown in WA. This virulence decreased with the age of seedlings. Late infection after complete emergence of sorghum seedlings reduced the virulence of the fungus. Symptoms sometimes were not clearly expressed in late infection and the pathogen was apparently systemic and mildly virulent.

4. In the early stage of germination the opening observed in the seed coat produced by the emerging radicle provided an important means of fungal penetration. Future investigations may need to observe the positive tropism of the growing hyphae towards this wound.

5. Resistance to F. moniliforme in selected cultivars of sorghum was low. However, the cultivars B OK11 (no. 3) Martin (no. 10) and R OKY78 (no. 14) (Tables 8 and 9) showed a certain level of resistance that should be considered in further investigation either in field testing or breeding programs.

6. Water stress, induced by PEG 6000, was a significant parameter and a predisposing factor for infection. Water stress produced an uneven seedling stand with low vigor. Reduction in root length was observed on water stressed sorghum seedlings.

7. Water stress also had an inhibitory effect on conidial production and mycelial growth of the pathogen.

8. In general, any practice that may delay penetration or infection such as crop rotation, ecofallow (13), seed treatment, germination of seed prior planting, breeding for resistance, wound reduction, vigorous growth of plant prior to fungal development or any other practices that may increase vigor and reduce stress, may greatly reduce F. moniliforme stalk and root rot.

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