PATHOGENICITY OF FUNGI ISOLATED FROM ROOTS OF SORGHUM SEEDLINGS

by

ROGER JONES

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Approved by:

Earl D. Hansing

Major Professor

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INTRODUCTION

One of the most serious problems in sorghum production is that of obtaining good stands. The replanting of whole fields or parts of fields one to three times is not unusual (Leukel & Martin 1943). According to Buckholtz (1946) yield is primarily a function of stand when the environment is unfavorable to the germination of sorghum. There are a number of factors which may alter stands. The peculiar characteristics of the seed, for example, may influence stands. Large seed varieties do not necessarily germinate any better than small seed varieties even though the former, by virtue of their greater food supply, might be expected to result in better stands. In fact, small seeded sorghums show a tendency to germinate better than the large seeded varieties (Swanson & Hunter 1936). Other hazards to stands are poor seed-bed, low grade seed, planting too early, the use of improper planting plates and torrential rains which may cover or wash away the young seedlings (Swanson & Hunter 1936). Crusting of the soil is supposedly another stand reducing factor (Leukel & Martin 1943).

Various workers have observed that sorghum seed shows a much higher viability when germinated in blotters or in sterilized soil than when it is germinated in unsterilized field soils or when grown under other unfavorable conditions (Leukel & Martin 1943, Swanson & Hunter 1936, Andersen 1952). Seed having a viability of 90% or more in the laboratory showed relatively low viability of 50-70% and also a high percentage of blighted seedlings in unsterilized field soils (Leukel & Martin 1943). The discrepancy between

laboratory tests and field germination is probably greater for sorghums than other cultivated crops (Swanson & Hunter 1936).

Experiments in the greenhouse and laboratory by Leukel & Martin (1953) showed that seed-borne and soil inhabiting fungi were capable of inflicting severe injury on germinating seeds and young seedlings of sorghum. Seed rot is most severe when the soil is cold and wet after planting, a common condition in the North and in other areas when seed is planted early. Sorghum needs a soil temperature of 70°F and above to germinate promptly. At lower temperatures germination is retarded with the result that seed-borne and soil inhabiting fungi have a chance to attack the seed. Furthermore, most seed-rotting fungi thrive at soil temperatures below 70°F (Leukel & Martin 1953).

In an effort to find out more about fungi which attack roots of sorghum seedlings, an investigation was undertaken to isolate and carry out pathogenicity tests on fungi from roots of such seedlings.

REVIEW OF LITERATURE

A disease of corn and sorghum caused by seed-borne species of Fusarium was described in 1916 but it was not indicated whether it caused seedling blight (Pammel et al. 1916). Later, Fusarium moniliforme Sheld. was shown to be internally borne in seed corn and also to cause damping off of seedlings under very humid conditions (Valleau 1920). Species of Fusarium and Penicillium have been found to be borne within sorghum seed (Leukel & Martin 1943). The

formation of prussic acid in sorghum and sudan grass, especially under unfavorable conditions of growth, has been cited as the cause of disease in seedlings of these plants (Harris & Goss 1934). The primary roots, and especially the mesocotyls reddened and then turned dark. The mesocotyl shrivelled to such an extent that some plants were killed by the interruption of the flow of soil solutes from the primary roots. This condition was aggravated by the action of certain fungi in the soil, especially species of Fusarium.

Stakman (1923) described in detail the effect of certain Fungi Imperfecti on roots of cereals grown in agar. The tests supported the theory that in addition to injuries caused by such well defined parasites as Fusarium culmorum (W. C. Sm.) Sacc., cereals are weakened by attacks of other less virulent imperfect fungi which primarily infect the roots.

Rhizoctonia bataticola (Taub.) Bull. was reported in India to cause, under favorable conditions a destructive seedling blight (Uppal et al. 1936). Rhizoctonia solani Kuhn. has been stated as causing damping off and destructive seedling blight of sorghum, especially under warm moist conditions, in the Phillipines (Quebral & Gibe 1958).

Pythium arrhenomanes Drechs. prevented, under certain conditions, germination or killed sorghum seedlings either as they emerged or after they had produced two or three leaves (Elliot et al. 1937).

Leukel & Martin (1943) investigated the causes and conditions favoring seed rots and seedling glights of sorghum. Both seed-

borne and soil-inhabiting fungi were indited. Seed-borne pathogens were usually found to respond to seed treatments much more readily than organisms occurring in the soil. Spores of Alternaria and Fusarium species were the ones most commonly found on sorghum seed. Often found were species of Penicillium, Aspergillus, Rhizopus and Trichoderma. Occasionally spores of Spacelotheca, Helminthosporium and unidentified sclerotial bodies were observed. Some seeds that failed to germinate after surface sterilization showed no fungus growth until after they had been cut open under aseptic conditions. Then they frequently yielded a Fusarium or Penicillium indicating that these were borne in the seed.

Leukel & Martin (1943) obtained soil from fields in which sorghum had been previously grown for 6 to 23 years. From subsequent experiments it was discovered that micro-organisms detrimental to germination and stand in sorghum are not restricted to soil in which sorghum has been grown for several years. There seemed to no consistent or striking relationship between the percentage of emergence and the length of time the soil had been cropped to sorghum. Further, it appeared that soil type, soil moisture, temperature, and soil flora were interrelated factors affecting emergence independently and in combination with one another. Some fungi are probably more virulent at higher soil moistures, but it appeared that soil temperature or soil type or both could alter this relation to some extent.

Isolations made by Leukel & Martin (1943) from roots, stem, ungerminated seeds and aborted seedlings grown in natural soil from

Manhattan, Kansas included species of Pythium, Fusarium, Trichoderma, Rhizopus, Penicillium, Aspergillus, Mucor, Alternaria, Stachybotrys, and Helminthosporium as well as some unidentified sclerotial and non-fruiting fungi. Isolates of Pythium, Fusarium and Penicillium were used to inoculate soil kept at 20°C. Five days later seeds of Spur feterita were planted in the infested soil. After two weeks it was noted that emergence was practically inhibited by all of the Pythium isolates - 1900 seeds producing only 19 plants. Only one of the Fusarium isolates showed any marked effect on emergence and subsequent growth, the percentages of emergence were somewhat depressed by others but no damping off or other disease symptoms followed. The Penicillium isolates also depressed emergence to some extent.

In experiments to determine the effect of different soil temperatures on emergence and damping off of seedlings by various fungi Leukel & Martin (1943) found that Pythium debaryanum Hesse was responsible for poor emergence more than any other fungus tested, especially at lower temperatures. Next in line of importance were species of Fusarium. Seed treatment was less effective in soil infested with species of Pythium than in soil infested with Fusarium spp.

MATERIALS AND METHODS

Two different locations were used for the collection of soil. They were a field at the Agronomy farm north of the campus which had been cropped to corn the previous year and the Experimental

field at Rocky Ford where alfalfa had been the previous crop. Both locations belong to the Kansas Agricultural Experiment Station. Samples were collected at random from the top six inches of an area approximately 50 yds. by 50 yds. in each field. Samples from the same field were mixed and divided into two parts. One part was autoclaved for 12 hr at 15 psi. Sterile 5 in. pots were filled to within .75 in. of the top with sterilized soil and a similar series filled with unsterilized soil. Twelve pots containing sterilized and 12 pots containing unsterilized soil were kept at $65^{\circ} \pm 5^{\circ}$F and another similar series were kept at 80° to $85^{\circ} \pm 5^{\circ}$F in the greenhouse. Half of the pots in each greenhouse were irrigated by pouring water into saucers upon which the pots were placed and the other half by pouring water onto the soil in the pots. Thus there were four different water treatments;

(1) sterilized soil, surface irrigated

(2) sterilized soil, sub-irrigated

(3) unsterilized soil, surface irrigated

(4) unsterilized soil, sub-irrigated.

Seed of the Midland variety, obtained through the Kansas Agricultural Experiment Station, was treated with Captan 75 in an effort to kill seed-borne fungi. Ten of these treated seeds were planted to a depth of .75 in. in each pot. After one month plants were removed and the roots washed under running tap water for one hour, rinsed in sterile water and examined for lesions. The following measurements and observations were made on 20 plants from each water treatment: Number of leaves and crown roots per plant;

height of plant; length of root systems; weight of fresh plants; weight of fresh root systems; weight of oven dried (24 hr at 120°C) root systems. Portions of roots showing lesions were cut into 1 mm lengths and plated onto 2% water agar and Rose Bengal agar (Johnson et al. 1960). Three days later hyphal tips were transferred from the developing colonies to potato dextrose agar slants. Later, pure cultures were obtained by placing a small portion of mycelium from a slant in warm melted Rose Bengal agar in a petri dish and swirling it around to disperse any spores. After 24 hr isolated germinated spores were removed and transferred to potato dextrose agar slants. These cultures were used for identification and pathogenicity tests.

Pathogenicity tests were initiated by placing surface sterilized Pink kafir seeds on potato dextrose agar slants and at the same time inoculating slants with a fungal isolate (Stakman 1923). It soon became apparent that this method was not suitable as conditions were so favorable for the fungus that even saprophytic fungi would appear to be pathogenic. Since many isolations were to be tested, a relatively simple and rapid test was required. It was decided to use vermiculite (Terra-lite brand) as the rooting medium. Six hundred ml of this material was placed in a half-gallon Lamb and Mason fruit jar stoppered with a cotton plug. Jars were autoclaved for 30 min. at 15 psi. Seed of Pink kafir was surface sterilized in commercial Clorox (Sodium hypochlorite 5.25% by weight) for 25 min and washed in sterile distilled water two times. Lots of 20 seeds were dropped at random into jars and each lot covered

to a depth of 2 cm by more vermiculite.

The fungal isolates were incubated for 14 days at 70°F on potato dextrose agar plates. Six plates of an isolate were blended in 750 ml of half strength Hoagland's nutrient solution (Hoagland & Arnon 1950). Another 750 ml of half strength Hoagland's solution was added, and 250 ml of this mixture of hyphae and spores were poured into each of 6 Lamb and Mason jars containing vermiculite and seed. Three jars were kept in an environmental chamber at 70°F and the other three were kept in a similar chamber at 80°. After 14 days seedlings were removed, examined and counted for evidence of pathogenicity. Plants were placed into 4 classes using a modification of the method suggested by McKinney (1923). The classes were given numbers depending upon the severity of the infection.

0 No signs of infection as evidenced by the absence of any lesions on the underground parts.

1 Slight infection as evidenced by small lesions on the coleoptile and /or roots.

2 Moderate infection as evidenced by partial or complete rotting of/or the presence of small lesions on the coleoptile, sub-crown internode and stem base.

3 Abundant infection as evidenced by complete rotting of the coleoptile, sub-crown internode and/or stem base.

4 Death of the seedling.

An infection rating on a percentage basis was assigned to each isolate according to the following formula:

$$\text{Infection rating} = \frac{\text{Sum of all numerical ratings x 100}}{\text{4 x Total number of inoculated plants.}}$$

This result is then the comparative infection rating for the given temperature, since 4 times the total number of plants (4 being the highest numerical rating) represents the highest possibility for disease under the conditions of the experiment.

The isolates which were considered to be pathogenic in this experiment were tested again by the same method with the exception that only 3 plates were blended. In other words, half as much inoculum was used.

The isolates were then tested in a sterile soil sand mixture (3 parts soil to one part river sand, autoclaved for 3 hr at 15 psi). Three varieties were used. They were Combine 7078, Pink kafir and Westland. Seed was obtained through the Kansas Agricultural Experiment Station. Four sets of 20 seeds of each of the 3 varieties were planted in rows .5 in. deep in three 9 in. x 5 in. x 3 in. flats containing sterile sand/soil mixture. Each flat contained 4 rows. The inoculum was prepared by homogenizing 3 plates of the isolate, grown for 14 days at 70°F on potato dextrose agar, in 750 ml of sterile water. Twenty-five ml of this mixture was poured onto the seed in each row. After covering the seed, three flats were placed in a greenhouse at 70°F and three in another greenhouse kept at 80° to 85° during the day and at 60° during the night. After 20 days counts were made of emergence and seedlings showing signs of blight or damping off.

Identification of fungi was made with the aid of the following:

Barnett 1960, Gilman 1957, Raper & Thom 1949, Thom & Raper 1945, Snyder & Hansen 1940, 1941, 1945.

EXPERIMENTAL RESULTS

Comparison of Plants Grown in Normal Field and Sterilized Soil

Seed of the Midland variety was planted in pots containing normal field and sterilized soil. Half of the pots of each soil type were sub-irrigated and the other half surface irrigated. Pots were kept at 65°F and 80°. After 1 month plants were removed and a number of their characteristics noted.

At both temperatures and for both soil sources the highest percentage of germination was with sub-irrigation (Tables 1, 2, 3, and 4). Plants were taller when grown in sterilized than when grown in unsterilized soil generally being nearly twice as tall in sterilized soil. Plants were also taller in the surface irrigated pots than those grown where sub-irrigation was practiced. In contrast, the primary root systems were longest in the pots which were sub-irrigated, with the exception of pots containing sterilized soil from the corn field and which were kept in an air temperature of 80 to 85°F.

The average number of crown roots per plant was generally greater when pots were surface irrigated than when they were sub-irrigated. Plants also tended to have more crown roots at 80 to 85°F. than at 65°. This is in agreement with Martin et al (1935) who obtained some indication of fewer crown roots as soil temperature decreased.

Table 1. Some characteristics of 1 month old plants of the Midland variety grown in the greenhouse at 65°F in soil obtained from Rocky Ford Experimental Field. Recordings are the average of 20 plants.

Water Treatment	Germination %	Height cm	Length of root system cm	No. of leaves per plant	No. of crown roots per plant	Weight of blotted dry root system gm	Weight of oven dried root system gm
Normal soil, sub-irrigated	83.3	17.1	41.4	5.0	4.5	0.38	0.040
Normal soil, surface irrigated	63.3	20.0	20.4	5.7	5.5	0.47	0.044
Sterilized soil, sub-irrigated	81.8	29.8	33.9	6.2	4.7	0.73	0.070
Sterilized soil, surface irrigated	53.3	44.2	27.6	6.0	8.1	2.17	0.155

Table 2. Some characteristics of 1 month old plants of the Midland variety grown in the greenhouse at 80°F in soil obtained from Rocky Ford Experimental Field. Recordings are the average of 20 plants.

Water Treatment	Germination %	Height cm	Length of root system cm	No. of leaves per plant	No. of crown roots per plant	Weight of blotted dry root system gm	Weight of oven dried root system gm
Normal soil, sub-irrigated	87.5	23.7	33.4	5.4	4.9	0.45	0.042
Normal soil, surface irrigated	69.2	29.1	23.7	6.2	7.0	0.88	0.080
Sterilized soil, sub-irrigated	72.2	41.8	44.8	7.1	6.8	1.17	0.126
Sterilized soil, surface irrigated	61.8	52.4	31.3	7.0	9.3	1.97	0.182

Table 3. Some characteristics of 1 month old plants of the Midland variety grown in the greenhouse at 65°F in soil obtained from the Agronomy Farm. Recordings are the average of 20 plants.

Water Treatment	Germination %	Height cm	Length of root system cm	No. of leaves per plant	No. of crown roots per plant	Weight of blotted dry root system gm	Weight of oven dried root system gm
Normal soil, sub-irrigated	85.8	18.6	24.9	4.1	6.2	0.23	0.030
Normal soil, surface irrigated	70.8	16.2	23.3	4.0	4.5	0.50	0.026
Sterilized soil, sub-irrigated	77.5	33.7	31.8	5.6	6.6	0.97	0.076
Sterilized soil, surface irrigated	70.8	39.3	25.2	5.9	7.9	0.70	0.090

Table 4. Some characteristics of 1 month old plants of the Midland variety grown in the greenhouse at 80°F in soil obtained from the Agronomy Farm. Recordings are the average of 20 plants.

Water Treatment	Germination %	Height cm	Length of root system cm	No. of leaves per plant	No. of crown roots per plant	Weight of blotted dry root system gm	Weight of oven dried root system gm
Normal soil, sub-irrigated	85.0	21.5	29.7	4.4	5.8	0.39	0.044
Normal soil, surface irrigated	76.6	21.0	20.9	4.8	5.8	0.38	0.043
Sterilized soil, sub-irrigated	76.0	32.1	33.5	5.5	6.3	0.76	0.085
Sterilized soil, surface irrigated	79.2	43.1	37.1	6.0	7.4	1.71	0.141

Plants had more leaves per plant on average when grown in autoclaved soil than when grown in normal field soil.

There were differences in the average fresh weights of plants. Those grown in sterilized soil were often 3 to 4 times the weight of their counterparts in normal field soil. At both temperatures, surface irrigation resulted in heavier plants than did sub-irrigation. Plants had greater fresh weight at 80 to 85°F than at 65° for the corresponding treatments. The average fresh weights of the root systems followed the same trend.

Differences were noted in the average dry weights of root systems. Systems from plants grown in sterilized soil had greater dry weight than those grown in normal field soil. Plants grown at 80 to 85°F had average dry root weights which were greater than those at 65°, though the differences were not very great. Water treatment had an effect in that surface irrigation resulted in a greater average dry root weight than did sub-irrigation.

Surface irrigation of the soil probably resulted in a certain amount of oxygen deficiency in the neighborhood of the seed, due to water logging. This would help account for the lower percentage in germination observed with this type of irrigation as compared to the higher percentage of germination when sub-irrigation was practiced. However, plants were taller on average when surface irrigation was used as opposed to sub-irrigation. In the former treatment an optimal amount of water was available in the vicinity of the crown roots whereas water had to rise by capillarity to this region in the latter case.

Symptoms of Plants Grown in Lamb and Mason Jars

Symptoms shown by plants grown in the Lamb and Mason jars are described for some of the fungal isolates.

Aspergillus niger van Tieghem caused moderate stunting at 70°F and severe stunting at 80°. At both temperatures the mesocotyls were colored red-brown. Some of the plants at 80°F had small brown lesions on the leaves from which conidiophores and conidia were growing. The seed at both temperatures was covered with a black mass of conidia.

Of the 7 isolates of Fusarium moniliforme Sheld. 6 caused varying degrees of stunting and underdevelopment of root systems at 70°F. Seminary root production was seriously retarded, crown roots were browned and stunted. Narrow elongate lesions up to 3 cm in length were present on the mesocotyls. At 80°F aerial and root systems were nearly as well developed as those of the control. The crown roots, however, in many cases were browned and showed signs of rotting. Often, the mesocotyls were dark red-brown and shrivelled. Some plants were killed.

The symptoms observed when seedlings were grown in jars infested with F. oxysporum Schl. varied from practically no observable effect on the host plants to severe stunting in other cases. In cases of severe stunting the mesocotyls were reddened at 70°F and at 80° they were rotted and shrivelled. In such instances root production was poor and crown roots were browned and stunted. In less severe cases very small elongate lesions were present on the mesocotyls. In severe cases the lesions were dark red-brown, about

1 mm wide, 1 cm long and extended one third of the way around the mesocotyl. Other isolates appeared to attack only the seed at 70°F and to attack both seed, mesocotyl and roots at 80°.

Fusarium roseum (Lk.) caused moderate to severe stunting at 70°F and none at 80°. Small elongate, reddish-brown lesions were present on the mesocotyls at 70°F. At 80°F the mesocotyls were rotted and shrivelled. Crown roots were being attacked at 80°F and were colored red-brown.

Aerial and root development was retarded by Fusidium sp. at 70°F but not at 80°. Mesocotyls were generally reddened at 70°F, and at 80° they were often times shrivelled. At the latter temperature crown roots were mostly stunted and their tips browned and shrivelled.

Stunting of aerial and root systems caused by Mucor racemosus Fresenius occurred at both temperatures. Mesocotyls had a bright red-brown coloration at 70°F and were shrivelled at 80°.

Penicillium rubrum Stoll. caused very slight stunting of the aerial and root systems. At 70°F there were occasional red-brown lesions about 1 mm in length of the mesocotyls and also at the point of emergence of roots from the sub-crown internode. In a few cases the crown roots were browned. The mesocotyl in the majority of plants had a bend in it just above the seed. Seeds were rotted at both temperatures and were covered with a mass of green spores. Mesocotyls were generally red-brown and shrivelled at 80°F. As at 70°F, the crown roots were often browned.

Trichoderma lignorum (Tode) Hartz caused slight stunting of

some seedlings at 70°F. Some plants showed small elongate lesions along with reddening of the mesocotyls. At 80°F the mesocotyls of some plants were reddened and shrivelled but in most cases the mesocotyls were reddened for about 1 mm above the seed. This organism rotted the seed at both temperatures.

Emergence and Infection Ratings

Emergence and infection ratings were determined for Pink kafir plants grown in vermiculite in Lamb and Mason half gallon fruit jars. The results when 6 plates of inoculum were used are shown in Table 5 and the results when 3 plates were used in Table 6. Tables 5 and 6 refer to isolates obtained from soil collected at the Agronomy farm. Tables 7 and 8 are for soil collected from Rocky Ford Experimental Field.

Two species of Fusarium, F. moniliforme and F. oxysporum were isolated from seedlings grown in soil from the Agronomy Farm. Both species reduced emergence at 70°F and at 80° when 6 plates of inoculum were used. Infection ratings for the two species were higher at 80°F than at 70°. Isolate 178 of F. oxysporum reduced emergence at 70°F to 11.7% whereas the control resulted in 92.1% emergence. Emergence was also considerably less at 80°F being 56.7%, the control was 92.4%.

Emergence was 63.3% at 80°F and 45.0% at 70° when A. niger was used.

Alternaria humicola Oudemans, Penicillium rubrum and the nonfruiting fungi did not reduce emergence to any great extent.

Table 5. Emergence and infection ratings of isolates obtained from soil collected at Agronomy Farm when 6 plates of inoculum were used.

Species	Isolation No.	70°F		80°F	
		Emergence %	Infection rating %	Emergence %	Infection rating %
Fusarium moniliforme	181	83.3	42.5	78.3	92.0
"	184	75.0	65.5	76.7	84.8
"	268	71.7	68.0	80.0	77.5
"	273	66.7	26.8	78.3	70.3
"	280	53.3	67.3	*	*
F. oxysporum	178	11.7	78.6	56.7	70.5
"	824	83.3	21.0	81.7	50.2
Aspergillus niger	155	63.3	34.8	45.0	90.8
Alternaria humicola	187	95.0	39.5	88.3	19.6
Penicillium rubrum	196	86.6	26.4	85.0	46.5
Trichoderma lignorum	822	63.3	27.0	88.3	44.8
Non fruiting	183	93.3	61.5	81.7	69.5
"	190	93.3	62.5	88.3	73.0
Control		92.1		92.4	

*spoiled by excessive heat due to malfunction of environmental chamber.

Table 6. Emergence and infection ratings of isolates obtained from soil collected at Agronomy Farm when 3 plates of inoculum were used.

Species	Isolation No.	70°F		80°F	
		Emergence %	Infection rating %	Emergence %	Infection rating %
Fusarium moniliforme	181	48.3	65.5	76.7	84.8
"	184	48.3	72.5	65.0	78.3
"	268	40.0	76.0	70.0	81.0
"	273	75.0	22.8	88.3	52.0
"	280	46.7	67.0	75.0	71.0
F. oxysporum	178	0.17	50.0	65.0	75.8
"	189	11.7	50.0	80.0	63.0
"	824	75.0	23.3	76.7	55.5
Penicillium rubrum	196	90.0	30.0	55.0	68.0
Trichoderma lignorum	822	68.3	15.3	86.6	43.8
Non-fruiting	183	90.0	65.5	95.0	71.0
"	190	80.0	54.3	78.3	74.0
Control		93.6		93.9	

Table 7. Emergence and infection ratings of isolates obtained from soil collected at Rocky Ford Experimental Field when 6 plates of inoculum were used.

Species	Isolation No.	70°F		80°F	
		Emergence %	Infection rating %	Emergence %	Infection rating %
Fusarium moniliforme	458	66.7	78.3	90.0	74.5
"	459	66.7	67.5	---	---
"	481	71.7	77.3	55.0	93.8
F. oxysporum	469	78.3	51.5	81.7	64.3
"	1256	60.0	50.0	66.7	57.0
"	1280	73.3	42.5	70.0	75.0
"	1281	73.3	33.5	88.3	74.0
"	1303	81.7	42.8	78.3	60.0
"	1304	91.7	31.0	93.3	64.3
F. roseum	1265	51.7	69.3	83.3	65.0
F. solani	1302	91.7	17.8	78.7	54.3
Fusidium spp.	454	60.0	70.8	75.0	75.0
"	467	71.7	75.0	60.0	72.3
"	468	61.7	63.5	73.3	76.3
"	480	61.7	68.3	68.3	78.0
"	572	---	---	66.7	75.0
Humicola nigrescens	1270	88.3	18.5	88.3	47.8
Mucor racemosus	1306	38.3	58.8	35.0	67.8
Rhizoctonia sp.	1304	53.3	58.5	80.0	82.3
Trichoderma lignorum	1260	85.0	17.3	86.7	44.3
"	1275	93.3	29.5	90.0	54.8
Verticillium sulphurellum	573	48.3	62.0	76.7	77.3
Non fruiting	571	85.0	75.5	78.3	76.0
"	1289	90.0	7.0	78.3	45.8
Control		92.1		92.4	

Table 8. Emergence and infection ratings of isolates obtained from soil collected at Rocky Ford Experimental Field when 3 plates of inoculum were used.

Species	Isolation No.	70°F		80°F	
		Emergence %	Infection rating %	Emergence %	Infection rating %
Fusarium moniliforme	458	66.7	57.5	63.3	77.8
"	459	68.3	67.8	66.7	87.5
"	481	76.7	54.3	75.0	75.5
F. oxysporum	469	78.3	21.8	88.3	52.8
"	1256	73.3	51.8	76.7	73.8
"	1280	78.3	30.8	76.7	71.3
"	1305	81.7	25.0	75.0	48.3
"	1303	80.0	26.0	83.3	53.5
F. roseum	1265	78.3	29.8	81.7	63.8
F. solani	1302	93.3	12.5	90.0	43.3
Fusidium spp.	454	66.7	61.5	66.7	87.0
"	467	51.7	70.3	80.0	82.8
"	468	63.3	71.8	66.7	89.0
"	480	---	---	71.7	66.3
"	572	63.3	51.3	66.7	68.8
Humicola nigrescens	1270	93.3	15.8	93.3	28.5
Mucor racemosus	1306	---	---	---	---
Rhizoctonia sp.	1304	80.0	66.3	76.7	76.7
Trichoderma lignorum	1268	86.7	11.0	90.0	35.8
"	1275	90.0	20.8	85.0	47.0
Verticillium sulphurellum	573	86.7	10.5	88.3	14.5
Non fruiting	571	76.7	49.0	83.3	70.5
"	1289	86.7	20.7	93.3	26.4
Control		93.6		93.9	

Trichoderma lignorum reduced emergence at 70°F and hardly at all at 80°.

The isolates of Fusarium moniliforme, F. oxysporum, the non-fruiting fungi and Aspergillus niger all had relatively high infection ratings at 80°F - generally above 60% and as high as 92% for isolate 181 of F. moniliforme at 80°. The remaining isolates were not particularly virulent.

When 3 plates of inoculum were used the isolates of F. moniliforme, with the exception of No. 273, caused emergence to be less than 50% at 70°F, emergence was not so low when 6 plates of inoculum were used. Emergence was essentially similar at 80°F with 6 plates and with 3 plates of inoculum.

Generally, infection ratings observed when 3 plates of inoculum were used were very similar to those obtained when 6 plates were used. Two isolates of F. oxysporum 178 and 189, were, however, particularly virulent at 70°F when 3 plates of inoculum were used. Emergence was only 0.17% with isolate 178 and 11.7% with 189. At 80°F emergence was 65% and 80% respectively.

The highest infection ratings in the majority of cases for isolates from Rocky Ford Experimental Field occurred at 80°F, indicating that disease development in the seedlings was greater at this temperature than at 70° (Tables 7 and 8). No trend indicating a reduction in emergence at 80°F compared with 70° is observable. In the vast majority of cases, however, more plants emerged in the control than in cases where the vermiculite had been infested with fungal isolates. Of the species of Fusarium, F. moniliforme reduced emergence more than any other, both at 70°F and at 80°. This

species also had higher infection ratings at these temperatures than did the other Fusarium species. F. solani (Mart.) App. et Wt. did not reduce emergence, or show much disease development at 70°F. At 80°F emergence was reduced. The infection rating increased from 17.8% at 70° to 54.3% at 80° for F. solani. When F. roseum was used emergence was 83.3% at 70°F and 51.7% at 80°. Disease development was similar at both temperatures.

The isolates of Fusidium reduced emergence by nearly 30% (points) at 70°F and 25% (points) at 80°. The infection ratings for the same isolates were some of the highest observed.

Of all the isolates tested, M. racemosus, caused the severest reduction of emergence. There was only 53.3% emergence at 70°F and 35.0% at 80°. Rhizoctonia and Verticillium sulphurellum Saccardo caused approximately 40% (points) reduction of emergence as compared with the control at 70°F. At 80°F, however, reduction was only 15%(points) less than the control. T. lignorum did not reduce emergence, however, the infection ratings were higher at 80°F than at 70°.

Reducing the amount of inoculum by a half did not have an appreciable effect on virulence of most of the isolates. The exception was V. sulphurellum where the infection rating dropped from 62% at 70°F when 6 plates of inoculum were used to 10.5% with 3 plates of inoculum. The drop at 80°F was from 77.3% to 14.5%. An increase in emergence was shown by F. roseum, Rhizoctonia sp. and V. sulphurellum at 70°F as compared to 80°.

Emergence and Blighting of Seedlings in the Greenhouse

Pathogenicity tests were conducted with 36 different isolates in the greenhouse at 60°F and at a temperature of 80-85° during the day time and 60° at night. Seed was infested at the time of planting in a sterile soil/sand mixture. Three varieties of sorghum were used. They were Combine 7078, Pink kafir and Westland. The results of tests made with isolates from the Agronomy Farm are shown in Tables 9, 10, and 11.

Fourteen isolates obtained from seedlings grown in soil from the Agronomy Farm were tested and none appeared to be highly virulent in the greenhouse at the temperatures used. A. niger did not affect emergence or cause any damping off of seedlings. A. humicola reduced emergence of Pink kafir at 60/80°F but not at 70°. This organism did not affect emergence of Westland or Combine 7078. Five isolates of F. moniliforme were tested and all varied somewhat in their effect upon emergence of the three varieties. All five reduced emergence of Pink kafir at 60/80°F, whereas at 70°, only isolate 181 reduced emergence. Isolates 181, 184 and 280 reduced emergence of Westland at 60/80°F and at 70° only isolate 184 caused an appreciable difference from that of the control. Emergence of Combine 7078 was reduced as compared with the control by isolates 268 and 280 of the same fungus at 60/80°F and by isolate 181 at 70°.

The three isolates of F. oxysporum did not reduce emergence of the three varieties. Similarly, neither did P. rubrum cause reduction in emergence. T. lignorum reduced emergence of Pink kafir at both temperatures and Westland at 60/80°F. Emergence of the

Table 9. Emergence of Westland seedlings in infested soil/seed mixture. Isolations from soil obtained at Agronomy Farm.

Species	Isolation No.	60/80°F Number of plants: 1/ Emerged	Dead	Emerged in control	70°F Number of plants: 1/ Emerged	Dead	Emerged in control
Aspergillus niger	155	51	0	46	---	--	--
Alternaria humicola	187	50	0	48	51	0	45
Fusarium moniliforme	181	35	0	48	40	0	45
"	184	38	0	60	38	0	48
"	268	45	0	48	40	1	45
"	273	37*	0	38*	36	1	35
"	280	49	0	60	53	0	48
F. oxysporum	178	45	0	48	45	0	45
"	189	43	0	48	43	0	45
"	824	58	0	60	51	0	48
Penicillium rubrum	196	33*	0	38*	39	0	35
Trichoderma lignorum	822	45	0	60	46	0	48
Non-fruiting	183	47	0	45	44	0	48
"	190	54	0	48	61	0	60

* 80° 1/ 80 seeds planted.

Table 10. Emergence of Combine 7078 seedlings in infested soil/sand mixture. Isolations from soil obtained at Agronomy Farm.

Species	Isolation No.	60/80°F Number of plants: 1/ Emerged	Dead	Emerged in control	70°F Number of plants: 1/ Emerged	Dead	Emerged in control
Aspergillus niger	155	46	0	48	--	--	--
Alternaria humicola	187	49	0	48	53	0	53
Fusarium moniliforme	181	42	0	48	43	0	53
"	184	54	0	55	51	0	42
"	268	38	0	48	43	0	53
"	273	34*	0	33	29*	0	42
"	280	42	0	55	46	0	42
F. oxysporum	178	45	0	48	50	0	53
"	189	48	0	48	48	0	53
"	824	57	0	55	53	0	42
Penicillium rubrum	196	33*	0	37*	38	2	42
Trichoderma lignorum	822	50	0	55	53	0	42
Non-fruiting	183	54	0	53	45	0	48
"	190	51	0	42	52	0	55

* 80° 1/ 80 seeds planted.

Table 11. Emergence of Pink kafir seedlings in infected soil/sand mixture. Isolations from soil obtained at Agronomy Farm.

Species	Isolation No.	60/80°F Number of plants: 1/ Emerged	Dead	Emerged in control	70°F Number of plants: 1/ Emerged	Dead	Emerged in control
Aspergillum niger	155	60	0	56	--	--	--
Alternaria humicola	187	53	0	62	52	0	50
Fusarium moniliforme	181	44	0	62	54	0	50
"	184	54	0	72	53	0	64
"	268	52	0	62	46	0	50
"	273	40*	0	46*	46*	0	38*
"	280	59	0	72	58	0	64
F. oxysporum	178	56	0	62	45	0	50
"	189	54	0	62	61	0	50
"	824	66	0	72	52	0	64
Penicillium rubrum	196	41*	0	46*	45*	0	38*
Trichoderma lignorum	822	59	0	72	52	0	64
Non-fruiting	183	61	0	50	55	0	62
"	190	50	0	64	55	0	72

*80°F. 60 seeds.

1/ 80 seeds planted.

latter variety at 70°F and of Combine 7078 was similar to that of the control.

Twenty-two isolates from seedlings grown in soil from Rocky Ford were tested the results of which are shown in Tables 12, 13 and 14. None of the isolates were highly virulent. One isolate of F. moniliforme, No. 459, caused some damping off of seedlings of all three varieties, especially of Westland at 70°F. This same isolate also caused reduction in emergence of Westland at 70°F and Combine 7078 at 60/80°. Of the other two isolates of F. moniliforme, No. 458 reduced emergence of Combine 7078 and Pink kafir at 60/80°F. F. roseum and F. solani did not reduce emergence of any of the varieties though F. roseum caused blighting of some seedlings of the three varieties. Three of the isolates of F. oxysporum, No. 1256, 1280 and 1305 did not reduce emergence or cause any damping off. Isolates 469, 1281 and 1303 reduced emergence of Combine 7078 at 60/80°F. The latter two isolates reduced emergence of Pink kafir and Westland at the same temperature but not at 70°F. Similarly the isolates of Fusidium varied somewhat in their virulence towards the three varieties. Isolate 454 was the most virulent in that it caused blight of seedlings of the three varieties. Sixteen out of 46 plants of Pink kafir, 9 out of 55 of Combine 7078 and 10 out of 56 of Westland were blighted at 60/80°F by isolate 454. A few plants were blighted at 60/80°F. Isolate 468 reduced emergence of all three varieties at both temperatures with the exception of Westland at 60/80°F. Mucor racemosus caused some reduction in emergence at 60/80°F of the three varieties. The other isolates did not

Table 12. Emergence of Combine 7078 seedlings in infested soil/sand mixtures. Isolations from soil obtained at Rocky Ford Experimental Field.

Species	Isolation No.	60/80°F Number of plants: 1/ Emerged	Dead	Emerged in control	70°F Number of plants: 1/ Emerged	Dead	Emerged in control
Fusarium moniliforme	458	41	0	58	46	0	46
"	459	39	0	58	42	4	46
"	481	48	0	48	--	--	--
F. oxysporum	469	38	0	58	44	0	46
"	1256	49	0	47	50	0	49
"	1280	49	0	47	45	0	49
"	1281	33	0	48	--	--	--
"	1303	35	0	48	--	--	--
"	1305	45	0	48	--	--	--
F. roseum	1265	58	0	47	53	2	49
F. solani	1302	53	0	48	--	--	--
Fusidium sp.	454	46	3	58	55	9	46
"	467	46	0	58	40	4	46
"	468	36	0	58	34	3	46
"	572	53	0	48	--	--	--
Humicola nigrescens	1270	52	0	55	50	0	42
Mucor racemosus	1306	42	0	48	--	--	--
Rhizoctonia	1304	56	0	48	--	--	--
Trichoderma lignorum	1275	45	0	49	49	0	49
Verticillium sulphurellum	573	48	0	48	--	--	--
Non-fruiting	571	45	0	58	--	--	--
"	1289	50	0	48	49	0	53

1/ 80 seeds planted.

Table 13. Emergence of Pink kafir seedlings in infested soil/sand mixture. Isolations from soil obtained at Rocky Ford Experimental Field.

Species	Isolation No.	60/80°F Number of plants: 1/ Emerged	Dead	Emerged in control	70°F Number of plants: 1/ Emerged	Dead	Emerged in control
Fusarium moniliforme	458	50	0	61	54	0	53
"	459	57	2	61	59	2	53
"	481	63	0	56	--	--	--
F. oxysporum	469	65	0	61	65	0	53
"	1256	52	0	50	53	0	53
"	1280	52	0	50	52	0	53
"	1281	41	1	56	--	--	--
"	1303	48	0	56	--	--	--
"	1305	60	0	56	--	--	--
F. roseum	1265	52	3	50	58	0	53
F. solani	1302	63	0	56	--	--	--
Fusidium sp.	454	50	2	61	46	16	53
"	467	45	0	61	61	0	53
"	468	34	0	61	38	2	53
"	572	62	0	56	--	--	--
Humicola nigrescens	1270	53	0	64	65	0	64
Mucor racemosus	1306	46	0	56	--	--	--
Rhizoctonia	1304	66	1	56	--	--	--
Trichoderma lignorum	1275	52	0	53	52	0	53
Verticillium sulphur-ellum	573	61	0	56	--	--	--
Non-fruiting	571	61	0	53	--	--	--
"	1289	59	0	62	59	0	50

1/ 80 seeds planted.

Table 14. Emergence of Westland seedlings in infested soil/sand mixture. Isolations from soil obtained at Rocky Ford Experimental Field.

Species	Isolation No.	60/80°F Number of plants:1/ Emerged	Dead	Emerged in control	70°F Number of plants:1/ Emerged	Dead	Emerged in control
Fusarium moniliforme	458	48	0	52	48	1	57
"	459	53	7	52	48	13	57
"	481	51	0	46	--	--	--
F. roseum	1265	48	0	49	46	3	43
F. solani	1302	56	0	52	--	--	--
F. oxysporum	469	56	0	52	44	0	57
"	1256	44	0	49	50	0	47
"	1280	51	0	49	41	0	47
"	1281	39	0	46	--	--	--
"	1305	63	0	46	--	--	--
"	1303	34	0	46	--	--	--
Fusidium	454	54	2	52	56	10	57
"	467	47	0	52	41	2	57
"	468	50	3	52	38	3	57
"	572	56	1	46	--	--	--
Humicola nigrescens	1270	49	0	60	53	0	48
Mucor racemosus	1306	32	0	46	--	--	--
Rhizoctonia	1304	49	4	46	--	--	--
Trichoderma lignorum	1275	54	0	49	51	0	47
Verticillium sulphurellum	573	52	0	46	--	--	--
Non-fruiting	571	46	0	55	--	--	--
"	1289	51	0	45	--	--	--

1/ 80 seeds planted.

cause noticable reduction in emergence or any blighting of seedlings.

DISCUSSION

According to Garrett (1944) non-pathogenic invasion of living roots may be a compensatory mechanism for organisms deficient in competitive saprophytic ability in that they can gain a prior foothold in these roots and thus achieve a head start in colonizing tissues when the host dies. Menzies (1963) is of the opinion that non-pathogenic invasion of a host is an important survival mechanism of root infecting fungi in a soil. The fact that the host of a particular pathogen does not grow in the field does not necessarily mean that survival of the particular pathogen is purely saprophytic. The pathogen may be established in native vegetation or weeds. Apparent non-pathogenic host invasion has been well demonstrated with the wilt producing Fusaria, when the fungus may be found in the stems of resistant varieties of the usual host plants without external or internal symptoms of wilt (Armstrong & Armstrong 1948, Hendrix & Nielsen 1958).

Root disease fungi may lie dormant in the soil as resting spores or sclerotia (Menzies 1963). These bodies may remain dormant until stimulatory substances liberated from host seedlings bring about their germination (Barton 1957, Jackson 1957). However, Menzies is of the opinion that highly specific germination stimuli restricted to preferred hosts are more the exception than the rule.

The relative frequency with which Trichoderma lignorum was isolated suggests that it may have been parasitizing some of the fungi. Weindling (1932) showed that T. lignorum was an active parasite of a number of soil fungi in culture. These included R. solani, Phytophthora parasitica, Pythium sp., Rhizopus sp., and Sclerotium rolfsii. However, because of its tendency to self digestion and because the vegetative form of the fungus is dependent on continuous moisture it would be difficult to keep Trichoderma dominant in the surface soil and thus control root-disease fungi (Weindling 1932). In the greenhouse, the field soil from which the isolates were obtained was kept well watered. Consequently, conditions were well suited for the growth of T. lignorum.

Some of the isolates, such as Aspergillus niger, Mucor racemosus, Penicillium rubrum and Trichoderma lignorum are normally considered to be saprophytic rather than parasitic (Leukel & Martin 1943). Leukel & Martin (1943) found that some species which were normally considered saprophytic reduced emergence and caused damping off of seedlings. It has been suggested that certain biproducts of these fungi are toxic to seedlings (Leukel & Martin 1943). Another way by which these saprophytes could inhibit germination or emergence or cause aborted seedlings would be for them to attack the seed and so deplete the food supply. Such an attack would take place very easily if the seed were damaged or cracked. In such cases the fungus has ready access to the endosperm. If conditions are more favorable for the growth of the fungus than the germination and growth of the seed then an aborted or very weak seedling

will result. Such a seedling would be very well disposed to attack by other soil fungi. Leukel & Martin (1943) demonstrated that emergence was less and death of seedlings due to damping off was greater in the case of nicked seed than in the case of corresponding lots of sound seed when they were inoculated with A. niger. This they found was also true for the following species - A. flavus, F. culmorum, F. moniliforme and P. oxalicum. F. moniliforme and P. oxalicum were the most injurious of the fungi tested.

None of the fungal isolates were highly virulent in the greenhouse at the temperatures used but under unfavorable conditions in the field some of the isolations could cause some reduction in sorghum stands.

SUMMARY

Fungi were isolated from roots of one month old plants of the Midland variety of sorghum which had been grown in soil from two locations, the Agronomy Farm and Rocky Ford Experimental Field of the Kansas Agricultural Experiment Station. The isolates included species of Aspergillus, Alternaria, Fusarium, Fusidium, Humicola, Mucor, Penicillium, Rhizoctonia, Trichoderma, Verticillium and some non-fruiting fungi.

Pathogenicity tests, in which seeds of Pink kafir were grown in vermiculite contained in half gallon Lamb and Mason fruit jars, were conducted at 70°F and 80° in environmental chambers. Isolates were given infection ratings depending upon the severity of disease. Emergence was generally lower at 60°F than at 80° for isolates of

Fusarium moniliforme and F. oxysporum obtained from the Agronomy Farm. Of the isolates from Rocky Ford Experimental Field F. moniliforme, Fusidium spp. and M. racemosus were responsible for the lowest emergence. Disease development was generally higher at 80°F than at 70°. Isolates were then tested in a sterile sand/soil mixture to determine whether they reduced emergence or caused seedling blight or both, of Combine 7078, Pink kafir, and Westland sorghums. None of the isolates were highly virulent in the greenhouse at the temperatures used but under unfavorable conditions in the field some of the isolates could cause reductions in sorghum stands.

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PATHOGENICITY OF FUNGI ISOLATED FROM ROOTS OF SORGHUM SEEDLINGS

by

ROGER JONES

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AN ABSTRACT OF A MASTER'S THESIS

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KANSAS STATE UNIVERSITY
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One of the most serious problems in sorghum production is that of obtaining good stands. Various seed-borne and soil fungi have been found to cause seed rots and seedling blights. This investigation was undertaken to find out more about fungi which attack roots of sorghum seedlings and to test such fungi for pathogenicity.

Soil was collected from the Agronomy Farm and Rocky Ford Experimental Field of the Kansas Agricultural Experiment Station. Seedlings of the Midland variety were grown in soil from the two locations and fungi isolated from roots showing lesions. The isolates included species of Aspergillus, Alternaria, Fusarium, Fusidium, Humicola, Mucor, Penicillium, Rhizoctonia, Trichoderma, Verticillium and some non-fruiting fungi.

Pathogenicity tests were carried out in half-gallon Lamb and Mason fruit jars with vermiculite as the rooting medium and using Hoagland's nutrient solution as a source of nutrients for the seedlings. Isolates were grown on potato dextrose agar plates and blended with the nutrient solution. This mixture was poured onto the vermiculite in which Pink kafir seed had been sown. Jars were kept at 70°F and 80° in environmental chambers for 2 weeks. Then isolates were given infection ratings, depending upon the severity of disease they caused. Emergence was generally lower at 60°F than at 80° for isolates of Fusarium moniliforme and F. oxysporum obtained from the Agronomy Farm. For one isolate of F. oxysporum, emergence was only 11.7% when 6 plates of inoculum and 0.17% when 3 plates of inoculum were used at 70°F. For isolates from Rocky

Ford Experimental Field, F. moniliforme, Fusidium spp., and Mucor racemosus were responsible for the lowest emergence. Disease development was generally higher at 80°F than at 70°.

Isolates were tested in a sterile sand/soil mixture to determine whether they reduced emergence or caused blighting, or both of Combine 7078, Pink kafir and Westland in the greenhouse. Isolates of F. moniliforme were responsible for most of the reduction in emergence observed. None of the isolates were, however, highly virulent in the greenhouse at the temperatures used but under unfavorable conditions in the field some of the isolates could cause reductions in sorghum stands.

Non-pathogenic invasion of a host is an important survival mechanism of root infecting fungi in the soil. Under adverse conditions for the host or favorable condition for the pathogen, disease could be induced in the host. Some fungi which are normally considered to be saprophytes can attack seed and so inhibit germination or emergence or cause aborted seedlings.