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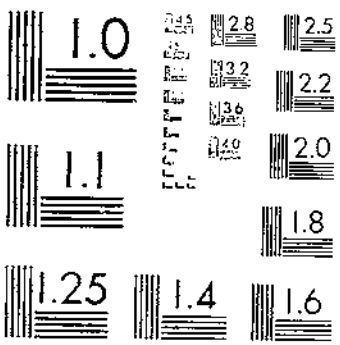
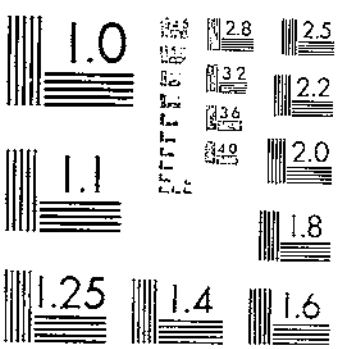


MICROCOPY RESOLUTION TEST CHART
NATIONAL BUREAU OF STANDARDS 1963-A



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Seed Rot and Seedling Blight of Sorghum

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SUMMARY

The production of sorghum, one of the important war-emergency crops, is seriously handicapped by poor stands. Up to the present time, these have been attributed almost solely to unfavorable environmental conditions. Studies in the greenhouse and laboratory, however, show that seed-borne and soil-inhabiting fungi, combined with certain environmental conditions, are largely responsible for poor germination and emergence of sorghum planted in the field.

Species of *Alternaria*, *Fusarium*, *Penicillium*, *Aspergillus*, and *Rhizopus* were found on seed in great abundance, and other fungi less frequently. Virulent strains of *Fusarium moniliforme* and *Penicillium oxalicum* were isolated from diseased seedlings grown in steam-sterilized soil, showing that these fungi are seed-borne. Species of *Fusarium*, *Penicillium*, *Rhizopus*, and *Aspergillus*, when used as inoculum, at times reduced emergence and caused subsequent seedling blight, especially at 15° and 20° C. Although seeds frequently were blackened with spores of *Alternaria*, cultures of this fungus showed little evidence of pathogenicity on sorghum.

Soil-inhabiting fungi, on the whole, reduced emergence more than did seed-borne fungi. When sterilized seed was planted in samples of unsterilized soil from the Arlington Farm and from six stations in the Great Plains, emergence was very poor, especially at low temperatures.

Isolations made from ungerminated seeds, aborted sprouts, and diseased seedlings, taken from unsterilized soil, yielded species of *Pythium* most frequently, with species of *Fusarium* next in order of occurrence. Nearly all isolates of *Pythium* proved extremely virulent and inhibited or drastically reduced emergence at the lower temperatures. This was true also of pure cultures of eight species of *Pythium*, obtained from other sources. *Pythium* frequently attacked the young plumule, thus inhibiting emergence, or destroyed the mesocotyl, seminal root, or subcrown rootlets before the crown roots developed. Species of *Fusarium* isolated from the soil showed much less pathogenicity than did species of *Pythium*, and also responded more readily to seed treatment. The pathogenicity of the isolates of species of *Penicillium*, *Rhizopus*, and *Aspergillus* varied with the quantity of inoculum used, the condition of the seed, and the environmental conditions after planting.

Although slight nicks or cuts in the seed coats caused some reduction in emergence from uninoculated sterilized seeds planted in sterilized soil, much larger reductions occurred when such seeds were first inoculated with certain fungi. This shows that seed-coat injuries, very common in threshed sorghum, give fungi ready access to the endosperm. Even saprophytic fungi were thus able to reduce emergence, presumably either by depleting the food supply needed by the young seedling or by producing toxins that tended to inhibit its development. The latter possibility was demonstrated by the failure of sorghum seedlings to develop normally when grown on potato-dextrose agar that had been sterilized after cultures of species of *Rhizopus*, *Penicillium*, or *Aspergillus* had been grown on it.

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DEPARTMENT OF AGRICULTURE
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Seed Rot and Seedling Blight of Sorghum[1]

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The experiments and results reported in this technical bulletin are considered important at this time. Since it is printed under war conditions, when paper, labor, and machinery are scarce, and time itself is at a premium, certain liberties have been taken with the form usually followed by the Department of Agriculture in scientific publications. The summary is placed at the beginning instead of at the end, and the discussion of results is combined with the introduction. This compact front matter, which will give any reader a quick grasp of the work and its practical implications, is printed in the usual 10-point type. The more detailed descriptions of experiments follow and are printed in 8-point type. In addition to this mechanical saving of space, the descriptive material has been presented as briefly as seems consistent with retaining its scientific value. The illustrations have been omitted. The tolerance of readers for this wartime arrangement is solicited.

COVE HAMBIDGE,
Agricultural Research Administration.

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INTRODUCTION AND DISCUSSION OF RESULTS

Sorghum (*Sorghum vulgare* Pers.) is an important war-emergency crop. It is the basic forage and grain feed crop in much of the Great Plains region. Certain varieties of sorghum are being processed extensively for sirup and alcohol and have possibilities as a source of cane sugar. Also, the seed of several domestic sorghum varieties contains a waxy starch that is being used in foods and adhesives to replace tapioca starch, made unavailable by the war.

[1] Submitted for publication May 1942.
[2] The writers gratefully acknowledge the assistance given by Aline F. Kempton, formerly junior pathologist, Division of Cereal Crops and Diseases, in the isolation and identification of soil-borne fungi.

In growing sorghum, failure to obtain good stands is one of the most serious problems. The replanting of whole fields or parts of fields one to three times is not unusual. In normal times this waste of seed and labor lessens the profits of the farmer. In wartime, it also curtails an important crop. Information regarding the causes of poor stands of sorghum and methods of improving stands is much needed.

The burying of seeds by soil washed into the lister furrows and the crusting of soil have been generally regarded as almost the sole causes of stand failures. However, in the greenhouse experiments described herein, seed-borne and soil-inhabiting fungi are shown to be capable of inflicting severe injury on germinating seeds and young seedlings of sorghum. That poor stands of sorghum under field conditions may also be due to such fungi seems evident from the following considerations.

Sorghum seed shown to have high viability (90 percent or more) in laboratory tests on blotters or in sterilized soil frequently showed a relatively low germination (50 to 70 percent) and a high percentage of blighted seedlings in unsterilized field soils or when grown under other unfavorable conditions (*12, 16*).[3] This is not true of equally viable seeds of certain other cereals of which good stands are usually obtained and which are known to be less susceptible to attack by rot-producing fungi at low temperatures than are the soft seeds of sorghum.

Sorghum stands usually were greatly improved when these harmful organisms were partly or totally eliminated by seed treatment or soil sterilization or both. On the other hand, severe reductions in stand, especially at lower temperatures, either because of poor germination or seedling blight in sorghum, usually followed inoculation of seed or soil with cultures of certain fungi isolated from sorghum seed or from soil.

Seed decay and seedling blight were greatly aggravated when the fungi had ready access to the starchy endosperm through breaks in the pericarp, although in soft-seeded types, such as feterita and hegari, invasion directly through the seed coats is not uncommon. This injury was not confined, as might be expected, to fungi found only in soil in which sorghum had been more or less continuously grown, but, on the contrary, in some experiments it was equally severe in soil not previously cropped to sorghum. It was found that seed decay and seedling blight can be caused by any one of a number of familiar saprophytes, or facultative parasites. Among these, probably the most common are species of *Pythium*, generally soil-borne only, and *Rhizopus, Aspergillus, Penicillium*, and *Fusarium*, found either on the seed or in the soil. On the whole, it seems from the data obtained that the soil-borne fungi, especially *Pythium*, play a more important part in seed decay and seedling blight in sorghum than do those that are commonly seed-borne. However, the relative importance of these two types of fungi may vary somewhat with the different types of seed and the relative susceptibility or resistance of different varieties of sorghum to this kind of injury.

Rotting of seeds and blighting of seedlings occur particularly when planting is done early, or in cold wet soil, or in a poorly prepared

[3] Italic numbers in parentheses refer to Literature Cited, p. 20.

seedbed. Using seed that is inferior because of immaturity, threshing injury, or improper curing and storing aggravates the trouble. It seems, therefore, that good stands in sorghum often depend largely on protecting the planted seed against attack by harmful organisms. It has been shown that this can be accomplished by using only sound, carefully selected, and highly viable seed of suitable varieties, treating it with an efficient fungicide, and planting it in a warm, mellow, well-prepared seedbed. The value of these preventive measures has long been recognized and frequently emphasized. Sorghums are of tropical origin and germinate and grow best at temperatures considerably higher than those frequently prevailing at planting time in the Temperate Zone; therefore, proper cultural practices should be employed to offset this disadvantage.

Thus it seems that, although unfavorable cultural and seasonal conditions are important contributing factors in causing poor stands in sorghum, fungi are the real cause of the trouble. The studies reported in this bulletin involved isolating and identifying seed-borne and soil-inhabiting fungi and determining their effect upon germination, emergence, blighting, and subsequent growth in sorghum and the effect of environment and seed treatment upon the injury caused by such organisms. The experiments were carried out in the laboratory and greenhouse at the Arlington Experiment Farm, Arlington, Va., over a period of more than 10 years. Much of the soil and seed used in the studies was sent in from the sorghum-growing areas of the Southwest.

Information presented here regarding the causes of and the conditions favoring seed rots and seedling blights in the sorghum crop should aid in reducing the losses from these diseases.

REVIEW OF THE LITERATURE

Despite extensive investigations of seed rot, root rot, damping-off, seedling wilt, and other seedling troubles of other cereals, very little attention has been given to such diseases in sorghum. In 1910 Pammel et al. (*10*) described a disease of corn and sorghum caused by a seed-borne species of *Fusarium* but did not state that it caused seedling blight. Valleau (*14*) demonstrated that *F. moniliforme* Sheld. is internally borne in seed corn and also showed that this fungus could cause damping-off in sorghum seedlings under very humid conditions.

Lippal et al. (*18*), in 1936, reported that in India *Rhizoctonia bataticola* (Taub.) Butl., under favorable conditions, may cause in sorghum a destructive seedling blight in addition to so-called "hollow stem" [4] in the mature plants. Harris and Goss (*2*), in 1934, described a seedling disease of sorghum and Sudan grass, which they attributed to the formation of prussic acid in the seedling, especially under unfavorable conditions of growth. The primary roots, and especially the mesocotyls, reddened and then turned dark. The mesocotyls shriveled 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*.

Elliott et al. (*1*), in studying the root rot caused by *Pythium arrhenomanes* Drechs., stated that in certain experiments this fungus prevented germination or killed the sorghum seedlings either as they emerged or after they had produced two or three leaves. The preemergence and postemergence virulence of the fungus was also reported by Kendrick and Briggs (*7*).

The occurrence of seed rot and seedling blight in sorghum plantings has been mentioned occasionally in publications dealing with sorghum culture or seed treatment, but the relation of causative organisms to these troubles generally has not been discussed.

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[4] In the United States a similar malady in sorghum is called "charcoal rot," and the causal organism is thought to be *Sclerotium bataticola* Taub.

MATERIAL AND METHODS

A total of 23 lots of sorghum seed were examined especially to determine what organisms are commonly seed-borne. Nine of these were obtained from Lawton, Okla., 5 from Chillicothe, Tex., and 9 were grown on the Arlington Farm. Among these were 7 lots of sorgo, 4 of kafir, 5 of feterita, 2 each of milo and hegari, and 1 each of Darso, Club, and Chiltex. Three methods were employed, which for convenience may be called (1) the centrifuge method, (2) the agar-plate method, and (3) the isolation method.

In the first method, 10 seeds from each sample were shaken in tubes of sterile distilled water to remove the surface-borne spores. The water was then centrifuged, the supernatant liquid poured off, and the residue examined microscopically for spores.

In the agar-plate method, separate portions from each lot of seed were sterilized with different degrees of severity and plated on plain water agar slightly acidified to suppress bacterial growth. The fungi thus obtained, after being transferred to nutrient media and identified, gave some indication of what organisms most frequently contaminate the seeds, and also, to some extent, their relative susceptibility to sterilization.

The isolation method consisted of planting unsterilized seed in different lots of sterilized soil kept at different temperatures. Isolations were then made from apparently diseased seedlings, aborted sprouts, and seeds that failed to germinate. The fungi obtained in this way were transferred to nutrient agar and identified after sporulation. Purified cultures were later used in pathogenicity studies.

The pathogenicity of a fungus thus isolated was determined in a preliminary way by growing seedlings on agar slants under sterile conditions and then placing transfers of the fungus at the bases of the seedlings. Fungi showing no indications of pathogenicity in this test were discarded. Others were tested further by planting surface-sterilized seed in soil that had been sterilized and then artificially infested, or by planting artificially infested seed in sterilized soil. Sterilized seed planted in sterilized soil was used for comparison. Standard feterita and Spur feterita were used in most of these pathogenicity tests because these varieties frequently produce poor stands when planted under unfavorable temperature and moisture conditions. Seed of other types of sorghum, such as kafir, milo, and sorgo, were used in some of the experiments. The relative percentages of emergence, the relative vigor of growth, and the severity of wilt, damping-off, or other disease manifestations were observed and recorded. Isolations were then made from seeds that failed to germinate and from parts of diseased seedlings, to see if the fungus used as inoculum could be recovered. Necessarily there were deviations from the above general procedure, and these will be described in connection with the individual experiments.

Materials used for disinfecting seeds, in order to eliminate seed-borne organisms, included the following: (1) Germisan, a mercuric cresol cyanide compound; (2) Semesan, a hydroxymercurichlorophenol; (3) Hyclorite, containing 4 percent of sodium hypochlorite; (4) formaldehyde as a 1:240 or 1:320 water solution of the commercial 37-percent product; (5) mercuric chloride as a 1:1,000 solution; and (6) hot water (52° to 56° C.) for 12 minutes.

The soil used in pathogenicity studies was first brought to a satisfactory moisture content, steam-sterilized, usually at 15 pounds' pressure for 2 hours on 2 successive days, and then stored for several weeks in large metal cans with tightly fitting covers. Separate portions of this soil inoculated with the different fungi being studied were stored for some time in sterilized metal containers at temperatures thought to be favorable for the growth of the respective fungi. Surface-sterilized seed was then planted in the different lots of inoculated or sterile soil.

In the determinations of the soil-inhabiting fungi presumably associated with seed rot and seedling diseases of sorghum, soil was obtained at several experiment stations from fields in which sorghum had been regularly grown. Surface-sterilized seed was planted in these soils and isolations were made from ungerminated seeds and diseased seedlings as described.

EXPERIMENTAL RESULTS

Seed-Borne Organisms

Because of the general effectiveness of recently developed seed treatments, diseases caused by seed-borne pathogens usually respond to control measures more readily than do those caused by organisms occurring in the soil. This simple and

economical method of control therefore is usually the first one tried in combating seedling diseases. Consequently in these studies attention was first given to the fungi that were thought to be seed-borne.

The results obtained from centrifuging seeds varied according to the source and the type of seed. Seeds grown at Arlington, Va., yielded more fungus spores per kernel when centrifuged than did those from Chillicothe, Tex., or Lawton, Okla. The more humid conditions at Arlington after heading time may explain the more abundant fungus development there. Seeds from varieties with compact heads, like feterita and certain kafirs, carried more fungus spores than did those from certain sorgos or other varieties with more open heads. Hard, smooth seeds and those threshed free from glumes were less contaminated, as a rule, than large, soft, rough seeds and those with the glumes retained.

On most seed lots, spores of *Alternaria* spp. were found most commonly and spores of *Fusarium* spp. ranked second in occurrence. Also frequently found were spores of species of *Penicillium*, *Aspergillus*, *Rhizopus*, and *Trichoderma*. Spores of species of *Sphacelotheca* and *Helminthosporium* and unidentified sclerotial bodies were found occasionally. The mere presence of certain spores on the seed, however, does not necessarily indicate that the fungi are pathogenic to sorghum. Thus seeds exposed to the weather until blackened by spores of *Alternaria* spp. germinated satisfactorily and the seedlings showed little evidence of disease or injury. A shortcoming of the centrifuge method for the identification of seed-borne fungi is its failure to reveal those present only in the form of mycelium.

Plating seeds on water agar yielded results somewhat similar to those obtained by the centrifuge method. Unsterilized seeds usually produced a preponderance of *Alternaria* spp., which frequently obscured the growth of other fungi. Species of *Fusarium* and *Rhizopus* also were very prominent. Species of *Penicillium* and *Aspergillus* survived light seed treatment in sodium hypochlorite, and species of *Fusarium* frequently were the only survivors after a slightly more severe treatment of the seeds, indicating that they were more deep-seated than the others. A number of the fungi failed to sporulate after being transferred to nutrient agar, and therefore were not identified. In many tests with surface-sterilized seeds no fungus growth appeared until after the seed had germinated. Some seeds that failed to germinate after the disinfection treatment 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 within the seed.

In connection with studies on seed transmission of diseases in corn, Valleau (*15*) observed that—

> organisms between the pericarp layers are often sealed and not capable of growing until the pericarp walls are broken down by other processes than those occurring during germination under aseptic conditions. It seems evident that the usual culture-plate method cannot be relied upon for the accurate determination of organisms carried by seed.

Isolations made from the seeds that failed to germinate and from diseased seedlings from nondisinfected seed grown in sterilized soil most frequently yielded species of *Fusarium*, *Penicillium*, *Rhizopus*, and *Aspergillus*, the last two being most often associated, particularly with ungerminated seeds and with sprouts that had failed to emerge.

FUSARIUM SPP.

Since species of *Fusarium* were generally most prominent in isolations from diseased seedlings grown in sterilized soil, they were regarded as probably among the more important seed-borne pathogens responsible also for poor emergence. In one test, for example, emergence in Kansas Orange sorgo from unsterilized seed planted in sterilized soil was only 49 percent, whereas that from seed soaked in 1:240 formaldehyde for 30 minutes was 89 percent. A high percentage of the seedlings from untreated seed, but none of those from formaldehyde-treated seed, succumbed to damping-off. Isolations from aborted sprouts and from the mesocotyls and lower stems of slightly affected seedlings consistently yielded a species of pink *Fusarium*. A number of these isolations were purified and used to inoculate sterile seedlings grown aseptically on agar slants. Within a week all the seedlings were dead, whereas the controls remained green and healthy. Likewise, when formaldehyde-treated seeds were inoculated with a heavy spore suspension and planted in sterilized soil, emergence was reduced to 42 percent, as compared with 83 percent from similar uninoculated seeds, and later 25 percent of the seedlings from inoculated seed were damped off. The fungus was reisolated from diseased

seedlings and was identified as *Gibberella fujikuroi* (Saw.) Wr. (*F. moniliforme* Sheld.).[5]

To test further the pathogenicity of this isolate of *Fusarium moniliforme*, formaldehyde-disinfected seed of four other types of sorghum was planted in flats of sterilized soil that had been inoculated with spores and mycelium from an agar culture. The seed in another series of flats was inoculated with a spore suspension, dried, and planted in sterilized, uninoculated soil. In half of the flats in each series the seed was treated with an organic mercury dust before planting. A temperature of about 27° C. was maintained in the greenhouse during and after emergence. The data on emergence and subsequent damping-off are shown in table 1.

TABLE 1.—*Emergence and damping-off in 4 varieties of sorghum as affected by inoculation of the soil or seed with Fusarium moniliforme (Gibberella fujikuroi) and by treatment of the seed with an organic mercury dust*

Variety	Emergence in—				Damping-off in—			
	Inoculated soil from uninoculated seed—		Uninoculated soil from inoculated seed—		Inoculated soil of plants from inoculated seed—		Uninoculated soil of plants from inoculated seed—	
	Untreated	Treated	Untreated	Treated	Untreated	Treated	Untreated	Treated
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Darso	68	86	41	73	44.1	37.2	20.3	6.8
Reed kafir	46	82	31	80	45.7	31.7	35.5	15.0
Dwarf Yellow milo	20	62	14	63	50.0	33.9	35.7	5.9
Freed	41	87	20	80	46.3	36.8	25.0	5.0
Weighted average	44	79	27	74	45.7	35.0	31.1	10.5
Increase		80		174	30.6		196.2	

Seed inoculation reduced the percentage of emergence more than did soil inoculation, but seed treatment combated the seed-borne inoculum more effectively than it did the inoculum in the soil. Damping-off was more severe in the plants grown in infested soil, and seed treatment was less effective in combating it than when only the seed had been inoculated.

The effect of soil temperature on this isolate of *Fusarium moniliforme* was studied in constant-temperature tanks (*9*) maintained at 12°, 16°, 20°, 24°, 28°, and 32° C. Seed of Kansas Orange sorgo, soaked in a 4-percent solution of sodium hypochlorite for 2 hours and then washed and dried, was immersed in a heavy suspension of spores and bits of mycelium. Then 100 seeds were planted in each of 6 lots of sterilized soil adjusted to 60 percent of its water-holding capacity, and 1 lot was placed at each of the above mentioned temperatures. Uninoculated seed was planted in a parallel series for comparison. The results are shown in table 2.

TABLE 2.—*Effect of soil temperature on emergence and subsequent damping-off of seedlings of Kansas Orange sorgo from surface-sterilized seed inoculated with conidia of Fusarium moniliforme*

[100 seeds planted in each lot]

Soil temperature (°C.)	Period before emergence	Emergence from seed—		Reduction due to inoculation	Damping-off in plants from inoculated seed	
		Uninoculated	Inoculated			
	Days	*Percent*	*Percent*	*Percent*	*Number*	*Percent*
12	30	59	14	76.3	0	0
16	16	62	28	54.8	1	3.6
20	8	74	53	28.4	5	9.4
24	4	70	51	27.1	12	23.5
28	3	76	61	19.7	16	26.2
32	2	80	65	18.8	20	30.8

[5] Since, so far as is known, only the conidial form of this fungus was dealt with in these studies, it will be referred to as *Fusarium moniliforme* throughout the bulletin.

In general, the lower the temperature the greater was the reduction in emergence from inoculated seed as compared with emergence from uninoculated seed, and the higher the temperature the greater was the subsequent damping-off.

On potato-dextrose agar the most rapid and abundant mycelial growth was made by this fungus at 24° C., although a fairly vigorous growth was made at all temperatures from 20° to 32°. Growth at 6°, 9°, and 12° was very slow and at 36° it was almost negligible. These temperature requirements in pure culture explain the virulence of the fungus as a damping-off organism at the higher temperatures but not its depressing effect on emergence at the lower temperatures.

The unfortunate loss of all cultures of this virulent strain of *Fusarium moniliforme*, through the failure of a temperature-control mechanism to function properly, prevented further studies on its pathogenicity.

Another pathogenic isolate of *Fusarium moniliforme* was obtained from seed of Dwarf Blackhull (Sharon) kafir grown at the Arlington Experiment Farm. This lot of seed when planted in sterilized soil gave an emergence of only 25 percent. When plated on agar, 40 percent of the ungerminated seeds yielded *F. moniliforme* and 20 percent yielded other species of *Fusarium*.

A purified culture of this isolate of *Fusarium moniliforme* was used to inoculate 100 carefully selected, formaldehyde-treated seeds of Dwarf Blackhull kafir, which were then planted in sterilized soil. The emergence was only 47 percent, whereas similar uninoculated seeds gave an emergence of 82 percent. Later, 28 of the seedlings from the inoculated seed were killed by damping-off, but none from the uninoculated seed.

Numerous other isolates from sorghum seed grown in the field at the Arlington Farm proved to be *Fusarium moniliforme*. A number of these greatly impaired emergence and later caused damping-off when used as inoculum on seed planted in sterilized soil. However, four of the isolates failed to manifest any pathogenicity. This variability in the pathogenicity of *F. moniliforme* has been discussed and demonstrated by Leonian (*8*).

A considerable number of isolations were made of species of *Fusarium* other than *F. moniliforme*. Some of these were purified and used to inoculate seed planted in sterilized soil, but none proved to be highly pathogenic, as evidenced by their failure to reduce emergence seriously or to cause seedling blight.

One isolation of a *Fusarium* from seed of feterita had the appearance of *F. culmorum* (W. G. Sm.) Sacc. when compared with a culture of this species isolated from wheat, and it was identified as *F. culmorum* by Helen Johann. These two cultures had the same color and other cultural characteristics on potato-dextrose agar, but, in pathogenicity tests, the one isolated from wheat was very virulent on sorghum, whereas the other, isolated from sorghum seed, was only moderately pathogenic. Further experiments comparing these *Fusarium* cultures with other organisms are described later.

PENICILLIUM SPP.

The most virulent isolate of *Penicillium* spp. obtained in these studies came from seedlings of Dwarf White milo grown from untreated seed in sterilized soil. These seedlings were observed to wilt severely when in the second- to fourth-leaf stage and, although some of the affected plants recovered, most of them died. Isolations from the discolored mesocotyls of affected plants consistently yielded a greenish *Penicillium*. Since the soil had been sterilized, it was assumed that the organism was seed-borne. When 100 lightly disinfected seeds from this lot of Dwarf White milo were plated on agar, 25 yielded this same type of *Penicillium*.

A typical isolate was purified and then cultured on potato-dextrose agar, on which it sporulated profusely. Seed of Dwarf White milo soaked in a 1:320 formaldehyde solution for 30 minutes and washed in sterile water was dusted heavily with spores, and 100 seeds were planted in sterilized soil along with an equal number of uninoculated seeds. Both flats were held at a temperature of 25° to 30° C. Emergence was 75 and 88 percent, respectively, from inoculated and uninoculated seed. Subsequently, 68 percent of the plants from inoculated seed and 5 percent of those from uninoculated seed wilted.

The symptoms of injury caused in sorghum seedlings by this isolate of *Penicillium* started as a grayish- or silvery-green hue in the leaves, followed by a gradual yellowing. Then the leaves became limp and finally curled and dried completely. The mesocotyl was reddish to black, and its base frequently was covered with the greenish *Penicillium*. The dead plants did not fall over, as those attacked by *Fusarium moniliforme* usually do, but remained upright. Larger plants sometimes

recovered, although the affected leaves usually did not. Whether or not these plants were permanently retarded was not determined, as they were not grown to maturity.

The foregoing symptoms were recognized as similar to those caused on corn by *Penicillium oxalicum* Currie and Thom, as described by Johann et al. (*4, 6*). A culture of *P. oxalicum* isolated from corn by Johann was obtained and used in parallel culture and inoculation tests with the isolate from sorghum. The two were identical as to color, type of growth on culture media, pathogenicity to sorghum seedlings, and other details. Later this isolate from sorghum was identified by Johann as *P. oxalicum*, and hereafter it will be referred to thus or as culture No. 601.

In a subsequent experiment, 240 surface-disinfected seeds of Dwarf White milo, uninoculated, and a similar number inoculated with spores of the above-mentioned isolate of *Penicillium oxalicum* were planted in separate lots of sterilized soil and maintained at the following temperatures: 15°, 20°, 25°, and 30° C. The results are given in table 3. Emergence from inoculated seed was reduced most severely at 15° and least at 30°, but the percentage of plants that subsequently wilted was almost the same at all four temperatures. The wilt in the uninoculated controls indicates failure of the seed treatment to kill all the seed-borne fungi.

TABLE 3.—*Effect of soil temperatures and inoculation on emergence and subsequent wilt of Dwarf White milo grown from seed uninoculated or inoculated with spores of Penicillium oxalicum and planted in sterilized soil*

[240 seeds planted in each lot]

Soil temperature (°C.)	Plants emerged from seed—				Wilted plants from seed—			
	Inoculated		Uninoculated		Inoculated		Uninoculated	
	Number	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*
15	115	47.9	192	80.0	99	86.1	8	4.2
20	173	72.1	204	85.0	156	90.2	13	6.4
25	204	85.0	220	91.7	176	86.3	16	7.3
30	216	90.0	226	94.2	184	85.2	18	8.0

The relative pathogenicity of this culture of *Penicillium oxalicum* and that of the culture of *Fusarium moniliforme*, isolated from Kansas Orange, were compared in the following experiment. One lot of Dwarf White milo seed was treated in a 1 : 320 formaldehyde solution for one-half hour and washed in sterile water. Another lot was left untreated. Hundred-seed samples of each lot were (1) uninoculated, (2) inoculated by dusting with spores of *P. oxalicum*, or (3) inoculated with conidia of *F. moniliforme*. The seeds were planted in flats of sterilized soil in a greenhouse maintained at 27° to 33° C. Data on emergence taken after 5 days and on diseased or dead plants taken after 24 days are given in table 4.

TABLE 4.—*Emergence and final stand of Dwarf White milo grown at 27° to 33° C. in sterilized soil from formaldehyde-treated or untreated seed, uninoculated or inoculated with cultures of Penicillium oxalicum or Fusarium moniliforme*

[100 seeds planted in each lot]

Inoculum	Plants from treated seed				Plants from untreated seed			
	Healthy after—		Wilted or dead after 24 days		Healthy after—		Wilted or dead after 24 days	
	5 days	24 days			5 days	24 days		
	Percent	*Percent*	*Number*	*Percent*	*Percent*	*Percent*	*Number*	*Percent*
None	72	69	3	4.2	77	66	11	14.3
Penicillium oxalicum	60	5	55	91.7	82	21	61	74.4
Fusarium moniliforme	55	45	10	18.2	78	57	21	26.9

Although dusting the seed with spores of *Penicillium oxalicum* did not greatly affect emergence in this case, most of the plants from seed thus inoculated eventually succumbed to wilt. This reduction in stand was much more severe than that due to damping-off by *Fusarium moniliforme*. The formaldehyde treatment, it seems, did not eliminate all of the seed-borne infection.

Seed of eight varieties of sorghum, disinfected as in the preceding experiment, was dusted with spores from this same culture of *Penicillium oxalicum*, and 100-seed samples of each lot were planted in flats of sterilized soil kept in a greenhouse where the temperature during the period of emergence ranged from 25° to 27° C. Corresponding 100-seed lots of uninoculated seed were planted as controls. Data on emergence and subsequent wilt of the seedlings are given in table 5. Wilt was less severe than in the previous experiment, possibly because of different environmental conditions. None of the varieties was immune from wilt, although some were affected less severely than others.

TABLE 5.—*Effect of inoculating seed of eight varieties of sorghum with spores of Penicillium oxalicum on emergence and wilt in sterilized soil*

[100 seeds of each variety were planted]

Variety	C. I.[1] No.	Emergence from—		Wilted plants from—			
		Uninoculated seed	Inoculated seed	Uninoculated seed		Inoculated seed	
		Percent	*Percent*	*Number*	*Percent*	*Number*	*Percent*
Freed	350	68	61	0	0	12	19.7
Kansas Orange sorgo	107	67	70	0	0	14	20.0
Darso	615	80	72	1	1.3	9	12.5
Dawn kafir	340	70	65	2	2.9	11	16.9
Reed kafir	628	73	70	3	4.1	7	10.0
Dwarf White milo	627	70	60	2	2.9	20	33.3
Dwarf Yellow milo	332	63	60	2	3.2	15	25.0
Feterita	182	52	46	4	7.7	16	34.8

[1] C. I. refers to accession number of the Division of Cereal Crops and Diseases.

Five other isolates of *Penicillium* were tested for pathogenicity by planting treated, inoculated seed in sterilized soil, but none exhibited any marked degree of virulence. At times, emergence was slightly affected or a few seedlings showed symptoms of wilt, but after a week or two of growth, neither roots nor tops were appreciably inferior to those in the uninoculated checks.

RHIZOPUS AND ASPERGILLUS SPP.

As previously stated, nondisinfected seeds when germinated on plain agar frequently yielded species of *Aspergillus* and *Rhizopus*, especially *A. niger* Van Tiegh. and *R. nigricans* Ehr. The same fungi frequently were isolated from seeds that failed to germinate in sterilized soil and were then plated after light disinfection. Since these species of *Rhizopus* and *Aspergillus* are generally regarded as saphrophytes, it seemed desirable to determine the extent to which they could reduce germination, emergence, and stand. Seed of feterita was soaked in a 0.5-percent solution of Germisan, an organic mercury compound, for 1½ hours and planted in three lots of previously sterilized soil that were (1) uninoculated, (2) inoculated with an isolate of *Rhizopus nigricans*, and (3) inoculated with a pure culture of *R. tritici* Saito. Unsterilized, uninoculated soil was used for comparison. Three hundred seeds were planted in duplicate flats of each of these lots of soil, and one flat of each lot was kept at 20° and one at 25° C. Data on emergence, taken 10 and 25 days after planting, are given in table 6. The results indicate that these fungi reduced emergence chiefly by rotting the seeds rather than by parasitizing the seedlings, as no diseased seedlings were found in the inoculated, sterilized soil such as occurred in the unsterilized soil. However, it was thought possible that enough Germisan solution had been absorbed by the seed to protect it somewhat against attack by the organisms in the soil. To determine this, untreated seed and seed treated with Germisan were planted in sterilized and unsterilized soil kept at 20°. In sterilized soil emergence from treated and untreated seed was 83 and 27 percent, respectively; in unsterilized soil it was 47 and 9 percent, respectively. This indicated that Germisan, while most effective against the seed-borne organisms, also was somewhat effective against the organisms present in the soil, and was, therefore, not considered suitable as a seed disinfectant in these studies.

In another experiment, seed of feterita was treated in a 1:240 formaldehyde solution for one-half hour and washed thoroughly; 300 seeds were planted in

TABLE 6.—*Emergence and stand of feterita as affected by two soil temperatures and by inoculation of the soil with Rhizopus nigricans and R. tritici*

[300 seeds planted in each lot]

Soil temperature (° C.)	Days after planting	Emergence in soil—				Reduction [1] in emergence in soil—		
		Sterilized and—			Unsterilized and uninoculated	Inoculated with—		Unsterilized and uninoculated
		Uninoculated	Inoculated with—			*Rhizopus nigricans*	*Rhizopus tritici*	
			Rhizopus nigricans	*Rhizopus tritici*				
		Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
20	10	44	33	30	4	25.0	31.8	90.9
	25	61	40	57	[2] 51	19.7	6.6	16.4
25	10	44	43	39	40	2.3	11.4	9.1
	25	65	52	52	[3] 58	20.0	20.0	10.8

[1] Compared with emergence in sterilized soil.
[2] 2 percent died.
[3] 12 percent died.

each of four separate lots of sterilized soil, uninoculated or inoculated separately with cultures of *Rhizopus nigricans*, *R. tritici*, and *Aspergillus niger*. A temperature of 25° C. was maintained during emergence.

The percentages of emergence in uninoculated soil and in soil inoculated as described above were 63, 51, 49, and 54, respectively. Emergence was 19 percent, 22 percent, and 14.3 percent less in soil inoculated with *Rhizopus nigricans*, *R. tritici*, and *Aspergillus niger*, respectively, than it was in uninoculated soil. The plants showed no disease symptoms when discarded 1 week after emergence.

In another experiment conducted at 20° and 25° C., formaldehyde-treated seed of feterita was planted in sterilized uninoculated soil, and seed from this same lot, without further treatment or dusted with an organic mercury disinfectant, was planted in sterilized soil inoculated with *Rhizopus nigricans*. Emergence in the uninoculated controls at 20° and 25° was 72 and 91 percent, respectively. In the inoculated soil, emergence from the undusted seed was 42 and 53 percent, respectively, while from the dusted seed it was 57 and 69 percent, respectively. The effect of the fungus on emergence at the two temperatures was clearly demonstrated, as also was the beneficial effect of the organic mercury treatment of seed.

In a subsequent series of experiments with these same fungi, *Fusarium moniliforme*, *F. culmorum*, and *Penicillium oxalicum* were included for comparison. Two flats of sterilized soil were inoculated with each fungus and kept at 25° C. for 3 weeks previous to planting. On February 19, 300 formaldehyde-treated seeds of feterita were planted in each flat. One set of flats was kept at 25° until after emergence and the other at about 17°. The flats kept at 25° were replanted on April 14 and again on May 19, and those at 17° on May 17. Additional inoculum of the respective fungi was added to each flat 3 weeks before each subsequent planting. The data on emergence are presented in table 7.

On the whole, in this experiment the fungi displayed no marked virulence, although some pronounced reductions in stand occurred. The somewhat erratic results may be explained in part, perhaps, by variations in soil moisture that may have affected the various fungi differently. Subsequent damping-off, wilt, or other disease symptoms in this case were not observed, because the plants were discarded shortly after emergence. It was thought that possibly some of the cultures had become attenuated from being carried too long in pure culture. Therefore, another pure culture of *Fusarium moniliforme*, more recently isolated, and a culture of *F. culmorum*, isolated from wheat, were used, along with fresh cultures of more recent reisolations of *Penicillium oxalicum*, *Rhizopus nigricans*, *R. tritici*, and *Aspergillus niger*.

Cultures of these fungi were used to inoculate separate portions of sterilized soil that had a water-holding capacity of 40 percent and was adjusted to 50 percent of this capacity. Spores of *Penicillium oxalicum* were applied also to the seed planted in the soil inoculated with this fungus. Seed of feterita, treated with formaldehyde as before, was planted in the different portions of soil, 480 seeds in

TABLE 7.—*Emergence of feterita from seed soaked 30 minutes in a 1:240 formaldehyde solution and planted periodically in inoculated sterilized soil*

[300 seeds planted in each flat]

Fungi	Emergence in—				
	Series 1 (25° C.)			Series 2 (17° C.)	
	Planted	Replanted		Planted	Replanted
	Feb. 19	Apr. 14	May 19 [1]	Feb. 19	May 17
	Percent	*Percent*	*Percent*	*Percent*	*Percent*
None	87	86	75	50	48
Fusarium culmorum	81	66	61	28	39
F. moniliforme	80	75	47	41	33
Penicillium oxalicum	84	79	67	44	44
Rhizopus nigricans	82	80	55	37	43
R. tritici	69	64	43	48	49
Aspergillus niger	82	73	65	32	42

[1] Placed outdoors (after planting); temperature ranged from 17° to 26° C.

each inoculated lot kept at 15° C., and 120 seeds in each inoculated lot kept at 20° and 25°. As controls, formaldehyde-treated seed was planted in sterilized soil and, with or without copper carbonate, also in unsterilized soil, while untreated seed was planted in sterilized soil.

Data were taken periodically over a period of 20 days after emergence, on stand and also on the number of seedlings that became diseased or died during that period. Final results are shown in table 8.

The preemergence virulence shown by *Fusarium culmorum* was outstanding, especially at 15° and 20° C., as was also the postemergence virulence displayed at all three temperatures by *Penicillium oxalicum*. With the exception of *F. culmorum*, the fungi most harmful to emergence seemed to be those present in the unsterilized soil. Copper carbonate was very effective against these at 25° and 20°, but not at 15°

It seems that either seed treatment with formaldehyde was not wholly effective in eliminating the seed-borne fungi or there was some recontamination, because

TABLE 8.—*Emergence and subsequent seedling blight in feterita as influenced by temperature, seed and soil sterilization and inoculation, and seed treatment*

Inoculum added to soil and seed treatment	Treatment of soil	Seeds planted and total plants grown at—						Dead or diseased seedlings occurring at—		
		25° C.		20° C.		15° C.				
		Seeds planted	Total stand	Seeds planted	Total stand	Seeds planted	Total stand	25° C.	20° C.	15° C.
		No.	*Pct.*	*No.*	*Pct.*	*No.*	*Pct.*	*Pct.* [1]	*Pct.* [1]	*Pct.* [1]
Rhizopus tritici	Sterilized	120	67.5	120	66.7	480	52.5	48.1	32.5	12.3
R. nigricans	do	120	81.7	120	90.0	480	68.6	43.0	31.5	14.0
Penicillium oxalicum [2]	do	120	81.7	120	65.0	480	33.5	96.9	98.7	88.6
Aspergillus niger	do	120	87.5	120	86.7	480	76.5	54.3	32.7	14.2
Fusarium culmorum	do	120	46.7	120	11.7	480	0	80.4	85.7	------
F. moniliforme	do	120	90.0	120	82.5	480	52.7	33.3	35.4	15.0
None (seed treated with formaldehyde).	do	40	85.0	80	78.8	250	64.6	23.5	12.7	12.4
None (seed not treated)	do	40	85.0	80	91.3	250	60.0	29.4	27.4	13.3
None (seed treated with formaldehyde).	Unsterilized	80	62.5	40	35.0	133	.8	30.0	14.3	0
None (seed treated with formaldehyde and copper carbonate).	do	80	82.5	40	75.0	133	17.5	24.2	20.7	34.9

[1] Percentage of total stand.
[2] Spores of this fungus were applied also to the seeds.

a considerable percentage of the seedlings from formaldehyde-treated seed planted in sterilized soil became diseased. Fifty seeds from this treated lot, when plated on agar, produced three colonies of *Fusarium*, two of *Penicillium*, and some of species of *Aspergillus* and *Rhizopus*. Diseased plants from treated seed planted in sterilized soil, when plated on agar, yielded mostly *Penicillium* and *Fusarium*.

Sections of diseased plants grown in the different lots of inoculated soil were dipped in 95-percent alcohol, immersed in a 1:1,000 solution of mercuric chloride, washed in sterile water, and plated on plain agar. *Penicillium* was recovered in 6 of the 10 reisolations from the *Penicillium* series, but 4 were sterile. The reisolations from the *Rhizopus* and *Aspergillus* series were less conclusive. *Rhizopus* spp. developed in 19 of the 40 plates from that series; 13 were sterile, and 8 developed other fungi. In 20 plates from the *Aspergillus niger* series, that fungus was recovered in only 9; 7 were sterile, and the rest contained other fungi. Isolations from the *Fusarium* series were mostly species of *Fusarium*. Isolations from plants grown in the unsterilized soil were mostly *Fusarium*, *Penicillium*, *Tricoderma*, *Rhizopus*, sterile mycelium, and occasionally *Pythium* when sterilization was very light.

In a similar temperature series, separate portions of sterilized soil were inoculated with the different fungi as before. One hundred seeds of the same lot of feterita treated in a 1:240 formaldehyde solution for 1 hour were planted for each fungus at each of three temperatures, 15°, 20°, and 25° C. Periodic emergence data were taken until there was no more increase in stand. A final count of plants was taken 7 weeks after planting, and the percentage of dead or diseased plants was computed as shown in table 9.

TABLE 9.—*Effect on emergence and seedling blight in feterita of temperature, seed and soil sterilization and inoculation, and seed treatment*

[100 seeds planted in each lot]

Inoculum added to soil and seed treatment	Treatment of soil	Total emergence and subsequent reduction [1] in stand at—								
		25° C.			20° C.			15° C.		
		Stand on—		Reduction	Stand on—		Reduction	Stand on—		Reduction
		Jan. 23	Mar. 12		Jan. 28	Mar. 12		Feb. 5	Mar. 12	
		Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Rhizopus tritici	Sterilized	85	57	33	82	63	23	64	40	38
R. nigricans	do	93	78	16	87	66	22	78	52	33
Penicillium oxalicum	do	90	19	79	79	16	80	58	15	74
Aspergillus niger	do	92	76	17	88	68	23	67	47	30
Fusarium culmorum	do	62	30	52	32	13	59	0	0	.....
F. moniliforme	do	91	63	31	76	54	29	41	18	56
None (seed treated with formaldehyde).	do	94	84	11	90	82	9	80	62	23
None (seed not treated)	do	88	70	20	92	70	24	71	49	31
None (seed treated with formaldehyde).	Unsterilized	76	70	8	26	20	23	4	2	50
None (seed treated with formaldehyde and copper carbonate).	do	96	88	8	72	66	8	17	3	82

[1] Based on stand on first date in each case.

The results were very similar to those obtained in the preceding experiment except that the percentages of diseased plants were relatively less at 25° and 20° and considerably greater at 15° C.

To test the behavior of different sorghum varieties in soil inoculated with these fungi, formaldehyde-treated seed of eight varieties was planted in flats of soil that had been sterilized and then inoculated separately with various fungi. Two flats planted to each variety were devoted to each fungus, and 50 seeds were planted in each flat. Flats of uninoculated sterilized and uninoculated unsterilized soil were used as checks. The flats were kept in a greenhouse where the temperature ranged from 16° to 21° C. until emergence began and thereafter from 20° to 25° C. Periodic emergence data were taken until no more plants emerged.

Forty days after planting, the number of healthy plants in each pair of flats was recorded, and from these the percentages of diseased or dead plants were computed, as shown in table 10.

TABLE 10.—*Emergence and subsequent disease*[1] *or death of plants grown (Jan. 23 to Mar. 12) from seed of 8 sorghum varieties planted in inoculated sterilized soil in flats in the greenhouse*

Inoculum used and items compared	Evergreen broomcorn	Chiltex	Standard feterita	Hegari	Blackhull kafir	Dawn kafir	Dwarf Yellow milo	Dakota Amber sorgo	Average
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Rhizopus tritici:									
Plants emerged	72.0	84.0	94.0	68.0	90.0	82.0	80.0	88.0	82.0
Plants diseased	33.3	23.8	21.3	20.6	6.7	12.2	12.5	13.6	18.0
R. nigricans:									
Plants emerged	64.0	96.0	82.0	78.0	96.0	82.0	98.0	80.0	85.0
Plants diseased	65.8	6.3	9.8	10.3	2.1	7.3	10.2	0	14.4
Penicillium oxalicum:									
Plants emerged	68.0	96.0	82.0	78.0	84.0	78.0	92.0	84.0	83.0
Plants diseased	52.9	64.6	63.4	51.3	14.3	30.8	78.3	0	44.5
Aspergillus niger:									
Plants emerged	68.0	86.0	82.0	78.0	90.0	84.0	86.0	88.0	83.0
Plants diseased	86.2	32.6	12.2	25.6	35.6	23.8	46.5	36.4	37.6
Fusarium culmorum:									
Plants emerged	12.0	34.0	40.0	32.0	32.0	14.0	34.0	36.0	29.0
Plants diseased	100.0	88.2	90.0	100.0	68.8	100.0	94.1	66.7	88.5
F. moniliforme:									
Plants emerged	64.0	86.0	84.0	70.0	94.0	80.0	76.0	84.0	80.0
Plants diseased	87.5	53.5	21.4	31.4	40.4	70.0	78.9	0	45.4
Average:									
Plants emerged	58.0	80.0	77.0	67.0	81.0	70.0	78.0	77.0	73.5
Plants diseased	71.8	44.8	36.4	39.9	28.0	37.4	53.4	19.5	41.4
None (sterilized soil):									
Plants emerged	76.0	84.0	70.0	80.0	94.0	78.0	80.0	80.0	80.0
Plants diseased	17.6	11.9	5.7	7.5	8.5	2.6	2.5	0	7.0
None (unsterilized soil):									
Plants emerged	68.0	70.0	52.0	70.0	94.0	56.0	80.0	60.0	69.0
Plants diseased	42.1	25.7	26.9	37.1	29.8	39.3	17.5	20.0	29.8

[1] Percentages based on total number of seeds (100) planted in each test.

Fusarium culmorum, in this case, was the only fungus that reduced emergence consistently and apparently significantly. Postemergence injury by the other fungi, however, was pronounced, ranging from an average of 19.5 percent in Dakota Amber sorgo to 71.8 percent in Evergreen broomcorn. From the results obtained in uninoculated sterilized soil, it is apparent that treatment of the seed with formaldehyde was not entirely effective, and that some of the seedling blight may have been due to seed-borne fungi. Sections of roots, stems, and mesocotyls of diseased seedlings and unemerged aborted sprouts, taken from the unsterilized soil, were dipped in 95-percent alcohol, sterilized in 1:1,000 mercuric chloride from one-half minute to 3 minutes, washed in sterile water, and plated on plain agar. About 75 percent of the isolations were species of *Fusarium*, with some species of *Penicillium*, *Rhizopus*, *Aspergillus*, and occasionally also *Pythium* when the sterilization was light. A species of *Pythium* was obtained most frequently from roots, mesocotyls, and aborted sprouts.

Because of the contradictory results occasionally obtained, it is difficult to draw definite conclusions from the foregoing experiments with fungi, some of which, it is thought, may be commonly seed-borne. The uncertainty as to whether the seed (*15*) or the soil used in these experiments had been entirely freed from harmful fungi by sterilization, or whether recontamination had not occurred, made it questionable at times whether the fungi used for inoculum were entirely responsible for the injury observed. Experiments of this type conducted in uncovered soil containers in the greenhouse over a period of weeks do not exclude the possibility of recontamination of soil or seed with fungi carried in the air or in the water applied to the soil. Despite this possibility, the results obtained in inoculated soil compared with results in similar uninoculated soil indicate that, under some conditions, the fungi used as inoculum impaired emergence and caused different types of seedling blight. The apparent resistance of some varieties of sorghum to these types of injury was also demonstrated.

Soil-Borne Organisms

Soil from fields that had been continuously cropped to sorghum for 6 to 23 years was obtained from six experiment stations in Texas, Oklahoma, and Kansas. The soil types, water-holding capacities, number of years the soil had been cropped to sorghum, the locations, and the names of the cooperators who supplied the soil are listed in table 11.

TABLE 11.—*Source, period in sorghum, type, water-holding capacity, and person supplying sample of different lots of soil used in isolation studies*

Source of soil	Period in sorghum	Type	Water-holding capacity	Person supplying sample
	Years		*Percent*	
Lawton, Okla.	20	Fine sandy loam	37	W. M. Osborne.
Woodward, Okla.	20	Sandy loam	22	J. B. Sieglinger.
Chillicothe, Tex.	6	Black clay loam	31	J. C. Stephens.
Hays, Kans.	23	Black silt loam	43	A. F. Swanson.
Garden City, Kans.	14	do	42	F. A. Wagner.
Manhattan, Kans.	7	Black clay loam	41	C. O. Johnston.
Arlington, Va.	0	Keyport silt loam	30	

In a preliminary test, seed of Spur feterita and Dwarf Yellow milo, treated in undiluted Hyclorite for one-half hour, was planted in sterilized and unsterilized soil from each of the above lots and also in Keyport silt loam from a part of the Arlington Experiment Farm on which sorghum had not been grown. The tests were made in a greenhouse where a temperature of about 20° C. was maintained until 5 days after emergence. No attempt was made to adjust all the lots of soil to the same definite percentage of saturation. The data on emergence are shown in table 12.

TABLE 12.—*Emergence of Spur feterita and Dwarf Yellow milo from treated seed planted in sterilized and unsterilized soil obtained from seven sources and kept at 20° C.*

Source of soil	Emergence of—			
	Spur feterita in soil—		Dwarf Yellow milo in soil—	
	Sterilized	Unsterilized	Sterilized	Unsterilized
	Percent	*Percent*	*Percent*	*Percent*
Arlington, Va.	91	1	46	0
Lawton, Okla.	89	25	59	21
Woodward, Okla.	85	13	55	5
Chillicothe, Tex.	94	46	47	11
Hays, Kans.	82	8	32	10
Garden City, Kans.	95	4	67	4
Manhattan, Kans.	84	28	47	8
Average	87	18	50	8

The poorest emergence occurred in the unsterilized Arlington Farm soil, showing that micro-organisms detrimental to germination and stand in sorghum are not restricted to soil in which sorghum has been grown for several years. The surprisingly poor emergence in unsterilized soil compared with that in sterilized soil emphasizes the damage done by soil-borne micro-organisms. However, poor emergence in some cases may have been aggravated by insufficient aeration due to excessive soil moisture.

In a second similar test in unsterilized soils at three different temperatures, with sterilized Arlington Farm soil as a control, the soil moisture was adjusted to about 45 percent of saturation. This was followed by a parallel test in which the soils were about 70 percent saturated. The data on emergence in both series are shown in table 13. No data on subsequent damping-off or wilting were obtained, as the plants were discarded shortly after emergence. There seems to be

TABLE 13.—*Effect of soil moisture and temperature on emergence in Spur feterita from treated seed planted in soils from seven sources*

Source of soil	Total emergence at indicated temperature in soils adjusted [1] to about—					
	45 percent saturation			70 percent saturation		
	15° C.	20° C.	25° C.	15° C.	20° C.	25° C.
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Arlington, Va. (sterilized)	59	75	82	35	55	75
Lawton, Okla.	34	69	95	0	3	72
Woodward, Okla.	10	56	80	0	4	60
Chillicothe, Tex.	40	78	90	30	42	80
Hays, Kans.	16	49	78	0	32	61
Garden City, Kans.	16	36	77	3	14	45
Manhattan, Kans.	49	70	97	0	6	92
Arlington, Va.	8	40	73	0	4	65
Average	30.1	58.0	84.0	8.5	20.0	68.8

[1] The experiment with the wetter soils was carried out several weeks after that with the drier soils.

no consistent or striking relation between the percentage of emergence and the length of time the soil had been cropped to sorghum. On the whole, emergence was consistently best in the black clay loam from Chillicothe and poorest in the silt loam from Arlington. Emergence was consistently poor also in the black silt loam from Garden City. Emergence in the remaining soils showed considerable variation. In every case, even in the sterilized soil, emergence was relatively less, at any given temperature, in soils adjusted to 70 percent saturation than in those adjusted to 45 percent saturation. The percentage reduction was greatest at 15° and least at 25° C.

The soil type, soil moisture, temperature, and soil flora are doubtless interrelated factors affecting emergence independently and in combination with one another. Some fungi probably are more virulent at higher soil moistures, but it seems that soil temperature or soil type or both can alter this relation to some extent.

Further data on the effect of temperature and soil sterilization both on emergence and on seedling blight were obtained from an experiment in which feterita was grown in 2 types of soil and at 6 temperatures. One was a sandy soil from Woodward, Okla., and the other a black prairie soil from Hays, Kans. Both soils had been cropped continuously to sorghum for 20 years or more. A portion of each lot of soil was steamed at 5 pounds' pressure for 1 hour just before planting. This milder sterilization was employed to avoid altering too much the organic material in the soils. Both lots of sterilized soil, along with unsterilized lots of the same soils, were adjusted to about 50 percent saturation. Forty seeds, soaked in 1:240 formaldehyde solution for one-half hour, were planted in each of 6 cans of each lot of sterilized soil and in each of 18 cans of each lot of unsterilized soil. One can of each type of sterilized soil and 3 of each type of unsterilized soil were kept at each of 6 temperatures, 10°, 15°, 20°, 25°, 30°, and 35° C.

Data on emergence or stand taken after all plants had reached the first-leaf stage, along with data taken later on the number of plants subsequently killed, are shown in table 14.

Emergence was much better in the sterilized soil except at 35° C. There was no emergence at 10° in either sterilized or unsterilized soil and none at 15° and 20° in the unsterilized soil. Postemergence damping-off or wilting increased in severity with an increase in temperature. The occurrence of diseased plants in the sterilized soil indicates that either the method of soil sterilization was not entirely effective or that subsequent contamination occurred. The seed also may have carried some fungi despite the seed treatment used.

To isolate fungi possibly responsible for the poor emergence and seedling blight in unsterilized soil, seed of Spur feterita, surface-sterilized for one-half hour in undiluted Hyclorite, was planted in pans of sterilized and unsterilized soil from near Manhattan, Kans., and three pans of each were kept at each of three temperatures, 15°, 20°, and 25° C. Manhattan soil was used first because an ample supply was available. At emergence electric lights were used to promote normal growth. The pans were removed to the greenhouse several days after emergence.

TABLE 14.—*Effect of temperature and soil sterilization on emergence and subsequent seedling blight in feterita grown from formaldehyde-treated seed planted in two types of soil taken from fields continuously cropped to sorghum for 20 years or more*

Source and type of soil	Temperature	Period before emergence	Emergence[1] in soil—		Blighted seedlings in soil—	
			Sterilized	Unsterilized	Sterilized	Unsterilized
	°C.	Days	Percent	Percent	Percent	Percent
Woodward, Okla. (sandy loam)	10		0	0		
	15	16	22.5	0	0	
	20	8	55.0	0	0	
	25	4	65.0	20.8	8.0	12.0
	30	3	87.5	44.2	12.0	17.0
	35	2	50.0	57.5	20.0	27.0
Hays, Kans. (black silt loam)	10		0	0		
	15	16	37.5	0	0	
	20	9	52.5	0	0	
	25	5	60.0	9.2	4.0	0
	30	3	42.5	27.5	0.0	15.0
	35	2	47.5	50.0	5.0	40.0

[1] 40 seeds were planted in each lot of sterilized soil and 120 seeds in each lot of unsterilized soil.

Percentages of emergence at 15°, 20°, and 25° were 48, 81, and 92, respectively, in the sterilized soil; and 22, 65, and 81, respectively, in the unsterilized soil. The ungerminated seeds, unemerged sprouts, and diseased seedlings were then removed from the unsterilized soil for plating. All ungerminated seeds that had started to decompose were discarded. The rest were thoroughly washed in water to remove all soil particles, dipped in alcohol, immersed in Hyclorite for 3 to 15 minutes, and transferred to plain agar containing a trace of potato dextrose. The roots and stems of diseased seedlings received somewhat less severe sterilization before plating.

In most cases, isolations from roots and lower stems produced cultures of species of *Pythium*. In many cases, the fungi may have been killed by the sterilization before plating. Isolations from ungerminated seeds frequently yielded two or more fungi, one of which frequently was a *Pythium*. Next to species of *Pythium*, species of *Fusarium*, *Rhizopus*, *and Trichoderma* were most frequently isolated, along with some of *Penicillium* and *Aspergillus*.

Frequently it was difficult to separate two or more fungi growing out of one seed or portion of a seedling; hence, in many cases, pure cultures were not obtained.

Several additional plantings of Spur feterita, hegari, Blackhull kafir, Dwarf Yellow milo, and Kansas Orange sorgo were made in unsterilized soil from near Manhattan, Kans., and kept at 20° C. until after emergence. Isolations were then made as before. A total of 310 isolates obtained from these plantings were classified as follows:

Pythium (*debaryanum* type)	103	*Mucor*	3
Pythium (other types)	30	*Alternaria*	13
Fusarium	38	*Stachybotrys*	3
Trichoderma	26	*Helminthosporium*	10
Rhizopus	28	Unidentified sclerotial fungi	13
Penicillium	18	Nonfruiting fungi	11
Aspergillus	14		

Similar plantings of Spur feterita seed, treated, as before, in 1:240 formaldehyde solution for one-half hour, were made in soil from the other stations. A temperature of 20° C. was maintained during emergence and for 5 days thereafter. Isolations were then made from seeds that failed to germinate, from unemerged aborted sprouts, and from roots, mesocotyls, and lower stems of apparently diseased seedlings. The results of these isolations are shown in table 15. They are somewhat similar to those obtained from the soil from Manhattan, Kans., except that a larger proportion of the isolations were species of *Fusarium*. This may have been due to slightly more severe sterilization before plating.

The pathogenicity of most of the isolates from the Manhattan soil was tested by inoculating agar slants on which sorghum seedlings had been grown under sterile conditions. Some of the cultures showing evidence of virulence were then used in soil tests.

TABLE 15.—*Isolations from roots, stems*[1]*, and ungerminated seeds*[2] *or aborted seedlings of Spur feterita from treated seed in soils taken from sorghum fields in different localities*

Source of soil	Part isolated from	*Pythium* spp.	*Fusarium* spp.	Mucoraceae[3]	*Penicillium* spp.	*Aspergillus* spp.	Sclerotium-forming fungi	Miscellaneous[4]	Unidentified fungi[5]
		Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
Arlington, Va.	Kernel	15	25	32	18	9	0	7	4
	Stem	0	30	0	6	0	0	0	1
	Root	5	12	1	12	5	5	3	5
Lawton, Okla.	Kernel	12	5	3	2	1	3	1	1
	Stem	1	6	0	0	0	0	0	0
	Root	3	0	0	1	0	2	2	1
Woodward, Okla.	Kernel	11	3	1	0	2	1	0	0
	Stem	2	2	0	0	0	0	0	0
	Root	4	0	0	0	0	0	1	2
Chillicothe, Tex.	Kernel	12	1	0	2	4	2	2	3
	Stem	6	6	0	0	0	0	0	2
	Root	5	1	0	1	2	1	3	5
Hays, Kans.	Kernel	9	4	3	4	5	3	3	5
	Stem	1	5	0	0	0	0	0	1
	Root	4	2	0	2	1	1	1	4
Garden City, Kans.	Kernel	11	9	5	5	3	1	5	6
	Stem	6	3	0	0	0	0	0	3
	Root	5	1	0	2	2	0	3	5
Manhattan, Kans.	Kernel	10	5	6	4	7	2	4	2
	Stem	5	2	0	2	1	0	1	1
	Root	2	2	0	2	3	1	2	4
Total	Kernel	80	52	50	36	31	12	22	21
	Stem	21	54	0	8	1	0	1	8
	Root	28	18	1	20	13	10	15	26
	All parts	129	124	51	64	45	22	38	55

[1] Includes isolations from mesocotyls.
[2] Includes isolations from aborted seedlings.
[3] Mostly *Rhizopus* spp., with some *Mucor* spp.
[4] Among these were species of Perisporiaceae, *Gliocladium*, *Helminthosporium*, *Stachybotrys*, *Trichoderma*, and bacteria.
[5] Mostly nonfruiting mycelia, including probably species of *Rhizoctonia* and *Sclerotium*.

In a preliminary experiment three isolates each of *Fusarium* and *Pythium* and one of *Penicillium*, along with a pure culture of *Fusarium culmorum* from wheat, were increased on steamed corn meal and sand and used for inoculating separate lots of sterilized soil in each of which 100 formaldehyde-treated seeds of Spur feterita were later planted. In one control, only the sterile agar corn meal and sand medium was added to the soil before planting, and in another control nothing was added. After 15 days at 20° C., final data on stand, height, and vigor of plants were taken and are shown in table 16. The *Pythium* isolate No. 2 was the most virulent and entirely inhibited emergence. The two other *Pythium* isolates, although less virulent than No. 2, reduced emergence, rotted the roots, and stunted the plants. *Fusarium* No. 301 was almost equally virulent, whereas *Penicillium* No. 600 was less so. *Fusarium culmorum* showed less virulence than in previous tests. However, it is possible that some of the differences in virulence may have been caused by differences in the amount of inoculum used.

A second soil-inoculation series included 19 isolates of *Pythium*, 16 of *Fusarium*, and 4 of *Penicillium*, from the Manhattan soil, and pure cultures of *F. culmorum* and *Penicillium oxalicum*. The inoculated soil was incubated at 25° to 30° C. for 5 days, after which seed of Spur feterita, previously treated with 50-percent Hyclorite for one-half hour, was planted and the soil kept at 20°. Two weeks later data on emergence were taken and general observations made on growth of tops and roots, as shown in table 17.

Emergence in the three uninoculated checks ranged from 94 to 98 percent and averaged 96 percent. No diseased plants were found in any of them. Emergence was practically inhibited by all of the *Pythium* isolates at 20° C., 1,900 seeds producing only 19 plants. The appearance of the recovered seeds indicated that growth frequently had been checked, shortly after the emergence of the plumule from the seed, by the attack of the fungus near the growing point. *Pythium* was repeatedly reisolated from brown lesions on the plumules.

Only one of the *Fusarium* isolates (No. 301) from the plantings in the Manhattan soil showed any marked effect on emergence and subsequent growth. The percent-

TABLE 16.—*Stand, height, and vigor of plants of Spur feterita grown from formaldehyde-treated seed planted in sterilized soil, different lots of which had been inoculated* [1] *with cultures of fungi isolated from plantings in soil from Manhattan, Kans.*

(100 seeds planted in each lot)

Culture No.	Fungus	Total stand	Average height of plants	Dead plants	General notes when plants were dug up and discarded
		Percent	*Inches*	*Number*	
300	*Fusarium* sp.	71	2–5	1	Almost as good as check.
301	do	44	1–4	8	Small stunted plants; no rootlets.
302	do	82	2–4	0	Fair plants; poor roots.
303	*Fusarium culmorum*	41	1–3	10	Very poor plants; no rootlets; stem lesions.
None (control)	Only sterile medium added to soil.	82	4–5	1	Vigorous plants and roots.
1	*Pythium* sp.	42	2–3	3	Stunted plants; rotted roots.
2	do	0			No emergence; unemerged sprouts brown and dead.
3	do	49	2–4	17	Stunted plants; rotted roots.
600	*Penicillium* sp.	59	3–5	8	Plants fair, poor roots; some lesions.
None (control)	Nothing added to soil.	95	4–6	2	Excellent plants and roots.

[1] Cultures of organisms mixed with the soil 4 days before planting.

TABLE 17.—*Emergence and condition of plants of Spur feterita grown at 20° C. in previously sterilized soil that had been inoculated with various fungi*

Culture No.	Fungus	Emergence	Observations on plants after 2 weeks
		Percent	
None (control)	No inoculation	94	Tops and roots healthy.
1	*Pythium* sp.	8	Tops stunted, roots decayed.
2	do	5	Do.
3	do	0	No emergence.
9	do	0	Do.
11	do	0	Do.
12	do	0	Do.
13	do	0	Do.
17	do	0	Do.
18	do	0	Do.
20	do	1	Small plant with short roots.
21	do	0	No emergence.
25	do	0	Do.
27	do	0	Do.
28	do	2	Tops stunted; poor roots.
29	do	0	No emergence.
30	do	1	Normal plant.
32	do	2	Stunted plants.
33	do	0	No emergence.
36	do	0	Do.
300	*Fusarium* sp.	73	Normal healthy plants.
301	do	64	Some stunting and damping-off.
None (control)	No inoculation	96	Normal healthy plants.
302	*Fusarium* sp.	81	Do.
303 [1]	do	2	Plants damped-off.
304	do	96	Normal healthy plants.
305	do	93	Do.
307	do	94	Do.
309	do	87	Do.
310	do	73	Do.
312	do	94	Do.
314	do	87	Do.
315	do	74	Do.
316	do	71	Do.
317	do	99	Do.
319	do	92	Do.
320	do	93	Do.
321	do	90	Do.
600	*Penicillium* sp.	65	Healthy; some wilted later.
601	do	32	Nearly all wilted later.
602	do	75	Healthy plants.
603	do	78	Do.
604	do	71	Some wilted later.
Control	No inoculation	98	Normal healthy plants.

[1] *Fusarium culmorum* from wheat.

ages of emergence were somewhat depressed by three others, but no damping-off or other disease symptoms followed. The pure culture of *Fusarium culmorum* (No. 303) from wheat again proved to be very virulent.

Penicillium isolates 600 and 604 seemed to depress emergence somewhat, and about 15 percent of the plants wilted later. *Penicillium oxalicum* (No. 601) reduced emergence to 32 percent, and most of the plants wilted subsequently. *Penicillium* isolates 602 and 603 showed no apparent pathogenicity in this test.

To test the effect of temperature on emergence in soil infested with certain isolates and pure cultures, Spur feterita was planted in sterilized and unsterilized soil from Manhattan, Kans., and from Arlington Farm, Va., and in portions of sterilized Arlington Farm soil inoculated with cultures of *Fusarium*, *Pythium*, and *Penicillium* isolated from plantings in Manhattan soil, or with pure cultures of certain species of these same genera. The fungi were grown on agar media and mixed with the soil, which was then incubated for 5 days at 25° C. before seed was planted. The plantings were made in triplicate, the three lots being kept at 15°, 20°, and 25°, respectively. Eight days after planting, emergence data were taken on the lots kept at 25° and 20° and 6 days later on those kept at 15°. The data are shown in table 18.

TABLE 18.—*Effect of soil temperature on emergence of Spur feterita grown from formaldehyde-treated seed in unsterilized soil and in sterilized soil uninoculated or inoculated with various fungi before planting*

Soil—			Emergence at—		
Source	Sterilization	Inoculation	15° C.	20° C.	25° C.
			Percent	*Percent*	*Percent*
Manhattan, Kans.	Unsterilized	None	0	0	70
Do.	Sterilized	do	66	81	85
Arlington, Va.	Unsterilized	do	0	2	37
Do.	Sterilized	do	79	87	91
Do.	do	*Fusarium* sp. No. 301 [1]	68	90	33
Do.	do	*Fusarium* sp. No. 350 [2]	8	72	40
Do.	do	*F. culmorum* No. 303 [3]	0	4	54
Do.	do	*F. culmorum* No. 335 [3]	1	46	48
Do.	do	*F. moniliforme* No. 331 [1]	79	78	83
Do.	do	*Penicillium* sp. No. 600 [1]	64	83	90
Do.	do	*P. oxalicum* No. 601	2	19	90
Do.	do	*Pythium* sp. No. 2 [1]	0	1	11
Do.	do	*Pythium* sp. No. 3 [1]	0	0	4
Do.	do	*Pythium* sp. (Nos. 5 to 15, inclusive) [1]	0	0	27
Do.	do	*P. arrhenomanes* No. 70	0	0	8
Do.	do	*P. debaryanum* Hesse No. 74	0	0	7

[1] Isolated from plants grown in soil from near Manhattan.
[2] Isolated from seed of feterita and later identified as *F. culmorum*.
[3] Isolated from wheat plants.

The organisms present in the unsterilized Arlington Farm soil reduced emergence somewhat more than did those in the Manhattan soil. Of the Fusaria, *Fusarium culmorum* (No. 303) again was the most virulent. The other strain of *F. culmorum* (No. 335) was equal to No. 303 at 15° and 25° but less injurious at 20° C. Results from *Fusarium* sp. (No. 350) were similar to those from *F. culmorum* (No. 335). The strain of *F. moniliforme* (No. 331) was apparently without any harmful effect on emergence at any temperature.

All the cultures of *Pythium*, both the mixtures and the pure strains, inhibited emergence at 15° and 20° C., and reduced it severely even at 25°. *Penicillium* sp. (No. 600) caused some reduction at 15° and none at 20° or 25°. *Penicillium oxalicum* was very severe on emergence at 15° and 20°, but showed no effect at 25°. Postemergence wilt, however, was very severe at this latter temperature.

The lots that had been kept at 20° C. until no more plants emerged were then transferred to a greenhouse where the temperature ranged from about 20° to 26°. In the soil inoculated with *Penicillium oxalicum*, 56 more seedlings emerged after the transfer to the higher temperature, but most of these soon showed typical wilt symptoms; that is, the leaves showed gray silvery streaks and became flaccid and limp. Some emergence also occurred in the *Pythium*-infested soil after the transfer to the higher temperature. After 16 days, further observations were made on stand, and the plants were then removed from the soil by washing. Those grown in sterilized uninfested soil were 5 to 6 inches tall and had a mass

of white fibrous roots. Those in the unsterilized soil and also those in the inoculated sterilized soil, with the exception of those inoculated with cultures No. 331, 350, and 600, were much smaller and had roots that were discolored and limited in extent. The plants that emerged in *Pythium*-infested soil after the transfer to the warmer greenhouse were small and stunted and had very few roots.

Another experiment involved tests of the pathogenicity of a number of fungi isolated from ungerminated seeds or diseased plants removed from Manhattan soil, in comparison with the pathogenicity of pure cultures of some organisms of the same genera from other sources. Separate lots of sterilized Manhattan soil were inoculated with eight pure cultures of species of *Pythium* obtained from the Division of Sugar Plant Investigations, along with two isolates of *Pythium*, two isolates and one pure culture of *Fusarium*, five isolates of *Rhizopus*, five isolates of *Penicillium*, three of *Alternaria*, and one of *Aspergillus*. Uninoculated sterilized and unsterilized soil from Manhattan, Kans., served as controls. The inoculated soil was kept at 25° C. and a relative atmospheric humidity of 90 percent for 5 days. Seed of Spur feterita, treated with formaldehyde as before, was then planted in three separate portions of each of the above lots of soil placed at 15°, 20°, and 25°, respectively, and left there until there was no further emergence. The data on emergence are shown in table 19.

TABLE 19.—*Effect of soil temperature on emergence of Spur feterita from formaldehyde-treated seed in soil from Manhattan, Kans., unsterilized and sterilized and inoculated with various fungi*

Inoculum added to soil	Emergence at—		
	15° C.	20° C.	25° C.
	Percent	*Percent*	*Percent*
None (control in sterilized soil)	74	74	87
None (control in unsterilized soil)	5	21	75
Pythium arrhenomanes No. 70	0	0	42
P. butleri No. 72	7	41	65
P. complens No. 73	29	71	82
P. debaryanum No. 74	0	11	45
P. dissotocum No. 75	13	59	70
P. graminicolum No. 76	17	35	67
P. mamillatum No. 77	0	31	72
P. ultimum No. 78	0	4	49
Pythium sp. No. 2 [1]	2	12	65
Fusarium sp. No. 301 [1]	63	68	64
Fusarium sp. No. 350 [1]	13	65	77
F. culmorum No. 303 [1]	1	62	83
Alternaria sp. No. 200 [1]	66	75	84
Alternaria sp. No. 201 [1]	71	60	71
Alternaria sp. No. 202 [1]	76	87	79
Aspergillus sp. No. 501 [1]	74	78	81
Penicillium oxalicum No. 601	53	71	81
Penicillium sp. No. 600 [1]	74	75	78
Penicillium sp. No. 602 [1]	74	85	73
Penicillium sp. No. 603 [1]	66	69	82
Penicillium sp. No. 604 [1]	69	71	84
Rhizopus sp. No. 1200 [1]	74	78	90
Rhizopus sp. No. 1201 [1]	71	78	84
Rhizopus sp. No. 1202 [1]	76	79	89
Rhizopus sp. No. 1203 [1]	62	62	73
Rhizopus sp. No. 1204 [1]	64	75	83

[1] Isolated from plants grown in soil from Manhattan, Kans.
[2] Isolated from seed of feterita and later identified as *F. culmorum*.
[3] Isolated from wheat plant.

All the species of *Pythium* greatly reduced the percentage of emergence at 15° C., and four of them inhibited it entirely. At 20° the effect was less severe, although *P. ultimum* Trow., *P. arrhenomanes*, and *P. debaryanum* Hesse reduced emergence to 4, 9, and 11 percent, respectively. Even at 25°, with one exception (*P. complens* Q. Fischer), there was an appreciable reduction in emergence in the *Pythium*-infested soil.

Fusarium cultures 303 and 350 were very injurious at 15° C., and culture 301 was slightly so. *Alternaria* culture 200 seemed to be somewhat harmful at 15° and culture 201 was most harmful at 20°. *Aspergillus* culture 501 produced no apparent injury.

None of the *Penicillium* isolates had any pronounced effect on emergence except *P. oxalicum*, which affected it significantly only at 15° C. But many plants

succumbed to wilt at all three temperatures soon after they had emerged in the soil infested with *P. oxalicum* and isolate 604. The two other *Penicillium* isolates seemed to cause but little wilt.

The different *Rhizopus* isolates, possibly with the exception of 1203 and 1204, showed no significant effect on emergence.

The differences in emergence in uninoculated soil that had been sterilized or not sterilized were very pronounced, especially at 15° and 20° C.

After the plants in the *Fusarium*-infested soil and the *Penicillium*-infested soil had been removed, these lots of soil were sieved to remove the ungerminated seeds, care being taken to disinfect hands and implements so as not to spread inoculum from one soil lot to another. The soil was brought to the proper moisture content and 100 seeds of Spur feterita were planted in each lot. The seed planted in each lot of *Penicillium*-infested soil was first dusted with spores of the same fungus originally used for inoculating that lot of soil. Likewise, the seed planted in the soil infested with *Fusarium culmorum* (No. 350) was inoculated with that fungus, whereas the seed planted in the soil infested with *Fusarium* cultures 301 and 350 was not inoculated. Uninoculated sterilized soil was included for comparison. Three temperatures, 25°, 20°, and 15° C., were again used. Data on the percentages of emergence and on the number of plants that subsequently died are shown in table 20.

TABLE 20.—*Effect of soil temperature on emergence and subsequent seedling blight of Spur feterita from seed inoculated with Penicillium spp. and Fusarium spp. in soil infested with the respective fungi*

[100 seeds planted in each lot]

Fungi used for inoculation	Seedlings					
	Emerged at—			Blighted at—		
	15° C.	20° C.	25° C.	15° C.	20° C.	25° C.
	Percent	*Percent*	*Percent*	*Number*	*Number*	*Number*
None (control in sterilized soil)	50	81	85	15	7	5
Penicillium oxalicum No. 631	4	48	84	4	44	75
Penicillium sp. No. 600	1	53	90	0	5	12
Penicillium sp. No. 602	20	74	92	20	30	50
Penicillium sp. No. 603	30	77	96	26	50	62
Penicillium sp. No. 604	17	77	86	10	40	52
Fusarium culmorum No. 303	0	31	78		18	62
Fusarium sp. No. 301 [1]	60	75	72	12	2	30
Fusarium sp. No. 350 [1]	52	59	88	14	24	38

[1] Seed not inoculated.

Inoculating the seed heavily with spores of *Penicillium* spp., or *Fusarium culmorum*, in addition to the inoculum already in the soil, resulted in severe reduction in emergence at 15° C., a considerable reduction in three cases at 20°, and, in the case of *Penicillium*, no apparent effect at 25°. Most of the plants that emerged in the *Penicillium*-infested soil at 15°, along with many at 20° and 25°, later died of wilt. A considerable number damped off in the *Fusarium*-infested soil. Since a number of the plants in the controls also died, it appears that either the soil became somewhat contaminated or the seeds carried some organisms.

Extensive pathogenicity tests of the isolates from soils other than that from Manhattan were not attempted. A few brief tests were made of a representative number of them, mostly *Pythium*, *Fusarium*, *Penicillium*, and *Aspergillus* spp., at 20° C., along with a few sclerotium-forming fungi and other miscellaneous isolates. Most of the *Pythium* isolates greatly reduced emergence at 20°. A number of *Fusarium* cultures caused some reduction in emergence and also some postemergence damping-off. None of the *Penicillium* isolates tested seemed to affect emergence, but three of them caused most of the young seedlings to wilt, while five others were less severe in this respect. The rest seemed to be nonpathogenic in these tests, as also were the *Aspergillus* isolates and the sclerotium-forming fungi. More extensive tests, under a wider range of environmental conditions, might have yielded more positive results. On the whole it seems that, in the soils studied, species of *Pythium*, probably more than any other organisms, were responsible for poor emergence in sorghum, especially at lower temperatures.

Pythium debaryanum seems to be the type most frequently encountered, although other species were present and undoubtedly played an active part in both preemergence and postemergence injury. Both of these types of injury in corn (*5*) and in sorghum (*1*) have been reported to be caused also by *P. arrhenomanes*.

Next to species of *Pythium*, certain species of *Fusarium* probably are most important in preventing emergence and in causing damping-off. Seed treatment was far less effective in soil infested with species of *Pythium* than in soil infested with *Fusarium* spp.

Miscellaneous Experiments

Although *Pythium* spp. and *Fusarium* spp. are generally recognized as being able to parasitize plants, species of *Rhizopus*, *Penicillium*, and *Aspergillus* are usually considered saprophytic. The mechanism by which these fungi cause seedling blight has not been fully determined. Johann et al. (*6*) show that *Penicillium oxalicum*, which "is essentially saprophytic in habit," can cause corn seedlings to die of wilt even in the fifth-leaf stage. It does this by producing oxalic acid, which weakens or kills the host cells in advance of the penetrating mycelium of the fungus. *Aspergillus niger* also is known to produce oxalic acid (*11*), and presumably its action may be somewhat similar to that of *P. oxalicum*. It might be assumed that *Rhizopus* and other nonparasitic organisms also may produce byproducts that break down living cells in advance of the mycelium and thus enable such fungi to attack the plant tissues saprophytically.

To determine whether certain species of *Rhizopus*, *Aspergillus*, and *Penicillium* can retard the growth of young sorghum seedlings, selected seeds of Spur feterita, sterilized for 1½ hours in a 0.5-percent solution of Semesan, were placed on slants of nutrient agar and the seedlings grown aseptically until the beginning of the second-leaf stage. Five separate lots of 10 seedlings each were selected for equal size and vigor. One lot of seedlings was left uninoculated, and the other four were inoculated with *Aspergillus niger*, *Penicillium oxalicum*, *Rhizopus nigricans*, or *R. tritici* by placing spores of the respective fungi on the agar surface at the bases of the seedlings. After 7 days five average seedlings from each lot were removed. Most of the inoculated plants were inferior to the uninoculated controls in size and root development. Their failure to produce abundant rootlets was especially noticeable. The other five seedlings in each group were transplanted to 2-inch pots of sterile sand and were watered with a balanced nutrient solution. After 18 days the five uninoculated plants ranged from 34 to 37 cm. in height and were growing vigorously. Three of the seedlings inoculated with *Rhizopus nigricans* and two from each of the other inoculated lots had died. The rest of the inoculated seedlings ranged from 10 to 28 cm. in height. Those inoculated with *R. nigricans*, *R. tritici*, *A. niger*, and *P. oxalicum* averaged 18, 18, 24, and 18 cm. in height, respectively.

The effect of the byproducts of some of these fungi on the growth of sorghum seedlings was demonstrated by growing seedlings aseptically in tubes of agar in which these fungi had been grown, the cultures having been autoclaved to kill the fungi. Seedlings were grown also as controls on tubes of clean agar. The seedlings were grown in the tubes for 12 days, when they were removed.

The seedlings in the controls ranged from 5 to 10 cm. in height, with an average of 7.3 cm., and their roots were well developed and abundantly branched. Those grown in the tubes containing the remains of the autoclaved cultures were much shorter, the average length of the tops being 4.5, 5.7, and 3.1 cm. for the byproducts of *Rhizopus nigricans*, *R. tritici*, and *Penicillium oxalicum*, respectively. The main roots of these were poorly developed and had relatively few branches.

The agar in the tubes in which *Aspergillus niger* had been grown failed to harden again after autoclaving, and a small amount of fresh sterile agar was added aseptically to each tube to harden the agar sufficiently to permit the growing of sorghum seedlings as in the other tubes. After 2 weeks the seedlings in these tubes were less than 2 cm. in length and were almost without roots, whereas the 10 control seedlings averaged over 10 cm. in length and had abundantly branched roots.

The ability of these fungi to inhibit germination or emergence or to cause aborted seedlings could easily be the result of their attack on and depletion of the food supply in the seed, especially when the seed coat is injured sufficiently to give the fungi ready access to the endosperm (*3*). Under conditions that are more favorable for the growth of the saprophytes than for the germination of the seed and growth of the seedling, the food supply in the endosperm may be

exhausted by the fungi before seed germination has progressed sufficiently to make the young plant independent.

The effect of injury to the seed coat on emergence and subsequent growth was demonstrated by an experiment in which 200 selected seeds of Spur feterita, after having been dipped in alcohol and then sterilized in Hyclorite for one-half hour, were nicked with a flamed scalpel while 200 similarly treated seeds were not mutilated. Four pans of sterilized soil (about 50 percent saturated) were each divided into two sections with a tight metal partition, and 50 of the nicked seeds were planted in one end and 50 sound seeds in the other. In two pans all the seeds were heavily inoculated with spores of *Aspergillus niger*, but in the other two no inoculation was used. One inoculated and one uninoculated pan were kept at each of two temperatures, 20° and 25° C. The data on emergence and the percentage reduction in emergence from the nicked seed, taken over a period of 6 days, are shown in table 21.

TABLE 21.—*Effect of nicking the seed and inoculating it with Aspergillus niger on emergence in Spur feterita germinated at two temperatures*

[50 seeds planted in each lot]

Temperature (°C.)	Period after planting	Emergence from seed—				Reduction due to nicking seed when—	
		Uninoculated and—		Inoculated and—			
		Sound	Nicked	Sound	Nicked	Uninoculated	Inoculated
	Days	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
25	3	94	80	80	40	14.9	50.0
	4	94	86	86	58	8.5	32.6
	6	96	86	90	66	10.4	26.7
	7	96	86	90	72	10.4	20.0
	6	78	46	68	32	41.0	52.9
20	7	84	60	78	42	28.6	46.2
	8	84	70	80	50	16.7	37.5
	9	90	74	78	52	17.8	33.3

Even in the absence of inoculation, a slight injury to the seed caused a reduction in the amount and speed of emergence, in part possibly because of incomplete seed sterilization, but the reduction was much greater when the seed was inoculated. The plants from sound uninoculated and inoculated seed averaged 5 and 4¾ inches in height, respectively, whereas the corresponding figures for plants from nicked seed were 3¾ and 2½ inches.

The inoculated nicked seeds that were recovered from the soil were found covered with mycelium and spores of *Aspergillus niger*. Mycelium was found to be invading the endosperm, which in most cases was discolored and disintegrating. The uninoculated nicked seeds, with few exceptions, were still clean and the endosperms were solid and white.

A similar experiment was carried out on a somewhat larger scale, and included *Aspergillus niger* and six other fungi. Two thousand carefully selected seeds of Spur feterita were soaked in water one-half hour, then in Hyclorite for 1 hour, and dried without being washed. A germination test on moist blotters showed the treated seed to be 88 percent viable. Half of the seeds were then nicked, as before, with a sterilized scalpel.

Sixteen small pans of soil were sterilized and each was divided into two parts, as in the previous experiment. Two of these pans were left uninoculated for controls, and the seed or the soil, or both, for two each of the others were inoculated separately with one of the following seven fungi: *Aspergillus niger*, *A. flavus*, *Fusarium culmorum*, *F. moniliforme*, *Penicillium expansum*, *P. oxalicum*, and *Sclerotium bataticola*. Nicked and sound seeds were planted in each pan on opposite sides of the partition. Uninoculated nicked and sound seeds were planted in uninoculated soil as controls. Two temperatures, 20° and 25° C., were again used. Periodic emergence data were taken until the maximum emergence was recorded. Later a count was made of the dead or diseased plants. The resulting data are shown in table 22.

At 25° C. emergence from inoculated sound seeds was from 4.3 to 47.8 percent less than from similar uninoculated seed, and emergence from inoculated nicked seed

was from 8.3 to 69.4 percent less than from the corresponding nicked controls. At 20° the corresponding percentages were 0 to 85 and 59.5 to 100. *Fusarium moniliforme* and *Penicillium oxalicum* were exceptionally injurious. In every case the percentage of emergence from nicked seeds was considerably less and the percentage of plants that subsequently died of seedling blight was greater than that from the corresponding lot of sound seeds.

TABLE 22.—*Effect of seed-coat injury and inoculation of seed or soil, or both, with cultures of various fungi on emergence and subsequent seedling blight in Spur feterita grown at 20° and 25° C.*

Organism used as inoculum	Emergence from seeds that were—		Reduction in emergence following—			Final stand and blighted seedlings from—			
			Inoculation [1]		Seed injury [2]	Sound seed		Nicked seed	
	Sound	Nicked	Sound seed	Nicked seed		Stand	Diseased [3]	Stand	Diseased [3]
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
GROWN AT 25° C.									
None (control)	92	72	----	----	21.7	80	13.0	50	30.6
Fusarium culmorum	88	64	4.3	11.1	27.3	68	22.7	36	43.8
F. moniliforme	64	40	30.4	44.4	37.5	10	84.4	0	100.0
Sclerotium bataticola	88	60	4.3	16.7	31.8	74	15.9	36	40.0
Aspergillus niger	84	66	8.7	8.3	21.4	64	23.8	18	72.7
A. flavus	78	48	15.2	33.3	38.5	56	28.2	18	62.5
Penicillium oxalicum	48	22	47.8	69.4	54.2	32	33.3	4	81.8
P. expansum	74	48	19.6	33.3	35.1	40	45.9	10	79.2
GROWN AT 20° C.									
None (control)	80	74	----	----	7.5	70	12.5	60	18.9
Fusarium culmorum	54	10	32.5	86.5	81.5	44	18.5	8	20.0
F. moniliforme	12	0	85.0	100.0	100.0	4	66.7	0	----
Sclerotium bataticola	76	30	5.0	59.5	60.5	66	13.2	24	20.0
Aspergillus niger	80	20	7.5	73.0	76.7	68	20.9	10	50.0
A. flavus	80	30	0	59.5	62.5	74	7.5	20	33.3
Penicillium oxalicum	30	0	62.5	100.0	100.0	24	20.0	0	----
P. expansum	70	4	12.5	94.3	94.3	64	8.6	2	50.0

[1] Based on emergence of uninoculated control.
[2] Based on emergence of sound seed as compared with that of nicked seed.
[3] Based on initial emergence in each case compared with final stand.
[4] Increase over control.

In a second similar series, an additional 100 sound and nicked seeds were dusted with copper carbonate before being planted at each temperature in each lot of inoculated soil along with undusted seeds. Data on emergence and on subsequent seedling mortality are shown in table 23. With few exceptions, injuring the seed coat again aggravated preemergence injury by the different fungi, although a few of these reductions in emergence were too small to be considered significant. This, it is thought, can be attributed to the lack of sufficient inoculum in these cases. Dusting the seed with copper carbonate, with one exception, improved emergence, the greatest benefits being to the nicked seeds. It also reduced the percentage of subsequent seedling blight in the inoculated series in 12 of the 14 cases at 25° C. and in 10 of the 13 cases at 20°.

TABLE 23.—*Effect of seed-coat injury, inoculation with various organisms, and dusting the seed with copper carbonate, on emergence and subsequent seedling blight in Spur feterita at 20° and 25° C.*

GROWN AT 25° C.

Organism used as inoculum	Emergence from seed—				Blighted seedlings from seed—			
	Treated		Untreated		Treated		Untreated	
	Sound	Nicked	Sound	Nicked	Sound	Nicked	Sound	Nicked
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
None (control)	94	86	80	83	2.1	4.6	3.4	8.4
Fusarium culmorum	76	70	68	58	15.8	37.2	44.1	42.9
F. moniliforme	78	76	66	28	43.6	57.9	78.8	92.9
Sclerotium bataticola	76	70	68	42	10.8	17.1	20.4	28.6
Aspergillus niger	76	64	74	52	27.0	61.5	28.9	81.3
A. flavus	88	74	82	74	11.4	59.5	24.4	51.4
Penicillium oxalicum	60	60	48	14	26.6	40.7	54.2	71.4
P. expansum	86	84	76	76	11.6	47.6	7.9	42.1
Average	77.1	71.1	68.9	48.9	21.0	46.8	38.2	58.7

GROWN AT 20° C.

None (control)	90	82	76	70	4.4	9.8	15.8	20.0
Fusarium culmorum	74	72	52	40	8.2	52.8	30.8	45.0
F. moniliforme	64	58	28	16	62.6	79.3	100.0	100.0
Sclerotium bataticola	72	64	68	26	19.4	50.0	29.4	38.5
Aspergillus niger	84	60	60	36	19.0	71.4	46.7	80.0
A. flavus	82	72	70	66	14.6	55.6	15.2	62.9
Penicillium oxalicum	46	6	26	0	65.2	100.0	76.9	------
P. expansum	74	64	64	60	19.0	31.3	12.5	71.9
Average	70.0	55.1	52.6	34.0	29.7	62.9	44.5	66.4

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