FUNGUS FLORA OF MUNGBEANS

(Phaseolus aureus Roxb.)

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PREFACE

Grateful acknowledgment is made to Dr. D. A. Preston for identification of the fungi isolated, and for his guidance throughout the entire series of experiments. Appreciation is expressed to Dr. K. Starr Chester for his helpful suggestions during the preparation of the thesis.

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INTRODUCTION

Mungbeans, although classed as a minor crop, are widely grown in Oklahoma for hay, seed, green manure, and as a summer cover crop. In the United States, Oklahoma ranks first in the production of mungbeans. Approximately 55,000 acres were planted to mungbeans in Oklahoma in 1948, the same amount in 1947, and 110,000 acres in 1946 (1).

One of the principal uses of mungbean seed is for sprouting purposes. Many of the commercial mungbean sprouters have been prejudiced against Oklahoma-grown mungbeans, claiming that the sprouts rotted. Until this study was begun, very little experimental work had been done on this problem, yet the survival of the Oklahoma mungbean industry is dependent upon eliminating or reducing the rotting of sprouted mungbeans.

This study of the fungus flora of Oklahoma-grown mungbeans has been undertaken as a contribution toward making Oklahoma-grown mungbeans more acceptable to the sprouting trade, through an investigation of the causes of sprout rotting.

LITERATURE REVIEW

A survey of the literature pertaining to mungbean diseases indicates that very little work has been done in this field. In searching for useful material, a survey was made of all bean diseases reported from the years 1918 to 1948 inclusive. Only a few reports of mungbean diseases have been published, and very few of them refer to fungus diseases.

Welles (9) reported that Cercospora cruenta Sacc. was the cause of a new leaf spot disease of mungbeans in the Philippines in 1925. Coons (2) reported that mungbeans were severely affected with an undetermined stem rot in Chester County, South Carolina. Sideris (7) found mungbeans susceptible to most species of Pythium. A leaf spot of mungbeans caused by Phyllosticta phaseolina Sacc., was reported in New York, and a root rot caused by Phymatotrichum omnivorus (Shear) Dug., was reported in Texas, both for the first time according to Dana (3). Larsh (4) surveyed Oklahoma mungbeans and found Sclerotium bataticola Taub. Preston (5, 6) listed Erysiphe polygoni D.C., Sclerotium rolfsii Sacc., Sclerotium bataticola, and Nematospora coryli Peglion as having been found on mungbeans in Oklahoma.

Tervet (8) mentioned that frost-injured seeds were more likely to be infected by fungi and bacteria than noninjured seeds in his work on soybeans. A situation very similar to that was found in Oklahoma-grown mungbeans.

MATERIALS AND METHODS

All of the experiments reported herein were conducted either in the laboratory or in the greenhouse at Oklahoma Agricultural and Mechanical College, Stillwater, Oklahoma. The varieties and selections of mungbeans used in the various experiments were furnished by Prof. L. L. Ligon of the Department of Agronomy of the same institution. The seeds used included Oklahoma Selection 44, variety Purdue, Oklahoma Selection 12, variety Golden, variety Stritzka, Indian 8257, and Oklahoma 12-4347.

Seed Tests.

Seeds were surface-sterilized and planted in potato dextrose agar in Petri plates. A method was devised whereby exact timing and agitation of

the seeds to secure the maximum effect of the solutions involved, was accomplished. Seeds were placed in a sterilized wire mesh strainer until a single layer of them covered the bottom. Sterilization consisted of soaking the seeds for two minutes in a 1:1000 mercuric chloride solution, followed by six rinses in sterile distilled water. The sterile water was placed in sterile, covered Petri plates just prior to use, and the seeds were transferred from one plate to another and poured from the strainer into the sixth plate. The seeds were then transferred aseptically to Petri plates of potato dextrose agar, and were stored at 30° C.

After two days, counts were made of the number of seeds sprouting, the number not sprouting, and those showing fungi, bacteria, and weevils, Callobruchus maculatus F. Those seeds not sprouting were kept at the same temperature for ten days and examined daily as a check on delayed germination. Usually, mungbeans will sprout within 36 hours after planting in agar.

Soil Tests for Soil-borne Agents.

Seeds were planted in sandy loam and in clay loam obtained locally. Those plants damping off or showing wilt symptoms were placed in sterile Petri plates and carried to the laboratory where sections of the stems were cut just above the soil line, surface-sterilized, and planted on potato dextrose agar in order to determine the agents involved. Isolations were made in triplicate of each fungus found growing from the seeds and stem sections, on the agar. A platinum hook was heated until red, cooled, and used as a means of transferring fungi from the Petri plate to potato dextrose agar slants. All of the fungi isolated were stored in an incubator at 30° C.

Tests of Fungi.

An attempt was made to determine the pathogenicity to mungbeans of representative fungi from each major group isolated. Sand was sterilized by placing it in six-inch flower pots and saturating it with 5% formaldehyde solution. The pots of sand were covered after adding the solution and left covered for a period of 18 to 24 hours. After removing the covers, the pots of sterile sand were allowed to air from two to four days, depending upon the humidity of the air in the greenhouse.

Pure cultures of the fungi to be tested were grown on potato dextrose agar in Petri plates until spores were abundant, at which time the spores and mycelium were scraped from the surface of the agar, and mixed with 15 cc of sterile distilled water in 250 ml. flasks. Seeds to be tested were selected for their healthy appearance and surface-sterilized in 1:1000 mercuric chloride solution. After rinsing in sterile distilled water, they were placed in sterile Petri plates. After two minutes agitation, the spore suspension was poured over the seeds in the Petri plate and allowed to remain for two hours. The seeds were planted in the pots of sterile sand using ten seeds in each pot with three replications for each fungus tested. Controls were prepared under similar conditions, with seeds soaked in sterile distilled water only for two hours prior to planting in the sterile sand. Counts were made on sprouts after 12 days.

EXPERIMENTAL RESULTS

Greenhouse tests were used as a preliminary check on sprouting seeds, and as a means of testing for soil-borne fungi. Tests were made on 125

seeds of Oklahoma Selection 12, using untreated seeds, planted in rows of 25 seeds each, grown in a sandy loam soil. The seeds were planted February 26, 1948, and emergence counts were made on March 4, 1948, at which time 91 had emerged and appeared healthy. The plants were observed at intervals and a final disease study was made on March 16, 1948 at which time 61 of the 91 seedlings were healthy and the other 30 had wilt and disease symptoms. Isolations were made from the diseased plants.

Fungi isolated from the diseased plants grown in the greenhouse included Chaetomium sp., Fusarium sp., Pythium sp., Rhizoctonia sp., and Verticillium alboatrum. Unfortunately, data on the frequency of occurrence of these diseases were not obtained.

One hundred seeds of the Golden variety were planted on April 26, 1948; by May 6, 1948, only 55 had emerged. The seeds used were not treated and had signs of weevil injury. Fourteen of the 55 sprouts were wilted in appearance and isolations from them were made on potato dextrose agar. Upon digging up the unsprouted seeds, several were observed to be infected with nematodes.

Other tests were made using a combination of varieties and selections of Oklahoma Selection 12, Oklahoma Selection 44, Purdue, and Stritzka. Ninety-three seeds were planted in a flat of sandy loam on April 30, 1948, using 33 seeds in two rows for Oklahoma Selection 44, and 20 seeds per row for the others. In this test, a count was made on May 6, 1948. Fifty percent of the Oklahoma Selection 12 seeds sprouted as compared to 39.9% of Oklahoma Selection 44, 60% of Purdue, and 65% of Stritzka. An overall average showed a sprout percentage of 53.7% as presented in Table 2.

Oklahoma Selection 44.

Seeds were planted on potato dextrose agar, using 246 seeds of Oklahoma Selection 44 harvested before frost, with six seeds per replication. Counts were made of the healthy sprouts, the diseased seeds, the fungus-infected seeds, the bacteria-infected seeds, and the weevil-infested seeds. This gave a general comparison of some of the factors interfering with the production of a healthy crop. Of the 246 seeds planted, 79.7% were healthy and 20.3% were diseased, including 6.1% fungi, 2% bacteria, and 12.2% weevils as shown in Table 3.

Oklahoma Selection 44 (frosted).

Tests of 188 seeds which had been harvested after a frost, were made on the basis of number of healthy plants, number of seeds per replication, seeds infected with fungi, seeds infected with bacteria, and seeds showing no signs of infection, yet failing to sprout. Table 4 lists a complete count of each replication. Of 188 seeds tested, 58.5% were healthy, 20.2% showed no growth, 16% had fungus diseases present, and 5.3% had bacterial diseases.

It was observed that 49 of the 188 seeds tested were of a beige to a light brown color due to the frost. The normal bean of this selection is a light green color. The seeds were checked as they appeared in the plates of healthy beans, using the healthy beans as a control. Of these frozen seeds, only 6.1% of the seeds sprouted, 39% had fungi, and 4% had bacteria. The remaining 50.9% had no sign of life and showed no organisms that would prevent their growing. This shows a decided reason for not using frosted seeds as a seed crop or for sprouting purposes.

Variety Purdue Seed Tests.

Tests were organized for Purdue mungbeans in the same manner as those

for Oklahoma Selection 44 with the exception that there were no frozen seeds in the test. A few weevil eggs were noticed on some of the seeds, so a count was made of those seeds not sprouting due to the weevil larvae within the cotyledons and other weevil damage. This information is listed under "weevils" in Table 5. With this variety, 330 seeds were tested. The seeds were surface-sterilized and rinsed in sterile distilled water prior to planting them on the agar plates. Fifty-five replications were used, with 6 seeds per replication. The seeds showed a fungus infestation of 5%, a bacterial infection of 8%, and a weevil infestation of 9.4%, making a total of 20.9% diseased and 79.1% healthy by actual count. Table 5 presents a detailed account of each replication. The overlapping of the number diseased and the total of the three causal agents, namely fungi, bacteria, and weevil, is due to the fact that some seeds were infested with weevil and fungi or weevil and bacteria at the same time.

Oklahoma Selection 12.

This material was based upon tests of 300 seeds using surface-sterilized seeds and planting them June 10, 1948. The emergence counts were made on June 14, 1948 and showed a percentage of 98.7 healthy plants and 1.3 diseased plants, including 0.3 with fungi present, 1.0 with weevil, and 0 with bacteria. The data for this variety are given in Table 6.

Variety Stritzka.

Three hundred seeds were tested on agar plates using 50 replications of 6 seeds each. The healthy plants showed a percentage of 95.7, the diseased 4.3, the fungi totaled 1.3, the bacteria 0.7, and the weevil 2.4. The seeds were surface-sterilized and planted on May 28, 1948, and checks were made May 31, 1948. The isolation and sprouting data for the variety Stritzka are given in Table 7.

Indian 8257.

Of three hundred seeds used, a total of 248 were healthy (82.7%); 52 were diseased (17.3%), 31 had fungi present (10%), 6 had bacteria present (2%), and 18 had weevil infestation (6%). Tests were started June 8, 1948, and the data shown in Table 8 were obtained June 10, 1948.

Oklahoma variety 12-4347.

The seeds were set out June 5, 1948, and counts were made June 7, 1948, as shown in Table 9. The total counts made of the three hundred seeds showed that 286 had sprouted (95.3%); 14 were diseased (4.7%), 1 had a fungus present (0.3%), 2 had bacteria present (0.7%), and 11 had weevil damage (3.7%).

Golden variety.

Fifty-four replications were used with six seeds per Petri plate of agar. The seeds were planted May 21, 1948, and counts were made on May 24, 1948. Of the 324 seeds planted, 284 (87.7%) had a healthy appearance, 40 (12.3%) were diseased, 10 (3.1%) were showing fungi present, 21 (6.5%) had bacteria present, and 8 (2.5%) had weevil damage. An account of each seed is shown in Table 10.

An overall average of the seed tests, excluding the frozen seeds, shows 88.4% healthy plants and 11.58% diseased, of which 3.74% were infected with fungi, 2.84% were infected with bacteria, and 5.3% were infested with weevils. A summary of diseased and healthy seeds is shown in Table 11. Since some of the weevil-infested seeds had fungi and bacteria present, there is a difference of 0.30% in the total of the three and the total diseased.

Seed-borne fungi isolated, according to the frequency of their occurrence, were: Aspergillus niger, other Aspergillus sp., Rhizopus stolonifer,

Alternaria sp., Curvularia sp., Mucor sp., Helminthosporium sp., Penicillium sp., and Fusarium sp.

Tests were arranged to learn the effects of the various fungi isolated upon the mungbean varieties and selections. Three replications of ten seeds each were used for each fungus, using a six-inch pot of sterile sand. Controls consisted of 30 uninoculated seeds planted in three replications. The seeds were selected for their healthy appearance, surface-sterilized, and planted under aseptic conditions. Those fungi tested were grown on potato dextrose agar in Petri plates and after sporulating were scraped into a flask of sterile distilled water where a spore suspension was made by agitation. Another group of seeds of the same variety were selected for their healthy appearance, surface-sterilized, and suspended in the spore suspension for two hours prior to planting. They were set out June 26, 1948, and results were recorded July 8, 1948.

Oklahoma Selection 44.

Tests were made of Alternaria sp., Curvularia sp., Mucor sp., and Rhizopus stolonifer using three replications of 10 seeds for the fungus and the control respectively.

The species of Alternaria tested for its effect on Oklahoma Selection 44 seeds showed little capacity to prevent the seeds from sprouting, as shown by the fact that in the three pots of sterile sand using ten inoculated seeds per pot, 9, 10, and 10 seeds sprouted favorably, averaging 9.7, while the same number of control seeds, soaked in sterile distilled water for two hours averaged 10 healthy sprouts.

Curvularia had no detrimental effect on the seeds inoculated and averaged 10 healthy sprouts both for the inoculated seeds and the control.

Mucor was tested in the same manner and showed no harmful results as an average of 10 was shown for the inoculated seeds and the control.

Rhizopus stolonifer showed a sprout count of 9, 10, 10, averaging 9.7 with the control averaging 10. None of the sprouting failures are significant, inasmuch as the seeds used are subject to internal diseases which could prevent at least one out of 30 seeds from sprouting.

Oklahoma Selection 12.

An olive green species of Aspergillus was used to inoculate the seeds, and when the sprouts were counted ten days later, they showed 10, 9, 8, averaging 9 sprouts for the inoculated, and 10 sprouts for the control.

Indian 8257.

Aspergillus niger was tested on this seed by the spore suspension method with the count of 9, 9, 10, averaging 9.3 for inoculated seeds and a control count of 10, 9, 10, averaging 9.7.

Purdue variety.

A test with Rhizopus stolonifer showed a count of 9, 9, 7, averaging 8.3 for the inoculated seeds and a control count of 10, 5, 10, averaging 8.3.

Chaetomium sp. showed a count of 8, 9, 10, averaging 9 for the inoculated seeds and a control count of 10, 10, 10, averaging 10.

Rhizoctonia solani was tested in three replications; however, one sterile pot had a solid bottom and the formaldehyde solution had not vaporized as it had in the other pots surrounding it. This caused a failure of the seeds to

germinate, which was verified by removing them for inspection. The two pots that had healthy seeds showed a count of 8, 5, averaging 6.5 for the inoculated seed and a control count of 10, 10, averaging 10 for the non-inoculated seed.

Fusarium sp., was subjected to the same tests as Rhizoctonia solani resulting in a count of 10, 8, averaging 9 for the inoculated seed and a control count of 9, 9, averaging 9.

Golden Variety.

Verticillium alboatrum was isolated from a stem section of this variety and it seemed wise to test its pathogenicity using a spore suspension to soak the seeds. Of three replications using 10 seeds per replication, planted June 28, 1948 and examined July 8, 1948, the spore-soaked seeds showed 9, 9, 10, averaging 9.3, as compared to a control count of 10, 10, 10, averaging 10.

A summary of the seed tests using spore suspensions shows that an injurious effect on mungbean germination is exercised by Rhizoctonia solani, Verticillium alboatrum, Aspergillus sp. and Chaetomium sp. Those fungi of doubtful pathogenicity include Aspergillus niger, Alternaria sp., and Rhizopus stolonifer. The harmless fungi include Curvularia sp., Mucor sp., and a seed-borne Fusarium sp. It is understood that other species of Fusarium have shown a decided harmful effect upon seedlings in other tests.

Table 1. Percentage of Diseased Plants of Oklahoma Selection 12

Replication	Number of seeds planted	Number sprouted	Number diseased	Percent diseased
1	25	21	7	28
2	25	13	2	8
3	25	21	8	32
4	25	20	4	16
5	25	16	9	36
Average	25	18.2	6	24

Table 2. Varietal Response in Sprouting Tests of Mungbeans

Variety or selection	Number of seeds planted	Number sprouted	Percent sprouted
Okla. Selection 12	20	10	50
Okla. Selection 44	33	13	39.9
Variety Purdue	20	12	60
Variety Stritzka	20	13	65
Average	23.2	12	53.7

Table 3. Isolation and Sprouting Data of Non-frosted Oklahoma Selection 44

Replication (6 seeds)	Healthy	Diseased	Fungi	Bacteria	Weevils
1	6	0	0	0	0
2	5	1	0	1	0
3	6	0	0	0	0
4	3	3	2	0	1
5	3	3	0	0	3
6	6	0	0	0	0
7	5	1	1	0	0
8	4	2	0	0	0
9	5	1	1	0	0
10	6	0	0	0	0
11	6	0	0	0	0
12	6	0	0	0	0
13	6	0	0	0	0
14	5	1	0	0	1
15	5	1	0	0	1
16	6	0	0	0	0
17	5	1	0	0	1
18	5	1	0	0	1
19	6	0	0	0	0
20	5	1	1	0	0
21	1	5	3	0	2
22	4	2	1	0	1
23	3	3	2	0	1
24	4	2	0	0	2
25	6	0	0	0	0
26	6	0	0	0	0
27	5	1	0	0	1
28	5	1	0	0	1
29	5	1	0	0	1
30	5	1	0	0	1
31	6	0	0	0	0
32	5	1	1	0	0
33	5	1	0	0	1
34	5	1	0	0	1
35	4	2	2	0	0
36	5	1	0	0	1
37	5	1	0	0	1
38	2	4	0	3	1
39	5	1	1	0	0
40	3	3	0	0	3
41	3	3	0	1	2
Total 246 seeds	196	50	15	5	30
Percentage	79.7	20.3	6.1	2	12.2

Table 4. Oklahoma Selection 44, Frosted Seeds

Replication	Number of seeds planted	Healthy	Fungi	Bacteria	No growth; frozen
1	6	3	1	1	1
2	6	3	2	0	1
3	6	4	2	0	0
4	10	6	3	1	0
5	10	4	2	3	1
6	10	7	1	0	2
7	10	7	2	1	0
8	10	3	2	1	4
9	10	7	1	0	2
10	10	5	2	0	3
11	6	2	0	2	2
12	10	4	1	0	5
13	10	4	3	0	3
14	10	4	0	0	6
15	10	9	0	0	1
16	10	6	2	0	2
17	9	7	1	1	0
18	10	7	1	0	2
19	10	10	0	0	0
20	8	5	2	0	1
21	7	3	2	0	2
Total	188	110	30	10	38
Percentage	100	58.5	16.0	5.3	20.2

Table 5. Frequency of Diseases in Variety Purdue

Replication (6 seeds)	Healthy	Diseased	Fungi	Bacteria	Weevils
1	5	1	0	1	0
2	4	2	0	0	1
3	5	1	1	0	0
4	5	1	1	0	0
5	6	0	0	0	0
6	6	0	0	0	0
7	0	6	0	3	3
8	5	1	0	1	0
9	5	1	0	0	1
10	5	1	0	0	1
11	2	4	0	4	0
12	3	3	1	1	1
13	5	1	1	0	0
14	6	0	0	0	0
15	6	0	0	0	0
16	5	1	0	1	0
17	6	0	0	0	0
18	5	1	0	0	1
19	4	2	1	1	0
20	4	2	1	0	1
21	4	2	2	0	0
22	4	2	2	0	2
23	4	2	1	0	2
24	5	1	0	1	0
25	6	0	0	0	0
26	6	0	0	0	0
27	4	2	0	2	0
28	6	0	0	0	0
29	4	2	0	0	2
30	6	0	0	0	0
31	5	1	0	0	1
32	5	1	0	0	1
33	5	1	0	0	1
34	5	1	0	1	0
35	3	3	0	1	2
36	6	0	0	0	0
37	5	1	1	0	1
38	4	2	0	0	2
39	6	0	0	0	0
40	6	0	0	0	0
41	6	0	0	0	0
42	5	1	0	0	1
43	5	1	0	1	0

Table 5. Continued:

Replication (6 seeds)	Healthy	Diseased*	Fungi	Bacteria	Weevils
44	4	2	0	2	0
45	0	6	2	4	0
46	3	3	1	0	2
47	6	0	0	0	0
48	4	2	0	0	2
49	6	0	0	0	0
50	5	1	0	0	1
51	3	3	1	0	2
52	6	0	0	0	0
53	5	1	0	0	1
54	6	0	0	0	0
55	6	0	0	0	0
Total 330 seeds	261	69	17	25	31
Percentage	79.1	20.9	5	8	9.4

*The total number of diseased plants is not always the same as the total number of the seeds diseased by fungi, bacteria, and weevils, since some seeds had both weevils and fungi or bacteria present at the same time.

Table 6. Isolation and Sprouting Data of Oklahoma Selection 12

Replication (6 seeds)	Healthy	Diseased	Fungi	Bacteria	Weevils
1	6	0	0	0	0
2	6	0	0	0	0
3	6	0	0	0	0
4	6	0	0	0	0
5	6	0	0	0	0
6	6	0	0	0	0
7	6	0	0	0	0
8	5	1	0	0	1
9	6	0	0	0	0
10	6	0	0	0	0
11	5	1	1	0	0
12	6	0	0	0	0
13	6	0	0	0	0
14	6	0	0	0	0
15	6	0	0	0	0
16	5	1	0	0	1
17	6	0	0	0	0
18	6	0	0	0	0
19	6	0	0	0	0
20	6	0	0	0	0
21	6	0	0	0	0
22	6	0	0	0	0
23	6	0	0	0	0
24	6	0	0	0	0
25	6	0	0	0	0
26	6	0	0	0	0
27	6	0	0	0	0
28	6	0	0	0	0
29	6	0	0	0	0
30	6	0	0	0	0
31	6	0	0	0	0
32	6	0	0	0	0
33	6	0	0	0	0
34	6	0	0	0	0
35	6	0	0	0	0
36	6	0	0	0	0
37	6	0	0	0	0
38	6	0	0	0	0
39	6	0	0	0	0
40	6	0	0	0	0
41	6	0	0	0	0
42	6	0	0	0	0
43	6	0	0	0	0

Table 6. Continued:

	Replication (6 seeds)	Healthy	Diseased	Fungi	Bacteria	Weevils
	44	6	0	0	0	0
	45	6	0	0	0	0
	46	5	1	0	0	1
	47	6	0	0	0	0
	48	6	0	0	0	0
	49	6	0	0	0	0
	50	6	0	0	0	0
Total	300 seeds	296	4	1	0	3
Percentage		98.7	1.3	0.3	0	1

Table 7. Isolation and Sprouting Data of Variety Stritzka

Replication (6 seeds)	Healthy	Diseased	Fungi	Bacteria	Weevils
1	6	0	0	0	0
2	6	0	0	0	0
3	6	0	0	0	0
4	5	1	1	0	0
5	6	0	0	0	0
6	6	0	0	0	0
7	6	0	0	0	0
8	6	0	0	0	0
9	4	2	0	0	2
10	6	0	0	0	0
11	5	1	0	0	1
12	6	0	0	0	0
13	6	0	0	0	0
14	6	0	0	0	0
15	6	0	0	0	0
16	6	0	0	0	0
17	6	0	0	0	0
18	6	0	0	0	0
19	6	0	0	0	0
20	6	0	0	0	0
21	6	0	0	0	0
22	6	0	0	0	0
23	6	0	0	0	0
24	5	1	0	0	1
25	6	0	0	0	0
26	6	0	0	0	0
27	4	2	1	0	1
28	6	0	0	0	0
29	4	2	1	1	0
30	6	0	0	0	0
31	6	0	0	0	0
32	5	1	1	0	0
33	6	0	0	0	0
34	5	1	0	1	0
35	6	0	0	0	0
36	5	1	0	0	1
37	6	0	0	0	0
38	6	0	0	0	0
39	6	0	0	0	0
40	6	0	0	0	0
41	6	0	0	0	0
42	6	0	0	0	0
43	6	0	0	0	0

Table 7. Continued:

	Replication (6 seeds)	Healthy	Diseased	Fungi	Bacteria	Weevils
	44	6	0	0	0	0
	45	6	0	0	0	0
	46	6	0	0	0	0
	47	5	1	0	0	1
	48	6	0	0	0	0
	49	6	0	0	0	0
	50	6	0	0	0	0
Total	300 seeds	287	13	4	2	7
Percentage		95.7	4.3	1.3	0.7	2.4

Table 8. Isolation and Sprouting Data of Variety Indian 8257

Replication (6 seeds)	Healthy	Diseased	Fungi	Bacteria	Weevils
1	5	1	0	1	0
2	5	1	0	0	1
3	6	1	1	0	0
4	5	1	1	0	0
5	4	2	0	2	0
6	6	0	0	0	0
7	3	3	0	0	3
8	5	1	1	0	0
9	5	1	0	1	0
10	4	2	2	0	0
11	5	1	0	0	1
12	5	1	0	1	0
13	6	0	0	0	0
14	5	1	1	0	0
15	5	1	0	0	1
16	5	1	1	0	0
17	6	0	0	0	0
18	5	1	1	0	0
19	6	0	0	0	0
20	4	2	2	0	0
21	6	0	0	0	0
22	4	2	2	0	0
23	6	0	0	0	0
24	4	2	0	0	0
25	6	0	0	0	0
26	4	2	2	0	0
27	5	1	0	0	1
28	5	1	0	0	1
29	5	1	0	1	0
30	6	0	0	0	0
31	4	2	0	0	2
32	5	1	1	0	0
33	5	1	1	0	0
34	5	1	1	0	0
35	6	0	0	0	0
36	5	1	1	0	0
37	5	1	1	0	0
38	4	2	1	0	1
39	4	2	2	0	1
40	5	1	1	0	0
41	6	0	0	0	0
42	5	1	0	0	1
43	5	1	1	0	0

Table 8. Continued:

	Replication (6 seeds)	Healthy	Diseased	Fungi	Bacteria	Weevils
	44	5	1	1	0	0
	45	5	1	1	0	0
	46	5	1	0	0	1
	47	4	2	2	0	2
	48	6	0	0	0	0
	49	4	2	2	0	0
	50	5	1	1	0	0
Total	300 seeds	248	52	31	6	18
Percentage		82.7	17.3	10	2	6

Table 9. Isolation and Sprouting Data of Variety Oklahoma 12-4347

Replication (6 seeds)	Healthy	Diseased	Fungi	Bacteria	Weevils
1	6	0	0	0	0
2	6	0	0	0	0
3	6	0	0	0	0
4	5	1	0	0	1
5	6	0	0	0	0
6	6	0	0	0	0
7	6	0	0	0	0
8	6	0	0	0	0
9	5	1	0	0	1
10	6	0	0	0	0
11	5	1	0	0	1
12	6	0	0	0	0
13	5	1	0	0	1
14	6	0	0	0	0
15	6	0	0	0	0
16	6	0	0	0	0
17	5	1	1	0	0
18	6	0	0	0	0
19	3	3	0	0	3
20	6	0	0	0	0
21	5	1	0	0	1
22	5	1	0	0	1
23	6	0	0	0	0
24	6	0	0	0	0
25	6	0	0	0	0
26	4	2	0	2	0
27	6	0	0	0	0
28	6	0	0	0	0
29	6	0	0	0	0
30	6	0	0	0	0
31	6	0	0	0	0
32	6	0	0	0	0
33	5	1	0	0	1
34	6	0	0	0	0
35	6	0	0	0	0
36	6	0	0	0	0
37	6	0	0	0	0
38	5	1	0	0	1
39	6	0	0	0	0
40	6	0	0	0	0
41	6	0	0	0	0
42	6	0	0	0	0
43	6	0	0	0	0

Table 9. Continued:

	Replication (6 seeds)	Healthy	Diseased	Fungi	Bacteria	Weevils
	44	6	0	0	0	0
	45	6	0	0	0	0
	46	6	0	0	0	0
	47	6	0	0	0	0
	48	6	0	0	0	0
	49	6	0	0	0	0
	50	6	0	0	0	0
Total	300 seeds	286	14	1	2	11
Percentage		95.3	4.7	0.3	0.7	3.7

Table 10. Isolation and Sprouting Data on Variety Golden

Replication (6 seeds)	Healthy	Diseased	Fungi	Bacteria	Weevils
1	5	1	1	0	0
2	5	1	0	0	1
3	5	1	0	1	0
4	6	0	0	0	0
5	6	0	0	0	0
6	5	1	0	0	1
7	5	1	0	1	0
8	5	1	0	1	0
9	4	2	0	2	0
10	5	1	1	0	0
11	6	0	0	0	0
12	4	2	0	1	1
13	5	1	0	1	0
14	6	0	0	0	0
15	6	0	0	0	0
16	6	0	0	0	0
17	4	2	1	1	0
18	6	0	0	0	0
19	6	0	0	0	0
20	4	2	0	1	1
21	5	1	0	0	1
22	6	0	0	0	0
23	6	0	0	0	0
24	5	1	1	0	0
25	5	1	1	0	0
26	6	0	0	0	0
27	5	1	0	1	0
28	6	0	0	0	0
29	5	1	1	0	0
30	6	0	0	0	0
31	6	0	0	0	0
32	5	1	0	1	0
33	6	0	0	0	0
34	5	1	0	1	0
35	4	2	0	1	1
36	6	0	0	0	0
37	6	0	0	0	0
38	4	2	1	1	0
39	6	0	0	0	0
40	5	1	0	1	0
41	6	0	0	0	0
42	6	0	0	0	0
43	6	0	0	0	0

Table 10. Continued:

	Replication (6 seeds)	Healthy	Diseased	Fungi	Bacteria	Weevils
	44	5	1	0	0	1
	45	5	1	0	1	0
	46	4	2	0	2	0
	47	6	0	0	0	0
	48	6	0	0	0	0
	49	3	3	1	2	0
	50	5	1	0	0	1
	51	4	2	1	1	0
	52	6	0	0	0	0
	53	6	0	0	0	0
	54	4	2	1	0	1
Total	324 seeds	284	40	10	21	9
Percentage		87.7	12.3	3.1	6.5	2.8

Table 11. A Summary of Diseased and Healthy Seeds

Selection or variety	Number of seeds tested	Healthy seeds percentage	Diseased seeds percentage	Fungi percentage	Bacteria percentage	Weevils percentage
Oklahoma Selection 12	300	98.7	1.3	0.3	0	1.0
Stritzka	300	95.7	4.3	1.3	0.7	2.4
Purdue	330	79.1	20.9	5.0	8.0	9.4
Indian 8257	300	82.7	17.3	10.0	2.0	6.0
Oklahoma 12-4347	300	95.3	4.7	0.3	0.7	3.7
Golden	324	87.7	12.3	3.1	6.5	2.8
Oklahoma Selection 44 (non-frosted)	246	79.7	20.3	6.1	2.0	12.2
(frosted)(1)*	188*	58.5*	41.5*	16.0*	5.3*	
Average	300	88.4(2)**	11.58	3.74	2.84	5.36

(1)* Not included in average.

(2)** 0.36 difference due to weeviled seed having fungi and bacteria.

DISCUSSION AND CONCLUSIONS

Greenhouse tests of Oklahoma Selection 12 seeds showed a sprouting average of 72.8% based on five replications of 25 seeds each. This would seem to be a low average for most seeds, and in this case it is even lower since 24% of the 72.8% seeds were diseased and would not have made a healthy planting. Another test was made with Golden variety seeds using 100 seeds and planting them in a clay loam characteristic of most Oklahoma topsoil. The seeds were not treated in any manner to remove organisms, and when checked ten days later only 55 had sprouted and 14 of these were wilted. Examination of the seeds that failed to sprout showed that a few were infested with nematodes. It is probable that wherever these nematodes are present in the soil they will tend to prevent a healthy mungbean crop, depending upon their distribution and the susceptibility of the variety or selection.

A test using a mixture of varieties and selections of untreated seeds in sandy loam indicated a sprouting average of 53.7%. This shows that seeds must be protected from the harmful factors in the soil and on the seeds to produce a good sprouting average.

Seed tests were made of six selections and varieties including Oklahoma Selection 44, variety Purdue, Oklahoma Selection 12, variety Stritzka, Indian 8257, and Oklahoma 12-4347. They were surface-sterilized in a 1:1000 solution of mercuric chloride and rinsed in sterilized distilled water prior to planting. An interesting test was made upon Oklahoma Selection 44. Seed samples were obtained that had been harvested after a frost. Comparisons were made with the sprouting tests of the other seeds tested, and particularly of the same selections, harvested before freezing. The frosted seed showed an increase in the number of fungi and bacteria as well as a 20.2% failure of the seeds to sprout

due to the conditions brought about by the frost. The comparison of the diseases found on Oklahoma Selection 44 and Oklahoma Selection 44 "frosted", showed a percentage difference of 3.7 for Alternaria sp.; 2.1 for Rhizopus stolonifer; 1 for Aspergillus niger, and .7 for other Aspergillus species. An examination of the seeds that had been discolored from the normal light green to a beige to light brown color by freezing, showed a percentage of 6.1 healthy seeds, 39 with fungus infection, 4 with bacterial infection, and 50.9 without viability. This indicates that frosted seeds should not be used for seed. Tervet (9) mentioned that frosted soybean seeds showed more fungi and bacterial infections than non-frozen seed. This is true of mungbeans as well.

Other observations show that in the majority of the seeds tested there was some damage caused by cowpea weevil, Callobruchus maculatus F. It was observed that invariably where the weevil count was high, the other diseases were also more abundant. This may be explained by the fact that weevils lay their eggs on the seeds and the larvae bore a hole in the seedcoat when they hatch. This serves as a direct channel for the entrance of fungi and bacteria. In the seeds tested it was noticed that invariably wherever a weevil larva had entered a seed, there was a small brown stained hole in the cotyledon. During the selection of disease-free seeds for the tests of the harmful fungi, it was observed that where the seeds were obviously diseased the weevils had by-passed them and had laid their eggs on the healthy-appearing seed only. From these observations it may be concluded that the cowpea weevil is one of the major factors to be considered in the selection of disease-free seed. An overall average of the seeds tested in agar plates shows a weevil infestation of 5.3% in 2,100 (non-frosted) seeds tested.

An overall average of the seed tests, excluding those with frozen seeds, shows 88.4% healthy plants, 11.58% diseased, 3.74% infected with fungi, 2.84% infected with bacteria, and 5.3% infested with weevils. This contrasts with the soil tests where the average healthy plants amounted to only 53.7%. The difference may be due to the fact that the seeds used in the agar plates were floated in water prior to surface sterilization and all seed fragments were floated off. The fact that they were surface-sterilized and grown under aseptic conditions indicates that, if a good sprouting average is needed, the conditions under which the seeds are sprouted must be taken into consideration. The use of clean seeds and weevil control will tend to increase the sprout percentage as much as five percent, according to the tests reported here.

Bacteria counts were made of the seeds only when the organism infesting the seed appeared to be the only factor that prevented the seed from growing properly. If the seed showed a zone of bacteria around it, and did not sprout, it was recorded as having a bacterial infection. The mode of entrance of the bacteria into the seed may be due, to some extent, to the activities of the weevils when laying their eggs on the seedcoat.

The agar plate tests of seed of variety Purdue showed more diseased plants than those of seed of the other varieties, and the results of the tests may be understood to accent the fact that where weevil is present, the disease rate will also be higher. Of 20.9% diseased plants, 5% had fungi present, 8% had bacterial infection, and 9.4% were infested with weevil.

Test plates of Oklahoma Selection 12 showed a high rate of healthy plants (98.7%) and a low rate of weevil (1%), and fungi (0.3%), with no bacteria. It must be remembered that the seeds were grown under aseptic conditions and

were surface-sterilized prior to planting. If they had been planted without surface sterilization, there would have been more fungi and bacteria.

Seed of variety Stritzka produced 95.7% healthy plants which indicates a good yield could be expected from the seeds if they were planted in suitable soil after being surface-sterilized. The weevil percentage was 2.4% as compared to 12.2% in Oklahoma Selection 44. The bacterial infection was only 0.7% as compared to 8% infection in variety Purdue, and the fungus infection was 1.3%.

Seed of Indian 8257 produced 82.7% healthy plants, ranking third from the lowest of the varieties and selections tested. The diseased seeds amounted to 17.3% and consisted of 10% with fungi, 2% with bacteria, and 6% with weevils.

Seed of variety Oklahoma 12-4347 produced 95.3% healthy plants and 4.7% diseased seeds, of which only 0.3% had fungi, 0.7% had bacteria, and 3.7% had weevils.

All of the experiments have shown that the cowpea weevil is one of the major causes of mungbean sprout failure. With proper control of this factor, there will be a smaller number of diseased seeds, since the experimental results show that there is an increase in the number of fungi and bacteria, in proportion to the number of weevils present.

SUMMARY

Mungbeans were examined in order to determine their seed-borne fungus flora and its effect on sprouting, at Oklahoma Agricultural and Mechanical College, Stillwater, Oklahoma, using the following varieties and selection: Oklahoma Selection 44, Purdue, Goldeh, Oklahoma Selection 12, Stritzka, Indian 8257, and Oklahoma 12-4347. Of all the seeds examined 88.4% were

healthy, and 11.58% were diseased, including 3.74% with fungi, 2.84% with bacteria, and 5.3% with cowpea weevils, Callobruchus maculatus F.

Seed-borne fungi isolated, according to the frequency of their occurrence, were: Aspergillus niger, other Aspergillus spp., Rhizopus stolonifer, Alternaria sp., Curvularia sp., Mucor sp., Helminthosporium sp., Penicillium sp., and Fusarium sp.

Sprouting tests of Oklahoma Selection 44 seeds, harvested after a frost, developed 58.5% healthy plants, 16% with fungi, 5.3% with bacteria, and 20.2% unable to sprout because of frost damage. In contrast, seeds of the same selection, which were not frosted, produced 79.7% healthy plants, and 20.3% diseased, of which 6.1% had fungi, 2% had bacteria, and 12.2% had weevils. This indicates that under Oklahoma growing conditions, mungbeans should be harvested before frost.

Untreated seeds grown in unsterilized soil developed disease symptoms caused by Fusarium sp., Chaetomium sp., Pythium sp., Rhizoctonia sp., and Verticillium alboatrum.

Preliminary tests of representative species of the major groups of fungi isolated indicated that an injurious effect on mungbean germination is caused by Rhizoctonia solani, Verticillium alboatrum, Aspergillus spp., and Chaetomium sp. Those of doubtful pathogenicity included: Aspergillus niger, Alternaria sp., and Rhizopus stolonifer. The harmless fungi included: Curvularia sp., Mucor sp., and a seed-borne species of Fusarium.

Planting seeds which have been harvested before frost, surface-sterilized, and protected from weevils, will aid in securing a better sprouting crop of mungbeans.

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