FUNGOUS FLORA, DISINFESTATION, AND GERMINATION OF FIELD LEGUME SEED

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By

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Marvin D. Whitehead

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FUNGOUS FLORA, DISINFESTATION, AND GERMINATION OF FIELD LEGUME SEED

In seed there is the potentiality for life. It is this potentiality that, through the ages, man has depended upon for his existence. Today, progressive technicians and farmers, who realize the importance of reasonably certain profitable crop production instead of only potential profit from their crops, are becoming convinced that the quality of the seed they plant is worthy of their most careful attention.

Disease infected seeds result in disease infected plants. Since, in most cases, a diseased plant can not be cured, control must be based upon the prevention of the disease and its spread. Many plant diseases are not readily controlled after they once get well established in the field or greenhouse, but it is often possible to limit their occurrence by measures designed to prevent infection from contaminated seed. It has long been known that fungi are disseminated with seeds of higher plants, either as spores or resting structures attached to or carried on the outside of the seed, sclerotia or infected plant parts mixed with the seed lot or as mycelium within the seed itself.

The author became interested in the organisms contaminating commercial seed lots while observing the effects of fungous flora on germination samples during official seed germination tests for the Federal-State Seed Control Laboratory at Montgomery, Alabama. It is the problem of the seed technologist to determine the planting value of seed. The percentage of germination is one of the main factors concerned

in determining that value. The technologist must determine, from laboratory tests, as accurately as possible the germination percentage that would be obtained if the seed under test were planted in the field. The contaminating organisms sometimes make this determination difficult. Recently many of the official testing laboratories are running germination tests on both disinfested and untreated portions of the seed lots. The commercial development of dust disinfestants for the control of crop diseases and the more extensive use of such materials by farmers not only affords an added opportunity for seed analysts to prescribe control measures, but also makes it possible, in many instances, to determine in the laboratory the probable value of seed disinfestation. Treating the seed of some of the staple crops such as corn and cotton is becoming universal in practice. But the experimental results as to the effect of seed treatment of some of the legumes are not extensive.

LITERATURE REVIEW

The idea that plant parasites are associated with seed dates back at least two hundred years. Orton[28] lists Jethro Tull of Bristol, England, in 1733, as the first recorder of seed transmission of disease in Tull's observation that seed wheat salvaged from the sea water was free from bunt. This record is also one of the first milestones in seed disinfestation, and it led to the use of salt-water steeps which continued in popularity until the nineteenth century.

A century elapsed before the first proof of seed transmission of a parasite, other than smut, was presented. In 1892, Beach[3] demonstrated the first bacterial plant pathogen proved to be seed borne. Since that time, the evidence that plant parasites are commonly carried with the seed has been steadily accumulating.

From a review of the literature, the author has assembled the following lists of organisms that have been found associated with the crops studied. Seymour's[31] Host Index was used as a basis for the literature review, and the completed list was assembled from the first reference of the organisms occurring in the literature. Bacteria listed embody recent changes in nomenclature as listed by Weiss and Wood[45].

CROTALARIA sp. - CROTALARIA

Botrytis cinerea Pers.[26]

Cercospora crotalariae Weber.[26]

Colletotrichum crotalariae Weber.[26]

Corticium vagum B. & C.[26]

Rizoctonia solani Kuhn.[26]

Diaporthe crotalariae Weber.[41]

Macrophomina phaseoli (Maubl.) Ashby.[42]

Sclerotium bataticola Taub.[42]

Sclerotium rolfsii Sacc.[26]

GLYCINE MAX MERR. - SOYBEAN

Alternaria atrans Gibson.[31]

*Alternaria tenuis auct. sensu Wiltshire.[11]

*Aspergillus flavus Link.[38]

*Aspergillus fumigatus Fresenius.[38]

*Aspergillus glaucus Link.[38]

*Aspergillus niger van. Tiegh.[38]

*Aspergillus ochraceus Wilhelm.[38]

Botryodiplodia pallida Ell. & Ev.[31]

Botrytis cinerea Pers.[31]

*Cephalothecium roseum Cda.[38]

Cercospora cruenta Sacc.[31]

*Cercospora daizu Miura.[31]

*Cercosporina kikuchii Mats and Tomo.[18]

*Chaetomium sp.[38]

Corticium vagum B. & C.[31]

Rhizoctonia solani Kuhn.[31]

*Cunninghamella echinulata (Thaxt.) Matr.[38]

Glomerella cingulata (Stoneman) Spaulding & v. Schrenk.[31]

Colletotrichum glycines Hori.[31]

Glomerella glycines (Hori.) Lehman & Wolf.[31]

Diaporthe sojai Lehman.[31]

Erysiphe polygoni D. C.[31]

Fusarium tracheiphilum (Erw. Sm.) Wr.[31]

Fusarium oxysporum f. tracheiphilum (E. F. S.) Snyder and Hanson.[18]

Heterodera marioni (Cornu.) Goodey.[18]

*Macrosporium sp.[28]

Metasphaeria carveri Ell. & Ev.[31]

Mycorrhiza of Legumes F. R. Jones.[31]

*Penicillium spp.[38]

Phymatotrichum omnivorum (Shear) Dug.[31]

Ozonium omnivorum Shear.[31]

*Peronospora manschurica (Naoumoff) Syd. in lit.[31]

*Peronospora sojae Lehman & Wolf.[31]

Peronospora trifoliorum D. By.[31]

Phoma terrestris Hansen.[23]

*Phomopsis sojae Lehman.[31]

Phyllosticta glycineum Tehon & Daniels.[31]

*Pseudomonas glycinea (Coerper) Stapp.[28]

Pythium debaryanum Hesse.[31]

*Rhizopus nigricans Ehr.[38]

*Sclerotinia libertiana Fuckel.[38]

Sclerotinia sclerotiorum (Lib.) Massee.[31]

Sclerotium bat aticola Taub.[17]

Macrophomina phaseoli (Maubl.) Ashby.[17]

Sclerotium rolfsii Sacc.[31]

*Septoria glycines T. Hemmi.[31]

Tielavia basicola (B. & Br.) Zoph.[31]

*Trichoderma viride (Pers.) Fr.[38]

*Xanthomonas phaseoli (E. F. Sm.) Dowson.[28]

*Xanthomonas phaseoli var. sojense (Hedges) Starr and Burkh.[18]

STIZOLOBIUM DEERINGIANUM BORT.-VELVETBEAN

Amerosporium oeconomicum Ell. & Tracy.[31]

Bacterium stizolobii (Wolf.) McCul.[45]

Cercospora mucunae Syd.[31]

Cercospora stizolobii Syd.[31]

Rhizoctonia solani Kuhn.[31]

Corticium vagum B. & C.[31]

Heterodera marioni (Cornu) Goodey.[12]

Heterodera radicicola (Greff.) Mull.[12]

*Phoma lingam (Tode) Desm.[28]

Phyllosticta mucunae Ell. & Tracy.[31]

Phymatotrichum omnivorum (Shear) Duggar.[13]

*Pseudomonas syringae van Hall.[28]

Sclerotium rolfsii Sacc.[31]

*Xanthomonas phaseoli (E. F. Sm.) Dowson[28]

VIGNA SINENSIS (L.) ENDL. - COWPEA

Alternaria atrans Gibson.[31]

Amerosporium oeconomicum Ell. & Tracy.[31]

Aristoma concentrica Tehn.[34]

Aspergillus niger van. Tiegh.[31]

Blakeslea trispora Thax.[31]

Botrytis rileyi Farl.[31]

Cercospora cruenta Sacc.[31]

Cercospora dolichi Ell. & Ev. 1887[31]

Cercospora vignae Ell. & Ev.[31]

Cercospora vignae Racib. 1898.[31]

Cercospora vignicaulis Tehon.[35]

Chaetoseptoria vignae Tehon[35]

Choanephora cucurbitarum (Berk & Tav.) Thax.[24]

Cladosporium vignae Gardner[31]

Colletotrichum gossypii Southw.[31]

Colletotrichum lindemuthianum (Sacc. & Magn.) Briosi. & Cav.[31]

Glomerella lindemuthiana Shear.[31]

Corticium vagum B. & C.[31]

Rhizoctonia solani Kuhn.[31]

Diplodia natalensis Evans.[27]

Erysiphe polygoni D. C.[31]

Fusarium aduncisporum Weimer & Harter.[43]

Fusarium martii, var. phaseoli Burk.[31]

Fusarium vasinfectum Atk.[31]

Fusarium vasinfectum var. tracheiphilum Erw. Sm.[31]

*Fusarium tracheiphilum (Erw. Sm.) Wr.[31]

Gleosporium fructigenum B.[31]

Helminthosporium molle B. and C.[31]

Heterodora marioni (Cornu) Goodey.[17]

Heterodora radicicola (Greef.) Mull.[17]

Leptosphaerulina vagnae Tehon. & Stout.[36]

Macrophoma subconica Ell. & Ev.[31]

Macrosporium leguminum Cke.[31]

Microsphaera alni (D. C.) Wint.[31]

Microsphaera euphorbiae (P. K.) B. & C.[31]

Nectriella tracheiphila Erw. Sm.[31]

Nematospora phaseoli Wingard.[31]

Necosmospora vasinfecta Erw. Sm.[31]

Neocosmospora vasinfecta var. tracheiphila Erw. Sm.[31]

Ozonium omnivorum Shear.[31]

Phymatotri chum omnivorum (Shear) Dug.[31]

Periconia pycnospora Fres.[31]

Phoma bakeriana Sacc.[2]

Phoma lathyrina Sacc.[31]

Phyllosticta phaseolina Sacc.[31]

Pleospora americana Ell. & Ev.[31]

*Pseudomonas glycineum (Coerper) Stapp.[17]

*Pseudomonas syringae van Hall.[45]

Pythium aphanidermatum (Edson.) Fitzpatrick.[31]

Pythium myriotylum Drechsler.[31]

Rhizoctonia dimorpha Matz.[31]

Rhizoctonia microsclerotia Matz.[31]

Macrophomina phaseoli (Maubl.) Ashby.[25]

Sclerotium bataticola Taub.[25]

Thielavia basicola (B. & Br.) Zoph.[31]

Caeomurus phaseoli (Reb.) Arth.[31]

Nigredo appendiculata (Fr.) Arth.[31]

Uromyces phaseoli (Reb.) Wint.[31]

Uromyces appendiculatus Fr.[31]

Negredo vignae (Barcl.) Fromme.[31]

Uromyces vignae Barcl.[31]

Xanthomonas vignicola Burk.[4]

* Reported Seed-Borne

In a review of the literature, no results were found on the effect of seed disinfestants on the germination of crotalaria, cowpeas, or velvetbeans. The results reported on the effect of disinfestants on the emergence of soybeans is meager and conflicting. The North Carolina Agriculture Experiment Station in 1925 and 1926, reported that the application of formalin solution, corrosive sublimate solution, and Bayer dust (Nitrophenolmercury) to two year old mammoth yellow soybean seed caused no appreciable reduction in the amount of bacterial leaf spot and downy mildew on the resulting plants. Formalin greatly reduced the stand of plants, whereas corrosive sublimate and Bayer dust greatly increased the stand as compared with that from untreated seed. It was concluded from these results that formaldehyde should not be used as a disinfesting agent for soybean seeds, and that the gain in germination due to the use of organic mercury disinfestants may be sufficient to make soybean seed treatment profitable entirely apart from any benefit obtained from the control of seed-borne diseases.

In 1929, the Delaware Agricultural Experiment Station reported negative results in the control of foliage diseases that are seed-borne. Heuberger and Manns[15] reported in 1943, that they received, under Delaware conditions, accelerated rate of emergence and increased final stand with treatments from Dow #5 (chloranil), Arasan (50% tetramethyl thiuram-disulfide), Spergon (tetrachloropara benzoquinone), and Ceresan (2% ethyl mercury chloride).

Johnson[18] has described greenhouse tests conducted at Illinois Agricultural Experiment Station in 1940 with Semasan Jr., (1% ethyl mercury phosphate), Cuprocide (cuprous oxide), Barbak C (8% mercury phenyl cyanamide.), and Spergon (tetrachloropara benzoquinone), as disinfestants. Cuprocide failed to increase emergence, but the other disinfestants caused a significant average increase in stand of twelve per cent.

Davy[7] reported in 1942 from the Oklahoma Agricultural Experiment Station that seed treatment of Virginia soybeans with Spergon and New Improved Ceresan (5% ethyl mercury phosphate) was effective in preventing seed rots and pre-emergence damping-off when the seed was sown in soil naturally infested with Rhizoctonia solani.

Tervet[38] in 1944, found that applications of maximum adhesive dosage of Arasan to soybean seed just prior to planting increased the total stand and resulted in a marked decrease in the percentage of stunted plants.

Hildebrand and Koch[16] working in Ontario in 1946, reported that with poor quality seed and the cracked-coat fraction of an otherwise high quality seed, Spergon accelerated and increased emergence of seedlings and increased yield. While the beneficial effect of Spergon was consistent so far as seed of low quality or damaged seed were concerned, such was not the case with regard to seed of high quality.

MATERIALS AND METHODS

The work herein reported was carried out with seeds of crotalaria, soybeans, velvetbeans, and cowpeas. Ten samples of each, except cowpea lots 1, 2, and 3, were official inspector samples obtained from the state and Federal seed testing laboratories. These lots represent seed taken by control officials from seed offered for sale in the respective states. Cowpea lots 1, 2, and 3 were obtained from the Oklahoma Agricultural Experiment Station. Pertinent data relating to these seed lots are shown in Table I.

Isolation experiments were conducted in an effort to determine the fungous flora of the seed lots of soybeans, velvetbeans, and cowpeas. Since the air is constantly laden with fungus spores, an effort was made to clean the air surrounding the working table of floating spores by heavily spraying the air with water from a small hand spray each time before isolations were made. As an added precaution, the work table was covered with a damp cloth while cultures were being made.

Forty-eight seeds to be cultured were selected from a portion of the test samples. Effort was made to obtain all types of possible fungal damage appearing in the sample from small or shriveled seeds to normal size seeds with visible seed coat or germ damage.

Ten samples were tested for each crop treated. The seeds were placed, by means of flamed forceps, in petri dishes of 2% water agar at the rate of six seeds per plate. Eight plates were made of each sample. Four plates were of non-treated seeds and four plates were of surface sterilized seeds.

Table 1. Variety, visible condition, and origin of seed lots tested

Seed Lots	Variety	Visual Observation	Origin				
			Alabama	Federal Beltsville	Florida	Mississippi	Oklahoma
Cowpea							
1	Blackeye	Good condition					x
2	Dixie Queen	15% Weevil Damage					x
3	Purple Hull	Slightly immature, 10% weevil damage					x
30901	Mixed (Brabham-Iron)	Weather + 5% weevil damage	x				
31153	Cream	Good condition, few cracked seed	x				
31218	Blackeye	10% badly discolored	x				
31339	Blackeye	Slight weather damage	x				
31390	Blackeye	3% Badly weather damaged	x				
31543	Blackeye	Slight weather damage	x				
31589	Blackeye	2% weevil and weather damage	x				
Soybean							
4	Mammoth Yellow	75% Purple discolored				x	
12403	Otootan	Slight weather damage	x				
31319	Otootan	Cracked seed coats, old seed	x				
31378	Otootan	Slight weather damage	x				
31602	Otootan	Weather damaged	x				
31609	Otootan	Good condition	x				
31610	Otootan	20% seed coats cracked	x				
31630	Laredo	Slightly immature, 5% cracked	x				
75189	Unknown (Large Yellow)	20% of seed, seed coats wrinkled and blistered		x			
75202	Unknown (Large Yellow)	Cracked seed coats		x			
Velvetbean							
4838	Early Speckled	Good condition			x		
4839	Early Speckled	Weather damaged			x		
7442	Early Speckled	Good condition, small seed			x		
7549	90 Day	Slightly immature, weather damaged			x		
13378	Early Speckled	Weather damage	x				
13422	Early Speckled	Good condition	x				
31396	Early Speckled	Good condition	x				
31397	Early Speckled	Slightly immature, weathered	x				
31552	Early Speckled	Slight immature	x				
39680	Early Speckled	Good condition	x				
Crotalaria							
4722	Crotalaria striata	Good condition			x		
4771	C. spectabilis	Slightly weathered			x		
7266	C. spectabilis	Very immature seed			x		
7468	C. spectabilis	Immature, badly weathered			x		
7471	C. spectabilis	Immature, badly weathered			x		
7573	C. spectabilis	Good condition			x		
7852	C. striata	Good condition			x		
12750	C. spectabilis	Slightly weathered	x				
12990	C. striata	Badly weathered	x				
12991	C. spectabilis	Badly weathered	x				

The seeds were surface sterilized by gently agitating twenty-four seeds, held in a piece of folded cheese cloth, for ten seconds in a 60% ethyl alcohol solution to pre-wet the seed surface, then for two minutes in 50% commercial Clorox (2.625% sodium hypochlorite).

The cultures were observed on the third, fourth, fifth, seventh, ninth, and fourteenth day after plating. Every organism which appeared to be new was isolated and grown in pure culture. In all cases where the fungus made rapid growth, the cultures were made from mycelium growing out from the seed by cutting off a small part of the mycelium at the tip of its growth with a scalpel. When it was not possible to obtain bits of mycelium from the agar surface, fragments of mycelium or bits of spore masses were taken from the fungus on the seed. The cultures were made on tubed potato-dextrose agar except those of the genera Penicillium, Aspergillus, and Fusarium which were plated in petri dishes upon Czapek's agar. Sometimes these three genera were not at first recognized and were cultured in potato-dextrose agar tubes until identifying characters appeared, and then they were transferred to the Czapek's agar plates. The cultures were stored in a sterile germination chamber held at room temperature until the organisms began to fruit and identification could be made.

Germination tests were made on disinfested and nontreated portions of the seed lots by the rag-doll and soil test methods to determine the effect of Arasan, a seed disinfestant, on percent of germination. The Arasan was applied to the seed lots by adding an excess, then thoroughly agitating the treated lot

in a mesh-wire sieve to allow the excess Arasan to be sieved out. A modified rag-doll method was used. The seeds were placed between double thicknesses of moist paper toweling underlain with a heavy sheet of parchment paper. The parchment paper was rolled with the toweling and seeds to prevent excessive loss of moisture. One hundred seeds were used (except velvetbeans, because of their large size, only 50 seeds were used) in each test and four replications of disinfested and four replications of nontreated seeds were made. The seed were exposed to a temperature of 20° C. for 16 hours and 30° C. for 8 hours each day. In the eight day germination period for soybeans and cowpeas and the fourteen day germination period for crotalaria and velvetbeans, the sprouts and dead seeds were removed at such close intervals in order to reduce the spread of fungal myceluim from contaminated seed to sound seed.

The soil tests were made in flats of sterile sandy loam soil. One hundred seeds were used in each test except in the velvetbean test. Two replications of disinfested and two replications of untreated seed were made. In the velvetbean test, 50 seeds were used in each test and four replications of disinfested and four replications of untreated seed were made. Due to the intense heat inside a greenhouse under Oklahoma-June temperature conditions, the test flats were placed under a lattice arbor between two wings of the greenhouse where the temperature conditions were thought to be near optimum and where the test would be shielded from the wind.

Germination counts were made of the cowpeas and soybeans on the fourteenth day and of the velvetbeans and crotalaria, on the twenty-first day after the seeds were planted.

EXPERIMENTAL DATA AND DISCUSSION OF RESULTS

The organism isolated in these tests are listed in Table 2. Of the fifty-two isolates reported, only eighteen have been previously reported as occurring on soybeans, cowpeas, or velvetbeans. There are no experimental data available as to the pathogenisity of most of the organism isolated in this test, but only a very small number of them can be regarded as having no pathogenic effect on plants in either the seedling or later stages. Seedling damping-off may be the combined effect of many organisms working together or in sequence; so careful study must be made before any organism contaminating seed can be eliminated as having no pathogenic significance. Members of the genus Aspergillus are seldom listed as pathogenic but Tervet[38] definitely found that microorganisms, especially fungi belonging to the genus Aspergillus, are important in the loss of viability of soybeans in storage and probably are responsible for much of the heating of soybeans up to temperatures of 40° - 45° C. By soaking seeds in filtered culture media on which species of fungi isolated from soybean seed had been grown, he demonstrated the sharp specificity of three species of Aspergillus (A. flavus, A. niger, and A. ochraceus) in causing abnormal seedling development and that Aspergillus flavus reduced the vigor of the seedlings more than any of the species tested. The author isolated Aspergillus flavus from twenty-four of the thirty seed lots tested, only Rhizopus nigricans surpassed it in frequency of occurrence.

Table 2. Organisms isolated from ten lots each of soybeans, velvetbeans, and cowpeas

Isolated Organisms	Soybean Seed Lots										Velvetbean Seed Lots							Cowpea Seed Lots									
	4	12403	31319	31378	31602	31609	31610	31630	75189	75202	4838	4839	7442	7549	13378	13422	31396	31397	31552	39680	[illegible]	[illegible]	B	30901	B1153	B1218	B1339	B1390	B1543	B1589
Actinomyces sp.			x														x	x											
Alternaria sp.											x																		
*Alternaria tenuis auct. sensu Wiltshire.	x					x	x		x		x	x		x		x						x	x	x		x		x	x
Ascochyta sp.				x			x																			x		x	x
Aspergillus sp.																													
Candidus Group														x															
Glaucus Group														x															
Aspergillus spp.																													
Flavus Oryzae Group	x	x																		x	x	x	x						x
Terreus Group							x																						
Aspergillus carneus (Van Tiegh.) Bloch.							x																						
Aspergillus clavatus Desm.			x					x																	x				
Aspergillus elegans Casp.																													
*Aspergillus flavus Link.			x	x		x		x	x	x	x	x	x	x	x		x	x	x		x	x	x	x	x		x		x
Aspergillus flavipes (Bain.& Sart.) Thom & Church.			x																										
*Aspergillus fumigatus Fresenius.				x						x																			
*Aspergillus niger (Van Tiegh.)		x		x		x		x			x		x					x					x		x				
Aspergillus niveus Bloch.							x																					x		x
*Aspergillus ochraceus Wilhelm.			x																										
Aspergillus sydowi (Bain. & Sart.) Thom & Church.				x																									
Aspergillus tamarii Kita.															x	x													
Aspergillus wentii Wehmer.										x																			
Brachysporium sp.		x																				x		x					
*Cephalothecium roseum Cda.																													
Chaetomium bostrychodes Zoph.									x																				
*Chaetomium sp.				x	x			x	x	x	x		x		x				x							x			
Cladosporium sp.						x																							
*Colletotrichum glycines Hori.	x						x																						x
*Colletotrichum sp.		x									x				x	x													
Curvularia inaequalis Shear.					x																								
Fusarium moniliforme Sheldon.	x	x			x	x		x								x			x				x	x					
Fusarium semitectum Berk. & Rav.		x			x	x	x				x						x	x						x					
Fusarium spp.																													
Discolor Section								x														x		x					
Elegans Section	x	x		x		x	x							x		x			x										
Gibbosum Section		x						x			x	x												x		x			
Hormodendrum sp.																												x	
Mucor sp.		x		x	x	x	x	x			x	x	x		x						x		x				x		x
Penicillium spp.																													
Asymmetrica-Velutina Section			x	x					x						x										x				
Biverticillata-Symmetrica Sect.					x		x				x			x	x	x								x					
Lanata Divaricata Section					x																								
Monoverticillata-Ramigena Sect.			x		x					x																			
Monoverticillata-Stricta Sect.	x					x						x								x		x		x	x			x	
*Phoma sp.		x																											
*Rhizoctonia solani Kuhn.	x																	x						x					
*Rhizopus nigricans Ehr.	x		x	x	x	x	x	x			x	x	x	x	x	x	x	x	x	x	x	x	x		x		x		x	x
*Sclerotium bataticola Taub.											x				x								x		x				
*Sclerotium Rolfsii Sacc.																		x											
Scopulariopsis brevicaule Sacc.		x		x																		x							
Scopulariopsis sp.						x																							
Stemphylium botryosum Wallr.																												x	
*Thielaria sp.		x												x	x														
Trichoderma lignorum (Tode) Harz.				x				x			x	x	x	x	x	x	x	x	x	x			x				x		
Trichoderma sp.													x			x													
Sterile Mycelium	x	x		x	x		x	x			x	x				x	x	x	x	x					x				

*Previously Reported Organisms

Table 2 lists the organisms occurring on each seed lot studied. No record was kept as to the percentage of seed within the seed samples that were contaminated by different isolates. Although the plates of surface sterile seed were, in all cases, relatively free of contamination, in the final analysis very few genera isolated from the untreated plates were not found to be among the list of the ones isolated from the surface sterile seed. This indicates that of the organisms present, most of the contamination is in the form of spores or resting structures attached to the seed coat; but in nearly all cases the organism has the ability to infect the seed.

It was observed that the hilums of all untreated seed of cowpea lots 1, 2, and 3 were covered with various species of Aspergillus and Penicillium. Aspergillus flavus, Rhizopus nigricans and Alternaria tenuis were the most prevalent isolates of the cowpea samples. Ascochyta sp., Mucor spp., and Penicillium spp. of the Monaverticillata section, were present on at least thirty percent of the seed samples. Twenty-one other isolates were listed as appearing on ten to twenty percent of the seed lots treated.

Of the twenty-six organisms reported isolated from velvetbean seed, Trichoderma lignorum was found most frequently, with Rhizopus nigricans second, and Aspergillus flavus third. Alternaria tenuis, Aspergillus niger, Chaetomium spp., Colletotrichum spp., Fusarium semitectum, Fusarium spp. of the Elegans Section, Mucor spp., Penicillium spp. of the Biverticillata section and sterile fungi, were listed as occuring in from thirty to forty percent of the seed lots.

Forty-one organisms were isolated from soybeans. A larger number of organisms were isolated from a high percentage of the seed samples tested than were isolated from cowpeas and velvetbans. Rhizopus nigricans was the most frequent isolate. Alternaria tenuis, Aspergillus flavus, Aspergillus niger, Chaetomium spp., Fusarium moniliforme, Fusarium semitectum, Fusarium Spp. of the Elegans Section, Mucor spp., and sterile fungi were reported isolated from forty to sixty percent of the seed lots.

In a comparison of Table I with Table 3 and 4, it can be observed that there is a high correlation between the visible condition of the seed lots and the percentage of germination obtained. Samples displaying visible damage in most cases proved to be of low germination quality. This indicates that special consideration should be given weather damaged seed in making recommendations for application of seed disinfestants to seed before planting.

The results of seed disinfestant tests expressed in percentages of germination are shown in Tables 3, 4, and 5. The germination value, or total percentage of live seed, of each sample was considered as the percent viable seed (percentage germination plus hard seed). It is shown in Table 5 that in all cases an increase in mean germination percent was obtained from the Arasan treatment over the check test. But a study of Tables 3 and 4 shows that there were no significant advantages obtained from Arasan disinfestation of Crotalaria, soybeans, and cowpeas, but a highly significant advantage was obtained from the treatment of velvetbeans.

Table 3. Effects of seed disinfestants on germination percent in modified rag-doll tests

Seed Lots	Arasan					Check				
	Replications				% Germ.	Replications				
	1	2	3	4	Ave.	1	2	3	4	Ave.
Cowpea										
1	76	83	83	—	81	72	71	73	—	72
2	78	75	74	—	76	81	76	77	—	78
3	91	86	90	88	89	79	90	86	79	83
30901	84	85	90	90	87	86	85	85	84	85
31153	80	92	90	89	88	85	85	86	80	84
31218	85	83	75	—	81	76	79	79	—	78
31339	94	94	93	—	94	87	92	90	—	90
31390	86	91	—	—	88.5	86	86	—	—	86
31543	89	89	94	—	91	88	87	87	—	87
31589	88	90	—	—	89	85	86	—	—	85.5
Soybean										
4	71	74	67	70	70.5	58	62	69	66	64
12403	81	84	85	84	83.5	83	85	82	86	83
31319	50	49	51	50	50	56	53	46	52	52
31378	84	88	87	84	86	84	82	77	77	80
31602	77	91	79	80	82	81	83	77	78	80
31609	90	89	80	85	86	85	91	91	84	88
31610	62	64	55	62	61	67	60	70	64	65
31630	76	84	80	86	81.5	85	85	86	87	86
75189	68	70	61	—	65	62	64	55	—	60
75202	55	59	61	—	58	50	47	44	—	47
Velvetbean										
4838	48	48	46	46	94	46	50	47	46	94
4839	48	42	42	43	87.5	32	40	42	39	76.5
7442	36	37	42	40	77.5	37	35	36	39	73.5
7549	17	20	22	24	41.5*	12	12	16	13	26.5
13378	20	25	23	27	42.5	20	20	20	20	40
13422	46	41	41	45	86.5**	42	38	39	41	80.0
31397	44	47	48	46	92.5*	42	46	44	38	85
31396	48	48	49	49	97*	45	43	42	44	87
31552	48	48	49	48	96.5	45	47	47	48	93.5
39680	49	46	48	50	96.5	48	47	47	47	94.5
Crotalaria										
4771	58	63	70	71	65.5**	56	52	50	50	52
4722	72	78	75	76	75	75	72	80	71	74
7266	79	67	72	64	71	72	64	69	73	69.5
7468	74	69	79	74	74	65	54	71	69	65
7571	59	68	70	72	67	71	57	72	61	65
7573	96	86	90	88	90	90	85	90	86	88
7852	69	65	69	76	70	64	75	67	75	69
12750	97	96	95	98	96	90	92	91	95	92
12990	68	74	70	63	69	72	68	73	82	74
12991	50	57	52	59	54	45	52	42	54	51

* Statistically significant at 1% level. **Statistically significant at 5% level

Table 4. Effects of seed disinfectants on germination percent in soil tests

Seed lots	Arasan			Check		
	Replications			Replications		
Cowpeas	1	2	Ave.	1	2	Ave.
1	71	77	74	80	78	79
2	87	78	82.5	78	77	77.5
3	76	73	74.5	83	75	79
30901	86	86	86	88	80	84
31153	75	66	70.5	73	67	70
31218	79	77	78	62	81	71.5
31339	85	87	86	81	93	87
31390	86	91	88.5	86	86	86
31543	72	88	80	86	76	81
31589	81	85	83	94	79	86.5
Soybean						
4	52	62	57.5	47	54	50.5
12403	72	72	72	73	75	74
31319	36	36	36	40	36	38
31378	70	72	71	72	72	72
31602	79	76	77.5	68	77	72.5
31609	82	82	82	85	80	82.5
31610	59	46	52.5	56	54	55
31630	80	83	81.5	76	86	79.5
75189	65	61	63	60	56	58
75202	57	54	55.5	53	51	52
Velvetbean						
4838	90	88	89	86	85	85.5
4839	82	88	86	82	82	82
7442	77	77	77	78	70	74
7549	40	39	39.5	32	35	33.5
13378	38	34	36	30	34	32
13422	86	84	85	76	80	78
31396	76	72	74	66	66	66
31397	84	90	87	85	73	79
31552	90	88	89	86	87	86.5
39680	98	90	94	92	90	91

Table 5. The effect of Arasan disinfestant on germination percent as shown from the mean germination percent of the ten lots tested of each crop.

Seed Type	Rag-Doll Test			Soil Test		
	Arasan	Check	% Difference	Arasan	Check	% Difference
Soybean	72.35	70.5	1.85	64.85	63.40	1.45
Cowpea	86.45	82.85	3.60	79.55	72.45	7.10
Velvetbean	81.2	75.05	6.15	75.65	70.75	4.90
Crotalaria	73.15	69.95	3.20	Test Discarded		

These results suggest that seed disinfestation of velvetbeans is a desirable practice. Treatment of crotalaria, soybean, and cowpea seed resulted in increases in healthy germination, and while these increases were not great enough to be statistically significant, they suggest that a more extensive study might reveal a significant benefit from disinfestation. In making recommendations as to the value of seed disinfestation to a seed technologist, the added work involved in making his germination report must be considered. The work required is more than doubled for the samples so treated because both treated and untreated lots must be tested for him to be able to give a farmer or seed merchant a true value report. In laboratory germination tests, seed must be placed in such close proximity that mycelium growing out from an infected seedling or from dead seed may spread over noninfected seedlings and viable seed making a true germination count difficult. In this study the author observed a larger amount of fungal growth on the untreated check tests and consequent greater ease in making germination counts on the Arasan-treated tests. This difference was more pronounced in the lots of visibly

damaged seed with subsequent low germination percent. From these tests it can be concluded that the seed technologist would find, aside from the increased value of his reports, that seed disinfestation would be economical on badly weather damaged seed or on lots in which a visual examination revealed probable low germination, while the treatment might be a waste of time on seed appearing to be of high germination quality. One must consider that a comparative test with a disinfestant would determine the probable value of treatment of the seed lot. If a farmer or seed merchant receives a report which shows that the germination of his seed can be increased five to twenty percent by seed treatment, he will be more likely to treat his seed than if only the presence of the disease is reported.

SUMMARY

Ten official state inspection seed lots of each of crotalaria, soybeans, cowpeas and velvetbeans were used for isolation of contaminating organisms and germination tests, by rag-doll and soil methods, with and without seed treatment. From the isolations fifty-two isolates were obtained: forty-one from soybeans, twenty-six from velvetbeans, and twenty-seven from cowpeas. Only eighteen of the organisms isolated had previously been reported as occurring on soybeans, velvetbeans, or cowpeas; the remaining thirty-four isolations represent new records of fungi associated with these crops.

Modified rag-doll and soil germination tests were run on Arasan disinfested and check test portions of the seed lots to determine the effect of Arasan or germination. A final analysis revealed that in all cases an increase of mean germination percent was obtained from the treatment with Arasan; but only with velvetbeans was the increase high enough to be statistically significant. There was a close correlation between the visible condition of the seed lots and the percentage of germination obtained. On the basis of these tests, it is concluded that seed disinfestation of velvetbeans is desirable and that, because of the germination increases from treatment of crotalaria, soybeans, and cowpeas, a more extensive study might reveal a significant benefit from disinfestation. The rag-doll germination tests show that seed treatment would be an

advantage to seed technologists where weather damaged or poor quality seed are concerned; but not with seed rated on visual inspection, to be of high quality.

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