SEED-BORNE DISEASES OF SOYBEAN IN TAIWAN

I. Factors Affecting the Isolation of Causal Organisms[1]

LUNG-CHI WU, WEI-CHEN TIEN, and YI-SHAN LIN[2]

(Received July 4, 1963)

Introduction

Recently, soybean has become an important food and industrial crop in Taiwan while it was cultivated as a soil-improving and a forage crop before World War II. The rapid increase in the acreage of soybean has correspondingly increased the seriousness of disease problem. General survey (Han, 1959; Chu and Chuang-yang, 1961) reveals the fact that seed-borne diseases constitute the major part of soybean diseases in Taiwan. To what extent seed treatment can be a general practice to control certain of the seed-borne diseases on this island seems to be one of the most important problems in connection with the soybean cultivation.

Extensive studies on seed treatment of soybeans have been done in North America. The results obtained from treated seed vary considerably at different locations. Heuberger and Manns (1943) and Hildebrand and Koch (1946, 1947) showed that application of seed treatment chemicals to soybean seed resulted in significant increases in seedling emergence and yield. Hildebrand and Koch (1947) in their literature review cited that Melhus *et al.* (1943) found the yield of soybean remained unaffected though the stand was increased by certain materials as the effect of seed treatment. Sherwin *et al.* (1948) reported that seeds obtained from Georgia, Mississippi, North Carolina, Maryland, and Virginia appeared to be more beneficial than those produced in Illinois by seed treatment. From the observations on six years' seed treatment of soybeans in

(1) The authors are greatly indebted to Professors T. Matsumoto and C. C. Chen of National Taiwan University; Drs. H. W. Li and R. J. Chiu, respectively, Director of the Institute of Botany, Academia Sinica, and Plant Pathologist of Plant Industry Division, Joint Commission on Rural Reconstruction for their encouragement.

Research was supported in part by subsidies from the National Council on Science Development and Joint Commission on Rural Reconstruction.

(2) Respectively, Associate Professor and Research Assistants of National Taiwan University, Taipei, Taiwan, China. The senior author is concurrently the Associate Reseach Fellow of the Institute of Botany, Academia Sinica.

Ontario, Hildebrand and Koch (1950) concluded that only extremely poor-quality seeds resulted in a statistically significant increase in yield which was correlated with an increase in early stand of plants as the profit of seed treatment. For other cases, the effect of seed treatment in modifying yield was that of favorable weather. Chamberlain and and Koehler (1951) and Johnson *et al.* (1954) also recommended the seed treatment for certain districts under limited conditions.

Nevertheless, the use of diseased seed is a means of introducing the disease into new fields and new communities. Lehman (1934) and Sherwin and Kreitlow (1952) found that many of the seedlings from the seeds discolored by the frogeye fungus initiated cotyledonary lesions and developed earlier infection on leaves in the field. In this connection, large number of soybean samples were studied particularly on the microflora by some investigators (Tervet, 1945; Kilpatrick, 1952; Pietkiewicz, 1959; Kurata, 1960). The results obtained in the different parts of the world revealed that many of the species isolated caused pre- and post-emergence seedling diseases. Seed encrusted with oospores of downy mildew fungus was found in 73% of the soybean seeds from 200 locations in 21 states of U.S.A. (Dunleavy, 1959). Seed transmission of tobacco-ringspot virus in soybean was found to be 54–78% (Desjardins *et al.* 1954) though no significant difference in yield occurred until 50% of the seeds was in the mixture (Athow and Laviolette, 1961). Lister (1960) demonstrated the transmission of soil-borne viruses, *i.e.* tomato black ring, raspberry ringspot, and arabis mosaic virus, through seed.

Despite of the fact mentioned above, Kurata (1960) indicated that seed treatment was beneficial to the control of soybean scab, anthracnose, pod and stem blight, purple seed stain, frogeye, downy mildew, charcoal rot, and Fusarium pod-rot in Japan. On survey of the available literature, it seems likely that an attempt to control soybean diseases by seed treatment is meaningful in Taiwan, though an appreciable effect on nodulation can be expected (Sherf and Reddy, 1952; Brinkerhoff *et al.*, 1954). In the present paper factors affecting the isolation of seed-borne pathogen from soybean seeds are studied in order to establish a reliable method for further investigations of this series on the seed-borne diseases of soybean on this island.

Materials and Methods

Seeds of six varieties, *e.g.* Acadian, Dortchsoy, Palmetto, Sankuo, Shihshih, and Wakashima secured by courtesy of the Tainan District Agricultural Improvement Station (TDAIS), were used in the first part of present investigations. Seven varieties of soybean seeds kindly supplied by the Kao-hsiung District Agricultural Improvement Station (KDAIS) were applied in the later part of

experiments. They were Acadian, Black bean, Chiputou, Palmetto, Pearl bean, Shihshih, and Wakashima. Randomized seed lots of the varieties were appropriately treated, transferred aseptically to agar plates, 10 seeds a Petri-dish, and incubated at the temperatures concerned. Five or 10 replicates were made in each treatment. Mercuric chloride and sodium hypochlorite of chemical pure (c. p.) grade were used.

The compositions and pH values of media tested are as follows:

1. Czapek's sucrose nitrate agar (pH 6.9)

Distilled water	1,000.00 ml.
Sodium nitrate	2.00 g.
Potassium dibasic phosphate	1.00 g.
Magnesium sulphate	0.50 g.
Potassium chloride	0.50 g.
Ferrous sulphate	0.01 g.
Sucrose	30.00 g.
Agar	15.00 g.

2. Nutrient dextrose agar (pH 6.2)

Distilled water	1,000.00 ml.
Beef extract	3.00 g.
Peptone	10.00 g.
Dextrose	10.00 g.
Agar	17.00 g.

3. Oat agar (pH 6.4)

Distilled water	1,000.00 ml.
Oatmeal	100.00 g.
Agar	17.00 g.

4. Onion agar (pH 6.4)

Distilled water	1,000.00 ml.
Peeled onions	200.00 g.
Agar	17.00 g.

5. Potato dextrose agar (pH 6.2)

Distilled water	1,000.00 ml.
Peeled and sliced potatoes	200.00 g.
Dextrose	20.00 g.
Agar	17.00 g.

6. Richard's agar (pH 5.4)

Distilled water	1,000.00 ml.
Potassium nitrate	10.00 g.

Potassium monobasic phosphate	5.00 g.
Magnesium sulphate	2.50 g.
Ferric chloride	0.02 g.
Sucrose	35.00 g.
Agar	15.00 g.

7. Soybean agar (pH 6.0)

Distilled water	1,000.00 ml.
Dried soybean	400.00 g.
Agar	20.00

Results

Incubation of seed on the different media.

Seeds of six varieties obtained from TDAIS were throughly mixed by hand and sampled at random. Each lot of soybean seeds was directly rinsed in 10, or 20 changes of sterile water, or rinsed in 5 changes of sterile water after surface-disinfection. For the surface disinfection, seeds were soaked in 0.1 per cent mercuric chloride or 1 per cent sodium hypochlorite for one minute. The number of colonies appeared on the different media was recorded every 24 hours, during the incubation of seed in Petri-dishes of given agar medium at 28°C.

Among the media applied Czapek's sucrose nitrate agar and potato dextrose agar were most suitable for the isolation of bacteria when the number of colonies was compared by using the data obtained from 2-day old cultures. In the case of fungi, use of potato dextrose agar and Richard's agar seemed to be promising when the number of colonies was compared by the data of 3-day old cultures (Table 1.). Most of bacterial colonies were observed in 24 hours. On the other hand, fungous colonies began to show up after 2-day incubation. Hilum seemed to be well furnished for fungi to grow, because turfy appearance of mycelia was observed there at first. Once the growth of fungi was noticed, the isolation of bacteria would be interferred. Furthermore, the rapid growing fungi such as *Rhizopus* spp., covered whole surface of Petri-dish before any other fungi came out.

It was also observed that bacteria and *Aspergillus* spp. were most frequently found, *i.e.* approximately one third of the isolates for each of them. *Cladosporium* and *Penicillium* spp. came to the second whereas the possible pathogenic fungi, *Alternaria* and *Fusarium* spp., were respectively only 1.4 and 0.8 per cent of the isolates. Besides those mentioned above, the fungi isolated from soybean seeds were *Chaetomium, Curvularia, Mucor, Nigrospora, Pestalozzia, Rhizopus,* and *Trichoderma* spp.

Table 1. *Comparison of seven different media in relation to microorganisms associated with soybean seed*

Medium	Treatment*	No. of bacterial** colonies after			No. of fungous** colonies after		
		1-day	2-day	3-day	2-day	3-day	4-day
Czapek's agar	CK	35	43	50	0	23	50
	1	20	42	50	0	24	48
	2	6	45	50	0	15	49
	3	0	0	0	0	1	2
	4	4	13	17	0	4	17
Nutrient dextrose agar	CK	31	44	50	4	39	50
	1	4	34	44	4	27	49
	2	7	29	34	7	15	42
	3	0	0	0	1	3	4
	4	4	6	10	3	5	13
Oat agar	CK	9	20	30	3	20	49
	1	0	7	11	0	19	40
	2	0	2	14	0	19	37
	3	0	0	0	0	0	2
	4	0	1	3	0	3	8
Onion agar	CK	26	50	50	3	29	50
	1	5	28	34	1	20	49
	2	2	20	26	2	21	38
	3	0	0	0	1	5	5
	4	0	10	12	0	2	6
Potato dextrose agar	CK	12	50	50	0	44	50
	1	13	40	50	2	36	50
	2	9	37	49	1	24	50
	3	0	0	0	0	0	0
	4	1	11	11	0	4	10
Richard's agar	CK	0	33	50	15	50	50
	1	0	32	47	7	42	50
	2	0	36	38	3	37	48
	3	0	0	0	0	2	5
	4	0	9	12	0	5	12
Soybean agar	CK	20	33	50	13	45	50
	1	4	19	24	5	24	32
	2	0	7	15	3	13	27
	3	0	0	0	0	1	9
	4	0	3	9	0	3	10

* Seeds, not treated (CK); rinsed in 10 (1) or 20 (2) changes of sterile water; surface-disinfected for 1 minute in 0.1% mercuric chloride solution (3) or 1% sodium hypochlorite solution (4) followed by rinsing in 5 changes of sterile water, were plated and incubated at 28°C.

** Total number of colonies found on 50 seeds of soybean.

It was thought likely that water agar might be suitable for this purpose since soybean seed itself could provide nourishment for the growth of the seed-borne pathogens in infected seeds. As shown in Table 2, however, this was found to be inferior to Czapek's sucrose nitrate agar and potato dextrose agar. When untreated seeds were used, there were 119 isolates of fungi from seeds incubated on potato dextrose agar, 104 from Czapek's sucrose nitrate agar, and 89 from water agar while no much difference was observed in terms of the number of isolates from each medium when seeds were treated with 1 per cent sodium hypochlorite. Similar results were obtained from bacteria.

Table 2. *Comparison of three different media in relation to microflora of soybean seed*

Medium	No. of Bacteria* from seed		No. of Fungi* from seed	
	Untreated	1% NaClO	Untreated	1% NaClO
Water agar	2	2	89	3
Czapek's agar	97	8	104	5
Potato dextrose agar	100	6	119	5

* Total number of bacteria and fungi found on 100 seeds of soybean.

Again, bacteria and *Aspergillus* spp. were shown to be most frequently found. Fungi isolated were *Alternaria, Aspergillus, Chaetomium, Curvularia, Fusarium, Mucor, Penicillium,* and *Rhizopus* spp. Each seed could yield more than one isolate in as much as more than one hundred isolates were yielded by one hundred seeds.

Effect of temperature.

The number of seeds yielding microorganisms seems to be related to the temperatures for the incubation of treated seeds when the comparison are made between 25°C and 35°C. It is possible to obtain more seeds yielding causal organisms at 25°C since the optimum temperatures for the growth of pathogens causing seed-borne diseases of soybean are very close to this temperature (Togashi, 1949; Kurata, 1960). Furthermore, temperatures higher than 25° are in favor of rapid growth of many saprophytes, *e.g. Aspergillus, Rhizopus,* etc. which may interfer the isolation of the causal organisms.

In order to clarify this point, soybean seeds untreated or treated with 1 per cent sodium hypochlorite for 1 minute were plated in the Petri-dish containing 20 ml. of potato dextrose agar and incubated at 25° and 35°C.

The experimental results (Table 3) showed that number of soybean seeds

Table 3. *Effect of temperature on the isolation of microorganisms associated with soybean seed*

Temperature (°C)	Treatment*	No. of seeds yielding							
		Bacteria incubated for				Fungi incubated for			
		1-day	2-day	3-day	4-day	1-day	2-day	3-day	4-day
25	Seed untreated	100	100	100	100	0	0	8	20
	With 1% NaClO	0	8	10	13	0	0	8	10
35	Seed untreated	72	100	100	100	0	29	69	100
	With 1% NaClO	0	19	22	22	0	1	5	5

* One hundered seeds were used for each treatment.

yielding fungi was greatly decreased by incubating untreated seeds at 25°C. Contrarily, more seeds infected by fungi were detected at 25°C than at 35°C when the seed treated with 1 per cent sodium hypochlorite were compared. Practically no *Aspergillus* spp. were found at 25°C. This caused less number of untreated seeds to yield fungi under the experimental condition. On the other hand, the treated seeds yielded less bacteria at 25° than at 35°C though there was no appreciable influence of temperture on the untreated seeds yielding bacteria.

Chemical treatment.

As already shown in Table 1, rinsing in sterile water was not very effective to decrease the number of colonies, mostly saprophytes, even after 20 changes whereas the decrease in the number of colonies upon surface disinfection with chemicals was very signicant. Treatment of seed with 0.1 per cent mercuric chloride completely inhibited the growth of bacteria on seven media tested while few fungous colonies were observed. Yet germination of the seeds was not affected. The sterility of seeds treated with 1 per cent sodium hypochlorite seemed to be low since the number of colonies either bacteria or fungi was still observed for a certain extent.

Since many saprophytes, such as *Aspergillus* and *Penicillium* spp. predominated to the number of isolates which consisted a relatively greater part of pathogenic bacteria and fungi, microorganisms possessed saprophytic nature might hinder the growth of pathogenic bacteria and fungi during the course of studies. Accordingly, to reduce the percentage of seeds which yielded storage fungi, *e. g.* *Aspergillus* spp., of natural contamination seemed to be plausible.

For this purpose, the aqueous solution of sodium hypochlorite were used at various concentrations, *i. e.* 0.5, 1.0, 2.0, and 4.0 per cent. Czapek's sucrose nitrate agar and potato dextrose agar were used in this experiment. According to the experimental results (Table 4), it was possible to reduce the percentage of seeds yielding storage fungi down to 5 per cent or less natural contaminations when the seeds were soaked in one or two per cent sodium hypochlorite for one minute followed by rinsing in 5 changes of sterile water and incubated on both Czapek's sucrose nitrate agar and potato dextrose agar.

At any concentration, number of fungous isolates decreased drastically down to less than 10 per cent of that from untreated seeds except one case which was not exceeding 15 per cent where seeds were treated with 0.5 per cent sodium hypochlorite and incubated on potato dextrose agar. It was also shown that the media for incubation of seeds took part in the soybean seeds yielding a number of microorganisms as already stated in the previous paragraph. It is worthy to mention that some seeds yield bacterial colonies cover-

Table 4. *Effect of sodium hypochlorite concentration on the isolation of fungi associated with soybean seed**

Medium	Czapek's agar					Potato dextrose agar				
Conc. of NaClO(%)	0**	0.5	1.0	2.0	4.0	0**	0.5	1.0	2.0	4.0
Alternaria	—	—	—	—	—	—	—	—	—	1
Aspergillus	65	5	1	1	1	106	8	1	—	1
Chaetomium	—	—	—	—	—	—	—	3	—	—
Curvularia	3	—	—	—	—	—	—	—	—	—
Fusarium	—	1	—	—	—	—	2	—	2	—
Mucor	3	—	—	—	—	2	—	—	—	—
Penicillium	8	1	1	1	—	—	2	—	—	—
Rhizopus	9	—	—	—	—	11	—	—	—	—
Unidentified***	16	—	3	2	1	1	3	1	—	—

* Isolation of fungi in each treatment was made from 100 seeds incubated for 4 days at 28°C.

** Seeds not treated

*** No spore formation was observed when the identification was made.

ing all over the surface of soybean seeds which are entirely different from those with bacteria only growing on the surface of seeds contacted with agar media. The bacteria of the former seems to be derived from the tissue of infected seeds and that of the latter is likely a natural contamination on the surface of the seeds. The number of the former seeds seem to increase when they are surface-treated at concentrations of 0.5 and 1.0 per cent sodium hypochlorite. In this respect, potato dextrose agar seems to be preferable.

In study of the sterility of seeds treated with sodium hypochlorite, soybean seeds were soaked in one per cent aqueous solution of sodium hypochlorite for 1, 2, 4, 8, and 16 minutes previous to rinsing in 5 changes of sterile water and then incubated on potato dextrose agar at 25°C.

The experimental results (Table 5) showed that number of soybean seeds yielding microorganisms was not entirely correlated to the length of time for chemical treatment. However, chemical injury was observed when the soybean seeds were treated for longer than 8 minutes in one per cent sodium hypochlorite solution, *e.g.* the color of the seed-coat was impaired. Seeds so appeared were found to be 27 and 66 out of 100 seeds soaked 8 and 16 minutes, respectively. With respect to the phytotoxic effect of sodium hypochlorite, the percentage germination of treated seeds was also slightly affected by the chemical with a lapse of time when the seeds were treated longer than 2 or 4 minutes.

Further investigation concering disinfection of soybean seeds was carried out with mercuric chloride. In the previous experiment, chemical treatment

Table 5. *Effect of time in treatment with sodium hypochlorite on the isolation of microorganisms associated with soybean seed*

Length of time treated (min.)	No. of seeds yielding*							
	Bacteria incubated for				Fungi incubated for			
	1-day	2-day	3-day	4-day	1-day	2-day	3-day	4-day
0**	100	100	100	100	0	0	8	20
1	0	8	10	13	0	0	8	10
2	0	7	9	10	0	0	4	10
4	0	0	1	3	0	0	4	9
8	0	3	6	6	0	0	10	12
16	0	2	2	3	0	0	7	10

* Isolation of microorganisms was made from 100 seeds incubated at 25°C.
** Seeds not treated.

was encountered to obtain the greatest number of seed-borne pathogen, however, the complete disinfection of soybean seed was desired in the present experiment to grow healthy plants for the inoculation purposes.

Under the experimental conditions, disinfection of soybean seeds seemed to be possible by soaking in a 0.1 per cent mercuric chloride solution even for 1 minute though there were one or two seeds yielding *Aspergillus* spp. which were known to be saprophytic fungi (Table 6). Seeds were treated with a 0.1 per cent mercuric chloride for 1, 2, 4, 8, and 16 minutes followed by rinsing in 5 changes of sterile water. The treated seeds were incubated on potato dextrose agar for observations of sterility and germinability of soybean seeds. In order to provide favorable conditions for the growth of pathogens causing

Table 6. *Percentage of seed germination, number of seeds yielding fungi, and treatment of soybean seeds with mercuric chloride**

Variety	No. of seeds yielding		Percentage of germination	Length of** seedling (cm)
	Bacteria	Fungi		
Acdian	0	0	100	5.2
Black bean	0	0	100	4.9
Chiputou	0	0	100	5.7
Palmetto	0	2	100	5.3
Pearl bean	0	0	96	2.6
Shihshih	0	0	100	4.8
Wakashima	0	1	100	4.2

* Seeds treated with 0.1 per cent mercuric chloride for 1 minute were incubated on potato dextrose agar for 4-day at 25°C.
** Length of seedling is an average of 100 seedlings.

seed-borne diseases of soybean and also the germination of soybean seeds, 25°C. was selected for the incubation of seed.

Germinability of soybean seeds was considerably impaired when the seeds of varieties, Pearl bean, Palmetto, and Shihshih were soaked in a 0.1 per cent mercuric chloride solution for 16 minutes. Namely, 7, 67, and 40 per cent germination were respectively recorded 4 days after incubation. The discoloration of seed-coat was also observed when the seeds of varieties, Acadian, Palmetto, Shihshih, and Wakashima, were treated in the same concentration for longer than 4, 1, 2, and 4 minutes, respectively.

With regard to the growth of soybean on potato dextrose agar, the seeds treated with 0.1 per cent mercuric chloride solution for 1 minute were the best among the seeds treated when the comparison was made with average length of seedlings after 4-day incubation. However, the length of seedlings was gradually shortened with a lapse of time when the seeds were treated longer than 2 minutes. It was significant that the growth of seedlings in control dishes was very much damaged by the saprophytic microorganisms associated with the seeds not treated with mercuric chloride.

Discussion

Seed treatment for the control of soybean was suggested elsewhere (Heuberger and Manns, 1943; Hildebrand and Koch, 1946, 1947; Kurata, 1960) though the controversy on the question of soybean seed treatment as a general practice was still remained (Hildebrand and Koch, 1950; Chamberlain and Koehler, 1951; Johnson *et al.*, 1954). Soybean diseases caused by seed-borne pathogens were found to be significant (Han, 1959; Chu and Chuang-yang, 1961). An attempt to clarify the benefit of seed treatment as a control measure seems to to be important in this particular area where the weather is tropical and subtropical. For this purpose, it is necessary to determine a method for isolation of possible pathogens from soybean seeds before proceeding the process of investigation on the seed-borne disease.

Potato dextrose agar was selected for the isolation purpose, since this medium revealed rapid growth of microorganisms and also yielded considerably large number of microorganisms isolated under the experimental conditions. However, Czapek's sucrose nitrate agar and Richard's agar were also respectively suitable to obtain a number of bacteria and fungi from soybean seeds.

The temperature was closely related to the number of seeds yielding microorganisms when the comparison was made between 25°C and 35°C. The microflora of soybean seeds seemed to be affected not only quantitatively. It was found to be useful to incubate treated seeds at 25°C for 4 days before any isolation of pathogens was attempted.

The growth of seedlings in control dishes was seriously damaged by the saprophytic microorganisms associated with the seeds not treated with chemical though the germination of seeds was remained unaffected. On the other hand, treatment of seeds with 0.1 per cent mercuric chloride completely impaired the growth of bacteria on the tested media while few fungi saprophytic in nature were observed. However, seeds treated with 1 per cent sodium hypochlorite were considered to be appropriate for the isolation of pathogenic bacteria and fungi since it was possible to reduce the percentage of seeds yielding storage fungi down to 5 per cent or less natural contaminations when the seeds were treated in one or two per cent sodium hypochlorite for one minute followed by rinsing in 5 changes of sterile water.

Summary

On account of investigations into the seed-borne diseases of soybean, methods for isolation of causal organisms from soybean seeds were considered in the first place. Studies on the rinsing of seeds in sterile water, selection of media, application of chemical with regard to concentration and length of time applied, and temperature for incubation were extensively carried out. Rinsing in sterile water was not effective to decrease the number of colonies appeared on the seeds even after 20 changes whereas disinfection with chemical solutions was very significant. Treatment of seeds with 0.1 per cent mercuric chloride completely inhibited the growth of bacteria on the tested media while few fungous colonies were observed. However, seeds treated with 1 per cent sodium hypochlorite were proved to be suitable for the isolation of pathogenic fungi and bacteria causing seed-borne diseases of soybean. Potato dextrose agar was found to be the best among the media tested and temperature for incubation was suggested to be 25°C.

臺灣黃豆種子傳染性病害

I. 影響黃豆種子病原菌分離之因子

吳龍溪 田蔚城 林逸生

研究黃豆種子傳染性病害，首先須由臺灣現有播種用之種子分離其病原菌。惟分離病原菌時，屢有各種因子影響病原菌在黃豆種子表面之出現。故若採用適合病原菌生長之培養基，在適當生長溫度培養時，可增高病原菌出現之機會。又藉藥劑之處理可除却種子表面之雜菌，促使種子組織內之病原菌滲出種子表面。

由試驗悉知，分離黃豆種子病原菌之最適方法爲：先將黃豆種子浸在百分之一濃度之次氯酸鈉一分鐘後，爲避免消毒劑遺留於種子，再以無菌水洗五次。此後，將黃豆種子擱置於馬鈴薯葡萄糖培養基上，在攝氏二十五度培養。

Literature Cited

ATHOW, K. L. and F. A. LAVIOLETTE. The relation of seed-transmitted tobacco ringspot virus to soybean yield. Phytopathology 51:341-342, 1961.

BRINKERHOFF, L. A., G. FINK, R. A. KORTSEN, and D. SWIFT. Further studies on the offect of chemical seed treatments on nodulation of legumes. Plant Dis. Reptr. 38: 393-400, 1954. (Abstr. in Rev. Appl. Mycol. 34: 123-124, 1955).

CHAMBERLAIN, D. and B. KOEHLER. Soybean diseases in Illinois. Univ. Ill. Circular 676: 29-30, 1951.

CHU, H. T. and C. CHUANG-YANG. Investigation on soybean diseases. Taiwan Sug. Exp. Sta. 25: 11-25, 1961.

DESJARDINS, P. R., R. L. LATTERELL, and J. E. MITCHELL. Seed transmission of tobacco-ringspot virus in Lincoln variety of soybean. Phytopathology 44: 86, 1954.

DUNLEAVY, J. Survey of races of *Peronospora manshuric* in the Unitod States. (Abstr.) Phytopathology 49: 537-538, 1959.

HAN, Y. S. Soybean diseases in Taiwan. Jour. Agr. Assoc. China. New Series 26: 31-38, 1959.

HEUBERGER, J. W. and T. F. MANNS. Effect of organic and inorganic seed treatments on rate of emergence, stand, and yield of soybeans. (Abstr.) Phytopathology 33: 1113, 1943.

HILDEBRAND, A. A. and L. W. KOCH. Seed treatment and other tested with soybeans in Ontario. (Abstr.) Phytopathology 36: 401, 1946.

HILDEBRAND, A. A. and L. W. KOCH. Soybean diseases in Ontario and effectiveness of seed treatment. Phytopathology 37: 111-124, 1947.

HILDEBRAND, A. A. and L. W. KOCH. Observations on six years' seed treatment of soybeans in Ontario. Sci. Agr. 30: 112-118, 1950.

JOHNSON, H. W., CHAMBERLAIN, anp S. G. LEHMAN. Diseases of soybeans and methods of control. U. S. Dept. of Agr. Circular 931: 34-35, 1954.

KILPATRICK, R. A. Fungi associated with soybean seeds within the pods at Stoneville, Mississippi, in 1951. (Abstr.) Phytopathology 42: 285, 1952.

KURATA, H. Studies on the fungal diseases of soybean in Japan. Bull. Nat. Inst. Agr. Sci., ToKyo, Ser. C 12: 1-154, 1960.

LEHMAN, S. G. Frogeye (*Cercospora daizu* Miura) on stems, pods, and seeds of soybean, and the relation of these infections to recurrence of the disease. Jour. Agr. Res. 48: 131-147, 1934.

LISTER, R. M. Transmission of soil-borne viruses through seed. Virology 10: 547-549, 1960.

PIETKIEWICZ, T. A. From studies of soybean seed microflora. Roczn. Nauk Rol., Ser. A. 79: 1077-1090, 1959. (Abstr. in Rev. Appl. Mycol. 40: 391, 1961).

SHERF, A. F., and C. S. REDDY. Seed treatment and inoculation studies on soybeans in Iowa fields. 1950-1951. (Abstr.) Phytopathology 42: 19, 1952.

SHERWIN, H. S., C. L. LEFEBURE, and R. W. LEUKEL. Effect of seed treatment on the germination of soybeans. Phytopathology 38: 197-204, 1948.

SHERWIN, H. S. and K. W. KREITLOW. Discoloration of soybean seeds by the frogeye fungus, *Cercospora sojina*. Phytopathology 42: 568-572, 1952.

TERVET, I. W. The influence of fungi on storage, on seed viability and seedling vigor of soybeans. Phytopathology 35: 3—15,1945.

TOGASHI, K. Biological characters of plant pathogens temperature relations. Meibundo Co., Tokyo, Japan, 478 p., 1949.