SEED-BORNE DISEASES OF SOYBEAN IN TAIWAN

II. Survey of the seed-borne pathogens from soybean seeds[1]

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Introduction

The fungi associated with soybean seeds and pods have been reported (Kilpatrick, 1952, 1953). Those which are most commonly recognized are *Cercospora kikuchii, Alternaria* spp., and some other fungi saprophytic in nature. Microflora of seeds varies from locality to locality. *Fusarium* spp. and *Botrytis cinerea* are frequently detected on 162 sample seeds from various parts of Poland (Pietkiewicz, 1959), whereas *Diaporthe phaseolorum* var. *sojae* and *C. kikuchii* are predominant in Japan (Kurata, 1960).

Factors affecting the isolation of organisms from soybean (Wu *et al.*, 1964) may be responsible for the difference in microflora. However, the effects of the maturity (Kilpatrick, 1952), quality (Tervet, 1945; Kurata, 1960), and water content (Tervet, 1945; Lehman, 1952) of seeds should not be ignored. Environmental conditions are also found to be very effective. Dry weather appears to reduce the percentage of fungus infection in soybean seeds (Kilpatrick, 1953) while percentage of seeds infected with *Aspergillus* spp. varies with the temperature under which seeds are stored (Tervet, 1945). In addition to the fact mentioned, loss of viability of fungus in seeds is also markedly accelerated by higher temperature (Lehman, 1952).

In the present paper, the results are obtained from soybean samples only limited to those representative areas in Taiwan, where most of soybeans are produced.

Materials and Methods

Sample seeds were collected from two sources: One is prior to harvest

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and the other post harvest soybeans. The former samples including two varieties, *i. e.* Palmetto, and Pearl bean, were directly taken from the soybean fields at Chiu-ju, Ping-tung. The latter samples were kindly collected by Kao-hsiung, Tainan, and Hsin-chu District Agricultural Improvement Stations (DAIS). These were stored by the local farmers and DAIS for a period of time before collection. The varieties are: Acadian, Black bean, Chiputou, Green bean, Kaoshi #6, Palmetto, Pearl bean, Shihshih, Wakashima from Kao-hsiung DAIS; Acadian, Koashi #201, KS-12, Palmetto, PT, Shihshih, Taita #114 from Tainan DAIS; and Dortchsoy, E-32, F-24, H-8, H-11 Kaoshi #86, Kaoshi #201, Kim, KS-12, Nungshih #157409, PI-232998, Shihshih from Hsin-chu DAIS. All the samples were kept in a cold room of about 2°C before use upon arrival of soybeans.

Soybean seeds were soaked in one per cent aqueous solution of sodium hypochlorite for one minute followed by rinsing in 5 changes of sterile water. The treated seeds were plated in a Petri-dish containing 20 ml potato dextrose agar, 10 seeds per Petri-dish, then incubated at 25°C for 4 days to isolate the causal organisms (Wu *et al.* 1964).

Results

Microflora of soybean seeds prior to harvest.

For the isolation of causal organisms soybean pods were directly collected in the 14 different fields before harvest. Dried pods and partially dried pods were picked and separately kept in vinyl bags. During the course of investigation, the partially dried soybean pods became moldy even though they were stored in a cold room at 2°C. Hence, further attempt of isolation was not made with the partially dried pods. There was no appreciable change in the dried pods stored under the same conditions. The isolation of causal organisms were, therefore, carried out only with seeds of dried pods from 14 fields.

Seeds were removed from the dried pods and grouped into four types: (1) clean, (2) slightly discolored, (3) extensively discolored, and (4) abnormally stunted seeds. Among the soybeans tested, extensively discolored seed occupied 44 per cent, slightly discolored came to next, *i. e.* 32 per cent, and abnormally stunted seeds were 16 per cent, while only 8 per cent of seeds were found to be clean. However, this is not the case usually found in the seed lots from post harvest soybeans and mostly clean seeds were found. This might be due to the fact that infection of seeds within pods occurred during the storage.

From 14 samples, 10 genera of known fungi were isolated (Table 1). They were *Alternaria, Cephalothecium, Cercospora, Cladosporium, Corynespora, Fusarium, Nigrospora, Penicillium, Pestalozzia,* and *Stemphylium.* Among the 10 genera named, *Alternaria, Cercospora, Corynespora,* and *Fusarium* were already reported to be pathogenic (Johnson *et al.*, 1954). *Cercospora* and *Fusarium* involved a

large number of isolates obtained. The *Corynespora* did not appear on the clean seeds, however, a few were found on the several samples of extensively discolored seed type. *Cephalothecium* was isolated from each type of seeds. Clean and slightly discolored seeds tended to yield more isolates of *Cercospora* whereas *Fusarium* inclined to be less in clean and slightly discolored seeds. The fact that more isolates of *Cercospora* and *Fusarium* are respectively obtained on slightly and extensively discolored seed is worthy of notice. Surprisingly, only two out of 14 samples yielded bacterial colonies.

Table 1. *The microflora and viability of soybeans* collected from the fields at Chiu-ju, Ping-iung, in 1962.*

Type of seeds	Clean	Slightly discolored	Extensively discolored	Abnormally stunted
No. of seeds tested	150(0-74)	587(0-110)	811(12-87)	299(7-37)
% of seeds germinated**	35.4(0-100)	27.7(0-87.8)	14.1(0-58.3)	19.1(0-85.6)
% of seeds infected**	76.9(0-100)	84.3(15.6-100)	96.4(58.3-100)	91.3(0-100)
No. of isolates:				
Alternaria	10(0-8)	5(0-4)	25(0-11)	3(0-1)
Cephalothecium	19(0-4)	170(0-36)	324(0-47)	109(0-21)
Cercospora	47(0-28)	98(0-54)	38(0-20)	21(0-10)
Cladosporium	—	5(0-5)	16(0-6)	11(0-3)
Corynespora	—	1(0-1)	69(0-27)	12(0-4)
Fusarium	8(0-5)	81(0-24)	244(2-41)	100(0-24)
Nigrospora	—	2(0-1)	—	—
Penicillium	—	2(0-2)	15(0-8)	3(0-2)
Pestalozzia	1(0-1)	—	—	—
Stemphylium	—	—	10(0-10)	—
Unidentified***	42(0-30)	65(0-39)	68(0-16)	32(0-14)
Bacteria	—	—	44(0-35)	1(0-1)

* The figures in parentheses indicate the range of number or percentage obtained from each lot of tested sample soybean.

** Percentage was calculated from the tested soybean lots.

*** Spore formation was not observed when the identification was made.

In comparing the germinability and percentage of infected seeds, it was shown that the germinability of seeds was greatly impaired after the seeds were heavily infected though the data varied in a vast extent of percentage. It was also found that less stand of soybean seedlings were observed when discolored seeds were sown in the pots with autoclaved soil. (15 pounds per square inch for 2 hours). Ungerminated seeds in the soil were mostly decayed while the germinated seeds yielded the stunting seedings which were heavily infected, particularly on the young cotyledons with necrotic spots.

In connection with this matter, relationship between seed treatment and germinability of seed was investigated by using the same soybean seeds. Fungicides available in the market, such as "Riogen Tablet", "Sankinon", "Sankyo Thiuram 80" and "Similton" were firstly taken into consideration. Their active ingredients are respectively phenylmercury acetate and ethylmercury chloride for "Riogen Tablet"; 2,3-dichloro-1,4-naphthoquinone and bis (dimethylthiocarbamoyl) disulfide for "Sankinon"; bis (dimethylthiocarbamoyl) disulfide (=tetra methylthiuram disulfide) for "Sankyo Thiurom 80"; and ethyl phenethynyl mercury for "Similton". Fifty seeds were soaked in a given fungicide for 1, 30, 60, and 120 minutes, respectively. The treated seeds were rinsed in 5 changes of sterile water or simply kept dry on a piece of sterilized filter paper without rinsing before incubating in a Petri-dish containing 20 ml potato dextrose agar, 10 seeds per Petri-dish, at 25°C.

The experimental results indicated that the percentage of gemination increased with decrease of the number of isolated microorganisms. However, the maximum germination was only 66 per cent where the recording was made 3 days after incubation. The fungicides tested could impair the growth of microorganisms not less than 30 per cent of the seeds examined after soaking the seeds for up to 2 hours. Bacterial colonies seemed to be increased when the number of fungal colonies was decreased by means of chemical treatment. It was conceivable that the fungal growth hindered the bacteria which were yielded by examined seeds. Rinsing seeds in sterile water after chemical treatment showed little influence on the chemical activity of the fungicides tested.

Microflora of post harvest soybean seeds.

The experiments concerning isolation of causal organisms from soybean seeds were carried out with seeds of good quality kept by the farmers and DAIS for planting purpose. Fifty soybean samples comprised 23 varieties from Kao-hsiung, Tainan, and Hsin-chu DAIS, which were kept in a cold room at 2°C for less than 3 month, were examined.

Eleven known genera of fungi were isolated, *i.e. Alternaria, Cercospora, Cladosporium, Colletotrichum, Corynespora, Curvularia, Fusarium, Penicillium, Pestalozzia, Phyllosticta,* and *Rhizoctonia.* High percentage isolation of *Alternaria, Cercospora, Cladosporium,* and *Fusarium* were detected on soybeans obtained from different localities. Relatively large number of bacteria were also isolated. Some pathogenic fungi, such as *Colletotrichum, Corynespora, Phyllosticta,* and *Rhizoctonia* were less frequently found. The germinability of soybean seeds seemed to be impaired by the infection of seed-borne pathogens though the striking cases were only obtained in a small number of the tested seed samples. Some of them might be attributed to the infection of bacteria isolated (Table 2).

Table 2. *The microflora and viability of soybeans* from Kao-hsiung, Tainan and Hsin-chu DAIS, in 1962–1963.*

Origin of samples	Kao-hsiung DAIS	Tainan DAIS	Hsin-chu DAIS
No. of samples	28	7	15
% of seeds germinated**	87.3(0-100)	99.7(99-100)	91.2(75-99)
% of seeds infected**	49.5(1-100)	34.6(27-41)	48.9(6-100)
No. of isolates:			
Alternaria	28(0-4)	13(0-4)	13(0-6)
Cercospora	26(0-9)	9(0-4)	132(0-32)
Cladosporium	298(0-100)	103(8-26)	83(0-25)
Colletotrichum	—	—	2(0-1)
Corynespora	—	—	1(0-1)
Curvularia	2(0-1)	—	5(0-1)
Fusarium	49(0-14)	9(0-4)	148(0-42)
Penicillium	8(0-1)	1(0-1)	13(0-6)
Pestalozzia	1(0-1)	—	—
Phyllosticta	1(0-1)	—	1(0-1)
Rhizoctonia	—	—	6(0-4)
Unidentified***	12(0-3)	—	104(0-32)
Bacteria	1,087(4-100)	159(9-39)	414(1-73)

* The figures in parentheses indicate the range of number or percentage obtained from each lot of tested sample soybean.

** Percentage was calculated from the tested soybean lots.

*** Spore formation was not observed when the identification was made.

In order to clarify the seed-borne diseases of soybean occurring under natural conditions, the same samples were sown on clay pots with autoclaved soil (15 pounds per square inch for 2 hours) collected in the field of the university farm. The soybean seeds were examined by means of three different treatments: (1) The seeds were soaked in a 0.1 per cent aqueous solution of mercuric chloride or (2) in 1 per cent acqueous solution of sodium hypochlorite for one minute followed by rinsing in 5 changes of sterile water, and (3) not treated. The seeds thus treated were sown on autoclaved field soil, 20 seeds per pot, to observe the disease development under greenhouse conditions (Temp. 10–32°C). Five replications were made for each treatment which consisted of 100 soybean seeds. Numbers of infected and germinated soybean plants were recorded at appropriate time during the period of experiments, that is when height of seedlings were about 30 cm high, then they were digged out, weighed, and examined.

From the observations made in greenhouse, 16 lots out of 25 lots of soybean samples showed typical symptoms of bud blight which was known to be incited by tobacco ring spot virus (Kahn and Latterell, 1955). The percentage of

soybean seedlings infected went up to 98 per cent as the highest disease incidence of bud blight. It was rather conspicuous that only two samples of soybean seeds indicated few cases of fungal infections, *e.g.* by *Botrytis, Fusarium,* etc. when the comparison was made in terms of the number of causal organisms isolated from the same lots of seeds which were incubated on potato dextrose agar. Young plants from seeds treated with mercuric chloride weigh less than those from seeds with other treatments. It might be due phytotoxic effect of mercuric chloride under the experimental conditions.

Discussion

The experimental results obtained from soybeans prior to harvest and post harvest showed that *Alternaria, Cercospora,* and *Fusarium* were the predominant causal organisms though a large number of saprophytes such as *Cephalothecium* and *Cladosporium* were isolated from soybeans prior to harvest and post harvest, respectively. These might be due to a varietal resistance, since the varieties of soybeans in Taiwan were mostly imported from the States and Japan where these fungi were most commonly isolated (Kilpatrick, 1952; Kurata, 1960). Kilpatrick (1952) reported that *Cercospora kikuchii* was isolated more frequently from the seed of late-maturing varieties than from early-maturing varieties. But whether this was a case of "disease escaping" still remained to be elucidated since dry weather also appeared to reduce the percentage of fungus infection on soybean seeds (Kilpatrick, 1953).

The clean and slightly discolored seeds yielded less isolates of *Fusarium* than extensively discolored seeds. On the contrary, the reverse was observed in the case of *Cercospora* which was reported to induce most frequently the discoloration of soybean seeds (Sherwin and Kreitlow, 1952; Jones, 1959). The less survival of *Cercospora* found in the extensively discolored seeds might be due to the higher moisture of seeds since the loss of viability of this fungus was found to be more rapid in seeds of high and medium moisture than in seeds of low moisture (Lehman, 1952). On the other hand, *Fusarium* was more saprophytic in nature and could cause Fusarium blight or wilt of soybean as well as cowpeas (Cromwell, 1917), rice (Matsuo *et al.*, 1959), and others. *Fusarium* might directly or indirectly lower the viability of *Cercospora.*

In comparing the germinability of seeds with isolation of fungi, low percentage of germination in the extensively discolored seeds seemed to accompany with a large number of isolated *Fusarium.* It was also found that less stand of seedlings were observed when discolored seeds were sown in the pots with autoclaved soil. Ungerminated seeds in the soil were mostly decayed while the germinated seeds yielded the stunting seedlings. These are possibly an indication of the effect of *Fusarium,* since seeds infected with *C. kikuchii* was known

to germinate normally and produce stand comparable to those produce by clean seeds (Sherwin and Kreitlow, 1952).

Seed treatment was shown to be beneficial to the germinability when the discolored seeds were used. The maximum germination of seeds was increased from 14–35 per cent to 66 per cent though the chemical treatment impaired the viability of microorganisms not less than 30 per cent.

From the observation made in greenhouse, the percentage infection of bud blight incited by tobacco ring spot virus went up to 98 per cent, while it was commonly found to be 80–90 per cent. On the other hand, only two samples of soybean seeds indicated a few cases of fungal infection. This was remarkably different from the results obtained from the same seed lots when isolation was made from soybean on potato dextrose agar. Temperature might be considered to be one of the factors which interfer the appearance of disease incidence in greenhouse since temperature was observed to have a marked effect on the response of soybeans to seed treatment (Sherwin *et al.* 1948).

Summary

A survey of the seed-borne pathogen from soybean seeds was made with seeds of good quality kept by farmers and DAIS for planting purpose. From 50 soybean samples comprised 23 varieties supplied by Kao-hsiung, Tainan, and Hsin-chu DAIS, *Alternaria, Cercospora,* and *Fusarium* were most frequently isolated. However, the seedlings of same lots of soybeans grown on autoclaved soil did not show comparable number of fungal infection. Stunting seedlings showing characteristic of bud blight were found in 98 per cent disease incidence. Microflora of soybean seeds prior to harvest was also determined. *Alternaria, Cercospora,* and *Fusarium* were the predominant isolates. With discolored seeds, seed treatment was shown to be beneficial to the germinability of discolored soybean prior to harvest.

臺灣黃豆種子傳染性病害

II. 黃豆"種子傳染"病原菌之調查

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所調查之黃豆種子，以農民及農業改良場，儲藏作播種用者爲主。由包括二十三個品種之五十個樣品，所分離之病原菌，以 *Alternaria, Cercospora*，以及 *Fusarium* 等屬者居多。但將此種黃豆種子播在殺菌土壤時，其發病之數目遠不及由種子所分離病原菌數目之多。芽枯病 (Bud blight)，在幼苗所表現之罹病率，可達百分之九十多。未收穫前，直接在

田間採集之種子調查結果，亦以 *Alternaria, Cercospora*，以及 *Fusarium* 爲最多。藥劑處理對收穫前採下之變色種子之發芽率甚有益處。

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