STUDIES ON THE BACTERIAL PUSTULE DISEASE OP SOYABEAN IN NORTHERN NIGERIA.

BY

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DECLARATION

The candidate hereby declares that the whole of the work now submitted as a Thesis for the Master of Science degree (Crop Protection) of Ahmadu Bello University is the result of his investigation (except where reference is made to published literature and where assistance is acknowledged) and has not been part of any presentation for any other qualification.

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SECTION 1

INTRODUCTION

SECTION 1. INTRODUCTION

(1) World distribution of Soyabean*

The origin and early history of the soyabean, Glycine max (L.) Merrill, are unknown (Hittle, 1975). According to Hymowitz (1970), current evidence for the antiquity of the soyabean lies in the pictographical analysis of the archaic Chinese word for soyabean (Shu), the book of Odes, and bronze inscriptions. He concludes that historical and geographical evidence point to the eastern half of North China as probably the primary centre, where it was first domesticated by around the 11th Century B.C., and Manchuria, the secondary centre.

In Europe, knowledge of soyabean as an exotic food plant dates as far back as the 17th century (Yule and Burnell, 1903).

The first mention of soyabean in the United States literature was by Mease (1804).

Mayo (1945) reported that the first recorded soyabean trial in Nigeria was conducted in 1910 by the Department of Agriculture. He also reported that by 1928, many varieties introduced from U.S.A. were being grown at Ibadan, Zaria, Kano and Yandev.

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* Soyabean (Soybean is American usage).

Currently, the main soyabean-growing area in Nigeria is the Benue State, followed by areas around Abuja in Niger State and southern Zaria in Kaduna State. Between 1967-1972, Nigeria produced between 63 and 93% of total soyabean production in Africa; the total export earning obtained was between ₦132,800 and ₦882,400 (Eden, 1975). The highest tonnage of soyabeans ever produced for export in Nigeria was 26,450 metric tons, from about 32,000 hectares of land (Anon, 1964).

(ii) Utilisation of Soyabean

Soyabean is a cheap source of protein and when added to starchy foods, it greatly reduces the problem of undernourishment often caused by shortage of protein in the diet. It has been observed that soyabean has a higher total digestible nutrient percentage (91.99%) than cowpeas (79.52%) and therefore a more metabolisable energy. It also has a higher lysine content (6.0 to 6.5%) than all other common vegetable protein sources (Onochie, 1965). During the Nigerian civil war (1967-1970) a food product made up of maize, soyabean and milk and called "Formula Two" helped to reduce malnutrition in the war-affected areas. Although soyabeans rank below fish, beef muscle, whole milk and whole eggs in nutritional value, it has a higher value

than sesame seed, cotton seed, peanut flour and cereal proteins. When properly processed, the soyabean protein is nearly as good in quality as casein, a major milk protein.

The main constraint with the use of soyabean as food in Nigeria is that it is difficult to cook in traditional Nigerian methods. Both the taste and colour after cooking are unattractive.

As a result of efforts directed towards removing the above constraints, the Federal Institute of Industrial Research, Oshodi, Lagos developed a form of cheap baby food (Soy-ogi) made by mixing soyabean and maize flour. (Ansa, 1977) Further efforts to avert the unpalatable taste of unprocessed soyabean can be made by processing the grains into a "milk" by crushing the soaked beans and filtering. This milk can then be utilised in enriching traditional foods such as "kwosei", "alele", "pancake" and "wara" in the Northern States or into "akara" ball, "moi-moi", "puff-puff" and "garri" or foo-foo during pounding in the Southern States of Nigeria.

Dr. Theodore Kay, a Biochemist of the Department of Pathology, Faulty of Medicine, Ahmadu Bello University, Zaria is currently working along these lines in an effort to popularise soyabean and to incorporate it into the Nigerian diet. (Ansa, 1977).

The soyabean has other potential uses. Its oil can be extracted to supplement groundnut oil. It can also be employed for preparation of margarine and salad oil. The lecithin from the oil is used in baked goods, candies, chocolate and cocoa. Insecticides, varnishes, enamels, ink and soap are also the by-products of soyabean oil. (Ansa, 1977)

In the animal industry, soyabean is a rich forage crop which can be preserved as hay or silage or fed green. Soyabean seed is a component of commercial animal feeds. (Ansa, 1977)

Although the production and local utilisation of the soyabean is at present small in Nigeria, an increase towards industrial and local consumption is expected in future, as Nigeria advances both in industrial sophistication and in population.

(iii) Diseases of Soyabean

Soyabean is susceptible to a number of diseases caused by bacteria, fungi, viruses and nematodes. Bailey's (1966) "Check-list of plant diseases in Nigeria" listed only the following diseases:- a leaf distortion and stunting, mosaic, both caused by viruses, a fungus, Myrothecium roridum (Tode ex Fr.) on leaves and a wilt caused by Sclerotium rolfsii (Tode).

Athow (1973) in his summary of major fungal diseases of soyabean in Nigeria recorded the occurrence of purple seed stain caused by Cercospora kukuchii (Mat. and Tomoy) Chupp; and pod and stem blight caused by Diaporthe phaseolorum (Cke. and Ell.) Sacc. var sojae (Lehman) Wehm.. At the International Institute of Tropical Agriculture, Ibadan, the following diseases have been found on soyabeans:- Cercospora pod and leaf spot, Web blight, caused by Rhizoctonia solani Kuehn, (Williams and Allen, 1976). Soyabean anthracnose, caused by Glomerella glycines (Hori)(Pers. ex Fr.) Grove var. truncata (Schw.) Arx, occurs in northern Nigeria (McDonald, personal communication, 1976).

Soyabean mosaic caused by soyabean mosaic virus has been reported in Nigeria (Dunleavy, 1973; Ansa, 1977).

Kennedy and Tachibana (1973) mentioned bacterial pustule disease caused by X. phaseoli var. sojense as one of the major bacterial diseases of soyabean in Nigeria.

(iv) Purpose of the present investigation

As the production of soyabean crop is likely to increase in Nigeria, the importance of bacterial pustule disease is also likely to increase. Very little work has been done in Nigeria to assess the importance of the bacterial pustule disease and

published literature on the subject is very sparse. Furthermore, most of the investigations were conducted in the humid forest zones of Nigeria. The purpose of the prosent investigation therefore was to make a study of the bacterial pustule disease on soyabean grown in the drier savannah areas of Nigeria, especially with regards to finding practicable means of control. Emphasis was placed on screening of varieties and lines for resistance to this disease because work carried out in Ibadan and elsewhere had shown that resistant varieties are available. It was however desirable to screen these and other genotypes for resistance to bacterial pustule under the differing climatic factors of northern Nigeria.

SECTION 2

REVIEW OF LITERATURE

SECTION 2. REVIEW OF LITERATURE

(i) The history of soyabean bacterial pustule disease.

Smith in 1904 isolated from soyabean leaves the bacterial pustule organism, and reproduced the disease on healthy soyabean plants by inoculation with pure cultures of the bacterium (Hedges 1924). He did not however, describe the causal organism which was later named Bacterium phaseoli E.F.Sm. Heald (1906) described very briefly a bacterial blight of soyabean. As he made no cultural studies, it was difficult to ascertain whether or not that disease was bacterial pustule or another leafspot disease later described by Johnson and Coerper in 1919 which resembles the pustule disease in certain stages, but is caused by a white bacteriium, Bacterium glycineum Coerper (Hedges 1924). There was also another soyabean disease which Wolf (1920) and Shunk and Wolf (1921) reported and the causal organism of which was named Bacterium sojae Wolf. This bacterium so much resembled Bacterium glycineum that it was questionable whether one or two diseases were involved. Clinton (1916) gave a more detailed description of the pustule organism first isolated by Smith though at that time, he considered it identical with the bacterial disease of wax and lima

beans caused by Bacterium phaseoli EFS. He made no cultural studies. Bryce (1918) reported the occurrence in Canada of a bacterial blight of soyabean leaves, but did not describe the disease. In 1918 the Plant Disease Survey of the United States Department of Agriculture mentioned a bacterial leafspot of soyabean in Pennsylvania and Indiana with no clues as to which bacterial disease was meant.

More precise information on the occurrence of bacterial pustule became available when Hedges (1924) isolated a yellow bacterium, closely resembling Bacterium phaseoli from infected soyabean leaves sent to her from Texas in 1917. She completed detailed cultural studies on the organism and named it Bacterium phaseoli var. sojense, currently known as Xanthomonas phaseoli var. sojense (Hedges) Starr and Burkholder 1942 (Dye and Lelliott, 1974).

(ii) Geographical distribution

Bacterial pustule appears to be one of the most important diseases of the soyabean plant as it is found almost everywhere the plant is grown.

Following the authenticated discovery of bacterial pustule in Texas, U.S.A. (Hedges, 1924) the occurrence of the disease was reported from other areas of the United States e.g., Delaware (Manns and Adam, 1925); ———————————— Minnesota and Dakotas (Tervet, 1943); Kansas (Pady, 1943): Arkansas and Oklahama (Larsh, 1944); and in Iowa (Crall 1950).

The disease has also been reported in other countries besides the United States such as in Formosa (Okabe, 1932); North China and Mongolia (Nakata, 1940); Sudan (Tarr, 1952); Pacific Isles (Litzenberger and Stevenson, 1957); and Malawi (Corbett, 1963).

Kennedy and Tachibana (1973) reported the occurrence of the disease in Nigeria.

(iii) Description of the bacterial pustule disease and its relation to other soyabean leafspots of bacterial origin

Bacterial pustule disease is caused by Xanthomonas phaseoli var. sojense (Hedges) Starr and Burkholder 1942 (Dye and Lelliott, 1974). The pathogen gains entry into soyabean leaves through stomata from where it moves into the intercellular spaces (Wolf, 1924). Bacterial pustule begins as small elevations on either or both surfaces of the leaf. The areas are pale green initially, but later turn brown to reddish brown.

A minute pustule (from which the name is derived) is usually present in the centre. The periphery of the spots changes from pale yellowish-green to a pronounced yellowing and the spots themselves may coalesce and cause defoliation of the plant. Rhoads (1944) in his summary of observations on soyabean diseases in Florida reported that both frog-eye disease and bacterial pustule cause defoliation. Pady (1943, 1944) reported that bacterial pustule also caused defoliation in Kansas.

Numerous workers have reported that bacterial pustule is primarily confined to soyabean leaves (Hedges, 1924; Wolf, 1924; Dunleavy, et. al. 1966; Laviolette, 1967; Hymowitz, et. al. 1976), though Hedges (1924) reported infections on pods of soyabeans and lima beans after artificial inoculations. Infection of stem in addition to leaves was reported by Matveeve and Ostrogskaya (1968).

Characteristic pustules normally arise by the reaction of the host cells, leading to a hypertrophy of the parenchymatous tissues (Wolf, 1924; Hedges, 1924). Wolf (1924) and Lehman (1931) reported that the absence of pustules from the lesions did not mean that bacterial pustule was absent. They had isolated

Xanthomonas phaseoli var. sojense from lesions on soyabean leaves showing no pustules.

Another bacterium, Pseudomonas tabaci (Wolf and Foster) F.L. Stevens, the causal organism of wildfire of tobacco and soyabean has been shown to enter soyabean leaves through lesions of bacterial pustule (Graham, 1953). This has been confirmed by other workers (Chamberlain, 1956 and Dunleavy et. al. 1966). The latter authors also reported that P. tabaci alone does not produce any disease on soyabeans.

Graham (1953) reported an antagonistic relationship between Xanthomonas phaseoli var. sojense, Pseudomonas glycinea and Pseudomonas tabaci isolated from soyabean. He found that in culture, Pseudomonas tabaci produces a diffusible substance antagonistic to the other two bacteria. The substance could not be separated from P. tabaci by the method he used. He also found that the tobacco isolate of P. tabaci possessed similar antagonism.

Hall (1973) observed that the symptoms of bacterial pustule very closely resemble those of bacterial blight caused by Pseudomonas glycinea Coerper. Symptoms on soyabean leaves are spots which are water-soaked and angular at first, but later turn

yellow, and then brown. The presence of the water-soaked border however distinguishes this disease from bacterial pustule, an observation which had also been made by earlier workers (Hedges, 1924; Wolf, 1924, and Laviolette, 1967).

(iv) Isolation and morphology of the bacterial pustule organism.

Xanthomonas phaseoli var. sojense can easily be isolated from infected soyabean by means of agar poured plates (Hedges, 1924). By using standard isolation and purification procedures for bacteria, the author obtained a good growth of a smooth, yellow, wet-shining culture of the pathogen on potato Dextrose nutrient agar. Wolf (1924) isolated the organism by simply macerating pustule lesions in a drop of sterile water, and then streaking the inoculum so obtained with a sterile loop, in zig-zag strokes on nutrient agar plates.

Hedges (1924) and Wolf (1924) both reported that the bacterium is yellow in mass. Cells are short-rods with rounded ends. They are either single or are in pairs, each measuring 1.4 to 2.3/u x 0.5 to 0.9/u and are actively motile with 1-2 flagella. They also observed that the bacteria stain readily with all the common bacteriological stains.

Wolf (1924) reported that cells stained with carbolfuchsin from 24 hour-potato-agar cultures, measured 1.3 to 2.1 μ X 0.6 to 0.75 μ. Hedges (1924) and Wolf (1924) also observed that Xanthomonas phaseoli var. sojense and Bacterium phaseoli EFS. resembled each other very closely both morphologically and culturally, but Hedges noted two distinguishing characteristics. One was the presence of internal markings in Xanthomonas phaseoli var. sojense, which were absent in Bacterium phaseoli EFS. The other was that soyabeans were not as readily infected by Bacterium phaseoli EFS, as they were infected by Xanthomonas phaseoli var. sojense. Wolf found that both organisms utilized a number of proteins e.g. gelatin, casein, blood serum and asparagine while Hedges observed that the following media were very suitable for the growth of Xanthomonas phaseoli var. sojense:- Whey agar, potato cylinders, potato agar plus 10% Dextrose and Kellerman's synthetic agar plus Congo red.

(V) Artificial inoculation with Xanthomonas phaseoli var. sojense

Hedges (1924) repeatedly reproduced the bacterial pustule disease on soyabean leaves in the glasshouse both by spraying and rubbing with absorbent cotton which had been dipped in bacterial suspensions. She obtained the best results when inoculations were

made on rapidly-growing plants kept moist for at least 36 hours after inoculation. Symptoms were evident in 5-9 days and pustules became visible in less than 10 days. The disease was induced on both seedlings about 3.6cm high with folded leaves and those which were 20.0 - 22.8cm high with unfolded leaves, using both spray and prick inoculation methods. She found different levels of infectivity when Bacterium phaseoli var. sojense was inoculated on soyabean, lima bean and Bush string bean. She also observed that this bacterium became more virulent to Phaseolus sp. after one passage through this same host. Wolf (1924) obtained rapid infection (within a week) when soyabean leaves were sprayed with a suspension of the bacterium using on atomizer. Symptoms developed within 4-6 days. The garden bean and lima bean failed to become infected under the same conditions.

Feaster (1951) inoculated soyabeans at different times of the day, and found that the most severe infections were obtained when most of the stomata were open at time of inoculation.

A method of obtaining a large quantity of inoculum for field inoculation was developed by Jones and Hartwiq (1959). Infected leaflets were put into a food chopper and then comminuted

in tap water with a food blender. The suspension was allowed to stand for about 2 hours before filtering and use. They also found that the diseased leaflets could be chopped and frozen for use the following year.

(vi) Some epidemiological aspects of the bacterial pustule disease

a) Disease transmission:

Hedges (1924) reported a series of experiments to determine whether or not the soyabean bacterial pustule organism could be transmitted by the spotted cucumber beetle (Diabrotica duodecim-punctata) which was found on the diseased plants. She fed these beetles on diseased leaves, on leaves smeared with the organism and on pure cultures prior to putting the beetles on leaves of healthy plants. All her results were negative.

She suggested that the organism could be spread by rain, but made no observations to support the view. This was subsequently shown to be so by other workers (Laviolette, 1967 and Hartwig, 1975). These workers found that the pathogen could be moved from an infected leaf to a considerable distance by wind-driven rain. She also was the first to show that the bacterium is seed-transmitted. This has since been confirmed by several authors (Piper and Morse, 1923; Kent, 1945; Graham, 1953; Dunleavy et. al. 1966; Laviolette, 1967).

b) Favourable weather conditions for disease development:

Lehman (1931) recorded more infection at 30-33°C than at a fluctuating temperature between 22 and 30°C. Graham (1953) observed that severe disease infection occured when the maximum and minimum temperatures ranged from 33° - 37°C and 15-21°C respectively in North Carolina. He observed that these ranges were obtainable in that area during the period of wind and rain storms. Scott and Aldrich (1970) referred to bacterial pustule as a warm weather disease. Based on investigations carried out at the I.I.T.A., Ibadan, Williams and Allen (1976) concluded that bacterial pustule spreads readily in monocrop soyabean during wet weather.

c) Survival of the Bacterium:

Piper and Morse (1923) found that infected seeds and leaves are the chief means by which the organism overwinters. Hedges (1924) found that the organism remained viable at room temperature for three to five months when cultured on a potato nutrient medium; stock cultures in an ice box were found to be viable up to one year and four months on both potato and beef agar in small-bore tubes of 1.5cm diameter. Graham (1952) found that the organism remained viable and pathogenic after 29 months storage on beef-

dextrose agar slant cultures covered with sterile mineral oil.

Lehman and Poole (1929) found that the organism was viable in dry diseased leaves as well as in partially decayed leaves left out of doors from one season to another.

Lehman (1929, 1931) showed that the organism remained viable and pathogenic for at least nine months in diseased leaves stored in the laboratory; and for three months in the leaves exposed to weathering and decay. Graham (1952) found that the bacterium could be plated from soyabean leaves kept dry in the laboratory for 8 months. Dunleavy et. al. (1966) and Laviolette (1967) reported that the organism overwinters in diseased leaves of the soyabean.

Pady (1943) found that the bacterial pustule organism is seed-borne. Kent (1945) reported that the pustule organism could be isolated from seeds as old as 17 months or more. Graham (1953) discovered that the bacterium could be detected in seeds up to 30 months after harvest. He further showed that the organism persisted in sterile soil for 9 months.

(vii) Control of bacterial pustule disease

a) Chemical control:

Little information is available on the effect of spraying or dusting soyabean plants to control bacterial pustule. Lehman (1944) obtained some measure of control of the disease by dusting the infected plants with copper-talc and copper-sulphur dusts. He also found that the sulphur dusts alone failed to reduce bacterial pustule. Later, Lehman (1946 and 1948) concluded that it was the dust containing copper that reduced the bacterial pustule. Petty (1944) working on soyabean diseases on the eastern shore of Maryland, U.S.A. could not obtain conclusive results from seed treatment experiments. Aureomycin, terramycin, streptomycin and chloramphenicol, each at the rate of 60/ug/ml inhibited the growth of Xanthomonas phaseoli var. sojense in vitro (Thirumalachar et. al. 1956). Oksent'yan and Gunina (1968) recommended the use of phytobacteriomycin and polymycin against both X. phaseoli var. sojense and P. glycinea. Rao and Patel (1973) showed that achromycin, aureomycin and chloromycetin inhibited the growth of the bacterium in vitro. Their foliar spray experiments with TF-130, vitavax and chloromycetin delayed symptom development by 7-14 days.

Barnes (1965) observed that ultraviolet irradiations of soyabean leaves inoculated with Xanthomonas phaseoli var. sojense killed the bacterium on leaf surface but not in the intercellular spaces of the leaf.

b) Use of resistant varieties:

The use of resistant varieties as a means of controlling bacterial pustule has attracted considerable attention because other methods have been found to be uneconomic and difficult on a large scale.

Hedges (1924) in 1921 artificially inoculated seven soyabean varieties, and obtained infection on all except Wilson Fine. She further grew 40 varieties under natural conditions and found that only three cultivars (Buckshot, Mandarin and Biloxi) had any resistance (slight in all cases) to the disease. According to Weiss (1950), resistant cultivars had become available in the southern United States, where pustule and wildfire usually caused a 10% yield reduction in plot trials. Feaster (1951) reported some investigations in which early-maturing susceptible varieties were crossed with Clemson Non Shatter (CNS), a variety which is known to carry high resistance or near immunity trait to bacterial pustule. Hartwig and Lehman (1951) reported that the resistance

response to the pustule organism in the CNS (soyabean) is due to a monogenic recessive gene. Hartwig and Johnson (1953) found that susceptible lines yielded 11% less than resistant lines at Stoneville, 8% less at Willard (both in U.S.A.) and also produced seeds 3% smaller than the resistant lines at Stoneville and 4% smaller at Willard. They also observed that the seeds of resistant lines were higher in protein content than those of susceptible lines. The varieties Bragg, Clark 63, Hampton, Hardee, Hill, Hood, Lee and Scott were reported to be highly resistant in Iowa (Dunleavy et. al. 1966). Further work at Stoneville and Mississippi have shown yield reductions of 15% when susceptible lines are grown (Hartwig, 1975). The authors also reported that all cultivars being grown on the 8 million hectares in southern U.S.A. were resistant to the disease. Hymowitz et. al. (1976) tested about 153 varieties of soyabean, for their responses to selected foliar, stem and root disease and reported that 31 varieties were resistant to bacterial pustule in the U.S.A. and Canada, 37 were reported under intermediate response and 85 were listed as susceptible to bacterial pustule.

SECTION 3.

EPIDEMIOLOGICAL ASPECTS OF BACTERIAL PUSTULE DISEASE

SECTION 3: EPIDEMIOLOGICAL ASPECTS OF BACTERIAL PUSTULE DISEASE.

A. Geographical Distribution of Bacterial Pustule in Nigeria.

Introduction

Work done in the United States of America and elsewhere has shown that bacterial pustule of soyabean occurs everywhere the crop is grown. According to Dr. D. Allen (personal communication, 1977) of the I.I.T.A., Ibadan, the disease has also been found everywhere the crop is grown in Nigeria and the following places were cited:- Gboko and Yandev in Benue State, Uyo in Cross River State, Bende in Imo State and Ibadan in Oyo State. All these areas are situated in the wetter parts of the country. An attempt was therefore made to determine the occurrence of the disease in the drier savannah areas of Nigeria.

Materials and Methods

Soyabean leaflets infested with the bacterial pustule disease were collected from five sites during the 1977 rainy season. The sites (Fig. 1) were Zaria (Kaduna State), Bokkos and Riyom (Plateau State), Ibadan (Oyo State) and Mokwa (Niger State). In addition to the leaflets, soyabean seeds of 20 different cultivars/lines were

obtained from Ibadan and Zaria in order to determine the incidence of the pustule pathogen in or on them.

Twenty seeds sampled from each of the 20 cultivars/lines obtained from Ibadan and Zaria were surface-sterilised in 0.1% solution of mercuric chloride for 1 minute, rinsed thrice in sterile distilled water, and allowed to soak in the latter for 1 hour. They were then teased apart with sterile needles in 3 ml of sterile water and allowed to stand for 2 hours for bacteria to migrate out of the tissues. A loopful of the suspension was streaked on nutrient agar (NA) plates. The plates were incubated for 24 hours at 27 $\pm$ 2°C. The same procedure was followed with pustule lesions from diseased leaflets collected from the various sites. Single colonies of the presumptive pathogen (greenish-yellow and smooth) were picked and re-streaked on nutrient agar until pure colonies were obtained.

Pathogenicity was determined routinely by spraying suspensions of colonies of the presumptive pathogen on healthy soyabean leaflets. The inoculated leaflets were incubated in 9-cm (diameter) Petri dishes containing 3 layers of moistened filter paper. Disease development on the leaflets was recorded after 7 days.

Results and Discussions

The bacterial pustule disease was reproduced several times on healthy soyabean leaflets with isolates from the diseased soyabean leaflets collected from each of the five sites.

Xanthomonas phaseoli var. sojense was detected in the seeds of three cultivars/lines out of the 20 cultivars/lines tested (Table 1). The isolates from these three cultivars/lines were found to reproduce the disease on healthy soyabean leaflets when inoculations were done with pure cultures. The above results have therefore confirmed earlier reports (Kennedy and Tachibana, 1973; and Allen, 1977) on the occurrence of bacterial pustule in Nigeria, Tanzania, Sudan and Malawi.

Some white bacteria were isolated from the materials from Ibadan, Bokkos and Zaria. These were thought to be Pseudomonads, but their pathogenicity on soyabean was not investigated.

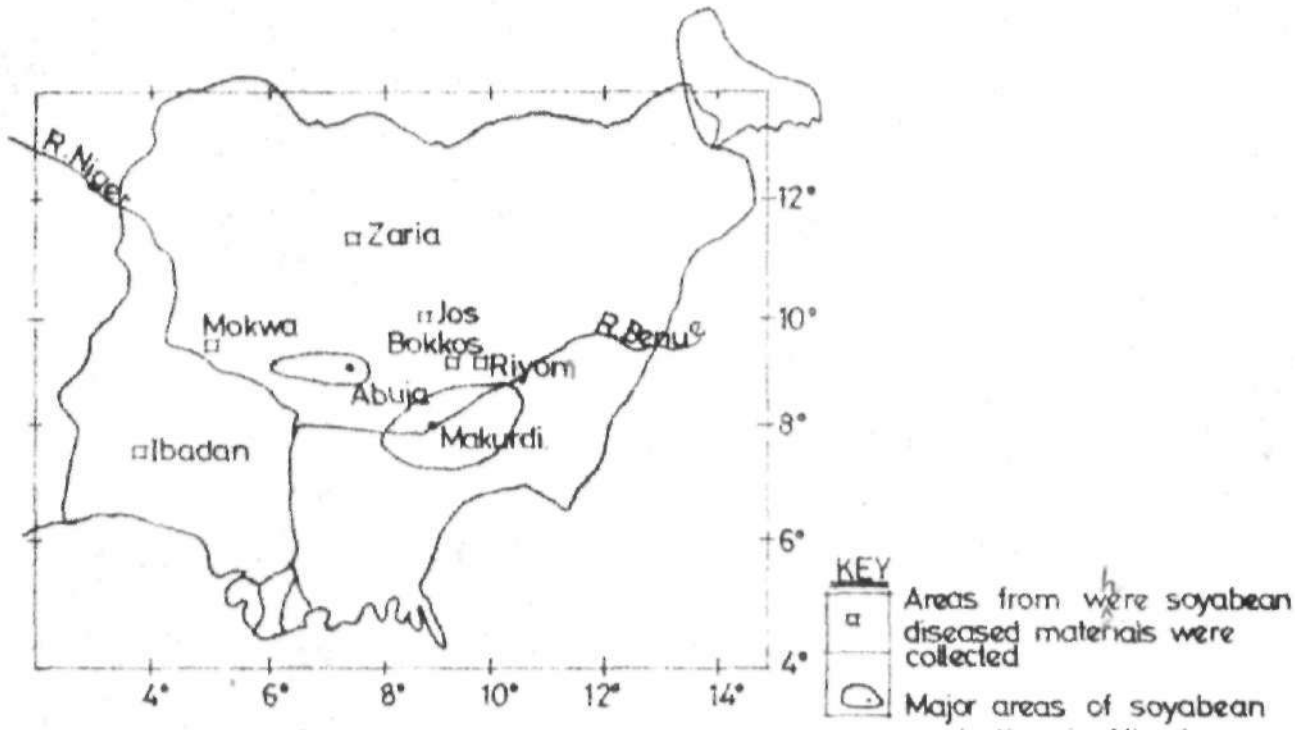


Fig 1.

Title : Map of Nigeria showing sites from where infected soyabean materials were collected.

Table 1: Isolation of Xanthomonas phaseoli var. sojense from infected soyabean materials collected from five sites in Nigeria.

Sites	Soyabean material:-		Presence of:-	
	Cultivar/ line	Plant part	X. phaseoli var. sojense	Pseudomo-nads
Zaria	K157	Seed	+	-
"	Janfield	"	+	-
"	X_2B7	"	-	+
"	Black malte Java	"	-	-
"	Congo yellow	"	-	-
"	Hernon 49	"	-	+
"	Fort Lamy	"	-	-
"	M216	"	-	-
"	K162	"	-	-
"	Bathel	"	+	-
"	Malayan	Leaflets	+	+
Bokkos	Malayan	Leaflets	+	+
Riyom	Malayan	Leaflets	+	-
Mokwa	Unknown	Leaflets	+	-
Ibadan	Improved pelican	Leaflets	+	+

Table 1 Contd.

Sites	Soybean material:-		Presence of:-	
	Cultivar/ line	Plant part	X. phaseoli var. sojense	Pseudomonads
Ibadan	SB6^{S} TGX 57-11^{C}	Seed	-	-
"	SB6^{S} TGX 62-2^{C}	Seed	-	-
"	SB6^{S} TGX 16-7^{E}	Seed	-	+
"	SB6^{S} TGX 43-4^{C}	Seed	-	+
"	SB6^{S} TGX 11-12^{E}	Seed	-	-
"	GES 407	Seed	-	+
"	SB6^{S} TGX 26-44^{D}	Seed	-	-
"	SB6^{S} TGX 57-11^{C}	Seed	-	-
"	SB6^{S} TGX 11-39^{E}	Seed	-	-
"	SB6^{S} TGX 19-1^{D}	Seed	-	-

+ = Presence of bacteria

- = Absence of bacteria.

B. Extent of Seed Infection by X. phaseoli var. sojense.

Materials and Methods

Seeds of the following five cultivars/lines, collected from 1976 harvests were tested for the incidence of infection by X. phaseoli var. sojense: Bathel, Janfield, K157, X_2B_7 and Hernon 49. Two hundred seeds of each cultivar/line were surface-disinfected and then washed as described above and then soaked in sterile water for 1 hour. They were plated on Nutrient agar in 9-cm Petri dishes (10 seeds per plate). The plates were randomly arranged in 10 replicates (two plates per replicate); incubated at room temperature ($27 \pm 2^{o}C$) and examined daily for growth of the bacterium.

One confirmed culture of X. phaseoli var. sojense isolated from pustule lesions was used as the reference for visual identification of any yellow bacterial colony isolated from the seeds.

Results and Discoussions

The results (Table 2) agree with earlier reports (Piper and Morse, 1923; Hedges, 1924; Pady, 1943; Graham, 1953, Dunleavy et al., 1966 and Laviolette, 1967), that X. phaseoli var. sojense can be seed borne. Lines X_2B_7 and Hernon 49 were, however not infected by X. phaseoli var. sojense.

Table 2: Extent of seed infection by Xanthomonas phaseoli var. sojense.

Cultivars/lines	Bacterial pustule infected seeds	Percent seed infection
K157	31	15.5
Jamfield	29	14.5
Bathel	5	2.5
X_2B_7	0	0.0
Hernon 49	0	0.0

C. Progress of bacterial pustule development in five cultivars/lines of soyabeans.

Materials and Methods

The cultivars/lines used in this experiment were $35^{S}/58$, 3H55F4/167/2, Black malte Java, M98 and Fort Lamy.

Each line was planted on a 4-metre ridge on 15th July 1977. Ridges were 1 metre apart. Seeds were sown 2-cm deep (2 seeds per hole) at 15cm spacing. Twenty days after sowing seedlings were thinned to a single plant per stand, that is 26 stands per line.

In order to study the rate of disease build-up, five plants were selected from the middle of the ridge. They were tagged with polyethylene ropes (obtained by cutting 30 x 20 cm polyethylene sheets into narrow strips) while the rest were left as discards. From the 6th week after sowing, the number of leaflets with bacterial pustule was recorded weekly for five weeks. Observations were made on the development of the pustule lesions, by use of a hand lense.

Results and Discussions

Very few plants were infected before 7 weeks from sowing. By the 9th week, however, the disease had become established. The greatest number of leaflets with bacterial pustule was recorded at the 10th week from sowing (Fig. 2). This was a period of rapid vegetative growth and flowering, corresponding with the time of high rainfall (199.5 and 302.7 mm in August and September respectively) and warm temperature (monthly means of 24°C and 25°C for August and September respectively)(Table 3).

The weather pattern in 1977 was rather peculiar; there were rains from mid-May to June, a break in July, then heavy rains in August and September. The observation on weather factors and bacterial pustule development agrees with the findings of Leham (1931) and Graham (1953). The sharp decline after the 10th week from sowing coincided with the dry weather of October which followed.

The rate of spread of bacterial pustule within the lines was found to be related to the plants' resistance (see Section 6). Disease spread was fastest in the two susceptible lines, $35^{S}/58$ and 3H55F4/167/2, intermediate in Black malte Java a tolerant cultivar and slowest in Fort Lamy and M98 which are resistant to bacterial pustule.

Observations made on the development of the pustule lesions indicate that the lesions start as small yellowish green portions on the upper surface of the leaflets. On the lower surface of these leaflets, small raised pustules are produced at the same time. The pustules measured after 7 days (from period of first visible symptoms) were found on the average to be 340 µm wide and 400 µm high at X60 microscopic magnification (Fig. 3).
This observation agrees with the report of Hedges (1924) that the young pustule is much less than 1mm in diameter. Seven days later, the lesions become prominent. They have angular edges and coalesce producing necrotic spots on the infected leaflets. The original small spots (pustular) can still be distinguished in these necrotic spots (Fig. 4 i). Twenty-one days after the appearance of first visible symptoms, the central portion of pustules drop out, leaving the leaflets ragged (Fig. 4 ii). Yellowing of severely infected leaflets occurs. There is no sign of water-soaking. This agrees with the reports of Hedges (1924); Dunleavy et al. (1966) and Laviolette (1967).

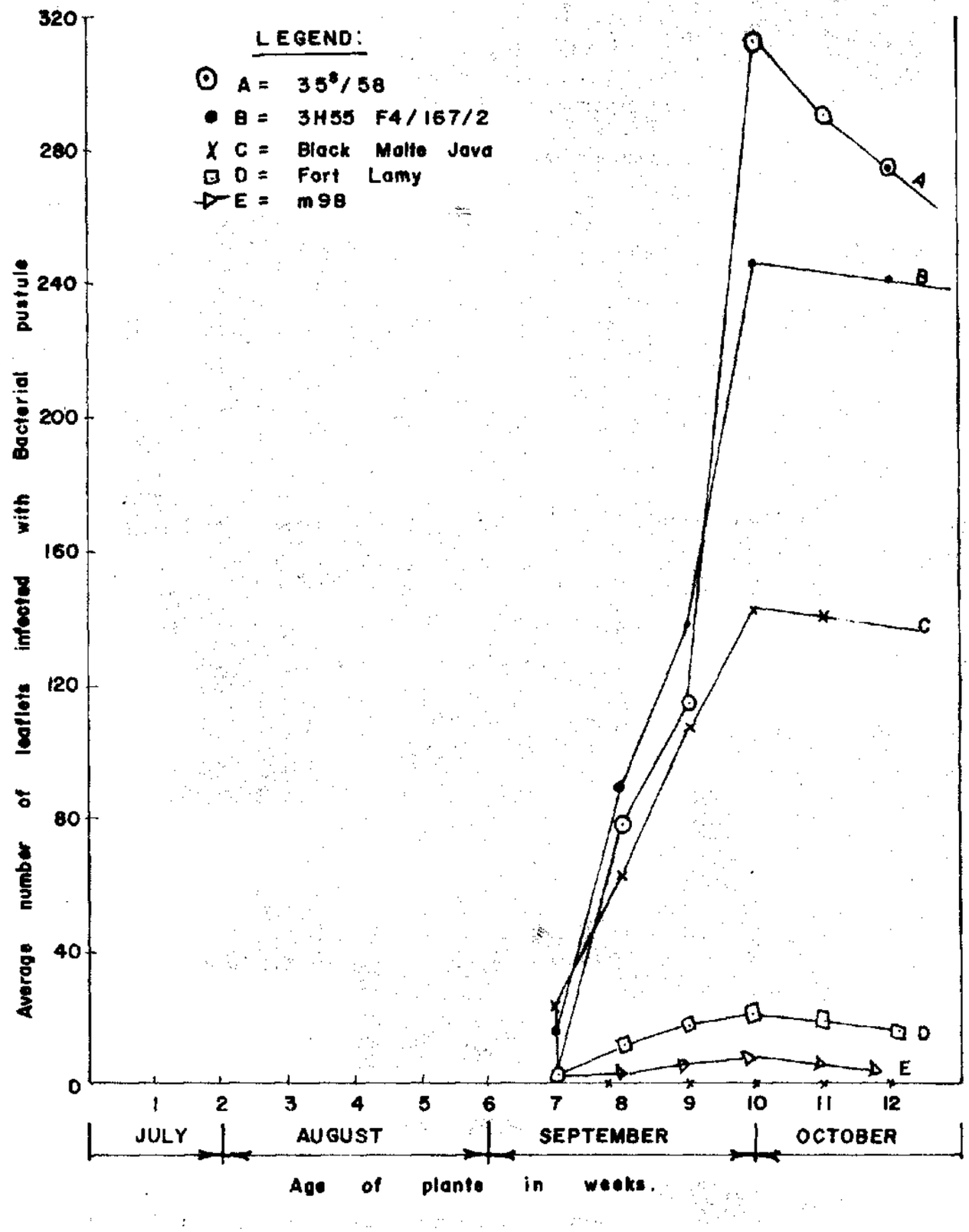

LEGEND:
A = 35⁸/58
B = 3H55 F4/167/2
C = Black Malte Java
D = Fort Lamy
E = m98
Average number of leaflets infected with Bacterial pustule
320
280
240
200
160
120
80
40
0
1
2
3
4
5
6
7
8
9
10
11
12
JULY
AUGUST
SEPTEMBER
OCTOBER
Age of plants in weeks.

Figure 2: Progress of bacterial pustule development in five cultivars/lines of soyabeans.

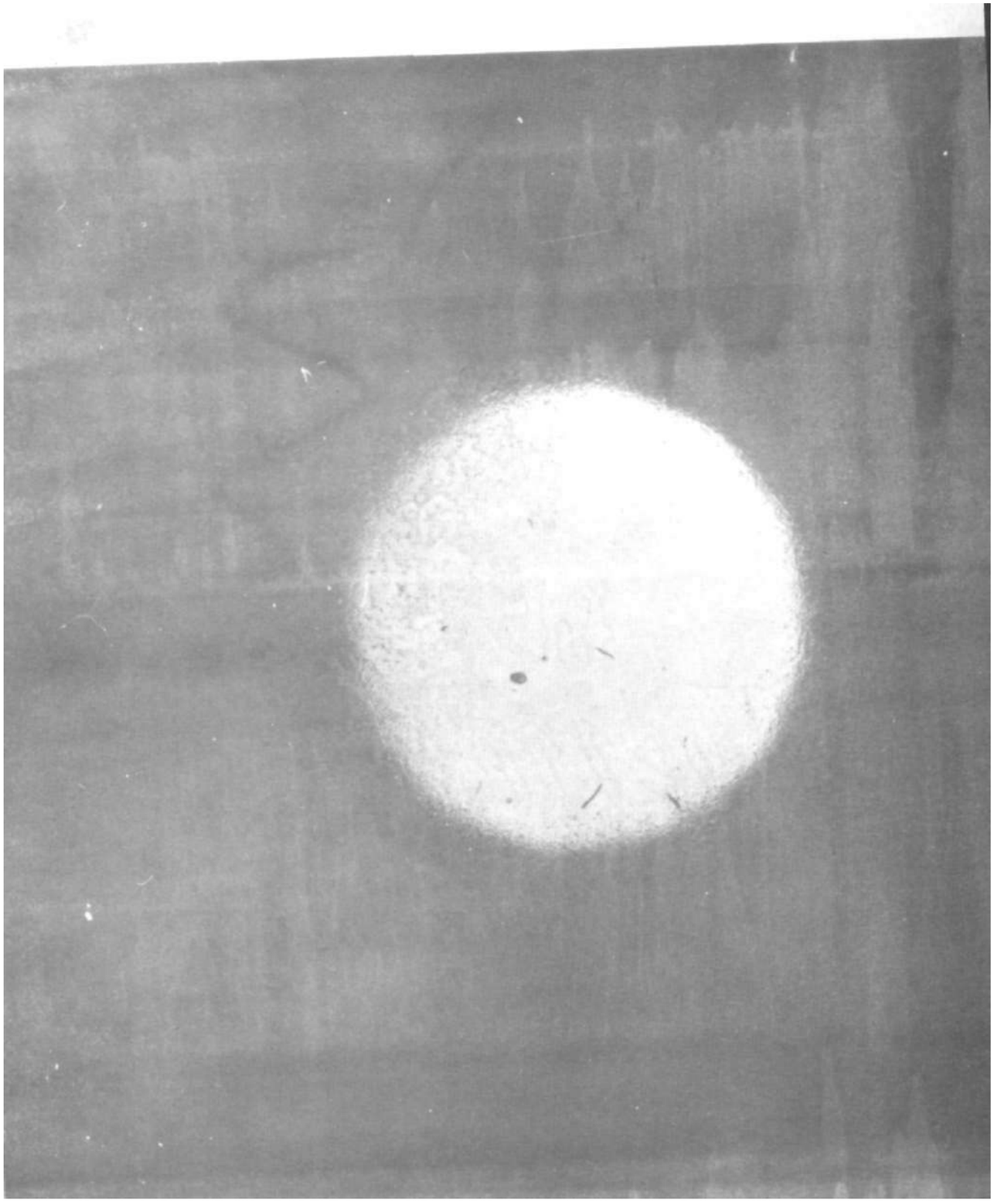
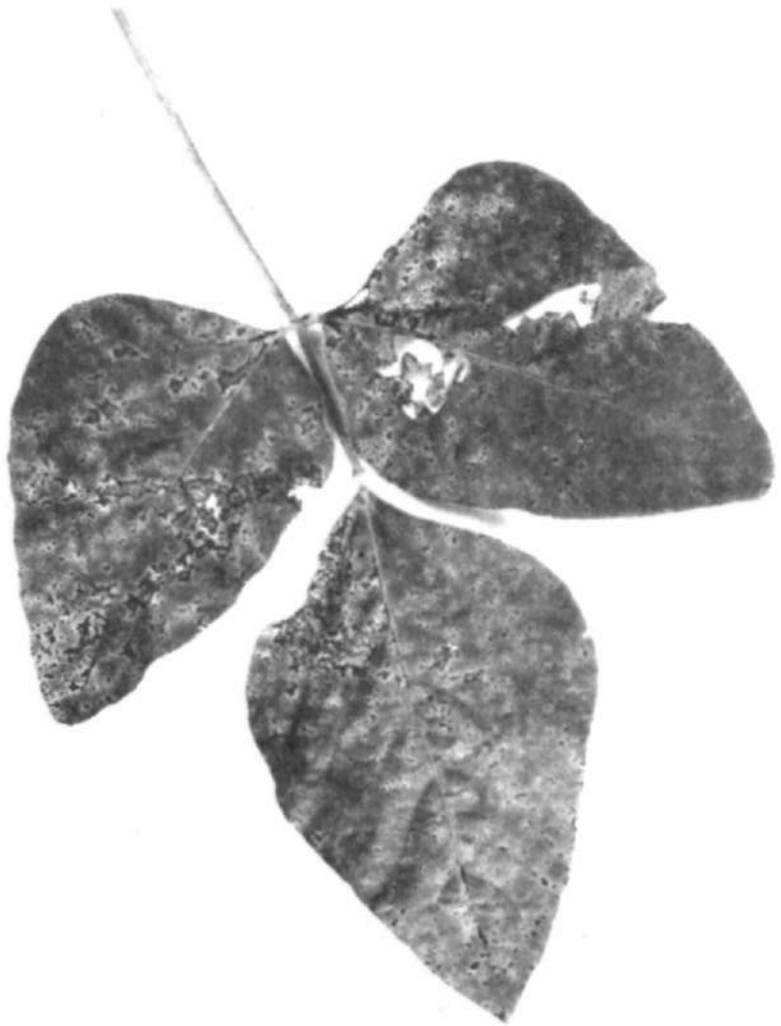


Figure 3: A young pustule lesion seen from the undersurface of the soyabean leaflet (photographed under light Ortholux II at x 60 magnification).

(ii)



(i)



Figure 4: (i) Pustules surrounded by necrotic and chlorotic borders.

(ii) Coalesced necrosis culminating in ragged leaflets.

Table 3: Weather records in Samaru during the field experimental periods, 1977.

Month	Mean Rainfall (mm)	Mean Tempt. °C (air)	Mean Tempt. °C (on plant surface	Sunshine (HRS)	R H %		Mean wind speed KLM/ Day
					A.M	P.M	
MAY	78.3	27.4	18.9	8.4	57.9	39.6	253.73
JUNE	101.7	25.5	19.2	7.7	74.2	58.8	233.00
JULY	59.0	25.0	17.8	6.0	77.9	62.0	205.82
AUGUST	302.7	23.8	16.3	4.6	82.1	72.1	160.93
SEPTEMBER	199.5	24.8	16.5	7.7	76.4	64.9	97.88
OCTOBER	4.3	23.9	14.5	8.8	65.2	45.4	105.90

SECTION 4

SURVIVAL OF *XANTHOMONAS PHASEOLI* VAR. *SOJENSE*

SECTION 4: SURVIVAL OF XANTHOMONAS PHASEOLI VAR. SOJENSE

A. Infectiousness of 1975 Infected Soyabean Trash

Materials and Methods

Investigations were conducted in order to establish the survival of the bacterial pustule organism in soyabean trash. Soyabean trash (leaves, stems) with the underlying soil was collected in May 1976 from a field at the Institute for Agricultural Research farm, Samaru in which a severe attack of bacterial pustule had occurred during the 1975 rainy season. The trash was stored in polyethylene bags on benches placed in a shaded area outside the laboratory until ready for use in August 1977.

(i) Preparation of inocula:

Twenty grammes of each type of trash was macerated with a mortar and pestle and added into 50 ml sterile distilled water contained in 500 c.c. conical flasks. The mixture was kept for three hours before larger particles of the trash were removed. In the case of the soil, organic matter debris were first removed before it was pulverised and then sieved through a mesh (No. 30) containing openings 595 /um in diameter, in order to remove all plant debris.

(ii) Inoculation.

A soyabean line, $35^{S}/58$, known to be susceptible to bacterial pustule was used. One hundred seeds of this line were soaked in each suspension prepared as described above for the following periods: 30 minutes, 1 hour, 2 hours, 3 hours, 6 hours and 12 hours. Each of the six controls consisted of 100 seeds soaked in 50 ml of sterile distilled water for the respective periods.

Four seeds from each treatment were sown in 0.8 kg sterilised soil contained in a 16cm plastic pot. Each treatment was replicated four times. The pots were arranged on a table in the open air outside the laboratory and watered daily. Seedlings were thinned to two per pot, 20 days after sowing. Disease records were made 30 days after sowing by which time each seedling had produced at least four true leaves.

Results and Discussion

The experiment showed that both infected soyabean leaves and stems yielded infective bacterial cells after about 2½ months of harvesting the crop; the trash was collected from the field about 6 months after the crop had been harvested and was then stored in polyethylene bags for 15 months before use. The infectiousness of both the stem and leaf trashes appeared high when seeds were soaked in inocula derived from them (bacterial suspensions) for

short periods (Fig. 5). The infectivity of inoculum derived from stem trash was highest when seeds were soaked in it for 1 hour, while that from leaf trash was highest when seeds were soaked in it for 2 hours. Infectivities of inocula from both types of trash were similar after seeds had been soaked in them for 3, 6 and 12 hours. Generally, prolonged soaking appeared to depress infectiousness of the trash.

Lehman and Poole (1929) found that soyabean bacterial pustule organism was viable in dry diseased leaves as well as in partially decayed leaves left outdoors from one season to another. Lehman (1929, 1931) showed that the pustule organism remained viable and pathogenic for at least 9 months in diseased leaves stored in the laboratory and for 3 months in the leaves exposed to weathering and decay. Graham (1952) found that the bacterium could be isolated from soyabean leaves kept dry in laboratory for 8 months. Graham (1953) again discovered that the bacterium could be detected in seeds up to 30 months after harvest.

There was no bacterial pustule infection on any of the seedlings grown from seeds soaked in the suspension derived from infested soil, at 30 days after sowing. This suggests that the bacterium Xanthomonas phaseoli var. sojense could not persist in the field

soil through the 24 months period. The bacterium has only been reported to persist in sterile soil for 9 months (Graham 1953). There was no bacterial pustule infection on any of the seedlings grown from seeds soaked in sterile distilled water.

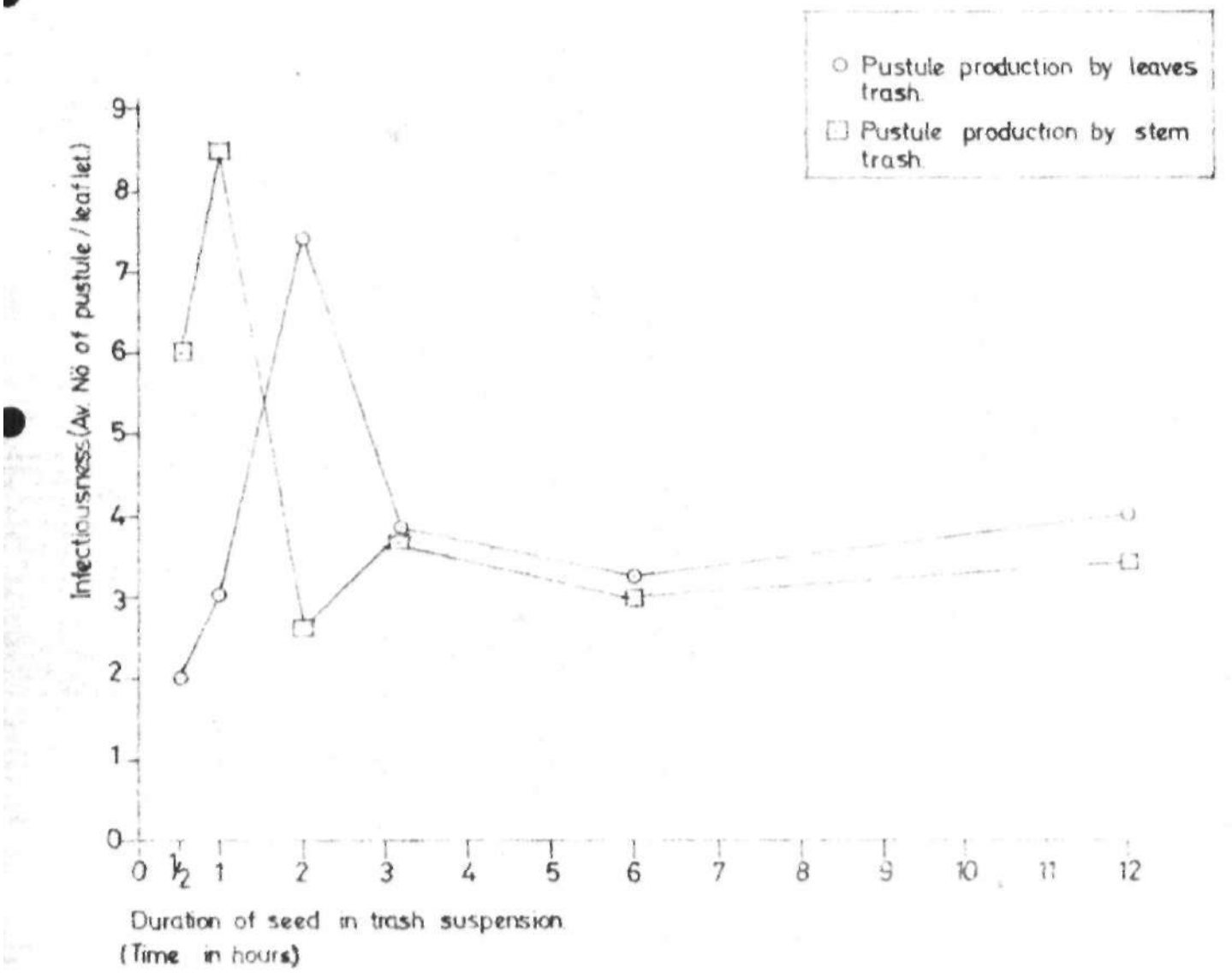


Fig 5 Infectiousness of 1975 infected trash (Pustule production by leaves and stem trash)

B. Comparison of Infectiousness of 1975 and 1977 Soyabean Trash Infected by X. phaseoli var sojense.

Materials and Methods

Soyabean trash from a 1975 crop severely infected by X. phaseoli var. sojense (see Section A above) was compared with trash obtained from line $35^{S}/58$ also severely attacked by bacterial pustule in Samaru in 1977.

(i) Preparation of inocula.

Fifteen grammes of leaves and stems were macerated with mortar and pestles and added into 25 ml sterile water contained in 100 ml conical flasks. After three hours, the fragments of trash were filtered off using a whatman No. 1 filter paper.

(ii) Test plants.

Excised soyabean leaves of the susceptible line $35^{S}/58$ were used for the tests. The leaves were detached from similar positions on stems so that they were all approximately of same age. The bases of the petioles were trimmed off with a sterile razor blade, they were dipped into rooting hormone No. 1 (manufactured by May and Baker Ltd.) before planting in 44 gm. of moist sterile sand contained in 7.5 x 2.5 cm glass tubes.

(iii) Inoculation.

The leaves were inoculated with the inocula 12 days after they had been detached, by which time new roots had developed from the petiole. About 20 ml of each inoculum was sprayed on five leaves (giving a total of 15 leaflets) using an atomiser. Spraying was directed more at the under-surface of the leaflets than the upper-surface.

Five leaves were inoculated with sterile distilled water to serve as control. Inoculated leaves were incubated for 7 days in a polyethylene covered humid chamber, 90 x 60 x 30 cm which was kept humid by placing two open beakers containing water in the enclosed space and by watering the leaves, twice daily.

After 10 days from inoculation readings were taken on the number of infected leaflets, and the number of pustules produced by each inoculum source for either year. On the basis of 15 leaflets inoculated with each infected trash, the percentage infection and the average number of pustules per leaflet were calculated.

Results and Discussion

The results showed that the 1975 infected leaf trash produced the same percentage infection (100%) as that produced by the freshly infected leaf trash of 1977. But the latter produced more pustules (22.3 per leaflet) than the 1975 infected leaf trash (10.3 per leaflet) (Table 4, Figs. 6 & 7).

The 1977 infected stem was more infectious (13.3% higher) than the 1975 infected stem. Also, the average number of pustules produced by the 1977 stem trash (5.3) was higher than that of the 1975 stem trash (3.5). No pustules were produced on leaflets sprayed with distilled water.

The reduced infectivity of the 1975 trash might be related to the antagonistic effects of saprophytes which may result from the environment and the period of storage. The viability of the organism itself had also probably been reduced.

Table 4: Comparison of infectiousness of 1975 and 1977 trash infected by X. phaseoli var. sojense.

Year	Type of trash	% of leaflets infected	Ave. No. of pustules/leaflets
1975	stem	66.7	3.5
1977	stem	80.0	5.3
1975	leaf	100.0	10.3
1977	leaf	100.0	22.3
Distilled water (control)		0.0	0.0

Figure 6: Bacterial pustules produced from 1975 soyabean leaf trash inoculum.



Figure 7: Bacterial pustules produced from 1977 soyabean leaf trash inoculum.
(Note the production of more pustules).

SECTION 5: EFFECT OF SOWING DATE ON THE INCIDENCE AND SEVERITY OF BACTERIAL PUSTULE.

A. Three Cultivars/lines Sown at Four Different Dates.

Materials and Methods

Three cultivars/lines $35^{S}/58$, M288 and CNS were sown on the flat in the field at Samaru, on four dates (D_1 - D_4) at two-week intervals beginning on the 17th of June, 1977. Two seeds were sown per hole 2.5 - 3.0 cm deep at a spacing 15cm within rows and 2m between rows. Each treatment was replicated three times.

Experimental design

A split plot design (Fig. 8) was used in which cultivars were independently randomised within each plot.

The land had received 63.75 kg/ha broadcast application of single superphosphate 10 days before planting. Seedlings were thinned down to one per stand 28 days after emergence; resulting in 12 plants per row. Incidence and severity of bacterial pustule following natural infection were recorded two times for each plot at 63 and 77 days after sowing (corresponding to periods of rapid vegetative growth and early flowering, respectively). Ten plants (one plant from the end of each row excluded) were scored according to the method described

in the I.I.T.A. scale (Williams and Allen, 1976) modified as follows:

Score 1 = No symptoms observed (immune)

Score 2 = Very few pustules scattered on less that ½ of the leaflets (highly resistant)

Score 3 = Very few pustules scattered on most of the leaflets (resistant)

Score 4 = Small areas (about 25%) of leaflet surface having heavy concentration of pustules, but on less than ½ of the whole leaflets (moderately resistant)

Score 5 = Small areas (about 25%) of leaflet surface having heavy pustule concentration and on most of the leaflets (moderately susceptible)

Score 6 = Most of the leaflet surface, of less than ½ of the leaflets having heavy pustule concentration (susceptible)

Score 7 = Most of the leaflet surface, of most of the leaflets covered with heavy pustule concentration, plus yellowing and leaf-fall (highly susceptible).

Results and Discussion

Results at 63 days after sowing show that the number of infected plants (Table 5) of lines $35^S/58$ and M288 was progressively higher in late-sown plots than in early-sown plots. There was no disease on CNS in plots sown between 17th June and 15th July, 1977.

Analysis of both the raw and square root transformed data of the disease severity scores revealed that there was a significantly higher ($p = 0.01$) disease severity on $35^S/58$ than on the other two cultivars (CNS and M288) for all sowing dates (Fig. 9; Appendix IA). The disease severity in the late plots (D_3 and D_4) of cultivar M288 was significantly greater ($p = 0.01$) than in early-sown plots (D_1 and D_2). Disease severity on CNS in the last sown plot (D_4) was found to be significantly higher ($p = 0.01$) than that in the other plots. There was a significant ($p = 0.01$) interaction between sowing dates and the cultivars/lines.

Sowing dates:

D_1 D_2 D_3 D_4 (for $35^S/58$)

D_1 D_2 D_3 D_4 (for M288)

D_1 D_2 D_3 D_4 (for CNS)

Sowing dates joined by the same line do not differ significantly in disease severity at $p = 0.01$.

At 77 days after sowing (Table 6), the disease incidence on lines $35^S/58$ and M288 was found to increase in late-sown plots than in early-sown plots. There was no disease in plots of CNS sown on 17th June and 1st July 1977.

Statistical analysis of the disease severity scores revealed that the disease severity on $35^S/58$ and M288 for all sowing dates was significantly greater ($p = 0.01$) than that on CNS (Fig. 10; Appendix IB). Disease severity on CNS, for the last two sowing dates (D_3 and D_4), was significantly higher ($p = 0.01$) than that in the plots sown earlier (D_1 and D_2).
The interaction between sowing dates and the cultivars/lines was significant ($p = 0.05$).

Sowing dates:

D_1	D_2	D_3	D_4	(for $35^S/58$)
D_1	D_2	D_3	D_4	(for M288)
D_1	D_2	$\underline{D_3}$	$\underline{D_4}$	(for CNS)

Sowing dates joined by the same line do not differ significantly in disease severity at the 1% level of probability.

Generally, the severity of the bacterial pustule disease was highest on the soyabean line $35^{S}/58$ in all sowing dates; it was intermediate on the cultivar M288 and least on the cultivar CNS. These differences may probably be due to the fact that the soyabean line $35^{S}/58$ is a highly susceptible line, while cultivar M288 is moderately susceptible (Section 6). The cultivar CNS is known to be resistant to bacterial disease (Hartwig and Johnson, 1953).

The yield data (Fig. 11) show that the earliest sown plot (D_1) produced the highest seed weight for all cultivars/lines while later sowing dates had lower seed weights.

The above data strongly suggest that the earliest sowing date (D_1) produced the best result for all the cultivars/lines grown.

Though there were other factors which might have contributed to the reduced yields, in late plots, disease severity might have contributed.

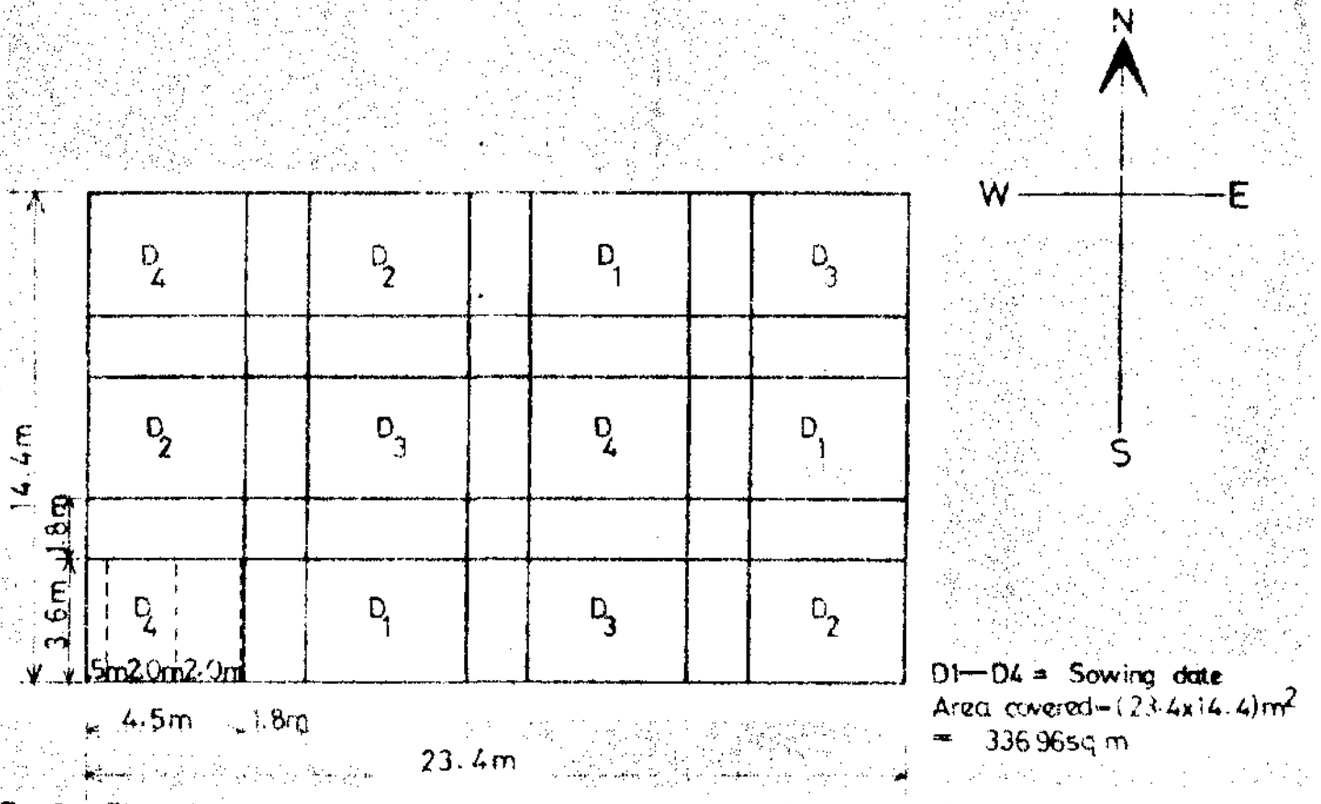


Fig 8 The effect of sowing date on the incidence and severity of the bacterial pustule of soyabeans

Table 5: Effect of sowing date on the incidence of bacterial pustule on three soyabean cultivars/lines, 63 days after sowing.

Sowing date	% infection on cultivars/lines:-		
	CNS	M288	$35^{S}/58$
D_1 (17/6/77)	0	4.2	40.4
D_2 (1/7/77)	0	5.9	56.3
D_3 (15/7/77)	0	18.8	59.2
D_4 (29/7/77)	13.3	31.3	82.4

Table 6: Effect of sowing date on the incidence of bacterial pustule on three soybean cultivars/lines, 77 days after sowing.

Sowing date	% infection on cultivars/lines:-		
	CNS	M288	$35^{S}/58$
D_1 (17/6/77)	0	8.3	55.5
D_2 (1/7/77)	0	29.4	69.4
D_3 (15/7/77)	20.0	37.5	86.1
D_4 (29/7/77)	23.3	46.9	88.2

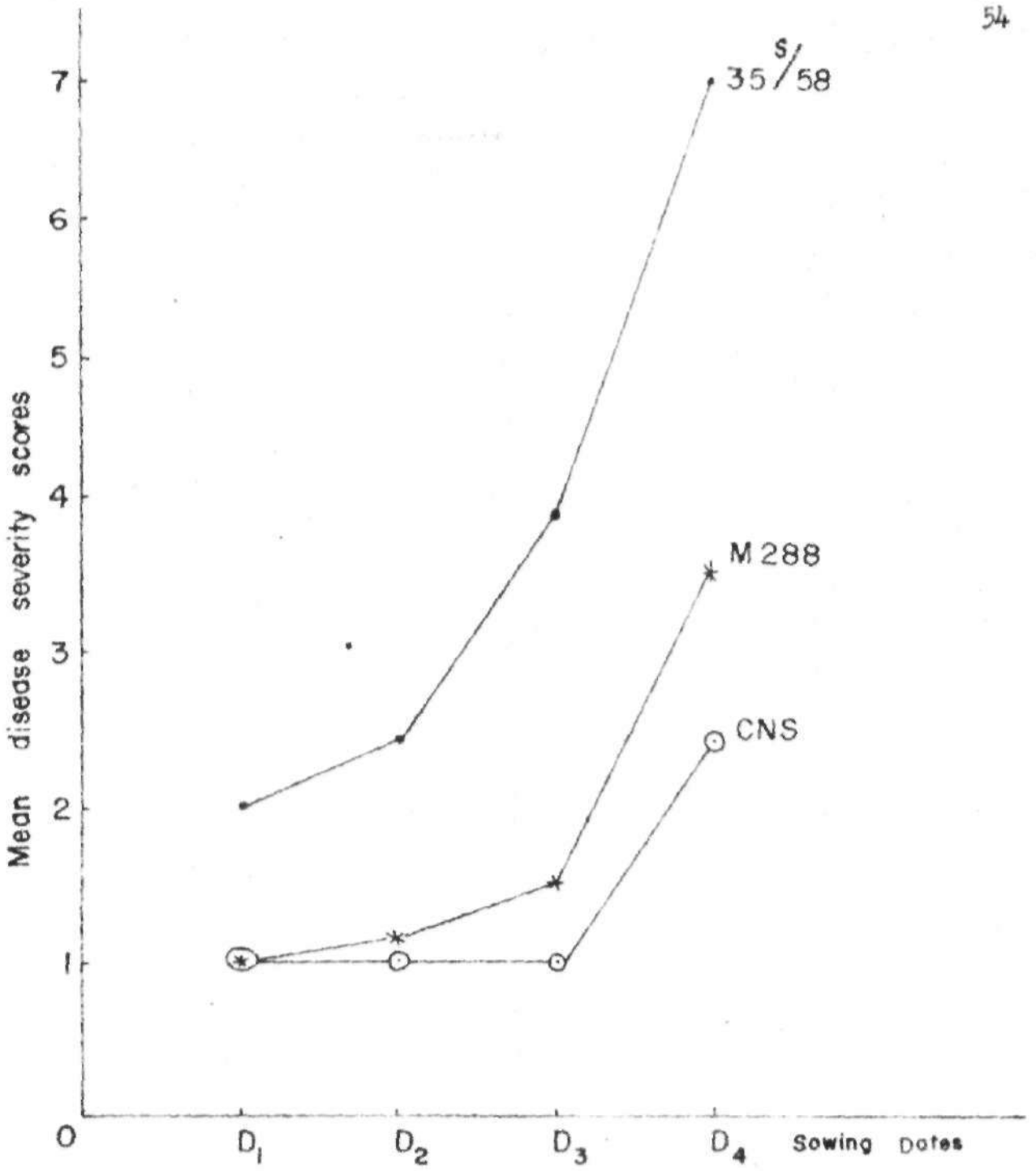


Fig 9 Effect of sowing date on severity of bacterial pustule on 3 Soyabean cultivars / lines at 63 days after sowing.

D_1 D_2 D_3 D_4 Plots planted on:
17-6-77, 1-7-77, 15-7-77, 29-7-77 respectively.

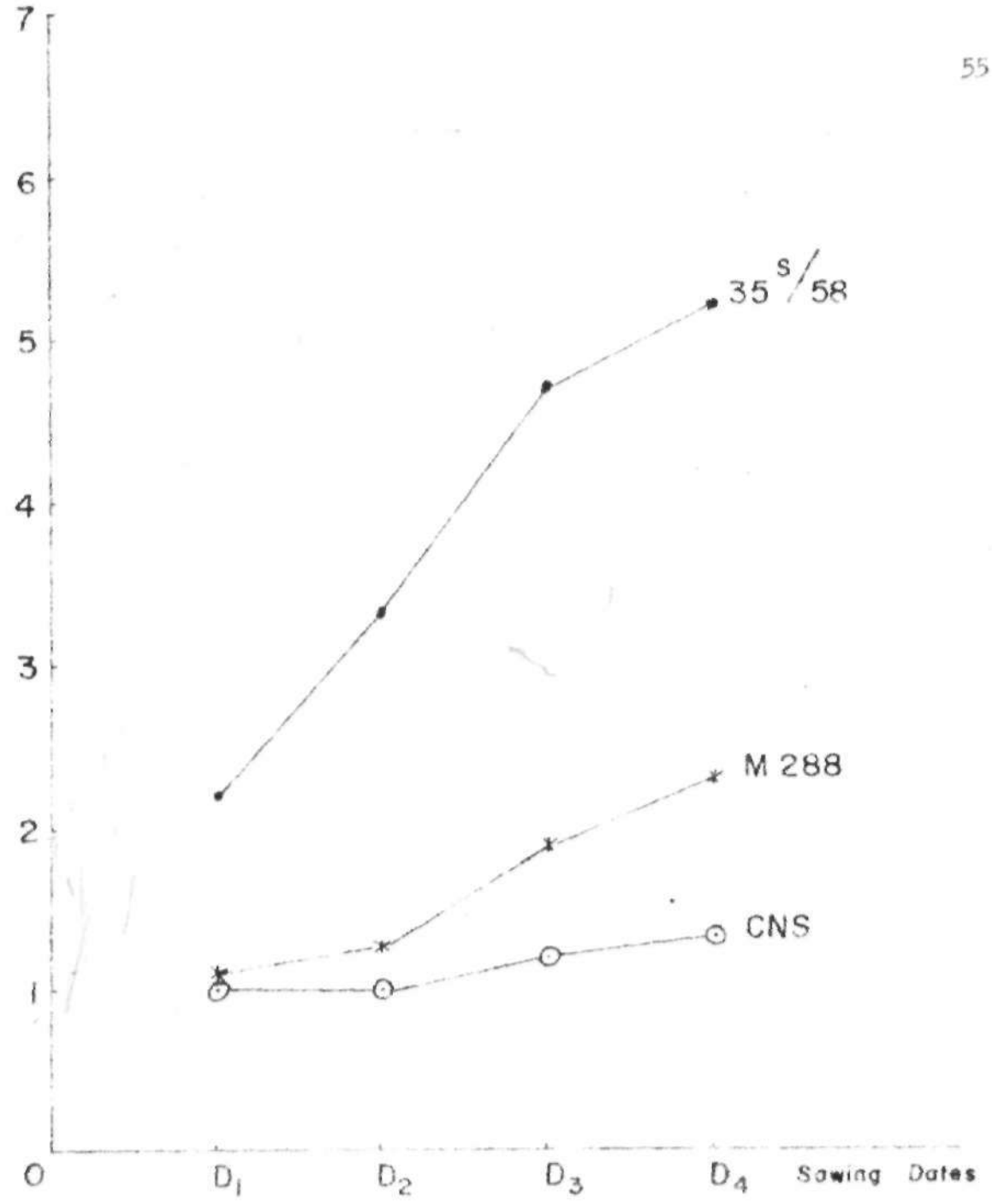


Fig 10 Effect of sowing date on severity of bacterial pustule on 3 Soyabean cultivars/lines at 77 days after sowing.

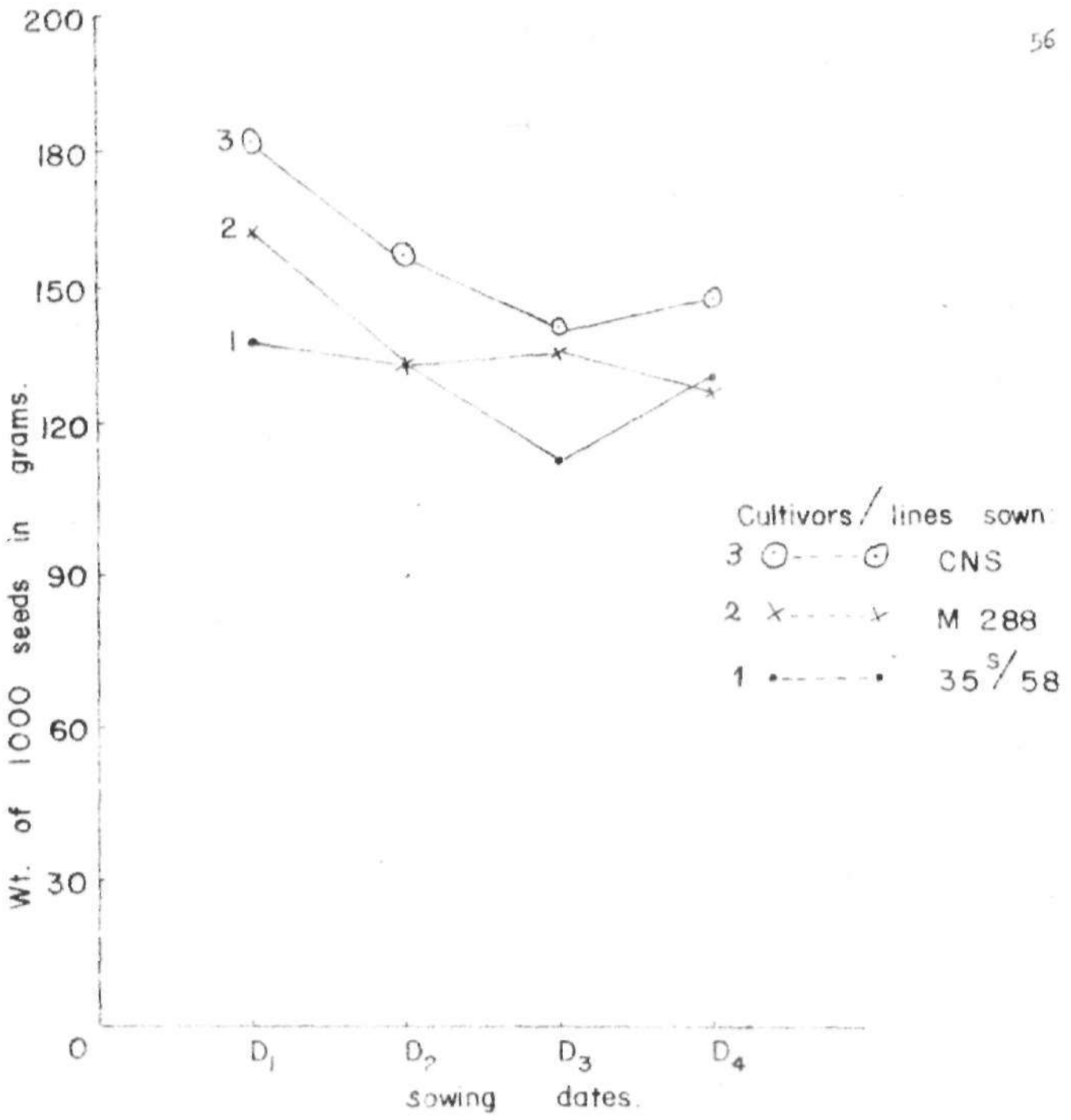


Fig. II Effect of sowing date on the seed yield of 3 Soyabean cultivars / lines.

B. <u>One Line Sown at Two Different Dates.</u>

It was thought necessary to establish an observation plot (near the Laboratory) for the same experiment described in Section 5A above, because of possible crop failure in field experiments which may result from adverse weather conditions.

<u>Materials and Methods</u>

A single soyabean line $35^{S}/58$ was chosen for this experiment, because it is a known susceptible line. The seeds were sown on a 21.6 x 7.2 sq m piece of land. The area was divided into two equal parts; one half was planted on 16th June and the other on 16th July, 1977. Two seeds of the soyabean line $35^{S}/58$ were sown per hole 2.5 - 3.0 cm deep, at a spacing 15cm within rows, on ridges 1 metre apart and 30cm high.

For each treatment, disease incidence was recorded twice at 74 and 88 days after sowing; and the disease severity scores were recorded six times at 46, 60, 74, 88, 102 and 116 days after sowing. Using the method described in Section 6, a total of 100 tagged plants were scored in each block at 10 plants per ridge.

At 140 days from sowing, the plants were harvested. Ten randomly selected plants (one from each ridge) were lifted. All pods from each plant were collected and bulked for each treatment. The pods were threshed in jute sacs and all the seeds were picked and weighed. Also, from each lot of the harvests, 1000 seeds were randomly picked, counted and weighed in order to provide an alternative method for assessing yield.

Results and Discussion

The results of this experiment (Table 7; Fig. 12) agree with those reported in Section 5A; in which the early sown soyabean plants had the least of the bacterial pustule while the late-sown plants had the most. The yields of early sown plants were also better than those of the late sown plants (Table 8).

Table 7: The incidence of bacterial pustule disease on the soyabean line $35^{S}/58$ sown in June and July, 1977.

Sowing Date	% Incidence of Infection	
	74 days after sowing	88 days after sowing
June 16, 1977	33	100
July 16, 1977	100	100

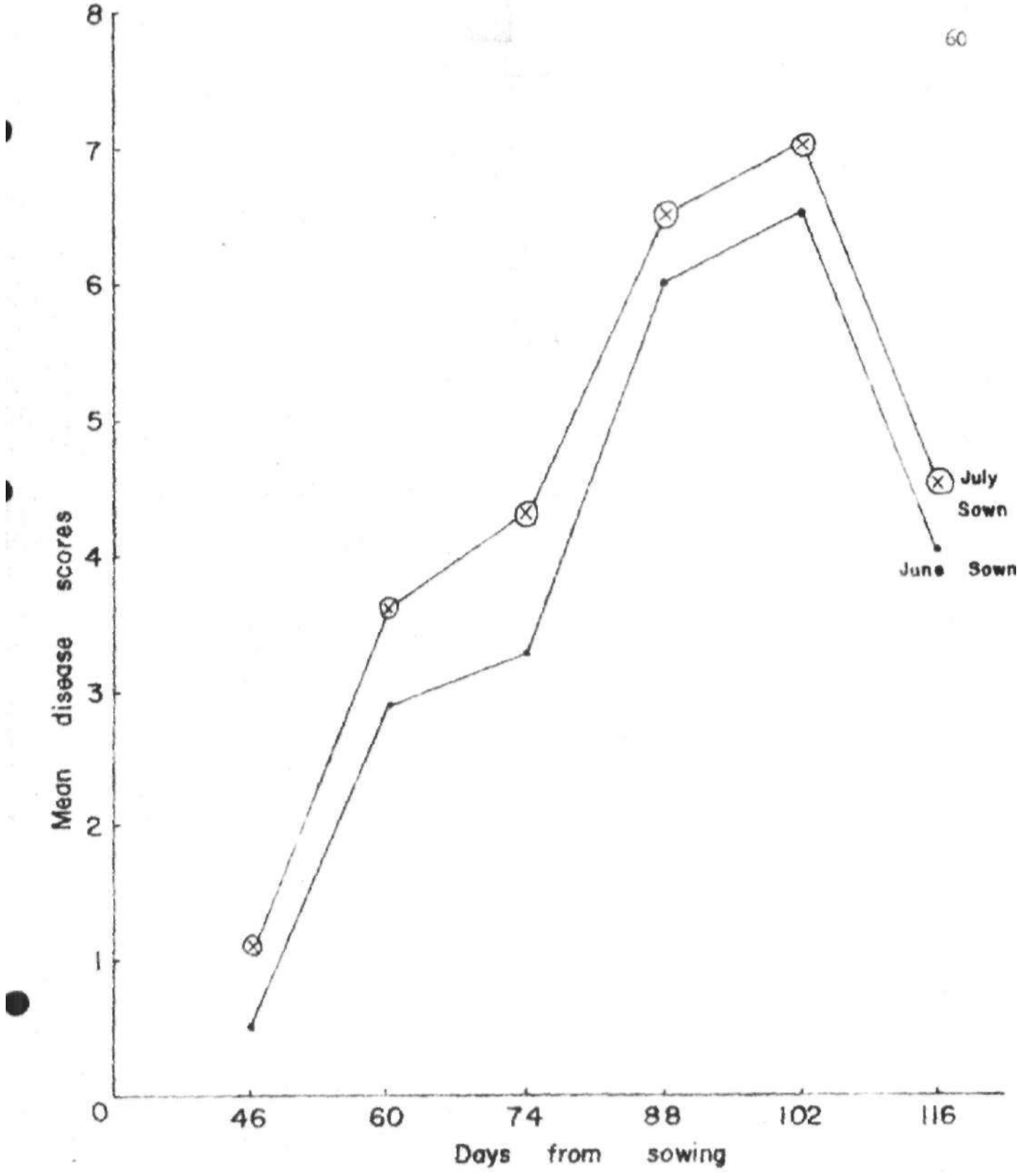


Fig. 12 Effect of sowing date on severity of bacterial pustule on the Soyabean line $35^{S}/58$

Table 8: The yields of $35^{S}/58$ soyabean line sown in June and July, 1977.

Sowing date	Seed wt. of 10 plants (gm)	Wt. of 1000 seeds (gm)
16th June, 1977	106.1	95.9
16th July, 1977	90.0	78.8

SECTION 6

SCREENING OF THE SOYABEAN CULTIVARS OR LINES FOR RESISTANCE TO BACTERIAL PUSTULE DISEASE

SECTION 6: SECREENING OF THE SOYABEAN CULTIVARS OR LINES FOR RESICTANCE TO BACTERIAL PUSTULE DISEASE

A. The Response Of 64 Soyabean Cultivars/Lines To The Bacterial Pustule Disease In The Field.

Introduction.

Many workers (Lehman, 1944; Petty, 1944; Lehman, 1946 and 1948 Thirumalachar et. al., 1956; Oksent'yan and Gunina, 1968; Rao and Patel, 1973) have investigated the use of chemical protectants for the control of bacterial pustule but found this method rather uneconomic. The testing of resistant cultivars/Lines has therefore attracted considerable attention and many resistant cultivars develop in other parts of the world have made a great impact on soyabean production in those areas. Investigations at International Institute of Tropical Agriculture, Ibadan have shown that some of these cultivars/Lines are resistant to bacterial pustule in southern Nigeria, similar investigations have not been conducted in the savannah zones of Nigeria where climatic and ecological factors are different from those around Ibadan. This Section is therefore an attempt to conduct similar investigation.

Materials and Methods

(i) Field preparations and planting.

A piece of land 820.8 sq. m. in an area located at the University Farm, Ahmadu Bello University, Zaria was used. The plot received 63.75 kg/ha of single superphosphate which was broadcasted and worked into the soil during tillage, 10 days before planting. A herbicide, metolachor ("Dual") manufactured by Ciba Geigy, was applied at the same time, at the rate of 5 litres of the chemical (2.5% a.i.) in 125 litres of water per hectare.

Seeds of 64 soyabean cultivars/lines were sown on 16th June 1977, on shallow ridges, 23cm high and 6.6 metre long; seeds (three per hole) were sown at a depth of 3cm. They were spaced 15cm within rows and 1.8 metres between the rows, giving a plant establishment of 44 seedlings per line or cultivar on each ridge.

The experimental design used (Fig.13) was the triple lattice (8 x 8). The randomisation was done in three stages:

The blocks were randomised separately and independently within each replication.

The treatments were randomised separately and independently within each block.

The treatments were alloted to the treatment numbers at random.

At both ends of each ridge, a disease spreader (line $35^{S}/58$, known to be susceptible to bacterial pustule disease) was sown. The experiment was replicated three times.

Twenty-eight days after planting, seedlings were thinned to a single plant per stand and were inoculated 2 days later.

(ii) Inoculation.

The inocula used were prepared from diseased leaflets collected from Ibadan and Zaria (during the 1976 planting season) and stored in polyethylene bags in the laboratory. The leaflets from each site were macerated in a mortar and soaked in tap water in beakers for 3 hours and then filtered through two layers of filter paper (Whatman No.1) into 500 ml flask. The presence of bacteria was confirmed under the high-power of a compound microscope. For each ridge, the disease spreader and the plant closest to it were inoculated by spraying with both a sprayer of 600 cc (BP, Canyon No.22712) and an atomizer (10 spraying/plant) at 5 p.m.

Spraying was concentrated on the undersurface of the leaflets. A total of three inoculations at 15 days interval, were made using fresh but severely infected leaflets for the second and third inoculations.

(iii) Scoring:

For each cultivar/line, a continuous row of 10 plants, selecting from about the middle portion of the ridge, were tagged with polyethylene ropes (obtained by cutting polyethylene sheets (30×20^2) into narrow strips or ropes). The tagged plants were scored five times at forthnightly intervals, through a period of 10 weeks using the method described in Chapter 5.

Bacterial pustule disease severity was found to correspond with the flowering stage and maximum vegetative growth. This occurred at about 98 - 106 days from sowing. Though a total of 5 scores were made, only the disease scores obtained at 98 days from sowing (at maximum disease build-up) were used for the calculations and the assessment of the plant reactions.

The disease severity scores obtained at the above period, for the individual plants in each cultivar/line, were added and divided by 10 (total number scored) to obtain the average score for the cultivar/line in a block. To obtain the mean score for the cultivar/line, the mean of the 3 block average scores of the cultivar/line was computed.

(iv) Defoliation.

Defoliation counts were taken in September, during maximum disease development in the field; scores were made at 7-day intervals for 28 days, starting from 1st September, 1977 (when the plants were 76 days old). The defoliation counts consisted of collecting and counting all bacterial pustule affected leaves which fell from the individual tagged plants of each cultivar/line in a block. After each picking the mean number of fallen leaves was computed for each cultivar/line in each block. Then the average of the three blocks was calculated. The mean defoliation values were arranged with the mean disease scores of the corresponding cultivar/line and correlation analysis was carried out.

(v) Harvesting and yield estimations.

Harvesting started 130 days after sowing when the majority of the cultivars/lines were mature and dry. For each mature cultivar/line, 5 plants (among those tagged) were randomly selected, uprooted and collected in jute bags. The yield characters studied included: estimated seed weight per hectare and weight of 1000 seeds.

The seeds which were threshed in the jute bags were picked and weighed. Average seed weight per plant was calculated and on basis of plant spacing (equivalent to a population of 37,037 plants per hectare) seed weight per hectare was estimated for each cultivar/line in a block.

For each cultivar/line in a block, 1000 seeds were counted (by repeatedly taking a handful from jute bags and counting all seeds obtained) and weighed. The seed weights obtained for each cultivar/line in the block were added up and then divided by the number of blocks to obtain the mean weight for the cultivar/line.

All the characters studied were statistically analysed for their correlation with the disease severity scores.

B. Pot Experiment

Materials and Methods

After the field planting of the 64 soyabean cultivars/lines described in Section A above, 60 cultivars/lines whose seeds were still available, were further tested in plant nursery-type bags.

Four seeds of each cultivar/line were planted in perforated 23cm x 6cm black polyethylene bag which contained 2 kg of a 1:3 sand/humus mixture. For each cultivar/line there were four bags arranged in completely randomised block design. Fifteen days after planting, the seedlings were thinned to two seedlings per bag, giving a total of 8 seedlings per cultivar/line. The pots were placed in the open on a table, 1 metre high, outside the glasshouse, and watered daily.

At 28 days from sowing, all the soyabean seedlings were artificially inoculated with the bacterium by spraying, using both the atomizer and a sprayer of 600 cc. (BP, Canyon No. 228712). Both the upper and lower leaf surfaces were sprayed.

(i) Inoculum preparation.

Inoculum was prepared from severely infected fresh leaflets of $35^{S}/58$ collected from the field. The leaflets were macerated in a food chopper and then suspended in tap water and left for

2 hours, before filtering and use.

(ii) Scoring.

From the 20th day of inoculation, the seedlings were scored three times at two-week intervals using the above scale. Disease scores obtained at 69 days from sowing (when disease severity was maximum) were used to judge the soyabean reactions to the bacterial pustule disease. The scores of the individual plants in each cultivar/line were finally added up and then divided by the total number of plants scored to obtain the average score for the cultivar/line.

(iii) Harvesting.

The cultivars/lines matured between 99-114 days from sowing and the yield data obtained were: number of pods per plant and seed weight.

All the dry mature pods of a cultivar/line, were plucked into a paper bag (40 x 20 sg. cm) and the average pod per plant was obtained. The average number of pods per bag was calculated for each cultivar/line.

For each cultivar/line, pods collected in the paper bags were threshed and all the seeds were picked and weighed.

The average weight (gm) of seeds per plant was calculated. The average seed weight per pot was also calculated for each cultivar/line. The average number of pods and the seed yield (gm) obtained per line were both analysed for correlation with the disease severity scores.

Results and Discussion

The disease severity scores of the 60 cultivars/lines planted both in the field and in pots are compared in Table 9. Table 10 shows the relationship between disease severity and leaf defoliation. Fig. 14 shows leaf defoliation caused by the severity of bacterial pustule disease in the field. Correlation between yield components and disease scores are shown in Table 11. Table 12 further shows the relationship between the yield components and defoliation for the field plants. Data from potted plants showing the correlation analysis between the disease scores and the yield components are shown in Table 13. Data on Table 14 show that bacterial pustule disease can reduce soyabean yield, and Tables 15 and 16 show the soyabean reactions to bacterial pustule disease in the field and in pots respectively. Seedling reactions of potted plants to bacterial pustule disease are shown in Fig. 15. In figures 16 and 17, resistant and susceptible soyabean plants are compared.

Statistical analysis (Appendix II) showed that mean disease scores of potted and field plantings (Table 9) were not significantly different ($p = 0.05$). The relationship (Table 10) between the mean disease severity scores and the mean number of defoliated leaves, from the field sown plants revealed that there is a highly significant ($p = 0.01$) and positive correlation ($r = 0.77$) between them (Appendix III).

Generally, higher seed weight per hectare and higher weight of 1000 seeds were produced by resistant soyabean cultivars/lines than by most of the susceptible cultivars/lines in the field. There was a highly significant, negative correlation (Appendixes IV & V) between the mean disease severity scores and both the yield per hectare and the weight of 1000 seeds ($r = -0.55$ and -0.56 respectively). In other words, cultivars/lines with high amounts of disease had low yields while those with the least amount of disease produced higher yields (Table 11). Correlation analysis (Appendices VI & VII) between leaf defoliation and yield components also show similar results (Table 12).

Also, similar results (Appendices VIII & IX) were obtained from the pot plantings. The mean seed weight per plant and mean pod yield per plant were found to correlate negatively with the mean disease scores (Table 13) though their correlations ($r = -0.23$ and -0.09, respectively) are not significant ($p = 0.05$).

It was only in few cases that high yields were recorded on resistant cultivars/lines and low yield on the susceptible lines.

This could be due to the fact that the disease severity was greater in field planted than in potted plants.

Field grown plants were planted in June and probably

were more exposed to conditions conducive for disease development. The pot experiment was sown in July and despite the artificial inoculations and watering, the dry weather conditions which affected the long maturing cultivars/lines, supressed maximum disease spread.

Spraying with bacteriocides could have been a useful method to assess loss in yield due to this disease but no such chemicals were available. An attempt to indicate the importance of the bacterial pustule disease was therefore made by considering the seed yields of different plots of the individual cultivars/lines. In the pot experiment, the four plots of each of 13 cultivars/lines showed varying disease scores, with correspondingly varying seed yield. For each cultivar/line low seed weight was consistent with high amount of the disease while high seed weight corresponded with low amount of the disease (Table 14).

These results suggest that the bacterial pustule disease can reduce soyabean seed yield. Hartwiq and Johnson (1953) working at two locations, have shown that susceptible soyabean lines yielded 8 and 11% less than the resistant at Stoneville and Willard respectively. They also found that the susceptible lines produced seeds 3% smaller in size than the resistant lines at Stoneville,

and 4% smaller at Willard. Work at Stoneville and Mississippi have also shown yield reductions of 15% when susceptible soyabean lines are grown (Hartwiq, 1975).

In the field, out of the sixty-four soyabean cultivars/lines screened againstthe bacterial pustule disease, 23 were susceptible, 31 were resistant and 10 showed intermediate reactions (8 were moderately susceptible and 2 moderately resistant) (Table 15). The results of pot experiment with sixty of these cultivars/lines showed that 18 were susceptible, 13 resistant, 17 moderately susceptible and 12 moderately resistant (Table 16). None of the soyabean cultivars/lines was immune.

The results of this work have shown, inter alia, that the cultivars Bethel, Improved pelican, Mamloxi and Ross were susceptible while Avoyelles was moderately resistant. These agree with the report of Hymowitz et. al. (1976) in Canada.

The majority of the cultivars/lines found to be resistant in this work, are the collections obtained from Ibadan (I.I.T.A.). The major problem I experienced with these collections was poor germination while the problem with some Zaria (Shika) resistant collections was poor yield or excessively small seed size.

The cultivar Malayan currently used by farmers in Abuja and Makurdi areas is highly susceptible to bacterial pustule and

should be replaced by a resistant variety that possesses many of its desirable agronomic and physiologic characters. This would require the co-operative efforts of plant pathologists and soyabean breeders.

Pooled results of the screening work done in the field and in pots have shown that the following eleven soyabean cultivars/lines are resistant to bacterial pustule disease:

SB6^{S} TGX 62-2^{C}

SB6^{S} TGX 26-48^{D}

SB6^{S} TGX 16-7^{E}

TGM294-4-4268

SB6^{S} TGX 26-33^{D}

SB6^{S} TGX 68-6^{C}

SB6^{S} TGX 16-17^{E}

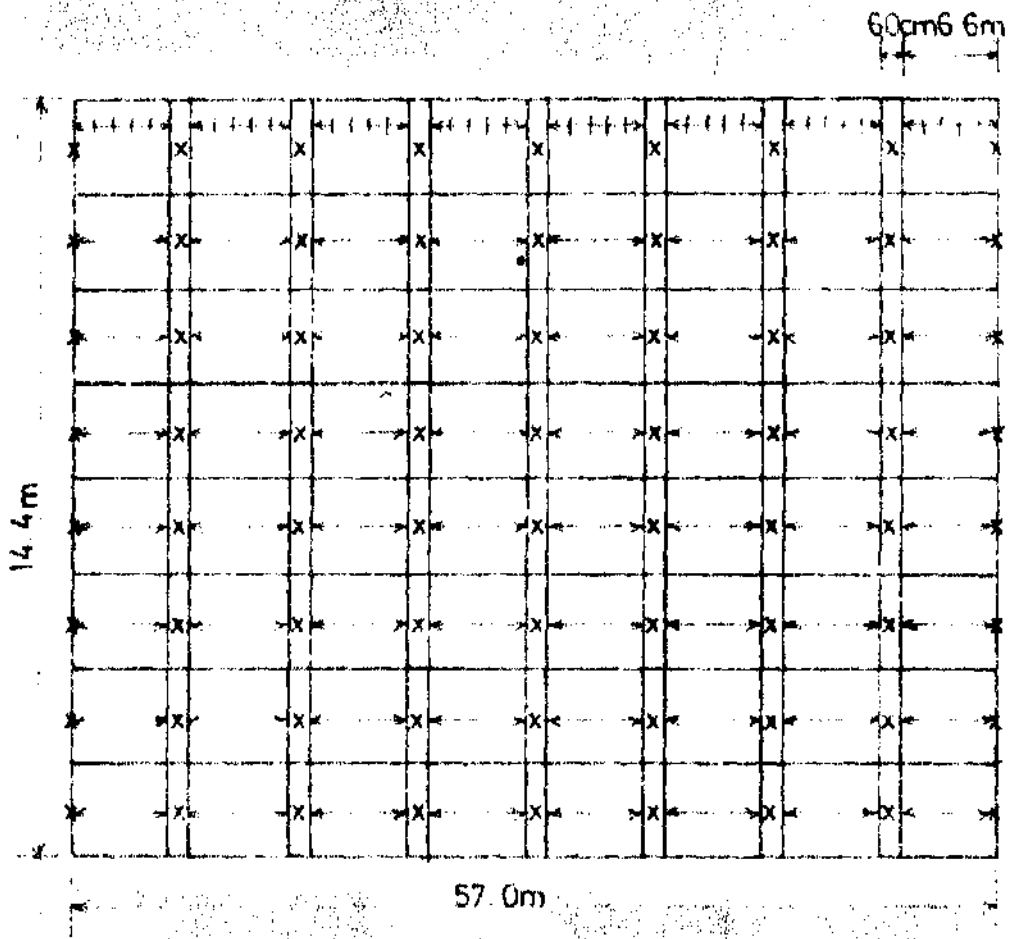
SB6^{S} TGX 57-1^{C}

Hale

SB6^{S} TGX 32-4^{D}

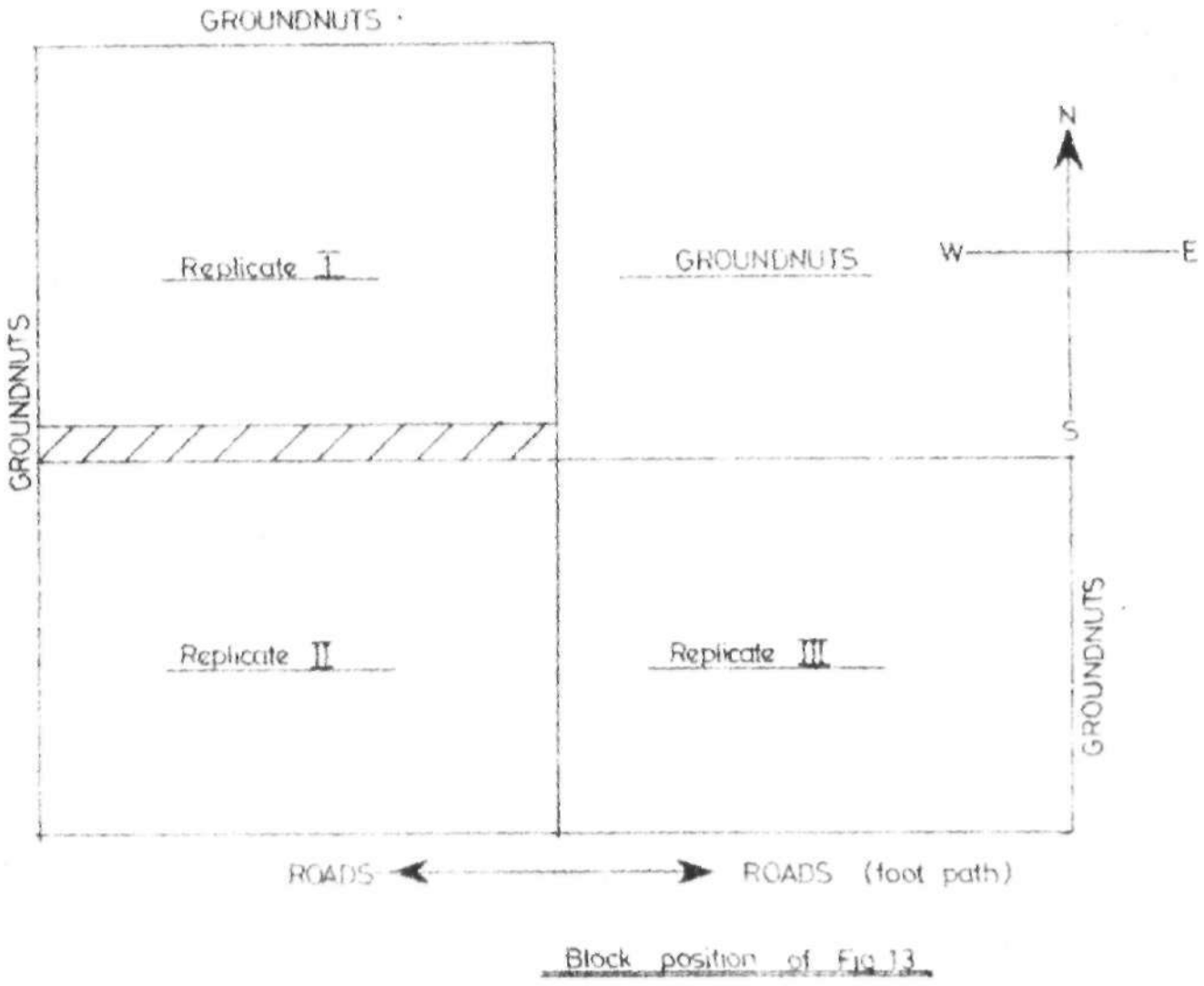
Congo yellow.

These can serve as sources of germplasm for the breeding and selection of cultivars/lines which can replace Malayan.



Between plant spacing 15cm
Between row spacing = 1.8m
Block size (57x14.4) m^2
= 820.8sq.m

Fig 13.
Field plan for the screening of soyabean cv. /Line5 resistant to bacterial pustle



Block position of Fig 13

Table 9: Comparison of the reactions to bacterial pustule disease of 60 soyabean cultivars/lines planted in the field and in pots.

Cultivars/lines	Mean disease Scores	
	From field plants (t_1)	From potted plants (t_2)
$SB6^S$ TGX 19-1 ^{D}	7.0	5.0
X_2B_7	7.0	6.1
Bathel	7.0	5.3
Malayan	7.0	6.8
Mamloxi	7.0	6.9
49-8	7.0	5.4
K138	7.0	6.0
K77	7.0	6.6
Improved Pelican	7.0	5.4
HLS-263	6.8	6.1
K197	6.7	5.6
Janfield	6.6	5.6
K157	6.6	5.1
M288	6.6	6.4
Hernon-49	6.5	6.8

Table 9 Contd.

Cultivars/lines	Mean disease Scores	
	From field plants (t_1)	From potted plants (t_2)
Ross	6.3	5.5
$35^S/58$	6.3	6.8
49-14	6.3	5.1
Trinidad	6.1	6.8
K39	5.9	4.5
K162	5.8	3.8
K53	5.8	4.5
Black matle Java	5.7	5.5
K152	5.2	3.3
49-9	5.1	6.8
3H55F4/167/2	4.9	3.0
$SB6^S$ TGX $2-22^E$	4.9	5.1
K12	4.6	6.3
3H55F4/1/2	4.5	4.3
Gilbert	4.3	3.6

Table 9 Contd.

Cultivars/lines	Mean disease scores	
	From field plants (t_1)	From potted plants (t_2)
$SB6^{S}$ TGX 11-4^{E}	3.4	2.9
$SB6^{S}$ TGX 26-33^{D}	3.3	5.4
$SB6^{S}$ TGX 11-13^{E}	3.3	4.3
Pitic GBX yalta	3.3	4.3
$SB6^{S}$ TGX 68-2^{C}	3.3	3.9
M281	3.2	5.4
M216	3.0	6.1
M79	3.0	3.6
TGM255-2-4341	3.0	4.9
$SB6^{S}$ TGX 11-39^{E}	2.8	5.3
M351	2.7	4.0
X_2S_{29}	2.5	2.6
$SB6^{S}$ TGX 32-4^{D}	2.5	3.5
Avoyelles	2.4	2.9
TGM 294-4-4268	2.4	3.9
$SB6^{S}$ TGX 57-2^{C}	2.4	2.8
$SB6^{S}$ TGX 68-6^{C}	2.4	2.8

Table 9 Contd.

Cultivars/lines	Mean disease score	
	From field plants (t_1)	From potted plants (t_2)
SB6^S TGX 57-1^C	2.4	5.5
SB6^S TGX 57-11^C	2.4	2.5
Congo yellow	2.3	3.4
SB6^S TGX 62-2^C	2.3	5.3
Java Forage	2.3	4.5
M353	2.2	5.0
M262	2.2	2.7
Hale	2.1	3.0
SB6^S TGX 16-7^E	2.1	3.4
SB6^S TGX 26-48^D	2.0	2.8
SB6^S TGX 16-17^E	2.0	4.0
M98	2.0	4.0
Fort Lamy	2.0	2.8

t = 1.342 Not significant.

(p = 0.05)

Table 10 : Relationship between disease severity scores and leaf defoliation of the 64 soyabean cultivars/lines planted in the field.

Cultivars/lines	Mean disease severity scores	Mean of defoliated leaves.
$SB6^{S}$ TGX 19-1^{D}	7.0	3.3
X_2B_7	7.0	3.7
Bathel	7.0	3.3
Malayan	7.0	2.7
Manloxi	7.0	3.8
49-8	7.0	2.9
K138	7.0	4.3
K77	7.0	5.7
Improved Pelican	7.0	3.4
HLS-263	6.8	3.4
K197	6.7	2.5
Janfield	6.6	2.8
K157	6.6	3.5
M288	6.6	3.5
Hernon-49	6.5	4.8

Table 10 Contd.

Cultivars/lines	Mean disease severity scores	Mean of defoliated leaves
Ross	6.3	2.9
$35^{S}/58$	6.3	3.4
49-14	6.3	4.5
Trinidad	6.1	2.4
K39	5.9	2.8
K162	5.8	3.3
K53	5.8	2.9
Black malte Java	5.7	2.4
$SB6^{S}$ TGX 11-12^{E}	5.3	2.2
K152	5.3	1.8
49-9	5.2	2.0
3H55F4/167/2	5.1	2.3
$SB6^{S}$ TGX 2-22^{E}	4.9	3.4
K12	4.9	2.8
3H55F4/1/2	4.6	2.9
Gilbert	4.5	1.5
$SB6^{S}$ TGX 11-4^{E}	4.3	2.3

Table 10 Contd.

Cultivars/lines	Mean disease severity scores	Mean of defoliated leaves
$SB6^{S}$ TGX 43-4^{C}	4.3	2.5
$SB6^{S}$ TGX 26-33^{D}	3.4	2.6
$SB6^{S}$ TGX 11-13^{E}	3.3	2.5
Pitic GBX yalta	3.3	2.1
$SB6^{S}$ TGX 31-5^{D}	3.3	1.7
$SB6^{S}$ TGX 68-2^{C}	3.3	1.8
M281	3.3	1.7
M216	3.3	2.7
$SB6^{S}$ TGX 11-11^{E}	3.1	1.9
M79	3.0	1.7
TGM255-2-4341	3.0	1.6
$SB6^{S}$ TGX 11-39^{E}	3.0	1.9
M351	2.8	1.6
X_2S_{29}	2.7	1.9
$SB6^{S}$ TGX 32-4^{D}	2.5	1.8
Avoyelles	2.5	1.9
TGM294-4-4268	2.4	1.9
$SB6^{S}$ TGX 57-2^{C}	2.4	1.8

Table 10 Contd.

Cultivars/lines	Mean disease severity scores	Mean of defoliated leaves
$SB6^{S}$ TGX 68-6^{C}	2.4	1.8
$SB6^{S}$ TGX 57-1 ^{C}	2.4	2.1
$SB6^{S}$ TGX 57-11 ^{C}	2.4	1.7
Congo yellow	2.4	1.7
$SB6^{S}$ TGX 62-2^{C}	2.3	1.9
Java Forage	2.3	1.9
M353	2.3	1.7
M262	2.2	1.7
Halo	2.2	2.1
$SB6^{S}$ TGX 16-7^{E}	2.1	2.3
$SB6^{S}$ TGX 26-48^{D}	2.1	1.6
$SB6^{S}$ TGX 16-17^{E}	2.0	1.9
M98	2.0	1.9
Fort Lamy	2.0	1.9

Correlation (r) = 0.77^{HS}

HS = Highly Significant.

(p = 0.01)



Figure 14: Defoliation caused by bacterial pustule disease.

Table 11 : Disease scores and yield components of 64 soyabean cultivars/lines planted in the field.

Cultivars/lines	Ranked mean disease score 98 days from sowing	Mean Yield (Characters studied)	
		Estimated mean seed yield/Hectare (kg) (Y_1)	Mean wt. of 1000 seeds (gm) (Y_2)
$SB6^S$ TGX 19-1^D	7.0	526.2	150.8
K_2B_7	7.0	271.2	92.1
Bathel	7.0	509.1	139.0
Malayan	7.0	299.5	83.5
Mamloxi	7.0	291.2	80.8
49-8	7.0	519.1	133.8
K138	7.0	272.3	88.3
K77	7.0	191.2	67.7
Improved Pelican	7.0	416.2	131.1
HLS-263	6.8	415.6	102.6
K197	6.7	340.6	119.6
Janfield	6.6	299.1	86.3
K157	6.6	284.1	102.6
M288	6.6	514.6	111.3
Hernon-49	6.5	381.2	141.0
Ross	6.3	351.2	101.3

Table 11 Contd.

Cultivars/lines	Ranked mean disease score 98 days from sowing	Mean yield (Characters studied)	
		Estimated mean seed yield/Hectare (kg) (Y_1)	Mean wt. of 1000 seed (gm) (Y_2)
$35^{S}/58$	6.3	345.6	93.4
49-14	6.3	483.4	124.1
Trinidad	6.1	347.6	83.4
K39	5.9	347.9	91.2
K162	5.9	499.1	137.2
K53	5.8	353.3	125.8
Black malte Java	5.7	324.5	94.1
$SB6^{S}$ TGX 11-12^{E}	5.3	479.5	153.6
K152	5.3	452.3	134.8
49-9	5.2	612.9	158.1
3H55F4/167/2	5.1	410.6	88.5
$SB6^{S}$ TGX 2-22^{E}	4.9	392.9	150.7
K12	4.9	386.2	118.0
3H55F4/1/2	4.6	357.9	84.6
Gilbert	4.5	379.5	126.8
$SB6^{S}$ TGX 11-4^{E}	4.3	430.1	157.7
$SB6^{S}$ TGX 43-4^{C}	4.3	467.9	124.9

Table 11 Contd.

Cultivars/lines	Ranked mean disease score 98 days from sowing	Mean yield (Characters studied)	
		Estimated mean seed yield/Hectare (kg) (Y_1)	Mean wt. of 1000 seeds (gm) (Y_2)
$SB6^S$ TGX $26-33^D$	3.4	534.6	150.3
$SB6^S$ TGX $11-13^E$	3.3	574.6	163.3
Pitic GBX Yalta	3.3	534.6	137.6
$SB6^S$ TGX $31-5^D$	3.3	587.3	158.7
$SB6^S$ TGX $68-2^C$	3.3	682.9	154.0
M281	3.3	905.2	148.9
M216	3.3	806.8	165.4
$SB6^S$ TGX $11-11^E$	3.1	659.0	178.8
M79	3.0	601.8	163.3
TGM 255-2-4341	3.0	560.7	161.0
$SB6^S$ TGX $11-39^E$	3.0	515.1	161.6
M351	2.8	366.7	112.4
X_2S_{29}	2.7	379.5	108.4
$SB6^S$ TGX $32-4^D$	2.5	807.4	206.7
Avoyelles	2.5	436.2	112.6
TGM 294-4268	2.4	397.3	150.3
$SB6^S$ TGX $57-2^C$	2.4	617.4	175.3

Table 11 Contd.

Cultivars/lines	Ranked mean disease score 98 days from sowing	Mean yield (Characters studied) Estimated mean seed yield/Hectare (kg) (Y_1)	Mean wt. of 1000 seeds (gm) (Y_2)
$SB6^S$ TGX 68-6^C	2.4	935.7	182.4
$SB6^S$ TGX 57-1^C	2.4	558.5	148.4
$SB6^S$ TGX 57-11^C	2.4	954.0	169.4
Congo yellow	2.4	510.7	111.3
$SB6^S$ TGX 62-2^C	2.3	486.2	156.6
Java	2.3	475.1	107.3
M353	2.3	697.4	114.3
M262	2.2	410.1	119.0
Hale	2.2	406.2	211.5
$SB6^S$ TGX 16-7^E	2.1	401.2	150.5
$SB6^S$ TGX 26-48^D	2.1	549.0	145.9
$SB6^S$ TGX 16-17^E	2.0	591.8	150.7
M98	2.0	557.3	147.0
Fort Lamy	2.0	477.3	106.5
Correlation = (r) =		-0.55^{HS}	-0.56^{HS}

HS = Highly significant.

($p = 0.01$)

Table 12: Defoliation and yield components of 64 soyabean cultivars/lines planted in the field.

Variety	Mean No. of Defoliated leaves (Y)	Wt. of 1000 seeds (gm) (X_1)	Seed yield (wt. in kg) (X_2)
$SB6^S$ TGX 19-1^D	3.3	150.83	526.22
X_2B_7	3.7	92.08	271.17
Bathel	3.3	138.98	508.99
Malayan	2.7	83.52	299.50
Mamloxi	3.8	80.83	291.17
49-8	2.9	133.80	518.99
K138	4.3	88.26	272.28
K77	5.7	67.66	191.15
Improved Pelican	3.4	131.10	416.19
HLS-263	3.4	102.58	415.64
K197	2.5	119.60	340.62
Janfield	2.8	86.26	298.95
K157	3.5	102.58	283.95
M288	3.5	111.28	514.55
Hernon-49	4.8	141.00	381.19
Ross	2.9	101.33	351.18
$35^S/58$	3.4	93.37	345.63

Table 12 Contd.

Variety	Mean No. of Defoliated leaves (Y)	Wt. of 1000 seeds (gm) (X_1)	Seed yield (wt. in kg) (X_2)
49-14	4.5	124.10	483.43
Trinidad	2.4	83.44	347.63
K39	2.8	91.23	347.83
K162	3.3	137.16	498.99
K53	2.9	125.77	353.40
Black malte Java	2.4	94.06	324.51
$SB6^{S}$ TGX $11-12^{E}$	2.2	153.57	479.54
K152	1.8	134.84	452.32
49-9	2.0	158.12	612.90
3H55F4/167/2	2.3	88.46	410.64
$SB6^{S}$ TGX $2-22^{E}$	3.4	150.71	392.86
3H55F4/1/2	2.9	84.55	357.85
K12	2.8	118.02	386.19
Gilbert	1.5	126.75	379.52
$SB6^{S}$ TGX $11-4^{E}$	2.3	157.69	430.09
$SB6^{S}$ TGX $43-4^{C}$	2.5	124.89	467.87
$SB6^{S}$ TGX $26-33^{D}$	2.6	150.28	534.55

Table 12 Contd.

Variety	Mean No. of Defoliated leaves (Y)	Wt. of 1000 seeds (gm) (X_1)	Seed yield (wt. in kg) (X_2)
$SB6^S$ TGX 11-13^E	2.5	163.27	574.56
Pitic GBX yalta	2.1	137.60	534.55
$SB6^S$ TGX 31-5^D	1.7	158.72	587.34
$SB6^S$ TGX 68-2^C	1.8	153.97	682.91
M281	1.7	148.59	905.18
M216	2.7	165.39	806.83
$SB6^S$ TGX 11-11^E	1.9	178.78	659.02
M79	1.7	163.26	601.79
TGM255-2-4341	1.6	160.97	560.67
$SB6^S$ TGX 11-39^E	1.9	161.60	515.10
M351	1.6	112.39	366.74
X_2S_{29}	1.9	108.36	379.52
$SB6^S$ TGX 32-4^D	1.8	206.73	807.38
Avoyelles	1.9	112.62	436.20
TGM 294-4268	1.9	150.28	397.30
$SB6^S$ TGX 57-2^C	1.8	175.30	617.35
$SB6^S$ TGX 68-6^C	1.8	182.40	935.74

Table 12 Contd.

Variety	Mean No. of Defoliated leaves (Y)	Wt. of 1000 seeds (gm) (X_1)	Seed yield (wt. in kg) (X_2)
$SB6^S$ TGX $57-1^C$	2.1	148.40	558.45
$SB6^S$ TGX $57-11^C$	1.7	169.40	954.00
Congo yellow	1.7	111.27	510.66
$SB6^S$ TGX $62-2^C$	1.9	156.58	486.21
Java Forage	1.9	107.25	475.10
M353	1.7	114.30	697.36
M262	1.7	119.00	410.08
Hale	2.1	211.53	406.19
$SB6^S$ TGX $16-7^E$	2.3	150.45	401.19
$SB6^S$ TGX $26-48^D$	1.6	145.89	549.00
$SB6^S$ TGX $16-17^E$	1.9	150.68	591.79
M98	1.9	147.04	557.33
Fort Lamy	1.9	106.49	477.32

Correlation = (r) = -0.46^{HS}, -0.53^{HS}

(HS) = Highly Singificant

($p = 0.01$)

Table 13 : Disease scores and yield components of 60 soyabean cultivars/lines, planted in pots.

Cultivars/lines	Ranked mean disease score at 69 days from sowing (X)	Characters studied	
		Mean seed wt. (in gm) (Y_1)	Mean pod yield (No.) (Y_2)
Mamloxi	6.9	2.3	12.4
Hernon-49	6.8	3.0	12.9
Malayan	6.8	0.7	7.5
$35^{S}/58$	6.8	0.10	3.0
3H55F4/167/2	6.8	1.0	10.6
Trinidad	6.8	1.7	16.3
K77	6.6	3.2	20.6
M288	6.4	0.3	5.3
3H55F4/1/2	6.3	0.7	14.9
HLS 263	6.1	0.2	6.5
$X_2{}^{B}7$	6.1	2.3	18.3
M79	6.1	1.9	10.1
K138	6.0	1.7	16.6
Janfield	5.6	3.4	23.3
K197	5.6	1.4	12.3
Ross	5.5	3.5	25.4

Table 13 Contd.

Cultivars/lines	Ranked mean disease score at 69 days from sowing (X)	Characters studied	
		Mean seed wt. (in gm) (Y_1)	Mean pod yield (No.) (Y_2)
$SB6^S$ TGX 57-11 ^{C}	5.5	0.2	3.6
Black Malte Java	5.5	3.5	26.5
Improved Pelican	5.4	2.6	16.3
49-8	5.4	3.1	11.1
M216	5.4	1.4	10.4
$SB6^S$ TGX 11-13 ^{E}	5.4	0.3	3.0
M351	5.3	2.4	14.7
Java forage	5.3	2.1	15.4
Bathel	5.3	3.6	13.5
K152	5.3	2.2	14.8
K157	5.1	3.2	21.4
49-14	5.1	2.8	12.1
K12	5.1	4.4	23.4
M262	5.0	1.8	17.1
$SB6^S$ TGX 19-1 ^{D}	5.0	2.2	11.4
$SB6^S$ TGX 11-39 ^{E}	4.5	1.1	7.3
M353	4.5	1.1	7.3

Table13 Contd.

Cultivars/lines	Ranked mean disease score at 69 days from sowing (X)	Characters studied	
		Mean seed wt. (in gm) (Y_1)	Mean pod yield (No.) (Y_2)
K39	4.5	3.8	20.6
K53	4.5	4.0	17.3
$SB6^S$ TGX $68-2^C$	4.3	1.3	8.0
Pitic GBX yalta	4.3	2.1	19.1
Gilbert	4.3	2.4	16.1
X_2S_{29}	4.0	2.9	24.9
M98	4.0	3.5	19.1
Fort Lamy	4.0	2.5	17.3
$SB6^S$ TGX $57-2^C$	3.9	1.3	6.4
M281	3.9	2.5	11.6
K162	3.8	2.3	14.5
TGM 255-2-4344	3.6	0.4	4.2
$SB6^S$ TGX $11-4^E$	3.6	4.4	18.5
Avoyelles	3.5	1.5	12.3
$SB6^S$ TGX $62-2^C$	3.4	2.6	11.9
$SB6^S$ TGX $26-48^D$	3.4	3.6	13.5

Table 13 Contd.

Cultivars/lines	Ranked mean disease score at 69 days from sowing (X)	Characters studied	
		Mean seed wt. (in gm) (Y_1)	Mean pod yield (No.) (Y_2)
49-9	3.3	3.1	10.4
$SB6^S$ TGX 2-22^E	3.0	1.2	15.0
$SB6^S$ TGX 16-7^E	3.0	0.3	3.4
TGM 294-4-4268	2.9	1.5	13.5
$SB6^S$ TGX 26-33^D	2.9	3.2	17.1
$SB6^S$ TGX 68-6^C	2.8	1.2	10.1
$SB6^S$ TGX 16-17^E	2.8	2.6	16.8
$SB6^S$ TGX 57-1^C	2.8	3.7	16.3
Halo	2.7	2.3	10.6
$SB6^S$ TGX 32-4^D	2.6	2.9	18.1
Congo yellow	2.5	3.4	22.5
Correlation = (r) =		-0.23^{NS}	-0.09^{NS}

NS = Not significant.

(p = 0.05)

Table 14: Data (from pot plants) showing that bacteria pustule disease can reduce soyabean yield.

Variety/line	Plot No. (or pot no)	Mean Disease Scores	Mean Seed wt/gm (Yield)
$35^{S}/58$	1	7.0	0.9
	2	7.0	0.08
	3	6.5	0.15
	4	7.0	0.10
3H55F4/1/2	1	5.9	1.3
	2	5.3	1.8
	3	7.0	0.83
	4	7.0	0.53
Hernon 49	1	7.0	3.03
	2	7.0	3.10
	3	6.2	3.35
	4	7.0	2.5
$SB6^{S}$ TGX 11-13^{E}	1	7.0	0.10
	2.	5.0	0.45
	3	5.5	0.35
	4	4.0	0.45
M288	1	7.0	0.13
	2	7.0	0.35
	3	5.0	1.6
	4	6.5	0.44

Table 14 Contd.

Variety/line	Plot No. (or pot no)	Mean disease scores	Mean seed wt/gm (Yield)
Improved Pelican	1	4.0	3.69
	2	7.0	1.54
	3	5.0	3.66
	4	5.5	1.55
$SB6^{S}$ TGX 19-1 ^{D}	1	3.0	2.45
	2	6.0	2.0
	3	5.0	2.25
	4	6.0	2.05
X_2B_7	1	6.0	2.27
	2	4.5	3.15
	3	7.0	1.87
	4	7.0	1.75
K138	1	7.0	1.11
	2	4.0	3.21
	3	6.5	1.25
	4	6.5	1.28
Jenfield	1	5.5	3.91
	2	7.0	2.56
	3	6.0	2.77
	4	4.0	4.29

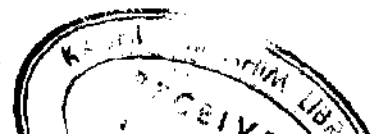


Table 14 Contd.

Variety/line	Plot No. (or pot no.)	Mean disease scores	Mean seed wt/gm (Yield)
K197	1	7.0	0.81
	2	4.5	1.92
	3	6.5	1.72
	4	6.0	1.81
Ross	1	5.0	4.19
	2	7.0	2.85
	3	7.0	2.87
	4	3.0	4.19
$SB6^{S}$ TGX $32-4^{D}$	1	2.0	2.70
	2	4.0	1.86
	3	2.4	2.60
	4	1.8	4.37

Table 15: Field reactions to bacterial pustule disease of 64 soyabean cultivars/lines at 98 days from sowing.

Highly susceptible	Susceptible	Moderately susceptible
$SB6^{S}$ TGX 19-1^{D}	Ross	$SB6^{S}$ TGX 11-12^{E}
X_2B_7	$35^{S}/58$	K152
Bathel	49-14	49-9
Malayan	Trinidad	3H55F4/167/2
Mamloxi	K39	$SB6^{S}$ TGX 2-22^{E}
49-8	K162	K12
K138	K53	3H55F4/1/2
K77	Black Malte Java	Gibert
Improved pelican		
HLS-263		
K197		
Janfield		
K157		
M288		
Hernon 49		

Table 15 Contd.

Moderately resistant	Resistant	Highly resistant	Immune
$SB6^{S}$ TGX 11-4^{E}	$SB6^{S}$ TGX 26-33^{D}	TGM294-4-4268	None
$SB6^{S}$ TGX 43-4^{C}	$SB6^{S}$ TGX 11-13^{E}	$SB6^{S}$ TGX 57-2^{C}	
	Pitic GBX Yalta	$SB6^{S}$ TGX 68-6^{C}	
	$SB6^{S}$ TGX 31-5^{D}	$SB6^{S}$ TGX 57-11^{C}	
	$SB6^{S}$ TGX 68-2^{C}	Congo yellow	
	M281	$SB6^{S}$ TGX 62-2^{C}	
	M216	Java Forage	
	$SB6^{S}$ TGX 11-11^{E}	M353	
	M79	M262	
	TGM255-2-4341	$SB6^{S}$ TGX 57-1^{C}	
	$SB6^{S}$ TGX 11-39^{E}	Hale	
	M351	$SB6^{S}$ TGX 16-7^{E}	
	X_2S_{29}	$SB6^{S}$ TGX 26-48^{D}	
	$SB6^{S}$ TGX 32-4^{D}	$SB6^{S}$ TGX 16-17^{E}	
	Avoyelles	M98	
		Fort Lamy	

Table 16: Reactions to bacterial pustule disease of 60 soyabean cultivars/lines planted in pots at 69 days from sowing.

Highly susceptible	Susceptible	Moderately susceptible
Mamloxi	M288	Improved pelican
Hernon-49	3H55F4/1/2	49-8
Malayan	HLS-263	M216
$35^{S}/58$	X_2B_7	$SB6^{S}$ TGX 11-13^{E}
Trinidad	M79	M351
3H55F4/167/2	K138	Java Forage
K77	Janfield	Bathel
	K197	K157
	Ross	K157
	$SB6^{S}$ TGX 57-11^{C}	49-14
	Black Matle Java	K12
		K262
		$SB6^{S}$ TGX 19-1^{D}
		$SB6^{S}$ TGX 11-39^{E}
		M353
		K39
		K53

Table 16 Contd.

Moderately resistant	Resistant	Highly resistant	Immune
$SB6^{S}$ TGX 68-2^{C}	$SB6^{S}$ TGX 62-2^{C}	None	None
Pitic GBX Yalta	$SB6^{S}$ TGX 26-48^{D}		
Gilbert	49-9		
X_2S_{29}	$SB6^{S}$ TGX 2-22^{E}		
M98	$SB6^{S}$ TGX 16-7^{E}		
Fort Lamy	TGM294-4-4268		
$SB6^{S}$ TGX 57-2	$SB6^{S}$ TGX 26-33^{D}		
K162	$SB6^{S}$ TGX 68-6^{C}		
M281	$SB6^{S}$ TGX 16-17^{E}		
TGM255-2-4341	$SB6^{S}$ TGX 57-1^{C}		
$SB6^{S}$ TGX 11-14^{E}	Hale		
Avoyelles	$SB6^{S}$ TGX 32-4^{D}		
	Congo yellow		



Figure 15: A set of potted soyabean plants inoculated with *X. phaseoli* var. *sojense*.

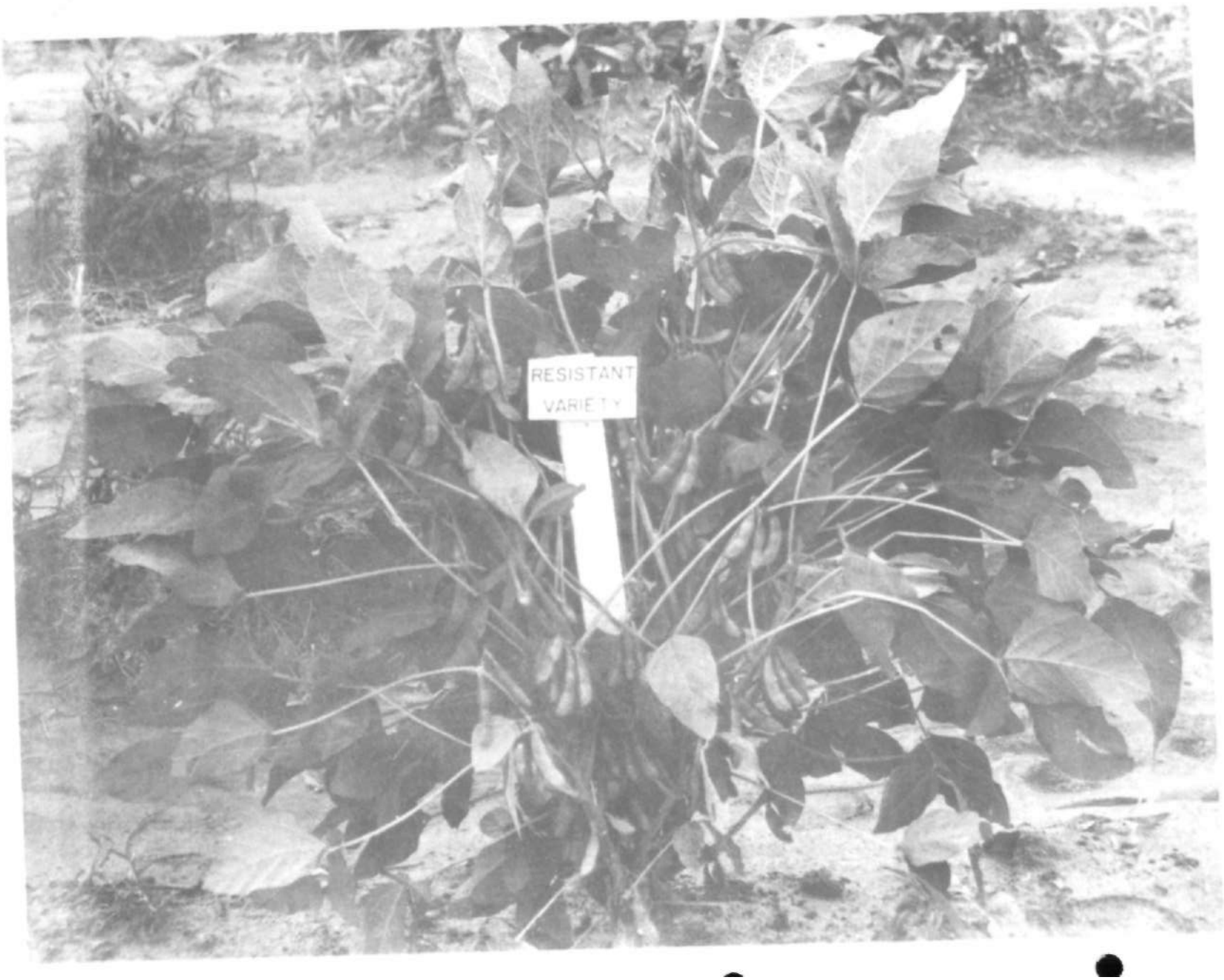

RESISTANT
VARIETY

Figure 16: A resistant soyabean cultivar.
(Note the absence of defoliation).



Figure 17: A susceptible soyabean cultivar.

SECTION 7

SUMMARY

SUMMARY

Bacterial pustule of soyabean caused by Xanthomonas phaseoli var sojense (Hedges) Starr & Burkholder was one of the serious diseases of that crop in all the areas visited.

The organism is seed-borne. Infected trash remained infectious after storage for 21 months. Infectiousness nevertheless declined with storage, as trash from a 1977 crop produced more disease than one from a 1975 crop. The leaves harboured a higher inoculum density than stems.

Soyabean cultivars/lines sown during the first two weeks of June had less bacterial pustule than those sown later.

Of the sixty cultivars/lines screened for resistance to bacterial pustule, 11 were resistant. A number of resistant cultivars/lines produced higher grain yields than susceptible (including Malayan, widely-grown in northern Nigeria) cultivars. Further research should therefore be conducted in order to select a better cultivar than the Malayan.

SECTION 8

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LITERATURE CITED

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SECTION 9

APPENDICES.

APPENDIX I: A: Severity of bacterial pustule on 3 soyabean cultivars/lines, 63 days after sowing.

Cultivars/lines	Sowing dates			
	D_1	D_2	D_3	D_4
$35^S/58$	4.53	4.91	6.16	8.40
M288	3.24	3.43	3.86	6.00
CNS	3.24	3.24	3.24	4.9

Analysis of variance for severity of bacterial pustule disease on 3 soyabean cultivars/lines ($35^S/58$, M288, CNS) at 63 days after sowing.

Source	D.F.	S.S.	M.S.	F
Replicates	2	0.08887	0.04443	1.49
Dates	3	43.478116	14.492705	485.65 **
Error A	6	0.17905	0.02984	
Variety	2	36.670827	18.335414	170.30 **
D x V	6	4.73472	0.78912	7.33 **
Error B	16	1.72264	0.10767	
Total	35	86.874218		

** Highly significant (p = 0.01)

Standard error of difference for sowing dates = 0.08143

Standard error of difference for cultivars/lines = 0.13396

LSD for sowing dates = 0.173

LSD for cultivars/lines = 0.1993

APPENDIX I: B: Severity of bacterial pustule on 3 soyabean cultivars/lines 77 days after sowing.

Cultivars/lines	Sowing dates			
	D_1	D_2	D_3	D_4
$35^S/58$	4.7	5.8	6.8	7.3
M288	3.3	3.7	4.4	4.8
CNS	3.2	3.2	3.5	3.7

Analysis of variance for severity of bacterial pustule disease on 3 soyabean cultivars/lines ($35^S/58$, M288, CNS) at 77 days after sowing.

Source	D.F.	S.S.	M.S.	F
Replicates	2	0.69432	0.34716	10.868
Dates	3	12.209116	4.06971	127.40 **
Error A	6	0.19166	0.03194	
Variety	2	48.937606	24.468803	135.47 **
D x V	6	3.99882	0.66647	3.69 *
Error B	16	2.89003	0.18063	
Total	35	68.921560		

** Highly significant (p = 0.01)
* Significant (p = 0.05)

Standard error of difference for sowing dates = = 0.08425
Standard error of difference for cultivars/lines = 0.17351
LSD for sowing dates = 0.21
LSD for cultivars/lines = 0.37

APPENDIX II: Analysis of variance for the comparison of disease scores from 60 soyabean cultivars/lines planted both in the field and in pots.

Sources of variation	Sum of squares	D.F	M.S.	F	Significance of F.
Treatments	0.249	1	0.249	1.801 NS	0.182
Residual	16.294	118	0.138		
Total	16.543	119	0.139		

NS = Not significant

APPENDIX III: Correlation analysis between disease severity scores and leaf defoliation of the 64 soyabean cultivars/lines planted in the field.

Analysis of variance	D.F.	S. squares	M.S	F
Regression	1	29.36249	29.36249	92.90502**
Residual	62	19.59501	0.31605	
Coeff. of variability	22.3			

Simple r = 0.7744**

** = Highly significant.

APPENDIX IV: Correlation analysis between seed yield (kg/h) and disease scores of the 64 soyabean cultivars/ lines planted in the field.

Analysis of Variance	D.F.	Sum of squares	M.S.	F
Regression	1	491 784.76851	491 784.76851	26.9385**
Residual	62	1131 861.336602	18255.82800	
Coeff. of variability	28			

Simple r = -0.5503536

** = Highly significant

APPENDIX V: Correlation analysis between disease scores and weight of 1000 seeds from each of the 64 cultivars/ lines planted in the field.

Analysis of variance	D.F.	Sum of squares	M.S	F
Regression	1	19863.67988	19863.67988	27.62903**
Residual	62	44574.42122	718.94228	
Coeff. of variability	20.4			

Simple r = -0.55521

** = Highly significant.

APPENDIX VI: Correlation analysis between seed yield in kg/h and the defoliation data of the 64 soyabean cultivars/ lines plant in the field

Analysis of variance	D.F.	Sum of squares	M.S.	F
Regression	1	10.54302	10.54302	16.52391**
Residual	62	39.55887	0.63805	
Coeff. of variability	31.8			

Simple r = 0.45873.

** = Highly significant.

APPENDIX VII: Correlation analysis between weight of 1000 seeds (gm) and defoliation data of the 64 soyabean cultivars/lines planted in the field.

Analysis of variance	D.F.	S.S.	M.S.	F
Regression	1	13.81926	13.81926	23.611444**
Residual	62	36.28263	0.58520	
Coeff. of variability	30.5			

Simple r = -0.52519

** = Highly significant

APPENDIX VIII: Correlation analysis between disease scores and pod yield (number of pods in each) of the 60 soyabean cultivars/lines planted in pots.

Analysis of variance	D.F.	S.S.	M.S.	F
Regression	1	15.99819	15.99819	0.46604 NS
Residual	57	1956.69130	34.32792	
Coeff. of variability	4.8			

Simple r = -0.0900546

NS = Not significant

APPENDIX IX: Correlation analysis between disease scores and seed yield (gm) of the 60 soyabean cultivars/lines planted in pots.

Analysis of variance	D.F.	S.S.	M.S.	F
Regression	1	4.17081	4.17081	3.25349^{NS}
Residual	57	73.07108	1.28195	
Coeff. of variability	51.2			

Simple r = -0.232719

NS = Not significant