

SCREENING FOR ROOT-KNOT NEMATODE
RESISTANCE IN MUNGBEANS

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CHAPTER I

INTRODUCTION

Most of the mungbeans, Phaseolus aureus Roxb., produced in the United States are grown in Oklahoma. Some production is found in north central Texas and small quantities in a few other states. Mungbeans are used mainly as canned or frozen bean sprouts. Before the 1940's, mungbeans were of interest to Oklahoma as a source of roughage and protein concentrate for animal feeding. The Oklahoma Agricultural Experiment Station conducted research in both the production and utilization of the crop for livestock production. During World War II, Oklahoma production shifted to the bean sprout industry.

The mungbean is a short season summer annual that can be seeded following wheat harvest to extend the use of the soil to obtain two cash crops per year. With adjustments, the same machinery required for wheat farming may be used with mungbeans. Following light tillage after wheat harvest, mungbeans are planted with the grain drill. At maturity, they are harvested with a combine, immediately cleaned by a local seed dealer, and marketed (17). Oklahoma's acreage has exceeded 50,000 acres in past years when mungbeans were used for livestock feed. In the 1960's, acreage varied from 35,000 to 45,000 acres.

July soil moisture in the producing counties is the primary determining factor in the acreage planted to mungbeans. Conditions are more favorable in areas having sandy soils that are capable of

taking up a maximum of moisture from intensive summer showers and where the humidity is relatively low (8).

Sandy soil is also a favorable habitat for the southern root-knot nematode, Meloidogyne incognita. No survey has been made to determine the loss of mungbeans due to root-knot nematode, but the symptoms exhibited by mungbeans are well known in the producing areas. Neither producers nor researchers have observed varietal differences in nematode damage, and no chemical treatments are used to reduce mungbean losses from nematodes. Occasionally, a mungbean crop is plowed under when it becomes apparent that the crop is a failure. A farmer sometimes substitutes another summer crop, such as forage sorghum, or summer fallows the land.

Nematode centers of infection are usually in spots over a field, and seldom do symptoms occur uniformly over large areas. Patch treatment can be uncertain, and general treatment is expensive. A high producing, nematode resistant variety of mungbeans would serve a growing need.

The southern root-knot nematode is a well known nematode, since its symptom of swollen roots is rather obvious. The northern root-knot nematode is also found extensively, particularly in the peanut growing area of Oklahoma. These two nematodes are of the same genus and have many hosts in common (16). From observational trials in the greenhouse, however, it appears that of the two, only the southern root-knot nematode attacks the mungbean.

The object of this study was to develop a rapid and reliable method for screening mungbean accessions and to identify nematode resistant germ plasm to use in developing improved resistant varieties.

Two years' results of screening 20 strains of mungbeans under field conditions are reported. Greenhouse trials were conducted on 156 accessions, using mixed and pure colonies of M. incognita. Many variations in technique were used, attempting to control pathogenic fungi in order to avoid high rates of mortality of mungbean seedlings.

CHAPTER II

REVIEW OF LITERATURE

The southern root-knot nematode is designated taxonomically as Meloidogyne incognita (Kofoid & White, 1919) Chitwood, 1949, according to A. G. Whitehead (19) in his taxonomic review of the genus Meloidogyne in 1968. Synonyms of this species include Oxyuris incognita Kofoid & White, 1919, and M. incognita var. acrita Chitwood, 1949.

Over 20 species of Meloidogyne have been classified and more will probably be recognized. All root-knot nematodes were considered to be one species, until Chitwood identified five species in 1949 (19). The earliest descriptions noted by Whitehead were made on a species attacking sugar cane in Java in 1855, and the other description in the same year in greenhouse cucumbers in England. There are hundreds of known host plants of the genus, Meloidogyne. Davidson and Townsend (5), of the Canadian Department of Agriculture, in testing possible weed hosts of M. incognita, found that 34 out of 56 species of weeds tested were susceptible. Among susceptible species were some common weeds: Amaranthus retroflexus, Bromus spp., Chenopodium album, Daucus carota, and Rumex crispus. In another Canadian study, Potter, Townsend and Davidson (12) found that 34 species of grasses in 20 genera were susceptible. They found that there was nematode reproduction in 27 species of both wild and cultivated grasses. Among the more common

ones were Bromus spp., Dactylis glomerata, Poa pratensis, Avena fatua, Agropyron repens, Elymus canadensis, Hordeum vulgare, Secale cereale, Triticum aestivum, Echinochloa pungens, Setaria spp., and Andropogon scoparius.

The Meloidogyne spp. are the best known of all plant parasitic nematodes because their symptoms are so easily seen. The first shipment of Japanese cherries for planting around the Tidal Basin in Washington, D. C., had to be destroyed because their roots were conspicuously swollen by the root-knot nematode. Thorne (16) pointed out that probably more nursery stock and seedlings are condemned because of Meloidogyne than all other types of nematodes, because of the obvious galls which distort the roots. At the same time, other plant parasitic nematodes whose symptoms are not so easily recognized may be permitted to cross boundaries.

The spreading of M. incognita under its own locomotion is probably not more than one foot in 12 months (16). For more distant dissemination, therefore, assistance is required. Machinery may scatter plant roots or soil containing eggs, larvae, or adult nematodes to other parts of a field. Lownsberry and Vigherchio (7) studied the mechanism of accumulation of M. incognita around tomato seedlings. Rather than random movement of the larvae, they found that areas of germinating seed attracted from three to ten times more larvae than the numbers accumulating in other areas. Apparently, the sense organs guide their movements more than does random motion. Even so, their own movement is restricted to small distances.

Brodie et al. (4) studied the nature of resistance to M. incognita by using known resistant and susceptible varieties of upland cotton,

Gossypium hirsutum. They suggested that resistant reaction in cotton involves three symptoms: retarded gall development, necrosis of entire small laterals, and lack of nematode reproduction. Larvae entered the roots of resistant and susceptible varieties at the same rate. They remained in the roots of the susceptible plants, but most of them soon left the roots of resistant plants. Of the few which remained in the resistant roots, some failed to develop and others appeared to develop more slowly than those in susceptible plants. It was found that water suspensions containing 900 larvae per milliliter gave the highest infection rate and the most uniform infection.

Allard (2) found additional sources of resistance to M. incognita in lima beans, Phaseolus lunatus L., by comparing strains with a well recognized susceptible variety. He found 12 strains out of 380 that demonstrated a high degree of resistance and included these in a breeding program of continuous accumulation of resistance. Allard conducted these tests in the greenhouse in order to avoid the more erratic results caused by the many variables under field conditions. In some cases, Allard found that the reaction of the control susceptible to the nematode was unaccountably low and could not be used.

Adeniji and Chheda (1) conducted experiments in field soil in greenhouse boxes, 20 cm x 45 cm x 25 cm, while studying resistance in Cynodon spp. Populations of nematodes and test plants were well maintained in the boxes. When these were changed for sterile soil and 15 cm pots, the inoculum failed. They also found that tomatoes following the grass, Cynodon nlemfuensis, produced over three times more fruit than tomatoes following tomatoes. This species of Cynodon was found to be resistant to root-knot nematode. It required 18 months

of continuous grass to reduce the nematode population. A problem in this field study was caused by a persistent creeping weed, Portulaca quadrifida, which was highly susceptible to M. incognita. Spot weeding was required until the grass had formed a dense cover.

Minton and Donnelly (10) found that yields from resistant strains of sericea lespedeza, Lespedeza cuneata, were high on soils containing a high or low population of nematodes. The susceptible check failed at a location of high Meloidogyne population one year, but produced about half as much as the resistant strain the following year. At other stations, where nematode populations were low, the susceptible at least equaled the yield of the resistant variety. Their results indicated low root galling and high forage yield to be closely correlated.

The possibility of the existence of physiological races of Meloidogyne incognita was suggested by Offutt and Riggs (11) in their work with strains of irradiated annual lespedeza seed, Lespedeza stipulacea Maxim and L. striata (Thumb). The races suggested were frequently considered to be two species, M. incognita incognita and M. incognita acrita. Significant differences were found in the virulence of these races on strains of lespedeza.

McCracken (9) tested 90 varieties of crops for resistance to four populations of M. incognita collected from widespread areas of Oklahoma. His results gave no indication of different races. The four populations acted as one. His study also pointed out that wheat, corn, cantaloupe, bush beans, barley, rye, sorghum, cowpeas, and watermelons--all crops that are often grown in mungbean areas--are capable of increasing or maintaining a population of M. incognita.

Three varieties of mungbeans, Golden mungbean, Kiloga, and Oklahoma 12, ranked as moderately to severely galled.

The difficulty of isolating and evaluating a particular biological event between two organisms is illustrated in many nematode studies. Powell (13) found that the death of nematode infested plants was greatly intensified by the fungi, Fusarium spp. and Rhizoctonia spp. Johnson and Littrell (6) found that Meloidogyne incognita increased the severity of Fusarium wilt on chrysanthemums. Taha and Racke (15) found that plant parasitic nematodes did not reduce the ratio of legume nodules to root weight. The reduction in root growth, however, did reduce the total nitrogen supply.

The mungbean Phaseolus aureus Roxb. has had a limited amount of study, compared to almost any other cultivated crop. The Oklahoma Agricultural Experiment Station has nearly 2,000 accessions listed in its experiment registry books (18). These accessions came from various sources. Many came from the United States Department of Agriculture Plant Introduction Station, others directly from other countries, and from experiment stations in the United States. The Oklahoma Agricultural Experiment Station has developed several varieties and made hundreds of selections in its breeding program.

Two rather extensive evaluations of many characteristics have been made. Banks (3) classified agronomic characteristics on 138 strains in 1958. Yohe et al. (20), working with 321 strains, studied agronomic characteristics, including virus and mildew tolerance, and protein content. The canning industry is encouraging production. The Ohio Agricultural Research and Development Center grew mungbeans in 12 locations in 1970 (14). Halo blight, carried on Oklahoma produced

Berken seed, reduced yields frequently over 50%. There appears to be no published data on nematode resistance. Matlock (18) conducted two years of field trials in 1960 and 1961. These data are included in this study.

CHAPTER III

MATERIALS AND METHODS

Mungbean strains were tested for possible root-knot nematode resistance in the field, greenhouse, and laboratory. The major problem in the field was in locating plots with a uniform population of Meloidogyne incognita. The next two series of tests were conducted in the greenhouse. The major problem in the greenhouse was a high mortality rate from fungal disease. Mungbeans were tested to a limited extent in the laboratory with the use of plastic growth packs. The growth pack method was employed as a possible means of avoiding serious plant mortality from fungal attacks.

Plant reactions to M. incognita infection were assigned root gall indices based on a one (1) to five (5) severity scale: one (1) - none; two (2) - trace; three (3) - moderate; four (4) - severe; and five (5) - very severe.

Meloidogyne incognita larvae were collected from Oklahoma fields that were producing mungbeans in Kingfisher and Garfield Counties.

One hundred fifty-six accessions from the collection at the Oklahoma Agricultural Experiment Station were screened for reaction to M. incognita infection (Table I). The field trials contained 20 accessions. The greenhouse tests were divided into two series, according to the experimental design used. The first series utilized a randomized complete block design with four replications. This series

TABLE I
 156 MUNGBEAN ACCESSIONS SCREENED FOR
 RESISTANCE TO ROOT-KNOT NEMATODE

Okla. M-No.	Variety or Other Identity
1	Kiloga, selection from Purdue
2	Purdue, from Purdue accessions
3	Okla. 12, OAEM Composite 57
6	OAEM 56 Comp. 3
7	OAEM 57-38
27	Yuba - California
45	OAEM 56, Jumbo
55	OK. 55 Jumbo
75	OK. 55 - 79
94	TH x P - 188
101	S - 125
115	PI 183065 - India
118	PI 164336 - India
130	PI 212909 - Afghanistan, <u>P. mungo</u>
135	PI 211612 - Afghanistan
143	PI 227041
145	PI 227248
146	PI 229708
151	Sel. 58 - 1, OK. 12
197	Sel. 58 - 47, OK. 12
200 to 251	Sel. 58 - 50 to 58 - 101, OK 12
312	PI 250164 <u>P. mungo</u> , Pakistan
317	PI 249552 - Iran
319	OAEM Comp. 58, Golden Mung
322	8 - B; Jumbo selection
323	26 - B; OK 55 - 44
324	18 - B; OK 55 bulk
325	23 - B; OK 55 - 26
326	128 - 2, Sel. from PI 223711
327	Sel. from PI 223711
328	PI 229708 - India
330	PI 223523 - Afghanistan
331	PI 214334 - India
332	OAEM 57 - 6
333	OAEM 57 - 6a
334	OAEM 57 - 8
335	OAEM 57 - 101
338	PI 223522-1 Afghanistan
339	Berken, Jumbo sel. from small green China
341	Sel. from PI 223523
342	Sel. from Chivel; M 92
343	Sel. from PI 217957
344	Sel. from Texas Jumbo
345	Sel. from M 116

TABLE I (CONTINUED)

Okla. M-No.	Variety or Other Identity
346	OAEM 56 - 1
347	OAEM 56 - 2
348	OAEM 56 - 3
349	OAEM 56 - 4
356	OAEM 56 - 11
357	OAEM 56 - 12
358	OAEM 56 - 13
379	OAEM 56 - 36
380	OAEM 56 - 37
384	OAEM 56 - 43
386	OAEM 56 - 45
414	OAEM 56 - 74
422	OAEM 56 - 83
425	OAEM 56 - 86
427	OAEM 56 - 89
430	OAEM 56 - 94
434	OAEM 56 - 99
435	OAEM 56 - 102
443	OAEM 56 - 111
445	OAEM 56 - 113
448	OAEM 56 - 116
464	OAEM 56 - 133
467	OAEM 56 - 136
468	OAEM 56 - 137
660	OAEM 59 - 6
732	PI 271401 - India
733	PI 271402 - India
735	PI 271406 - India
736	PI 271407 - India
739	PI 271491 - India
740	PI 271492 - India
771	PI 288585 - Peru
835	Sel. 3 - B
849	B 119, Sel. from PI 222116
865	B 104, Sel. from 215650
882	Lincoln - Philippines
888	B 108, from PI 217956
898	PI 323282 - Pakistan
900	PI 323284 - Pakistan
901	PI 323285 - Pakistan
902	PI 323286 - Pakistan
903	PI 323287 - Pakistan
908	PI 323292 - Pakistan
920	Sel. from M 16, 63 - 1
921	Sel. from M 18, 63 - 1
930	PI 163110 - India
931	PI 163113 - India

TABLE I (CONTINUED)

Okla. M-No.	Variety or Other Identity
932	PI 164644 - India
934	PI 171435 - China
936	PI 180311 - India
938	PI 183407 - India
944	Sel. 14-63 - 1 - 66
955	PI 305075 - Thailand
957	PI 317463 - Afghanistan
958	PI 317464 - Afghanistan
959	PI 317465 - Afghanistan
CJ	Jumbo (Commercial seed)
CO	Oriental (Commercial seed)
CB	Berken (Commercial seed)

contained 51 entries, which included 19 entries that had previously been in the field tests. The second greenhouse series utilized a 12 x 13 lattice design with three replications. The 156 entries included the 51 which had been in the previous tests, plus 105 which had not been tested.

Field Tests

Field tests were conducted for two years at the Perkins Agronomy Research Station. The sandy loam soil had been used several years for screening strains of sweet potato, Ipomoea batatas, for reaction to M. incognita. Three replications of 20 mungbean entries were grown in a randomized complete block design. A replication consisted of a single 20-foot row with 40-inch spacing between rows. Plantings were made in the summer and plants were harvested after 68 days of growth. Seed spacing in the row was four inches, but due to germination and survival rates, the actual spacing and number of plants varied considerably at harvest time. The plants were lifted individually by shovel, washed, and examined to determine the root gall index of each plant.

Greenhouse Experiments

Randomized Complete Block Design, 51 Mungbean Accessions

Five tests were attempted in this series. Three tests were discontinued before completion because of a high rate of plant mortality from pathogenic fungi.

Test 1, July 5 to August 4, 1970.

A. The four-inch clay pots, soil, and greenhouse bench were disinfected with methyl bromide gas.

B. The potting soil was composed of three parts of sandy loam field soil mixed with one part of coarse sand.

C. The pots were lined with plastic bags.

D. Five seeds were planted in the pots at the time of inoculation. If necessary, seedlings were thinned to three shortly after germination.

E. The inoculum consisted of galled roots of tomato, Lycopersicum esculentum, which had been washed and chopped. The tomato plants had been produced in the greenhouse in soil collected from a mungbean field during the previous March. A modified Perry-Christie soil extraction, using a tub technique, yielded the following nematode population per 100 milliliters of aliquat of field soil:

<u>Nematode</u>	<u>No.</u>
<u>M. incognita</u>	20
<u>Pratylenchus</u> spp.	60
<u>Aphelenchus</u> spp.	44
<u>Aphelenchoides</u> spp.	12

The last two, while characterized as plant parasitic nematodes, appear to feed more on fungi than on plants of economic importance. The Pratylenchus spp. are primarily migratory endoparasites. It is assumed that they were secondarily involved in all tests employing roots or soil from this field collection.

Two grams of the chopped tomato roots were mixed into the soil of each pot.

F. The night temperature was about 80^o F., and the maximum day temperature reached 95^o F. for short periods in the afternoon.

G. Test was discontinued after 30 days because of high plant mortality due to fungal attack.

Test 2, August 5 to September 14, 1970. Test 2 was treated with the same methods as Test 1, except that the seeds were treated with Captan for the control of fungi, and the root inoculum was reduced to one gram. The plant mortality rate was high. Forty days after planting, the test was discontinued, again because of fungal attack.

Test 3, October 5 to November 23, 1970. The following modifications were made:

A. The seeds were given, in addition to the use of Captan, a treatment of Vitavax 75 and Arasan 50 in a one-to-one mixture.

B. Inoculation was delayed eight days. Tomato root inoculum was introduced into a hole in the soil, made by removing a small vial that had been placed in the soil at planting time. The hole was about 3/4 inch in diameter and about 2½ inches deep.

C. This test was discontinued after 48 days because of the high mortality rate from fungal disease.

Test 4, January 9 to February 28, 1971. Further modifications were made as outlined below:

A. Seeds were started in transplant bands in sterile soil.

B. Twelve days after planting, the seedlings were transplanted to four-inch pots with about 300 grams of unsterilized field soil containing an estimated 300 larvae.

C. The night temperature was reduced to 70° F.

D. Fifty days after planting, the roots were washed free of soil and gall ratings were assigned.

Test 5, April 23 to June 24, 1971. Further modifications were employed in this test.

A. The seeds were planted directly in pots containing sterile

soil.

B. An aliquot containing approximately 750 larvae from a pure colony was placed near the roots, using the vial technique previously described, 12 days after planting.

The stock colony was initiated by transferring an eggmass from a mungbean root to a crock of sterilized soil containing established tomato seedlings. Species identification was verified on the basis of the perineal pattern of the parent female. After galling of these initial seedlings, about 90 days were required to produce sufficient inoculum for use in testing.

Large numbers of larvae were accumulated by incubation of the galled tomato roots in flasks of water aerated by forced air streams. Larvae continued hatching in the roots for weeks, but the peak hatching was reached by the end of the first week. Optimum storage temperature appeared to be from 60° to 68° F. At room temperature of about 73° F., there was a noticeable decrease in vigor and survival. The number of nematode larvae per milliliter was estimated by aliquot count under a binocular microscope.

C. Sixty-two days after planting, the plants were removed from the pots, the roots were washed and gall indices were assigned.

Lattice Design, 156 Mungbean Accessions

The use of this design was to permit screening greater numbers of mungbean accessions at one time. A 12 x 13 lattice design with 156 entries and three replications was used. The 51 strains previously tested were included in this series. To accommodate the 156 entries, two greenhouse benches were moved together.

Replication 1, May 5 to June 15, 1971.

A. Key methods again involved inoculating with chopped galled tomato roots, after the mungbean seedlings were 11 days old. Plastic lining for pots was not employed in these tests.

B. The plants in 100 of the 156 pots were killed by fungi within 41 days and the test was discontinued.

Replication 2, May 21 to July 1, 1971.

A. Galled tomato roots were dipped in a 10% solution of Clorox before being prepared as inoculum for a ten day delayed application.

B. Plants were harvested after 41 days, roots were washed and root gall indices were assigned.

Replication 3, June 10 to July 20, 1971.

A. Treated as above.

B. Plants were harvested after 40 days, roots were washed and assigned root gall indices.

Replication 1 Repeat, June 15 to August 2, 1971.

A. Treated as were replications 2 and 3.

B. Plants were harvested after 48 days, roots were washed and root gall indices were assigned.

Following the above screening, during the week of September 12, 1971, the three replications were repeated simultaneously. All replications were given the same treatment, including "A" under Replication 2 above. All replications were discontinued in 30 days because of the high plant mortality from fungal attack.

Laboratory

Commercially available diSPO seed pack pouches (manufactured by

Scientific Products, Evanston, Illinois) were used in this study. The see-through plastic pouch is approximately $6\frac{1}{2}$ inches wide by 7 inches high. A double thickness of highly absorbent paper is inside the pouch as a moisture wick. A fold near the top of the paper serves as an area where seeds are placed.

A preliminary test was first conducted employing 20 diSPo pouches. Following this limited trial, one replication of the 12 x 13 lattice design, 156 entry test, was conducted.

As additional precaution against fungi, the seeds were dipped in a 50% Clorox solution as they were placed in the packs. Twenty-five milliliters of distilled water was introduced into each growth pouch. The pouches were kept closely fitted in a box, with the upper one-third of the pouch protruding above the sides of the box. Temperature was maintained at about 75° F., and artificial lights simulated a 14-hour day.

Larvae extracted from soil were introduced at the rate of 900 per pouch on the third day after planting. Plants were watered on an as-needed basis. Root gall indices were recorded on the 30th day.

CHAPTER IV

RESULTS AND DISCUSSION

Field Tests

Twenty mungbean strains were tested for reaction to root-knot nematode at the Oklahoma Agronomy Research Station near Perkins, Oklahoma, in 1960 and 1961. The numbers of plants harvested in each entry were those that emerged and survived. Resultant scores were ranked by percentage of plants in the two lowest and the two highest infection indices. The comparative ranks are shown in Tables II, III, and IV.

Table II gives the percentage of all surviving plants of each entry ranked in the two lowest gall indices, 1 and 2, of the 1-to-5 index scale. For example, entry M-55 scored 100% because all plants in the three replications fell within the non-galled and slightly galled categories. Two entries, M-2 and M-7, occurred in the five least susceptible ranks in both years. Further examination in the midranks reveals similar relationships. Likewise, three entries, M-3, M-101, and M-130, were included in the six most susceptible ranks in each of the two years.

Table III gives the percentage of plants from each entry in the two most severe gall indices, 4 and 5. In this rank, two entries occurred in the five most resistant of each year. These, again, were M-2 and M-7.

TABLE II
 MUNGBEAN REACTION TO ROOT-KNOT NEMATODE;
 PERCENTAGE OF EACH ACCESSION IN LOW
 GALL (1 and 2) INDICES

1960			1961	
<u>Okla.</u> <u>M-No.</u>	<u>Percent</u>	<u>Rank</u>	<u>Okla.</u> <u>M-No.</u>	<u>Percent</u>
55	100	1	197	100
2	96	2	2	94
6	95	3	7	93
324	95	4	325	93
7	93	5	45	90
1	93	6	317	88
317	92	7	151	87
44	91	8	319	85
45	90	9	27	81
94	89	10	115	79
319	84	11	1	77
197	83	12	312	72
312	82	13	94	64
151	78	14	55	64
325	76	15	101	63
130	76	16	44	59
3	76	17	130	58
27	73	18	6	54
115	71	19	324	48
101	69	20	3	45

TABLE III
 MUNGBEAN REACTION TO ROOT-KNOT NEMATODE;
 PERCENTAGE OF EACH ACCESSION IN HIGH
 GALL (4 AND 5) INDICES

1960			1961	
<u>Okla.</u> <u>M- No.</u>	<u>Percent</u>	<u>Rank</u>	<u>Okla.</u> <u>M-No.</u>	<u>Percent</u>
2	0.0	1	197	0.0
55	0.0	2	7	0.0
6	0.0	3	2	3.0
317	0.1	4	325	3.7
7	0.3	5	151	4.3
324	0.3	6	319	6.3
94	0.5	7	45	7.0
27	0.7	8	317	7.7
45	1.4	9	27	9.3
1	3.6	10	130	12.0
151	7.8	11	115	16.3
319	8.5	12	55	16.3
44	8.9	13	101	18.3
130	9.0	14	1	20.0
312	11.1	15	94	20.3
3	11.4	16	312	22.0
197	13.7	17	3	29.3
325	15.3	18	6	30.0
101	16.7	19	44	31.3
115	25.0	20	324	38.0

TABLE IV
 SUMMARY OF TABLES II AND III, MUNGBEAN
 REACTION TO ROOT-KNOT NEMATODE,
 FOR 1960 AND 1961

Rank of Entries According to Percentages				
<u>Low Gall Indices, 1 and 2</u>			<u>High Gall Indices, 4 and 5</u>	
<u>Okla.</u> <u>M-No.</u>	<u>Percent</u>	<u>Rank</u>	<u>Okla.</u> <u>M-No.</u>	<u>Percent</u>
2	95	1	7	0.1
7	93	2	2	1.0
197	91	3	317	3.8
45	90	4	45	4.2
317	90	5	27	5.0
319	85	6	151	5.6
325	85	7	197	6.8
1	83	8	319	7.3
151	83	9	55	8.2
55	82	10	325	9.5
312	77	11	94	10.4
27	77	12	130	10.5
94	76	13	1	11.8
44	75	14	6	15.1
115	75	15	312	16.6
6	75	16	101	17.5
324	71	17	324	19.1
130	67	18	44	20.1
101	66	19	3	20.4
3	60	20	115	20.7

Table IV contains a summary of the two year field study. The left side of the table lists the percentage of plants in each entry which were assigned the lower gall indices, 1 and 2, for the two years. This summarizes Table II. Likewise, the right side of the table makes the similar consolidation of the data in Table III.

Because of the problem of non-uniform infestation in field plots, it is difficult to arrive at comparable degrees of root galling. In continuing this study in 1970, it was decided that the work would be carried on in the greenhouse.

Greenhouse Experiments

Randomized Complete Block Design, 51 Mungbean Accessions

Of the five tests attempted in this series, only two were completed. The first three tests were discontinued before completion because of plant losses from fungal disease. The predominant fungi, identified from dying and dead seedlings, were three: Rhizoctonia spp., Fusarium spp., and Pythium spp. Neither the fungicide treatment of the seeds nor sterilization of the soil appeared to be effective in protecting the mungbean seedlings from fungal invasion.

On a preliminary basis, it appeared that survival of the seedlings was influenced by two, or possibly three, factors. One was lowering the night temperature approximately 10° F., from 80° to 70° F. A second factor appeared to be delayed introduction of the inoculum. Later results, however, indicated that the mungbean seedlings might be killed by fungi even though these precautions are employed. It appeared that avoiding the direct introduction of tomato roots may be indicated.

Forty-nine of the 51 entries survived in the fourth test. All 51

entries survived in Test 5. (These tests included 19 of the 20 entries tested in the 1960 and 1961 field tests.) Strains most commonly used now by Oklahoma growers were also included. These were the varieties Berken, Kiloga, Oklahoma 12, and commercial collections golden, jumbo, and oriental.

Plants in Tests 4 and 5 were attacked by fungi, but they were less severely attacked than those of the three previous tests. One unexplainable feature of these tests, however, was the extremely low gall mean developed by replication 1 of the fourth test. Figure 1 shows the low gall mean of 3.5 for replication 1, compared to other replications in Tests 4 and 5. The mean gall ratings of the other three replications were over 4.2.

The analysis of variance for galling in Test 4 showed entries to be significantly different at the 5% level of probability (Table V). The first 11 strains were not significantly different in mean gall reaction (Table VI). The next test, Test 5, provided a reexamination of all entries.

The analysis of variance for galling showed that entries in Test 5 were not significantly different (Table VII). The mean gall score of all 51 entries fell within one range of Duncan's New Multiple Range Test. Entries rating lowest in gall indices in Test 4 were dispersed throughout the ranking in Test 5, from the 10th position to the 51st. Figure 2 plots the gall ratings of the 20 least galled entries of Test 4 and also plots their respective ratings in Test 5. These indices of galling do not indicate a degree of resistance, as the grand mean for Test 5 was 4.5 (severely galled), and the lowest index was 3.8 on the 1-to-5 scale.

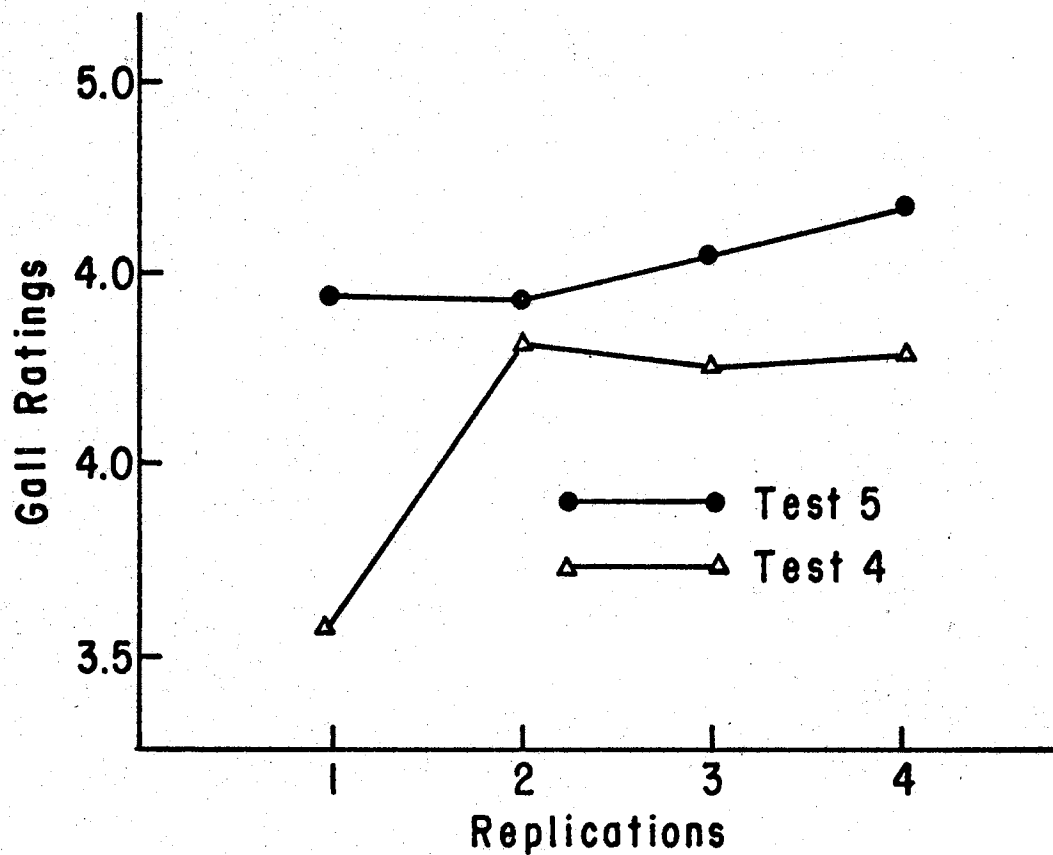


Figure 1. Mean Gall Rating by Each of Four Replications of 51 Mungbean Entries in Randomized Block Tests 4 and 5

TABLE V
ANALYSIS OF VARIANCE FOR ROOT GALLING
OF 51 MUNGBEAN ACCESSIONS IN TEST 4

Source	df	SS	MS	F Value
Total	195	183.8469		
Replications	3	20.9591	6.9864	9.089**
Entries	48	56.8469	1.1843	1.473*
Error	144	106.0409		

CV = 20.97%, Grand Mean = 4.0918

*, **Significant at the 5% and 1% levels of probability, respectively.

TABLE VI
 MUNGBEAN REACTION TO ROOT-KNOT NEMATODE;
 MEAN GALL RATING FOR THE 51 ACCESSIONS
 IN TESTS 4 AND 5*

Test 4			Test 5	
<u>Okla.</u> <u>M-No.</u>	<u>Mean</u>	<u>Rank</u>	<u>Okla.</u> <u>M-No.</u>	<u>Mean</u>
957	2.375	1	930	3.825
312	3.000	2	75	3.825
3	3.125	3	934	3.900
151	3.250	4	771	4.000
339	3.250	5	921	4.000
835	3.375	6	740	4.125
27	3.500	7	901	4.275
955	3.500	8	735	4.300
94	3.625	9	101	4.300
900	3.625	10	3	4.325
908	3.750	11	944	4.325
CB	3.875	12	732	4.325
740	3.875	13	898	4.375
902	3.875	14	932	4.375
932	3.875	15	957	4.375
958	3.875	16	920	4.400
55	4.000	17	151	4.400
115	4.000	18	1	4.400
319	4.000	19	115	4.425
324	4.000	20	835	4.450
735	4.000	21	736	4.450
930	4.000	22	908	4.450
CO	4.125	23	6	4.450
1	4.125	24	900	4.500
7	4.125	25	7	4.500
660	4.125	26	CO	4.525
921	4.125	27	959	4.550
931	4.125	28	660	4.550
75	4.250	29	312	4.575
934	4.250	30	936	4.600
CJ	4.375	31	45	4.600
101	4.375	32	938	4.600
732	4.375	33	130	4.625
736	4.375	34	955	4.625
771	4.375	35	317	4.650
197	4.500	36	339	4.675
733	4.500	37	CJ	4.750
882	4.500	38	319	4.750
6	4.625	39	903	4.750
901	4.625	40	197	4.750
903	4.625	41	94	4.750
936	4.625	42	733	4.750

TABLE VI (CONTINUED)

Test 4			Test 5	
<u>Okla.</u> <u>M-No.</u>	<u>Mean</u>	<u>Rank</u>	<u>Okla.</u> <u>M-No.</u>	<u>Mean</u>
45	4.750	43	325	4.750
325	4.750	44	931	4.775
898	4.750	45	882	4.800
944	4.750	46	902	4.875
317	4.875	47	55	4.925
920	4.875	48	324	4.925
938	4.875	49	CB	4.950
959**		50	958	5.000
130**		51	27	5.000

*Means enclosed by the same line are not significantly different at the 5% level of probability, according to Duncan's New Multiple Range Test.

**Plants died.

TABLE VII
ANALYSIS OF VARIANCE FOR ROOT GALLING OF
51 MUNGBEAN ACCESSIONS IN TEST 5

Source	df	SS	MS	F Value
Total	203	73.3269		
Replications	3	1.3241	0.4419	1.2180 NS
Entries	50	16.6069	0.3336	.9350 NS
Error	150	54.3959	0.3626	

CV = 13.20%, Grand Mean = 4.509

NS indicates non-significance at the 5% level of probability.

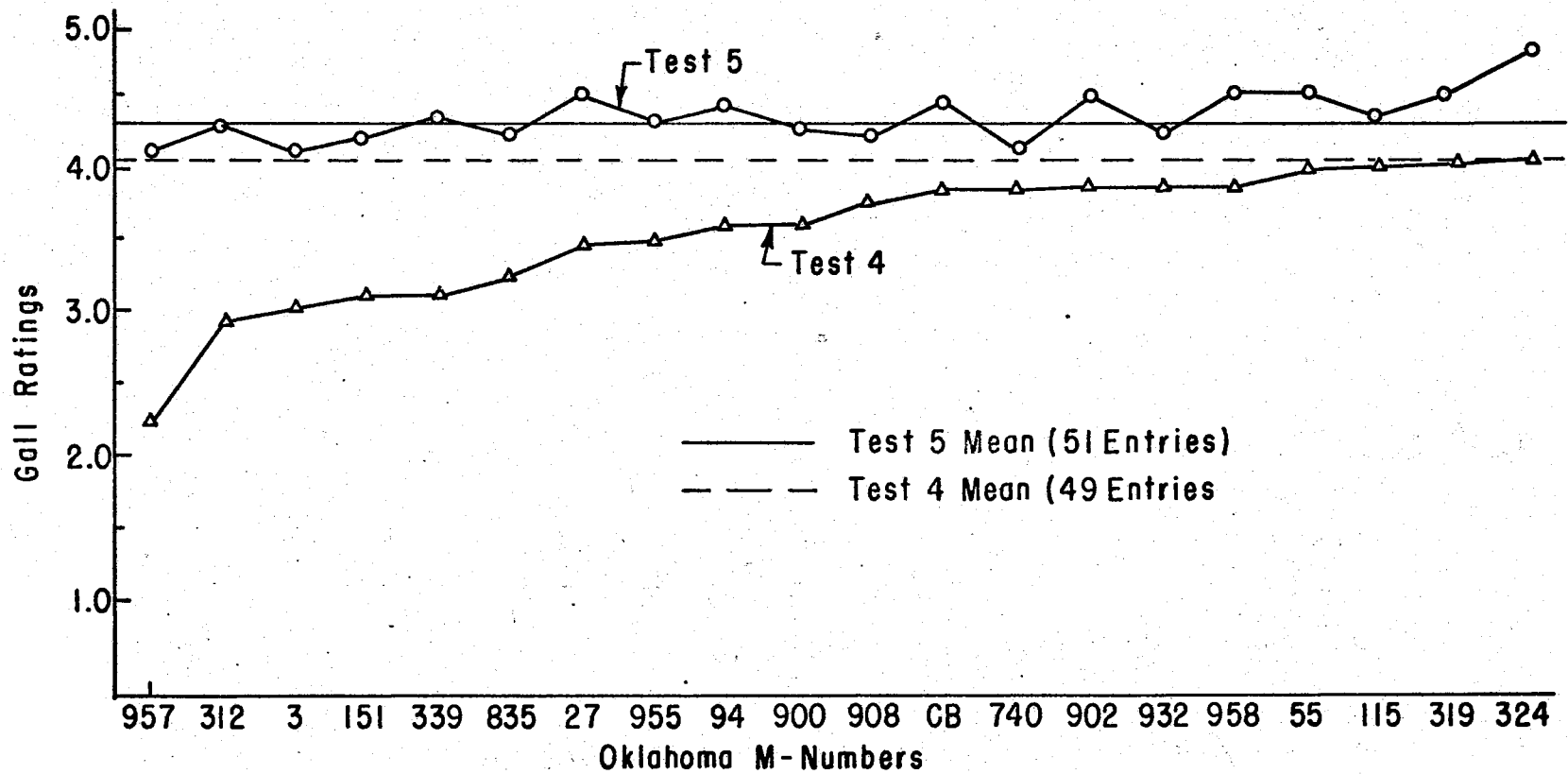


Figure 2. Comparison of Gall Ratings of the 20 Least Galled Mungbean Accessions in Test 4 with Their Respective Ratings in Test 5

Lattice Design, 156 Mungbean Accessions

The 156 mungbean strains in this test included the 51 that were evaluated in the randomized complete block tests 4 and 5, and 105 other accessions and selections of the Oklahoma Agricultural Experiment Station. This experiment was conducted in three parts, with the replications planted on different dates. Replication 1 failed in its first planting due to fungal disease. Although all precautions of previous tests against pathogenic fungi were observed, two-thirds of the entries were dead within 41 days. This replication was later repeated, although not without being threatened again by fungi.

Replication 2, a second randomization of the 156 entries, was planted about two weeks after the planting of the unsuccessful replication 1. Replication 2 grew with vigor from emergence to harvest. All entries survived, and the test was evaluated 41 days after planting. The additional precaution taken with this planting was the treatment of galled tomato roots with a 10% solution of Clorox before chopping. Plants in replication 2 were practically free from fungal damage both above and below the soil. It appeared at this point in the study that careful watering, fungicidal treatment of seed, sterilization of pots and soil, delayed inoculation, reduction of temperature, and Clorox treatment of tomato roots would give excellent control of all types of troublesome fungi. These precautions, however, gave poor protection to replication 3, and to the repeat planting of replication 1. Necrosis of roots was very severe in the last two tests. In some instances, poor root formation interfered with rating the nematode galling. There was some damping off before the inoculum was introduced, which suggested sources of contamination other than

the tomato root inoculum.

The analysis of variance for galling of the three completed replications of 156 mungbean strains indicated that there were significant differences in degree of galling (Table VIII) between entries. Table IX, which lists the entries, indicates that the first ranked entry, M-386, was not significantly different from the next five entries, but was significantly different from M-319 at the 5% level of probability. Entry M-210, with a root gall index of 4 (severe), and ranking second, only by chance does not rate as low as 29th, according to Duncan's New Multiple Range Test. Although there is a significant difference in galling indices in this test, the indices are at such high levels of susceptibility that the value of such entries in breeding for nematode resistance would be questionable.

Three of the six entries making up the first range were strains from the earlier tests. The third ranked entry, M-930, with a mean gall rating of 4.033, had placed first in one test of 51 entries in which there was no significant difference in root gall means. Fourth ranked M-735 had previously ranked eighth, and M-317 ranked 35th in the 51 entry test referred to above.

The other three entries in the first range, M-386, M-210, and M-204, were in the test program for the first time. Of these, M-210, with a gall rating of 4.033, scored a gall rate of 3.00 and ranked 11th among the same 156 entries in the one-replication growth pack test conducted later. The other two new entries did not rank in the top 10% of that test. Except for M-210, the other five of the first range developed a gall rating of 5.00 in the exploratory one-replication test, shown in Table X.

TABLE VIII
 ANALYSIS OF VARIANCE FOR ROOT GALLING
 OF 156 MUNGBEAN ACCESSIONS INFECTED
 WITH M. INCOGNITA

Source	df	SS	MS	V Value
Total	467	56.5100	1.2100	1.133
Replications	2	24.5000	1.2250	1.157
Blocks within Replications	36	29.8202	.8283	.776
Entries	155	21.8300	1.4083	1.321*
Intrablock Error	274	29.2479	1.0674	

CV = 6.72%, Grand Mean - 4.8621

*Significant at the 5% level of probability.

TABLE IX

MUNGBEAN REACTION TO ROOT-KNOT NEMATODE;
MEAN GALL RATINGS FOR 156 ACCESSIONS*

Rank	Okla. M-No.	Mean
1	386	3.833
2	210	4.033
3	930	4.033
4	735	4.166
5	317	4.200
6	204	4.333
7	319	4.366
8	212	4.433
9	223	4.466
10	351	4.466
11	250	4.500
12	230	4.500
13	238	4.500
14	335	4.500
15	229	4.533
16	226	4.533
17	740	4.533
18	341	4.533
19	232	4.533
20	220	4.533
21	231	4.566
22	228	4.600
23	217	4.600
24	352	4.600
25	216	4.600
26	118	4.600
27	227	4.600
28	324	4.600
29	145	4.600
30	234	4.633
31	237	4.633
32	215	4.666
33	205	4.666
34	201	4.666
35	203	4.666
36	151	4.666
37	339	4.666
38	882	4.666
39	250	4.666
40	240	4.666
41	200	4.666
42	353	4.700
43	849	4.700
44	342	4.700

TABLE IX (CONTINUED)

Rank	Okla. M-No.	Mean
45	233	4.700
46	322	4.700
47	384	4.700
48	936	4.700
49	736	4.700
50	218	4.733
51	357	4.733
52	246	4.766
53	224	4.766
54	380	4.766
55	739	4.766
56	219	4.766
57	247	4.766
58	348	4.766
59	835	4.800
60	248	4.800
61	207	4.800
62	235	4.800
63	944	4.800
64	959	4.800
65	3	4.833
66	347	4.833
67	346	4.833
68	333	4.833
69	45	4.833
70	101	4.833
71	955	4.833
72	197	4.833
73	414	4.833
74	6	4.833
75	957	4.833
76	214	4.833
77	379	4.833
78	143	4.866
79	325	4.866
80	865	4.866
81	331	4.866
82	903	4.866
83	908	4.866
84	202	4.866
85	209	4.866
86	435	4.866
87	326	4.866
88	55	4.933
89	356	4.933
90	332	4.933
91	94	4.933

TABLE IX (CONTINUED)

Rank	Okla. M-No.	Mean
92	208	4.933
93	334	4.933
94	211	4.933
95	242	4.933
96	445	4.933
97	222	4.933
98	206	4.933
99	427	4.933
100	958	4.933
101	245	4.933
102	733	4.933
103	732	4.933
104	75	4.933
105	146	4.933
106	27	4.933
107	932	4.933
108	327	4.933
109	888	4.933
110	467	4.933
111	323	4.933
112	225	4.933
113	241	4.933
114	901	4.933
115	312	4.933
116	236	4.933
117	335	4.933
118	7	5.000
119	1	5.000

All remaining entries scored 5.000: 213, 344, 221, 330, 338, 934, 422, 900, 771, 921, 243, 350, CO, 358, 938, 244, 902, 343, CB, 898, 443, 648, 328, 430, 135, 425, 130, CJ, 349, 239, 660, 931, 920, 434, 448, 464, 115.

*All means enclosed by the same line are not significantly different at the 5% level of probability, according to Duncan's New Multiple Range Test.

TABLE X

MUNGBEAN REACTION TO ROOT-KNOT NEMATODE;
 SINGLE ENTRY GALL INDICES OF 156
 ACCESSIONS GROWN IN PLASTIC
 GROWTH POUCHES

Rank	Okla. M-No.	Gall Index	Rank	Okla. M-No.	Gall Index
1	CB	1.0	22	351	4.0
2	328	1.0	23	380	4.0
3	959	1.5	24	414	4.0
4	6	2.5	25	443	4.0
5	101	2.5	26	448	4.0
6	145	2.5	27	733	4.0
7	237	2.5	28	955	4.0
8	422	2.5	29	143	4.5
9	660	2.5	30	146	4.5
10	3	3.0	31	214	4.5
11	210	3.0	32	225	4.5
12	224	3.0	33	229	4.5
13	345	3.0	34	239	4.5
14	1	3.5	35	326	4.5
15	434	3.5	36	341	4.5
16	353	3.5	37	346	4.5
17	7	4.0	38	358	4.5
18	222	4.0	39	888	4.5
19	236	4.0	40	27	5.0
20	242	4.0	41	45	5.0
21	350	4.0			

All remaining entries scored index 5.0: 55, 94, 115, 118, 130, 135, 151, 197, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 211, 212, 213, 215, 216, 217, 218, 219, 220, 221, 223, 226, 227, 228, 230, 231, 232, 233, 234, 235, 238, 240, 241, 243, 244, 245, 246, 247, 248, 250, 251, 312, 317, 319, 322, 323, 324, 325, 327, 330, 331, 332, 333, 334, 335, 338, 339, 342, 343, 344, 347, 348, 349, 352, 356, 357, 379, 384, 386, 425, 427, 430, 435, 445, 464, 467, 468, 732, 735, 736, 739, 740, 771, 835, 849, 865, 882, 898, 900, 901, 902, 903, 908, 920, 921, 932, 934, 936, 938, 944, 957, 958, CJ, CO.

Further screening might be desirable with the first 16 entries of this test. A lower inoculation rate could possibly bring out greater differentiation in gall indices between entries. The entries from 7 to 156 were not significantly different in their reaction to Meloidogyne incognita in this test (Table IX).

Statistical analysis of the results of this 12 x 13 lattice design showed no advantage over the randomized block design employed in Tests 4 and 5. This design was considerably more difficult to handle in the greenhouse. It required the combination of two benches to form an area of about 9½ x 10 feet. This large area made caring for the plants in the center portion of the two benches quite difficult because of the distance from any of the sides.

It became quite evident with this series of tests that consistently successful precautions for producing mungbeans in the greenhouse using tomato roots as inoculum, had not been found. While one replication of 156 entries grew out to maturity without apparent fungal disease, one replication was lost and two others were marginal.

Shortly after the completion of the foregoing experiment, another 12 x 13 lattice design experiment in pots was initiated with all three replications at one time. Again, all the precautions previously employed, including treatment of tomato roots, were followed. Each of the three replications underwent heavy fungal losses, and the test was ended without galling results.

Laboratory

The strains grown in this growth pouch trial were the 156 accessions tested in the lattice design experiment. The growth of

mungbeans in this exploratory test was satisfactory. Further tests should be run using this method, which appeared to be most reliable. Seeds, dropped in the top fold of the paper wick, germinated and grew with a minimum of problems. Before plant growth began to slow from lack of nutrients, the nematode larvae had entered the roots, and galls were observed. Although fungi growth was apparent on some ungerminated seed, no interference with normal seedling development was apparent in this study.

The 156 pouches were kept closely packed in a box. Minor problems arose which might be eliminated by providing partitioned spaces for the pouches, which tended to cling together. This made it possible to overlook watering a pack which was stuck to another. This was discovered by finding plants wilted shortly after it was thought that all pouches had been watered. The close proximity also permitted the foliage of neighboring plants to tangle, which, at times, caused breakage of the tops.

The evaluation of galling was greatly facilitated by the immediate visibility provided by the growth pack. The 900-larvae aliquats were introduced by pipette on the third day, at the time the seeds were germinating. On the 17th day, galling became easily visible on most entries. The gall indices of this one-replication observation are shown in Table X. The non-gall index, 1, for entries M-CB and M-328 most likely resulted from failure to inoculate the growth pack. The M-CB entry is the variety Berken, from a commercial seed source. It was included in all greenhouse tests and had not rated less than moderately galled. In most tests, M-CB had a gall index above 4.0, severely galled. Both M-CB and M-328 scored very

severely galled, 5.0, in the 12 x 13 lattice test discussed previously.

No significance can be given to differences in this one-replication test. It appears, however, that the growth pouch method would be reliable for preliminary screening for resistance to Meloidogyne incognita in mungbean seedlings.

CHAPTER V

SUMMARY AND CONCLUSIONS

The objective of this study was to develop a rapid and reliable method for screening mungbean accessions for reaction to southern root-knot nematode and to identify nematode resistant germ plasm to use in developing resistant varieties.

Twenty mungbean accessions were tested in the field in the summers of 1960 and 1961. Three mungbean accessions of the 20 tested in the two year field study were among those with the least amount of root gall in both years. Seed of one of these entries was not available for later testing, but the other two were included in subsequent tests and did not show resistance to root-knot nematode.

Nineteen field tested entries were included in the 156 accessions in these tests. Two randomized complete block design experiments conducted under similar conditions contained 51 accessions of mungbean and included the 19 field tested entries. The analysis of variance of the first of these experiments (Test 4) gave a difference in degree of galls developed that was significant at the 5% level of probability. The mean gall index of the least galled entry was 2.375 on the 1-to-5 gall indices. This least galled entry, according to Duncan's New Multiple Range Test, was not significantly different from the 10 following entries which approached the severe gall index. The second randomized complete block design experiment (Test 5) repeated the

screening of the 51 entries. The analysis of variance between varieties in the severity of root gall indices indicated no significance at the 5% level. The Duncan's New Multiple Range Test included the 51 entries within one range.

The screening was expanded to 156 entries and conducted in a 12 x 13 lattice design. The analysis of variance indicated a significant difference at the 5% level for entries in mean gall indices. The six least galled entries fell within the first range of Duncan's New Multiple Range Test, but had gall indices from 3.8 to 4.3 on the 1-to-5 gall indices. All except the 3.8 scoring entry were fully within the severely galled category.

A serious problem of high mortality from fungal infection in mungbean seedlings being grown in sterilized soil in four-inch pots existed throughout the study. No combination of precautions against fungi gave consistent results. Precautions of lowering temperatures, dipping galled tomato roots in Clorox solution in preparing the inoculum, and delaying inoculation, at given times appeared to be answers to the problem, only to become ineffective in subsequent tests. Seedlings grown in plastic growth pouches with distilled water suffered no losses. The growth pouch should be used in further screening as a preliminary method from which the least susceptible accessions could be selected for more extensive testing.

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