INFECTION OF SORGHUM BY *SCLEROTIUM BATATICOLA* TAUB

By

LII-JANG LIU

Bachelor of Science

Taiwan Provincial College of Agriculture

Taichung, Taiwan, China

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INFECTION OF SORGHUM BY SCLEROTIUM

BATATICOLA TAUB

Thesis Approved:

Thesis Advisor

Head of Department

Dean of the Graduate School

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INTRODUCTION

Charcoal rot caused by Sclerotium bataticola Taub. or Macrophomina phaseoli (Maubl.) Ashby or Botryodiplodia phaseoli (Maubl.) Thirumalachar comb. nov. is one of the most destructive diseases of sorghum. This fungus causes seedling blight as well as root rot and stalk or charcoal rot in sorghum. The later disease is particularly serve on grain sorghums throughout the central plains. In Nebraska, 60 percent lodging due to this disease had been reported (10), and reports of 50 to 100 percent lodging are not uncommon in Texas, Oklahoma and Kansas, especially in fields of newer hydrid sorghums. The disease is unpredictable, more or less sporadic in its appearance, and seems to be associated with soil and weather conditions that subjected the sorghum crop to extreme heat or drought at a critical stage in its development (9). Some studies of invasion of sorghum roots by this fungus have been made (11), but the developmental stages of stalk infection are still not definitely known. The rot of stem tissues of sorghum usually does not occur until late in the growing stage of the plants. In immature plants, roots are often observed rotted up to the point of transition into stem tissues but there the progress of infection stops abruptly. However, as the plants approach maturity the stem tissues apparently become more susceptible to the rotting action of the fungus and the disease resumes development.

Since, in most cases, the soil moisture level cannot be controlled in the field, the most feasible method of controlling this disease is by the

use of resistant varieties. Attempts to produce artificial disease epidemics to test for resistance in mature sorghum plants at Oklahoma State University, using the toothpick method of inoculation (20), have not been satisfactory.[1] Consequently the purpose of this investigation was to determine the effect of Sclerotium bataticola Taub. on the seedlings of two sorghum varieties, one resistant and the other susceptible to charcoal rot and lodging in the field, and then to study the relationship between seedling infection and the development of the charcoal rot phase of the disease in later stages of plant growth in the greenhouse.

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[1] Unpublished data. Department of Botany and Plant Pathology, Oklahoma State University, Stillwater, Oklahoma.

REVIEW OF THE LITERATURE

Taubenhaus (14) first described Sclerotium bataticola as the casual agent of a disease of sweet potato which he called charcoal disease. Somewhat later Butler (3) renamed the organism as Rhizoctonia bataticola, and later Ashby (2) discovered the pycnidial stage and named the organism Macrophomina phaseoli, and still later Thirumalachar (16) transferred the charcoal-rot-inciting fungus under Botryodiplodia as B.plaseoli (Maubl.) Thirumalachar comb. nov. because the fact that the hyaline glassy 1-celled spores which become reddish brown and 2-celled at maturity. Uppal et. al. (17) were the first to describe a disease of sorghum caused by this fungus. Since that time many investigators have studied various phases of the diseases of sorghum caused by this fungus (1), (4),(5),(6),(8),(11).

Macrophomina phaseoli or Sclerotium bataticola also has been described as the causal agent of diseases of corn, soybean, bean, peanuts, cowpeas, and many wild plants (7),(13),(15),(19).

Livingston (11) has given the most comprehensive report of the charcoal rot disease of sorghum. He found that high temperatures (38° C to 42°C) were most favorable to the organism, and this is supported by the work of Young (21) and Norton and Frank (12). He also found that the greatest amount of lodging due to this disease follows period of drought during the time of heading. Young (21) reports similar findings and states that the varieties of sorghum in his tests were generally susceptible. Wadsworth and Seiglinger (18) however, report differences in lodging due to this

disease in the varieties they studied, and Livingston (11) thought that the differences in resistance he observed between varieties might be distinguishable in the seedling stage. Semeniuk (13) held the same view for determining resistance to charcoal rot in dent corn.

MATERIALS AND METHODS

The culture of Sclerotium bataticola used throughout these experiments was originally isolated from charcoal rotted sorghum collected in Oklahoma. It was isolated by Dr. H. C. Young, Jr. and has been used for several years as a pathogen in broomcorn charcoal rot studies where it has proved to be extremely virulent. However, attempts to produce charcoal rot in sorghum by the toothpick method of inoculation with this culture have not been suscessful. Nevertheless, in the studies reported here it has been quite pathogenic.

Inoculum for the experiments was prepared in two ways. In one case, the organism was grown on potato-dextrose broth. Approximately 30 ml of broth were added to each 500 ml Erlenmeyer flask and inoculated from a tube culture. After 20 days when a mycelial mat had formed over the surface of the broth, the mat was removed, macerated in a blendor with 100 ml of water and this inoculum was used to pour over the seeds at the rate of 200 ml per 100 seeds at the time of planting.

The other method used for inoculum preparation was to grow the organism on a mixture of 7 parts oats and 5 parts wheat which had been moistened and sterilized twice. After the organism had grown on this medium for 30 days this inoculum was mixed with the soil at the rate of 1 part of inoculum to 500 parts of soil in which the sorghum seed was planted.

In certain experiments, washed, steam sterilized river sand was used to grow the seedlings, and in other experiments a soil mixture consisting

of one part loam soil, one part sand and one part manure was used. This mixture was steam sterilized just prior to planting.

Two varieties of grain sorghum, Redlan and Texas 7078 were selected for use in these tests. They were chosen because they have been at opposite extremes of susceptibility to charcoal rot under natural conditions in the field. Texas 7078 has been quite susceptible to lodging caused by charcoal rot and Redlan has been very resistant.[1] The seeds of these two varieties were obtainted from Prof. J. B. Sieglinger, Department of Agronomy, Oklahoma State University. One seed lot of each variety was used throughout the series of experiments. The seeds were surface sterilized for 3 minutes in a 1 to 1000 solution of bichloride of mercury, after which they were washed in sterile distilled water.

Four measurements of infection were made in the seedling stage. Two weeks after planting the number of plants showing discoloration or browning of the leaves was counted and in some cases the height of seedlings above ground was measured. Since the infection seemed to take place almost entirely on the primary root or at the point of seed attachment, the extent of discoloration up the hypocotyl from the seed attachment was measured. Then, the seedlings were divided into classes as follows; 1) No infection; 2) Primary root infection slight, hypocotyl not infected; 3) Moderate infection of primary root and slight infection on hypocotyl; 4) Moderate infection of both primary root and hypocotyl; and 5) Severe infection of both primary root and hypocotyl (plants dead or practically so). From these classes an index was computed by multiplying the frequency of plants

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[1] Unpublished data. Department of Botany and Plant Pathology, Oklahoma State University, Stillwater, Oklahoma.

in each class by the class value, summing, and dividing the total by the number of plants.

Three measurements of infection of mature plants were made. The root volume was measured by water displacement, the number of lesions on major roots of the secondary root system was counted, and the extent of discoloration of crown tissue was measured.

RESULTS

First an experimant was carried out to determine the seedling reaction of the two varieties of sorghum. For this purpose a flat of sterilized sand was planted with 300 seeds in 6 rows of 50 seeds for each variety. Each flat was inoculated by pouring broth inoculum over the seeds at the time of planting. A third flat was planted with 150 seeds of each variety as an uninoculated control. The flats were held in the greenhouse at a temperature of approximately 70°F. Two weeks after planting a count of the emerged plants showing discoloration or browning of the leaves was made (Table I).

TABLE I

THE PERCENT OF SEEDLINGS OF SORGHUM SHOWING DISCOLORATION OR BROWNING OF THE LEAVES FOLLOWING INOCULATION WITH SCLEROTIUM BATATICOLA AT PLANTING TIME

Variety	No. of seeds planted	No. of plants emerged	No. of plants with symptoms	Percent
Redlan	300	285	39	13.5
Texas 7078	300	152	17	11.0

The percent of the plants showing these symptoms was about the same for both varieties, but it will be noted that the total emergence in the variety Texas 7078 was only slightly over 50 percent while the emergence of the variety Redlan was over 90 percent. The emergence of both varieties was over 95 percent in the control flat and no symptoms were observed on the leaves.

Four weeks after planting the seedlings were removed from the flats and a random sample of 30 plants from each variety in the inoculated and in the check flats were carefully examined for the progress of infection in the hypocotyl tissue. In almost every case the initial infection appeared to take place at the point of seed attachment and then develop up the hypocotyl. In only rare instances were seedlings found where the organism had penetrated the hypocotyl above the point of seed attachment. The extent of lesion development is shown in Table II. In most of the seedlings the organism had penetrated from 2 to 4 mm up the hypocotyl from the point of infection at the seed attachment. There seemed to be very little difference between the two varieties in this respect. There were no lesions on any of the plants from the noninoculated control flat.

TABLE II

THE EXTENT OF INFECTION OF THE HYPOCOTYL TISSUE OF TWO-WEEK OLD SORGHUM SEEDLINGS INOCULATED WITH SCLEROTIUM BATATICOLA AT THE TIME OF PLANTING

Size of lesion in millimeters	Redlan		Texas 7078	
	No. of plants	Total lesion growth (mm)	No. of plants	Total lesion growth (mm)
1	0	0	3	3
2	4	8	10	20
3	15	45	5	15
4	7	28	9	36
5	2	10	1	5
6	2	12	1	6
7	0	0	1	7
Total	30	103	30	92

At the same time (4 weeks after planting) a 50 plant sample of each variety was examined for the severity of infection of the primary root system. Each plant was placed in a class as described previously. An index

for each variety was then computed by summing the products of the frequency times the class value and dividing by 50 (the total number of the plants). These data are presented in Table III. Here again there was little difference between the two varieties. The index of infection for Texas 7078 was

TABLE III

THE RELATIVE SEVERITY OF INFECTION ON THE PRIMARY ROOT SYSTEM OF FOUR-WEEK OLD PLANTS OF TWO VARIETIES OF GRAIN SORGHUM INOCULATED WITH SCLEROTIUM BATATICOLA AT PLANTING TIME

Infection Class	Frequency of plants of: Redlan	Texas 7078
1 /1	0	0
2	4	1
3	15	11
4	20	19
5	11	19
Index	3.8	4.1

/1 Class values range from 1= No infection to 5= dead plants.

slightly higher and reflected principally the number of plants which were dead (class 5). This difference was also noted in Table I where the preemergence seed-rot of Texas 7078 was higher than with Redlan. None of the noninoculated control plants were infected.

Following these observations, 10 plants of each variety in the inoculated series which had been classified into infection class 4, and 10 healthy plants of each variety from the noninoculated control flat were transplanted to one gallon glazed crocks filled with the soil mixture described previously. The plants were grown to maturity in a greenhouse where the daytime temperature was gradually raised from about 70°F at the time of transplanting to an average of slightly over 100°F at heading time. This temperature was maintained until the plants were mature. When the majority

of the plants were in the boot stage of the development each series of 10 plants was divided into two groups of 5. One group was watered regularly and kept at optimum soil moisture until mature. The other group was allowed to dry to the point of wilting and then was maintained at approximately that moisture level for a period of two weeks. Following this drought period those plants were again watered regularly at optimium soil moisture until mature.

When all of the plants were mature they were carefully removed from the soil and the total volume of the roots of each plant was measured by water displacement. The roots of each sorghum plant were dipped into a 500 ml beaker filled with water. . Then, the overflowing water from the beaker was measured in mls with a graduate cylinder. The results are presented in Table IV.

These data were analyzed statistically and all of the possible comparisons were found to be significant at odds of over 99:1. The means for all of the possible comparisons are presented in Table V. Inoculation with S. batatícola caused an appreciable reduction in total root volume, particularly in the variety Redlan. The reduction of root volume in the variety Texas 7078 may have been masked by the fact that several of the check plants of this variety had to be replaced after the original plants failed to survive the transplanting operation. Under all conditions the variety Redlan had the largest root system, on the average almost twice the size of the Texas 7078 variety.

The drought period of two weeks at heading time also caused a severe reduction in the root volume. The effect on both varieties was about the same. In the series of plants that were held at optimium moisture inoculation seemed to have very little effect, but in the

TABLE IV

THE VOLUME OF ROOTS OF MATURE PLANTS OF TWO SORGHUM VARIETIES INOCULATED AT THE TIME OF PLANTING WITH SCLEROTIUM BATATICOLA

Plant Number	Redlan				Texas 7078			
	Inoculated		check		Inoculated		check	
	dry[1]	wet	dry	wet	dry	wet	dry	wet
1	19[2]	58	59	142	21	33	25	--[3]
2	26	109	53	69	8	95	32	22[4]
3	66	82	29	90	15	46	55	44
4	94	116	40	99	--[3]	76	33	43
5	31	39	80	87	23	23	55	18[4]

[1] Dry = Two week drought period at heading stage; Wet = optimum soil moisture maintained throughout growth period.

[2] Measured in ml of water displacement.

[3] Plants died about boot stage of development and were discarded.

[4] Plants died immediately after transplanting and were replaced approximately one week after the other plants were transplanted.

TABLE V

THE MEAN ROOT VOLUME IN MILLILITERS OF TWO VARIETIES OF SORGHUM INOCULATED WITH SCLEROTIUM BATATICOLA AT THE TIME OF PLANTING AND SUBJECTED TO DIFFERENT MOISTURE LEVELS AT HEADING TIME

Varieties Vs Inoculation

Varieties	Inoculated	Check
Redlan	63.9	74.7
Texas 7078	35.4	35.8

Varieties Vs Moisture

Varieties	dry[1]	wet
Redlan	49.7	88.9
Texas 7078	28.2	43.0

Inoculation Vs Moisture

Inoculation	dry[1]	wet
Inoculated	21.7	67.5
check	46.1	64.3

Varieties Vs Inoculation Vs Moisture

Varieties	Inoculated		Check	
	dry[1]	wet	dry	wet
Redlan	47.1	80.6	52.5	97.1
Texas 7078	16.4	54.4	40.0	31.5[2]

[1] dry = 2 weeks drought period at heading stage; wet = Optimium moisture level maintained.

[2] Several plants in this series died and were replaced approximately one week after the other plants in the experiment were transplanted.

series that were subjected to drought the inoculated plants had notably smaller roots. The reduction of root volume in the variety Texas 7078 was especially noticeable in the series of inoculated plants which were subjected to drought.

Subsequently these plants were examined for disease lesions on the major secondary roots to determine if there was a correlation between such lesions and the reduction in total root volume. These data are presented in Table VI. Although there were more lesions on the inoculated plants, more lesions on the plants subjected to drought and more

TABLE VI

THE NUMBER OF LESIONS ON THE MAJOR SECONDARY ROOTS OF MATURE SORGHUM PLANTS INOCULATED WITH SCLEROTIUM BATATICOLA AT SEEDLING TIME

Varieties	Inoculated		Check	
	dry	wet	dry	wet
Redlan	6.2/1	5.2	3.4	1.0
Texas 7078	8.2	5.0	3.0	2.0

/1 Each figure is an average of 5 plants.

lesions on Texas 7078 than Redlan the number of lesions was not proportional to the reduction in root volume. Isolation from the lesions found on the inoculated plants yielded only S. batatícola. Isolations from the lesions on the check plants however, yielded primarily Fusarium moniliform but no S. batatícola.

Finally, the extent of infection into crown tissue of each plant was measured. All of the inoculated plants of Texas 7078 that were subjected to drought showed typical charcoal rot symptoms in the crown tissue (Figure 1) and S. batatícola was consistently isolated from this

area. There was some discoloration of the tissue at the base of the crowns of plants of this variety held at optimum moisture, and some of the plants of the Redlan variety were discolored somewhat, but the typical retting symptoms and sclerotia of charcoal rot did not develop. The extent crown infection in the mature plants is presented in Table VII. It will be noted

TABLE VII

THE EXTENT OF DISCOLORATION IN MILLIMETERS OF THE CROWN TISSUE OF MATURE SORGHUM PLANTS FOLLOWING INOCULATION WITH SCLEROTIUM BATATICOLA IN THE SEEDLING STAGE

Varieties	Inoculated		check	
	dry	wet	dry	wet
Redlan	2.2[1]	4.4	0	0
Texas 7078	21.5	4.2	6.2	2.2

[1] Each figure is an average of 5 plants.

that the only appreciable crown infection developed in the plants of Texas 7078 variety which were subjected to drought.

Following this study, and experiment was designed to determine if drought would affect the seedling reaction of these varieties as significantly as it had the mature plant reaction. For this purpose the varieties Redlan and Texas 7078 were again used. In this experiment the seeds were planted in 4-inch pots filled with the soil mixture mentioned previously at the rate of 102 seeds for the three pots used as a replication unit. All of the pots were watered the same amount and allow to germinate. The experiment was planted in duplicate and one replicate was held in a greenhouse at 68°F and the other one at 95°F. Following emergence, half of the pots of each variety at each temperature were watered to maintain

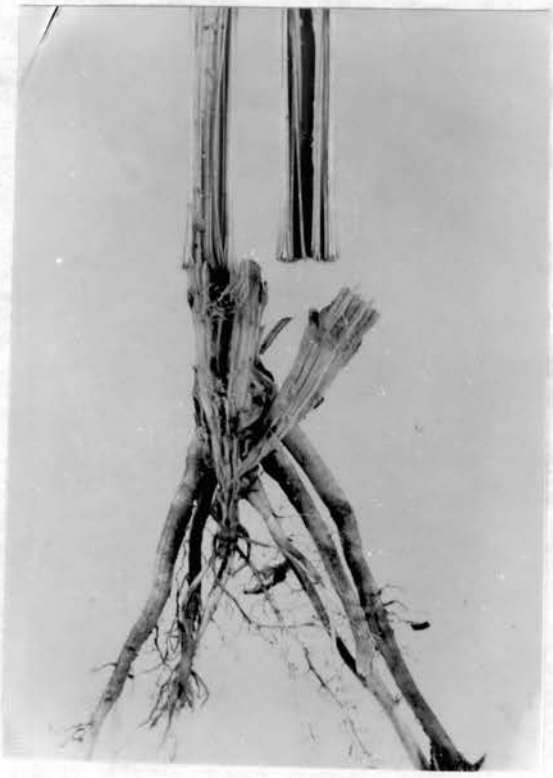

Fig. 1. Charcoal rot of Texas 7078 grain sorghum which developed from a seedling infected with Sclerotium bataticola and subjected to a 2-week drought period at heading time. (1/2.5 normal size)

optimum soil moisture. The other half was only watered half as often and half as much as the optimum soil moisture series. This entire experiment was again duplicated and the broth inoculum was used in one series and the oat-wheat inoculum was used in the second series. Three weeks after planting all of the pots were emptied and the plants were classified for disease reaction according to the system previous described. An index for each treatment was then calculated. No difference was found between the two sources of inoculum, and therefore only the data for the plants inoculated with broth inoculum are presented in Table VIII. It was found

TABLE VIII

THE DEGREE OF INFECTION OF THREE WEEK OLD SORGHUM SEEDLINGS INOCULATED WITH SCLEROTIUM BATATICOLA AT PLANTING TIME AND HELD AT DIFFERENT LEVELS OF TEMPERATURE AND MOISTURE

Varieties	Inoculated				Check			
	dry[1]		wet		dry		wet	
	68°F	95°F	68°F	95°F	68°F	95°F	68°F	95°F
Redlan	4.9	4.4	4.7	3.7	2.6	3.4	1.6	2.4
Texas 7078	5.0	4.9	4.6	4.5	1.9	1.7	1.2	3.4

[1] Wet = Optimum moisture level maintained; dry = watered half as much and half as often as "wet" series.

that neither temperature nor moisture had any appreciable influence upon the development of seedling blight caused by S. bataticola. Both varieties were infected to approximately the same degree, regardless of temperature or moisture conditions.

During the course of this experiment it was noted that 15 days after planting, all of the plants which had been inoculated with S. bataticola were taller than the plants in the check pots. This was true regardless of the type of inoculum used. At this time two random samples of 100

plants of each of the inoculated and check series were measured and it was found that the inoculated plants averaged over twice as tall as the check plants (Table IX).

TABLE IX

THE HEIGHT IN INCHES OF SORGHUM SEEDLINGS 15 DAYS AFTER INOCULATION WITH SCLEROTIUM BATATICOLA AT PLANTING TIME

Varieties	Inoculated	Check
Redlan	3.6[1]	1.4
Texas 7078	3.1	1.1

[1] Each figure is an average of approximately 100 plants

An experiment was then designed to determine whether this apparent stimulation was caused by the media used to produce the inoculum, the presnece of organism itself or to some metabolic product produced by the organism as it grew in the inoculum media. For this purpose 4 flats of 100 seeds of each variety were planted in sterilized sand. At the time of planting one flat of each variety was inoculated with sterile water, a second was inoculated with sterile potato-dextrose broth, a third flat was inoculated with a broth culture of S. bataticola and the fourth flat was inoculated with the broth from a culture of S. bataticola after it had been filtered to remove the inoculum. Two weeks after planting the height of the plants was measured and the results of this test are presented in Table X. The only stimulation observed was when the plants were inoculated with a broth culture of S. bataticola or when they were inoculated with the broth of a culture in which this organism had been growing. These plants were not discarded immediately after these observations were made, and when

TABLE X

THE HEIGHT IN INCHES OF 14 DAY OLD SORGHUM SEEDLINGS PLANTED IN STERILE SAND AND INOCULATED IN VARIOUS WAYS

Variety	Inoculated with			
	Sterile water	Sterile potato-dextrose broth	Broth culture of S. bataticola	Broth with S. bataticola filtered out
Redlan	1.1[1]	1.0	2.6	2.1
Texas 7078	1.1	1.2	2.2	2.1

[1] Each figure is an average of approximately 100 plants.

they were examined again, approximately 5 weeks after planting, it was noted that the stimulating effect was no longer visible. In fact, those plants in the flats inoculated with the culture of S. bataticola and those inoculated with filtered culture were smaller and were showing definite symptoms of seedling blight.

CONCLUSIONS

As a result of these investigations it can be concluded that Sclerotium bataticola causes a severe injury to seedlings of certain grain sorghum varieites. The attack of this organism in the seedling stage is principally on the primary root system and the lower portions of the hypocotyl tissue. The conditions of temperature and moisture utilized in these experiments had little effect upon the developed of the seedling infection.

In the field, stalk rot caused by S. bataticola has never been observed in the variety Redlan at this station, while in the same areas and often in the same tests the variety Texas 7078 has been infected 100 percent. In these studies, however, there was very little, if any, difference in the degree of seedling infection caused under which the seedlings were grown.

When the infected seedlings were grown to maturity, crown and stalk infection developed, apparently as a continuation or extention of the seedling infection, under certain enviromental conditions. It appeared from these studies that a period of drought of at least two weeks at approximately the heading stage was required before crown and stalk rot would develop. This is in agreement with the reports of field observations made by Livingston (11) and Young (21). When the crown and stalk infections of the two varieties were compared, it was found that in the mature stage Redlan was infected only slightly and did not develop the retting and sclerotia of typical charcoal rot whereas the plants of the variety Texas

7078 which were subjected to the drought period did develop these symptoms. The difference in susceptibility of these two varieties which had been observed under conditions of natural infection could be duplicated in the mature plant stage but not in the seedling stage. It would appear from these studies that the reaction of varieties in the seedling stage would not be indicative of their reaction in the later stages of plant development.

These studies also demonstrated that the charcoal disease of the crowns and stalks of susceptible grain sorghums will develop, under certain conditions, from infected seedlings. This method could be used, then, for testing sorghum varieties and selections for resistance to charcoal rot in place of relying upon natural infection in the field.

In the seedling tests a stimulation in growth of seedlings occured following inoculation with S. bataticola. This stimulation was evident from the time of emergence until about 3 weeks after planting, when the effect of the root and hypocotyl infections become dominant. An investigation showed that this stimulating effect was a result of some metabolic product produced by the growth of the organism on potato-dextrose broth. Further studies would be required to determine the nature of this metabolic substance.

SUMMARY

1. The browning and discoloration of the leaves of sorghum seedlings inoculated with Sclerotium bataticola was about the same for the varieties Redlan and Texas 7078.

2. Infection of the hypocotyl tissue of sorghum seedlings took place almost exclusively at the point of seed attachment. The progress of the disease infection upward through the hypocotyl tissue was essentially the same for the two varieties, Redlan and Texas 7078.

3. An index of infection of the primary root system indicated that the Redlan and Texas 7078 varieties were infected to about the same degree. The later variety had more seedlings actually killed than did Redlan.

4. When infected seedlings were carried to maturity; 1) The root volume of Redlan was almost twice that of Texas 7078 regardless of moisture or infection; 2) Plants from infected seedlings had noticeably less root volume than the check plants of either variety regardless of moisture; 3) A two week period of drought at heading time reduced the root volume regardless of variety or infection; 4) The root volume of infected plants of Texas 7078 subjected to drought was smaller than all other combinations of variety moisture and infection.

5. The number of disease lesions on the major secondary roots was related, but not proportional, to the total root volume.

6. Infection of the crown tissue and the development of typical charcoal rot symptoms in the mature plants were found only in the variety

Texas 7078 grown from infected seedlings and subjected to a drought period at heading time.

7. Temperature and soil moisture had little or no effect upon the development of seeding infections of the two varieties Redlan and Texas 7078.

8. A stimulation in growth in the initial stages was observed when seeds were inoculated with potato-dextrose broth culture of S. bataticola at planting time. This stimulation was later marked by the development of infection in the seedlings.

9. The stimulation of seedling growth was apparently due to a metabolic product produced by the growth of S. bataticola on the potato-dextrose broth medium.

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VITA

Lii-Jang Liu

Candidate for the Degree of

Master of Science

Thesis: INFECTION OF SORGHUM BY SCLEROTIUM BATATICOLA TAUB.

Major: Botany and Plant Pathology

Biographical:

Born: July 17, 1929. at Foochow, Fukien, China

Undergraduate Study: Taiwan Provincial College of Agriculture Taichung, Taiwan, 1947-1951.

Graduate Study: Ohio State University, Columbus, Ohio, 1956-1957. Oklahoma State University, Stillwater, Oklahoma, 1957-1958.

Experience: Assistant Agronomist, Taiwan Sugar Experiment Station Tainan, Taiwan, 1952-1956.

Date of Final Examination: August, 1958.