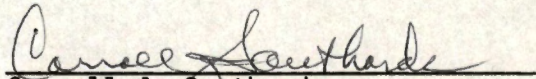
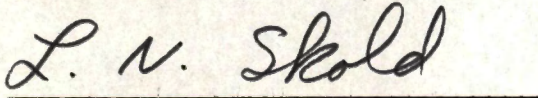


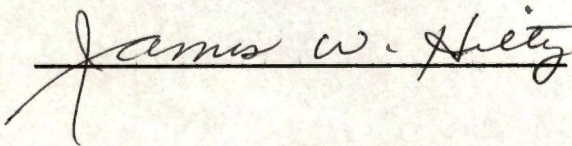
To the Graduate Council:

I am submitting herewith a thesis written by Margaret Leibowitz Hedrick entitled "An Interaction Between Meloidogyne incognita (Kofoid and White) (Nematoda: Heteroderidae) with the Fungus Sclerotium rolfsii (Saccardo) or Cylindrocladium crotalariae (Bell and Sobers) on Three Soybean Varieties." I recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Agricultural Biology.

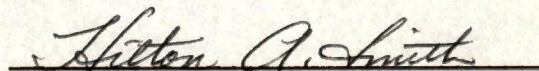

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AN INTERACTION BETWEEN MELOIDOGYNE INCOGNITA (KOFOID AND WHITE)
(NEMATODA: HETERODERIDAE) WITH THE FUNGUS SCLEROTIUM ROLFSII
(SACCARDO) OR CYLINDROCLADIUM CROTALARIAE (BELL AND SOBERS)
ON THREE SOYBEAN VARIETIES

A Thesis
Presented for the
Master of Science
Degree
The University of Tennessee

Margaret Leibowitz Hedrick

June 1974

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The author is also indebted to her parents and especially to her husband, Gale, for his encouragement and guidance.

ABSTRACT

The interaction on soybeans between the nematode Meloidogyne incognita and the fungus Sclerotium rolfsii, and between the nematode Meloidogyne incognita and the fungus Cylindrocladium crotalariae was studied under greenhouse conditions. Three soybean varieties chosen for their resistant and susceptible qualities to M. incognita were used: 'Lee,' 'Hill,' and 'Mack.' Treatments involved pathogens inoculated alone or in various combinations. Predisposition by nematodes was studied by nematode inoculation 14 days prior to fungus inoculation and by simultaneous nematode-fungus inoculation. Significant nematode-fungus interactions were found to occur as indicated by measurements of plant height, top weight and root weight and by ratings of nodule formation, nematode gall and egg mass formation and root necrosis.

Using root necrosis as a measurement of disease development, the greatest necrosis for treatments using C. crotalariae occurred where M. incognita was inoculated at planting and C. crotalariae was added after 14 days. Not only does this indicate the occurrence of an interaction between the pathogens, but it also indicates that predisposition by nematodes had occurred. Evidence that formation of nematode galls is associated with root nodules was also found. Greatest nodule formation was found on treatments where nematodes were inoculated alone; on these plants extensive gall formation was evident. Disease development in plants was also measured by percentage plant mortality.

Highest mortality for plants treated with either fungus occurred where the nematode and fungus were combined simultaneously.



TABLE OF CONTENTS

CHAPTER	PAGE
I. INTRODUCTION	1
II. MATERIALS AND METHODS	3
III. RESULTS	7
IV. DISCUSSION	27
V. SUMMARY AND CONCLUSION	35
LITERATURE CITED	37
VITA	41

LIST OF TABLES

TABLE	PAGE
I. Variety Comparisons by Treatments for Nematode Gall Formation Ratings on Those Plants Treated with <u>M. incognita</u> and <u>C. crotalariae</u>	23
II. Necrosis Ratings by Treatment and Variety for Plants Treated with <u>M. incognita</u> and <u>C. crotalariae</u>	24

LIST OF FIGURES

FIGURE	PAGE
1. Comparison of Percent Mortality by Treatments	8
2. Mycelium and Sclerotium Formation on Plants Treated with <u>S. rolfsii</u>	9
3. Treatment Comparisons of Percent Mortality for Plants Treated with <u>S. rolfsii</u> and <u>M. incognita</u>	10
4. Treatment Comparisons of Percent Plant Mortality for Plants Treated with <u>C. crotonariae</u> and <u>M. incognita</u>	11
5. Comparison of Percent Mortality by Treatments	12
6. Percent Mortality by Variety for Plants Treated with <u>S. rolfsii</u> and <u>M. incognita</u>	13
7. Percent Mortality by Variety for Plants Treated with <u>C. crotonariae</u> and <u>M. incognita</u>	14
8. Root Weight by Treatments Involving <u>M. incognita</u> and <u>S. rolfsii</u>	16
9. Treatment Comparisons of Root Nodulation Ratings for Plants Treated with <u>S. rolfsii</u> and <u>M. incognita</u>	17
10. Treatment Comparisons for Nematode Gall Formation Ratings Where <u>S. rolfsii</u> and <u>M. incognita</u> Were Used	18
11. Variety Comparisons of Nematode Gall Formation Ratings for Plants Treated with <u>S. rolfsii</u> and <u>M. incognita</u>	19
12. Treatment Comparisons of Root Necrosis Ratings for Plants Treated with <u>S. rolfsii</u> and <u>M. incognita</u>	20
13. Treatment Comparisons of Root Nodulation Ratings for Plants Treated with <u>C. crotonariae</u> and <u>M. incognita</u>	22
14. Root Necrosis on Soybean Plant Resulting from Inoculation with <u>Cylindrocladium crotonariae</u>	25
15. Treatment Comparisons of Root Necrosis Ratings for Plants Treated with <u>C. crotonariae</u> and <u>M. incognita</u>	26

CHAPTER I

INTRODUCTION

Soybeans are Tennessee's leading crop, both in value and in acreage. Income in Tennessee from soybean production in 1973 was 202.9 million dollars, and the acreage of soybeans planted that year was 1.6 million acres (31).

Several diseases affect soybeans including Cylindrocladium crotalariae (Bell and Sobers), Sclerotium rolfsii (Saccardo), and the root-knot nematode, Meloidogyne incognita (Kofoed and White). Many studies concerning the individual effects of each of these organisms have been made using various plant varieties. Rather extensive investigations on the effect of S. rolfsii have been made, but few studies have been concerned with its effects on soybeans. Cylindrocladium crotalariae is a relatively new pathogen which was first described in 1966 on peanuts (3). It has been observed on soybeans although no extensive research on its pathogenicity on soybeans has been reported. The root-knot nematode has been studied on soybeans as well as on numerous other plants. There are several reports of combinations of root-knot nematodes with other nematodes (14, 15), with fungi (24, 32), and with bacteria (19, 28) on host plants, but there are few reports on these kinds of interactions on soybeans.

Nematode-fungus interactions have been known to exist for some time (1). The fungus-nematode complex may have a depressing effect on the disease by decreasing disease severity, it may have an additive

effect where the extent of disease is equal to the respective damage of each pathogen combined, or finally, it may have a synergistic effect where the disease extent is greater than the additive effect. In some instances fungi which when alone are normally nonpathogenic on a particular plant, may actually colonize plant tissue and cause necrosis in the presence of nematodes. The majority of these fungus-nematode complexes involve species of the root-knot nematode. This nematode causes profound changes in the host plants including hypertrophy, hyperplasia, root proliferation, blocking of vascular elements, and an alteration in the plant's nutritional balance (4). In addition the females produce gelatinous egg masses on the surface of galls. There is evidence that nematode egg masses and associated plant metabolites attract fungi to the affected root area (10).

An association between nematodes and S. rolfsii was thought to exist in soybean fields in Western Tennessee, since southern blight was observed only in fields where nematodes were present (J. M. Epps, personal communication). No research has been done to confirm the hypothesis.

The objectives of this study were: (1) to determine the effect of M. incognita, S. rolfsii, and C. crotalariae on three different soybean varieties when inoculated alone and in certain combinations, and (2) to determine the difference in host response to a pathogen when inoculated simultaneously at seeding or by adding the fungus 14 days after inoculation with nematodes.

CHAPTER II

MATERIALS AND METHODS

This study was conducted in the greenhouse at Knoxville, Tennessee, from August 3, 1973, until November 26, 1973. Three soybean varieties were selected: (1) 'Hill,' which is resistant to M. incognita, (2) 'Lee,' which is susceptible to M. incognita, and (3) 'Mack,' which is susceptible to M. incognita. Five seeds of each variety were planted in sterile soil in four-inch plastic pots and five pots of each variety were used for each treatment. All data were recorded separately for each pot. Treatments consisted of: (1) a control to which sterile media was added, (2) M. incognita added alone, (3) S. rolf sii added alone, (4) C. crotalariae added alone, (5) M. incognita-S. rolf sii added simultaneously, (6) M. incognita-C. crotalariae added simultaneously, (7) M. incognita-S. rolf sii where the fungus was added 14 days after the nematodes, and (8) M. incognita-C. crotalariae where the fungus was added 14 days after the nematodes. The entire study was conducted in 1973, once on August 3 through October 19 and again on September 17 through November 26. An additional treatment was introduced in the second test whereby the fungus alone was added 14 days after the seed was planted.

Cylindrocladium crotalariae cultures were obtained from Dr. R. C. Rowe, North Carolina State University, Raleigh, North Carolina, who had designated them as the K-3 variety pathogenic to soybeans. Sclerotium rolf sii cultures were extracted from infected plants as sclerotia and

surface sterilized in a 10 percent Clorox solution before being plated on potato dextrose agar. Eggs of M. incognita were extracted from tomato roots by agitating by hand in a 10 percent Clorox solution for four minutes followed by a 1:1 dilution and sieving through a series of screens where the eggs were collected on a 400 mesh screen. Each pot of plants was inoculated with a calibrated suspension of 5,000 eggs. Plants grew for approximately 70 days in greenhouse conditions where the temperature range was between 75° F and 100° F.

The fungus inoculum in the first test was cultured on potato dextrose agar and each pot was inoculated with a circular mat of mycelium 11 cm in diameter using the inoculum layering technique of Schmitthenner and Hilty (29). Sterile wheat seeds were also added to provide an organic media for the fungus. On the second test, however, wheat seeds were placed in 500 ml Erlenmeyer flasks (approximately two-thirds full) and autoclaved. Under sterile conditions mycelial blocks of the fungus were added and seeds were incubated for several weeks. By this method the wheat seeds became well infected with mycelium and were then used as inoculum.

Epps et al. (7) showed that pure cultures of fungus grown on autoclaved wheat seeds can be held for many months with no apparent loss in viability. Mycelium, however, grown on sterile agar is usually considered persistent for only short periods of time. They also showed that the vegetative stage of S. rolfsii survived for only a few weeks in the greenhouse.

Approximately 25 infected wheat seeds were added to each pot and blended with the sterile soil. Sterilized wheat seeds were used in

Results of Test 1 and Test 2 of this experiment were combined in the analysis of variance as the differences between the two were not statistically significant.

CHAPTER III

RESULTS

Significant differences occurred in the percent mortality among varieties and treatments. Tests involving S. rolfsii showed a mortality range of 83.8 percent to 14.7 percent. The greatest mortality, 83.8 percent, occurred where M. incognita and S. rolfsii were combined simultaneously (Figures 1 and 2). Where the fungus was applied alone to plants the mortality was 80.3 percent (Figure 3). Tests involving C. crotalariae showed a mortality range of from 35.3 percent to 14.7 percent. The greatest mortality occurred where M. incognita and C. crotalariae were combined simultaneously (Figures 4 and 5). Where the fungus alone was used the mortality was 25.7 percent. The lowest mortality occurred where M. incognita was applied alone, 16.6 percent and in the uninoculated control, 14.7 percent. Similarities in mortality between treatments of C. crotalariae and S. rolfsii occurred even though the magnitude of mortality was quite different. 'Mack' exhibited the greatest mortality of all varieties, 35.2 percent for C. crotalariae and 62.7 percent for S. rolfsii. 'Lee' and 'Hill' varieties had 21.6 percent and 24.9 percent, respectively, for C. crotalariae and 50.4 percent and 46.0 percent, respectively, for S. rolfsii (Figures 6 and 7).

No significant differences in height or in top weight were found in treatments in which S. rolfsii was used. Root weights were significantly greater at the 5 percent level of probability for all treatments other than where M. incognita and S. rolfsii were combined



Figure 1. Comparison of percent mortality by treatments. Rows from left to right: M. incognita alone, S. rolfii alone, M. incognita and S. rolfii combined simultaneously, and the control.

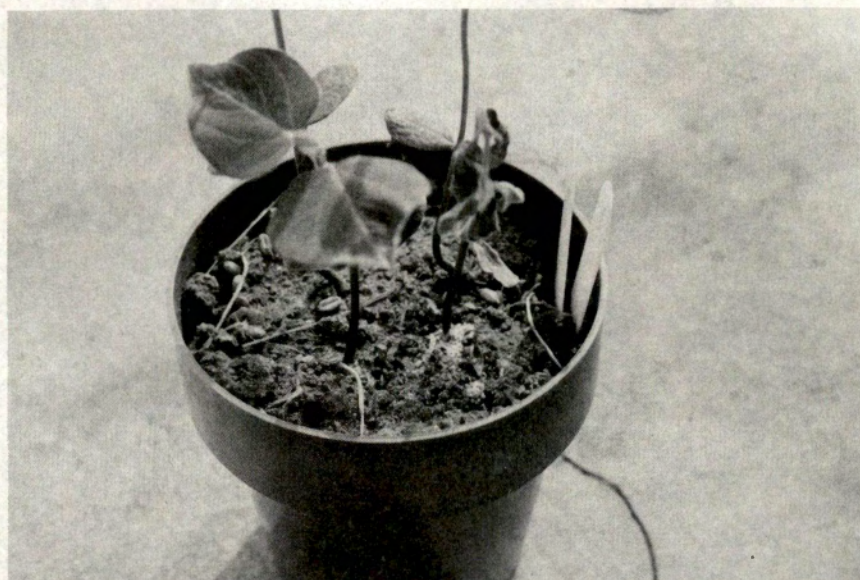


Figure 2. Mycelium and sclerotium formation on plants treated with S. rolfsii.

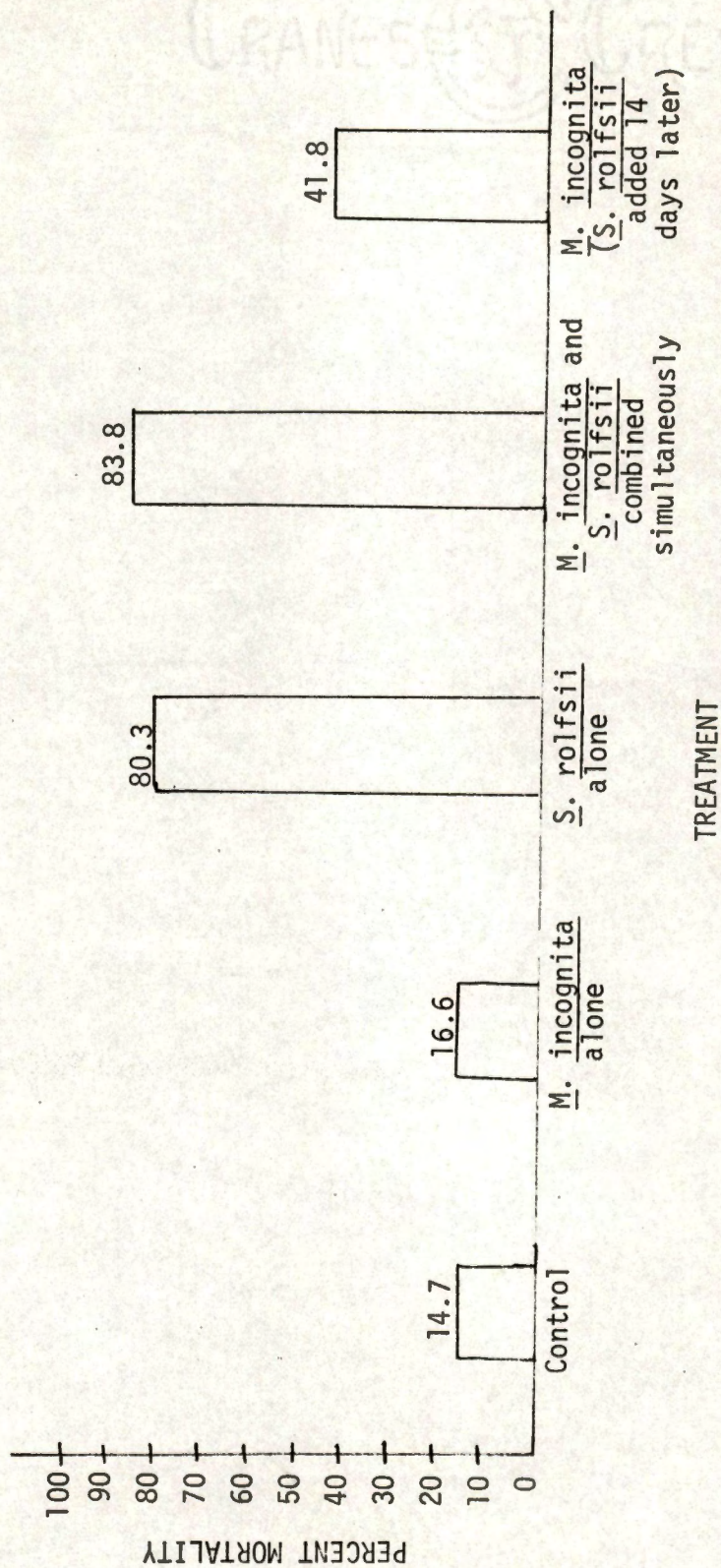


Figure 3. Treatment comparisons of percent mortality for plants treated with *S. rolfsii* and *M. incognita*.

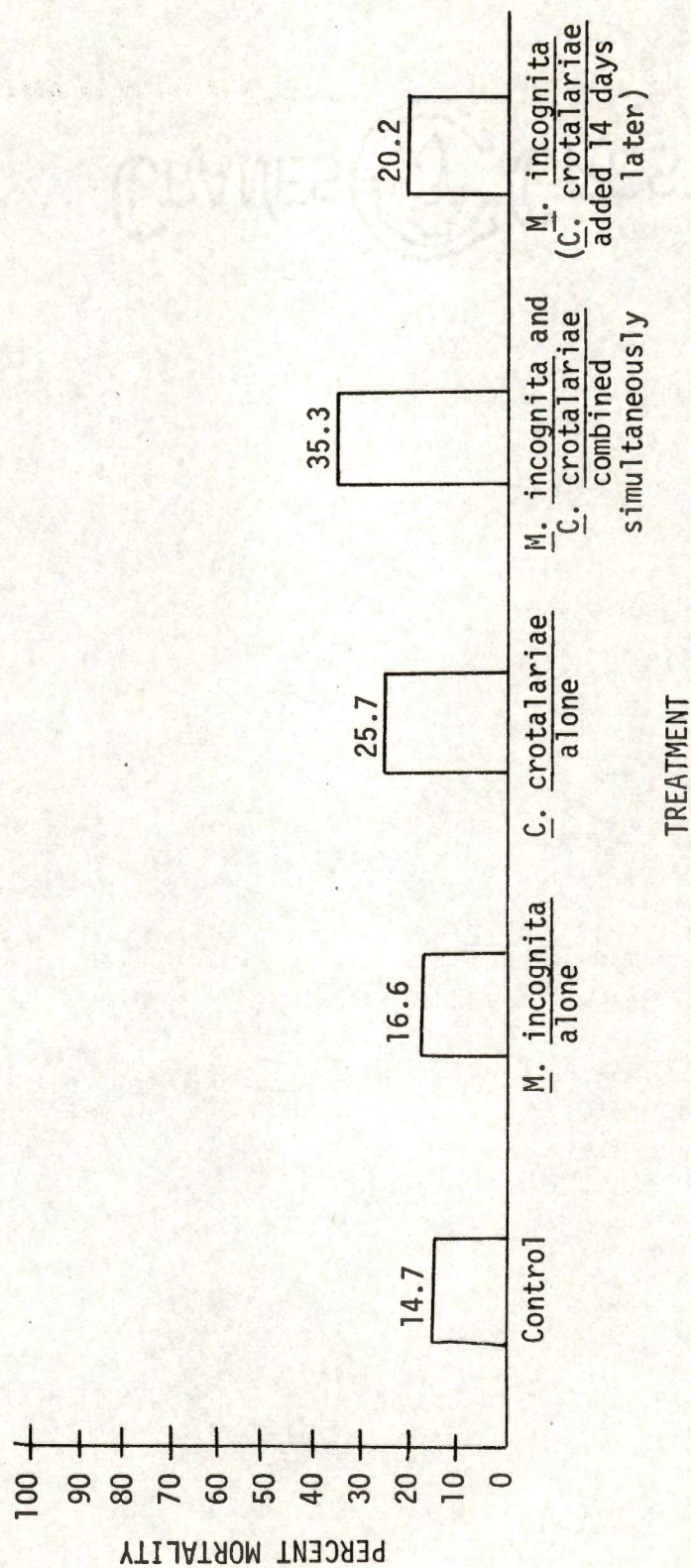


Figure 4. Treatment comparisons of percent plant mortality for plants treated with C. crotonariae and M. incognita.



Figure 5. Comparison of percent mortality by treatments. Rows from left to right: M. incognita alone, C. crotalariae alone, M. incognita and C. crotalariae combined simultaneously, and the control.

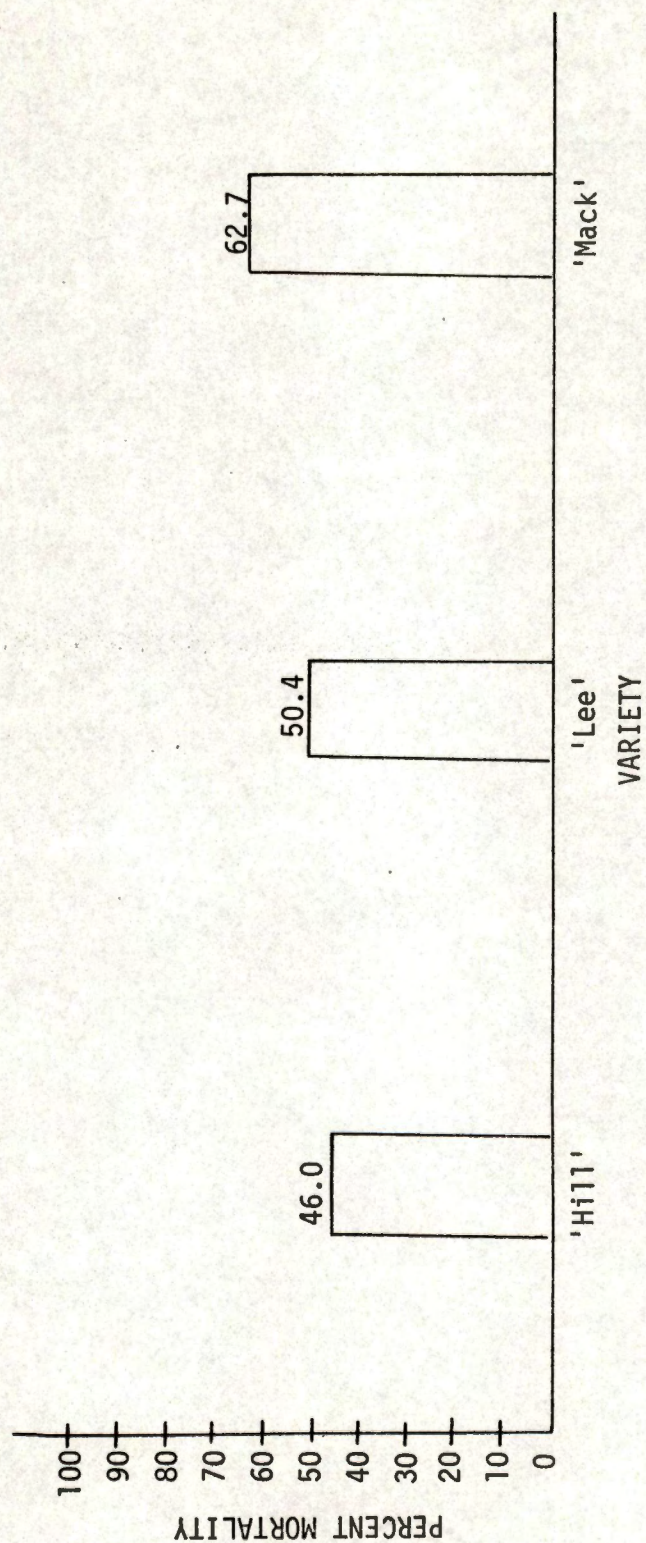


Figure 6. Percent mortality by variety for plants treated with *S. rolfsii* and *M. incognita* ('Hill' is resistant to *M. incognita*; 'Lee' and 'Mack' are susceptible to *M. incognita*.)

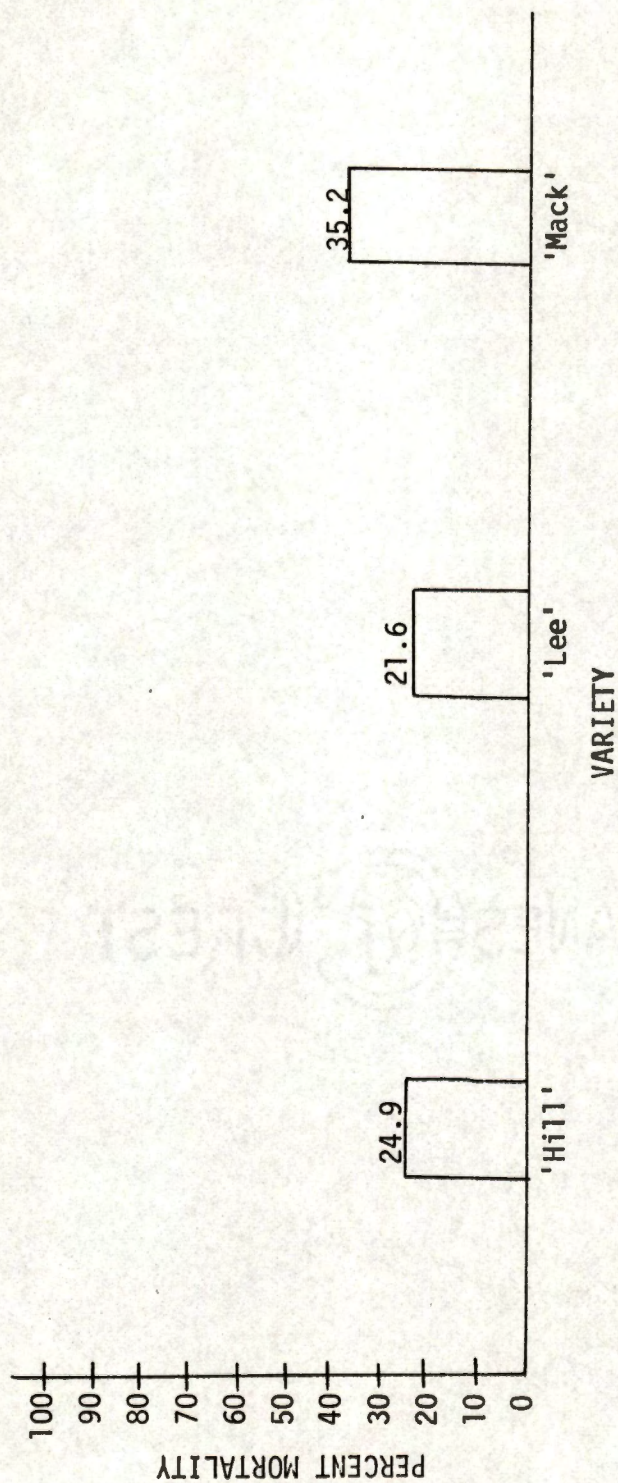


Figure 7. Percent mortality by variety for plants treated with *C. crotalariae* and *M. incognita*. ('Hill' is resistant to *M. incognita*; 'Lee' and 'Mack' are susceptible to *M. incognita*.)

simultaneously (Figure 8). Root nodulation was affected by some treatments. Greatest nodulation occurred where nematodes alone were used and in the uninoculated control (Figure 9). The nodule index for these treatments was 4.0 and 3.2, respectively. The lowest nodule index (2.0) was found when M. incognita and S. rolfsii were added simultaneously (Figure 9). Gall ratings were made for extent of gall formation on those roots inoculated with nematodes. By treatment, plants where the root-knot nematode alone and the root-knot nematode and S. rolfsii were combined after 14 days had significantly more galls and egg masses than when root-knot nematode and S. rolfsii were inoculated simultaneously (Figure 10). Where the nematode was added alone, 'Mack' variety had significantly fewer galls than 'Lee' or 'Hill.' In all treatments where the nematodes and S. rolfsii were combined, 'Hill' had fewer galls than either 'Lee' or 'Mack' (Figure 11). Nematode egg masses associated with galls were most prevalent where M. incognita-S. rolfsii were combined with the fungus added after 14 days. Necrosis measurements showed all treatments to be significantly more necrotic than the uninoculated control (Figure 12).

Data for C. crotalariae were also analyzed using Duncan's Multiple Range Test. No significant differences in height at the 5 percent level of probability were shown with the exception of the combination of M. incognita-C. crotalariae where the fungus was applied 14 days after the nematode. There were no differences in top weight and root weight among treatments. Differences were found in nodulation among treatments, however. Treatments of M. incognita alone resulted in a significantly greater number of nodules (4.0 on the nodule index) than all

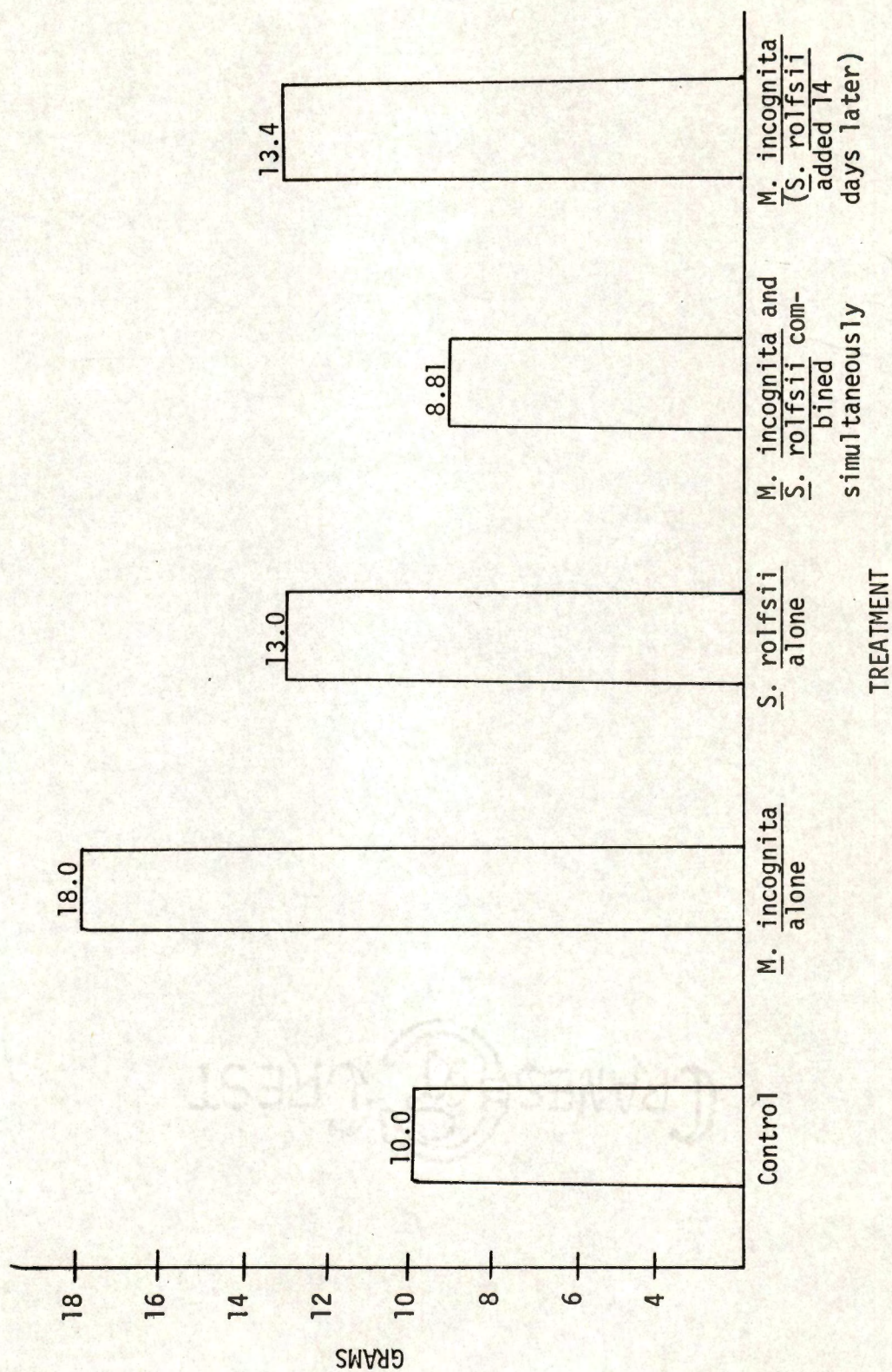


Figure 8. Root weight by treatments involving M. incognita and S. rolfsii.

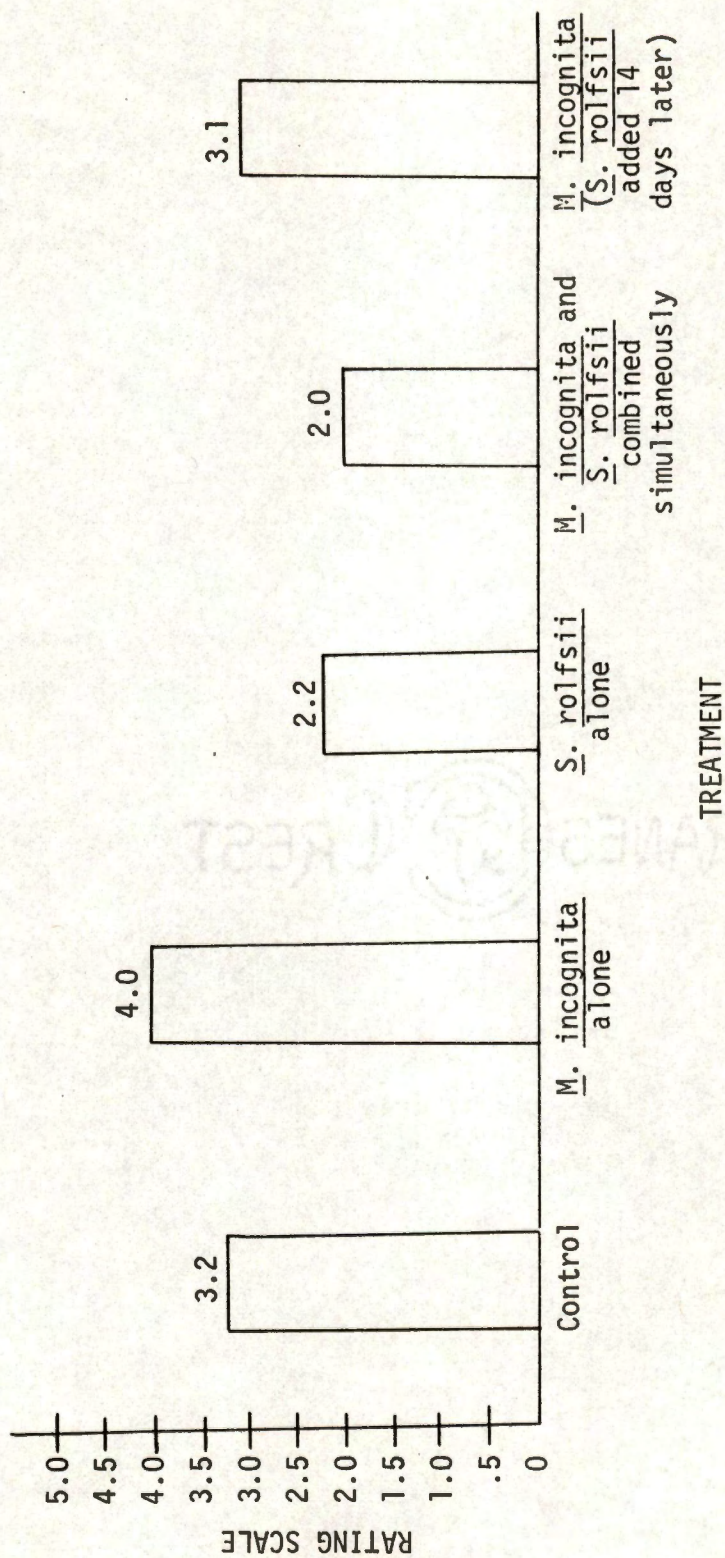


Figure 9. Treatment comparisons of root nodulation ratings for plants treated with *S. rolfsii* and *M. incognita*. Data expressed as nodule index (number of nodules present): 0 = none; $\frac{1}{1-5}$ = 1-5 nodules, 2 = 6-12 nodules, 3 = 13-25 nodules, 4 = 26-100 nodules, 5 = over 100 nodules.

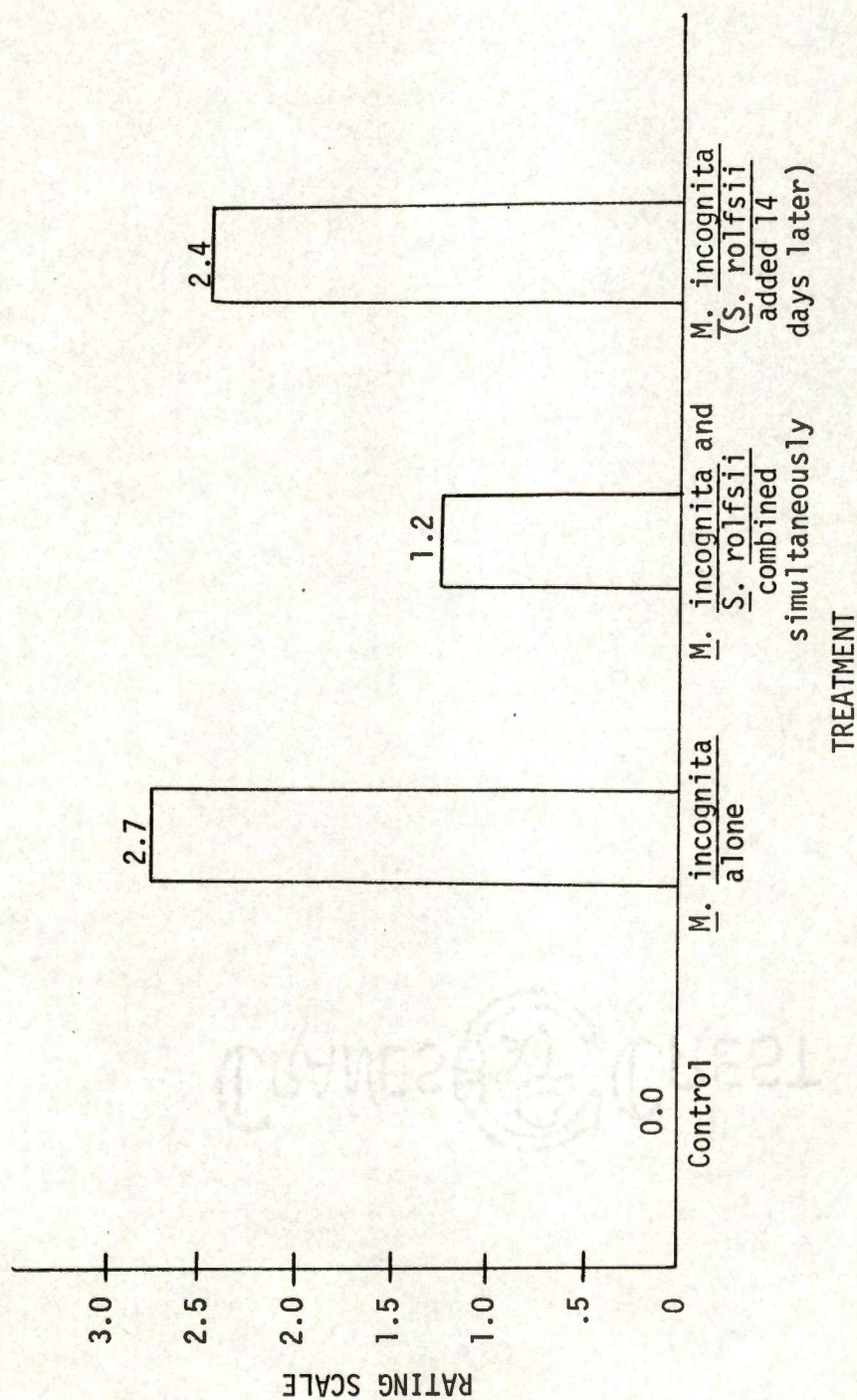


Figure 10. Treatment comparisons for nematode gall formation ratings where *S. rolfsii* and *M. incognita* were used. Data based on root knot index (number of galls and egg masses present): 0 = none, 1 = 1-5 galls and egg masses, 2 = 6-12 galls and egg masses, 3 = 13-25 galls and egg masses, 4 = 26-100 galls and egg masses, 5 = obviously over 100 galls and egg masses.

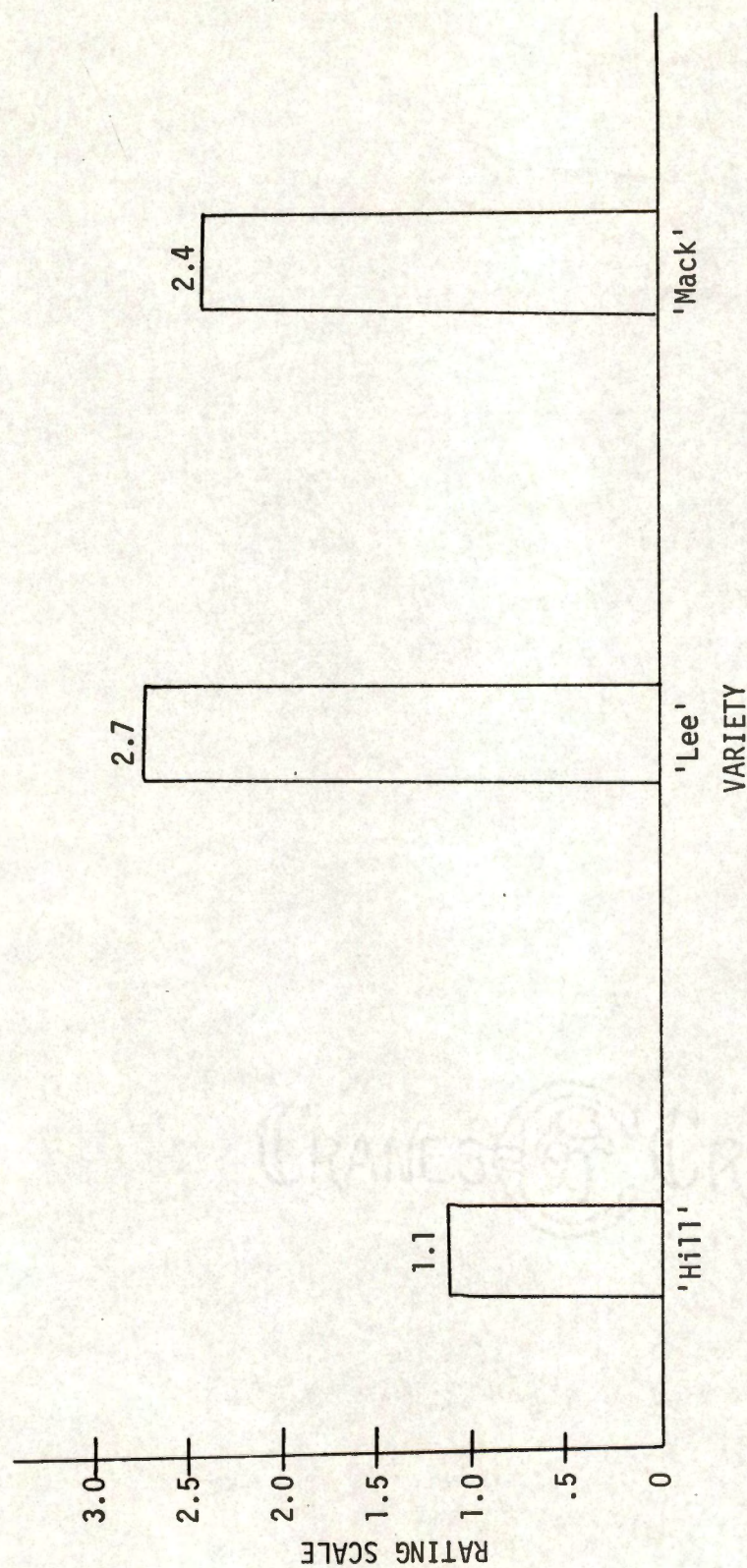


Figure 11. Variety comparisons of nematode gall formation ratings for plants treated with *S. rolfsii* and *M. incognita*. ('Hill' is resistant to *M. incognita*; 'Lee' and 'Mack' are susceptible to *M. incognita*.) Data based on root knot index (number of galls and egg masses present): 0 = none, 1 = 1-5 galls and egg masses, 2 = 6-12 galls and egg masses, 3 = 13-25 galls and egg masses, 4 = 26-100 galls and egg masses, 5 = over 100 galls and egg masses.

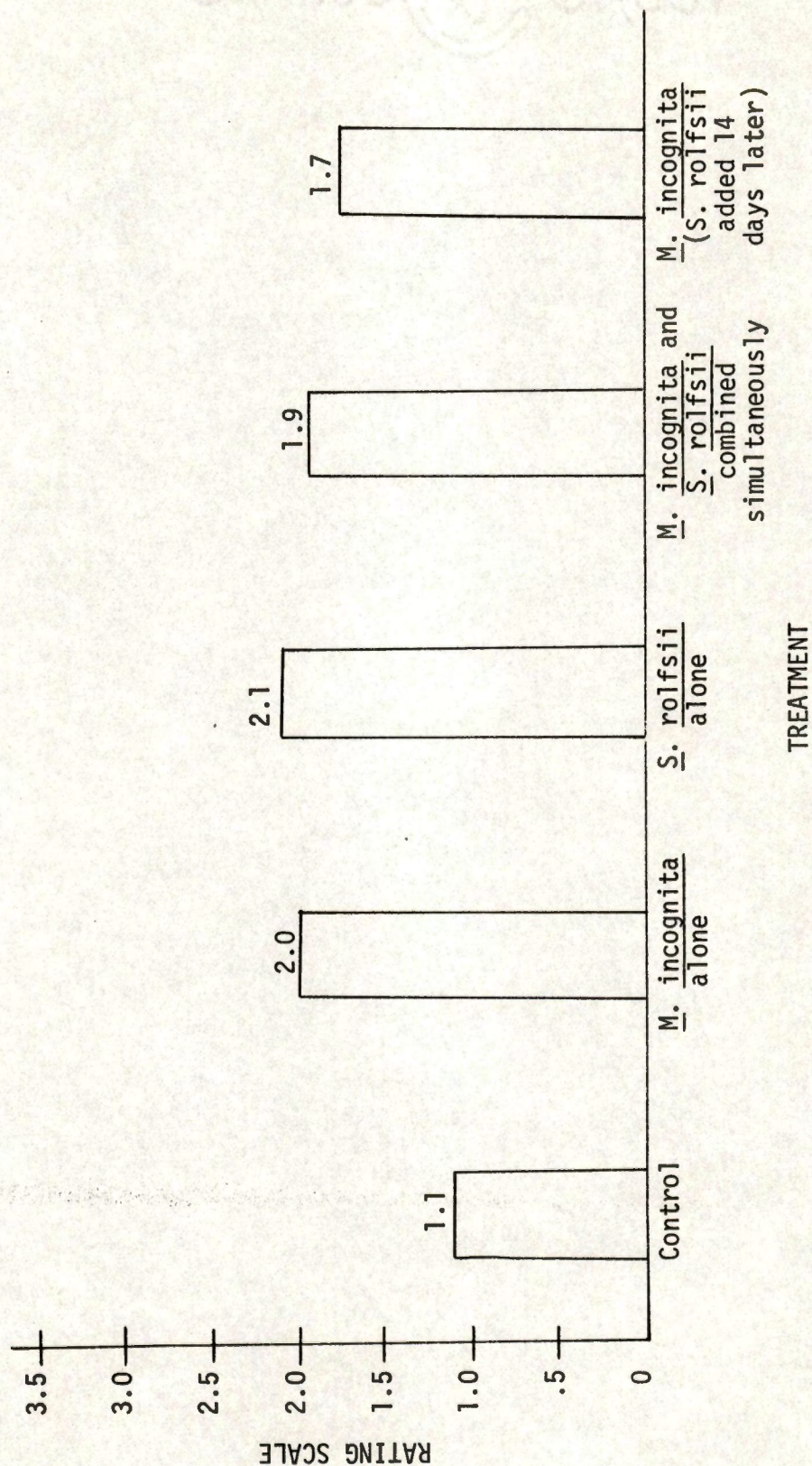


Figure 12. Treatment comparisons of root necrosis ratings for plants treated with *S. rolfsii* and *M. incognita*. Root necrosis index or disease index (percent visibly necrotic): 0 = none; 1 = Less than 10 percent, 2 = 11-25 percent, 3 = 26-50 percent, 4 = 51-75 percent, 5 = 76-100 percent.

other treatments. There was no significance among the following treatments: (1) M. incognita and C. crotalariae combined simultaneously (3.2), (2) the control (3.2), and (3) M. incognita alone (3.1).

Nodulation was significantly lower than M. incognita alone but greater than M. incognita and C. crotalariae combined in 14 days whose rating was 2.3 (Figure 13).

Gall ratings were also made for treatments of M. incognita and C. crotalariae. No significant differences by treatments for number of galls was observed. By variety, however, 'Lee' and 'Mack' varieties exhibited significantly more galls than 'Hill.' Where the nematode was added alone, 'Mack' variety had significantly less galls than 'Lee' or 'Hill.' In treatments where the nematode was in combination with the fungus, 'Mack' and 'Lee' had more galls than 'Hill.' The same was true for treatments which involved S. rolfsii (Table I). Egg masses formed on galls were more numerous in those treatments where nematodes and fungi were combined than where nematodes were inoculated alone. Root necrosis was also rated. The following showed significantly greater necrosis than all other treatments: (1) all varieties treated with M. incognita and C. crotalariae added in 14 days, (2) M. incognita alone on 'Lee' and C. crotalariae alone on 'Lee' (Table II). It was evident that the greatest necrosis of all treatments occurred when M. incognita and C. crotalariae were combined and the fungus was added 14 days after the nematode (Figure 14). The necrosis rating for the 14 day combination was 3.2 compared to 2.0 for the nematode alone and 2.1 for the fungus alone (Figure 15). 'Lee' variety was also more necrotic than either 'Hill' or 'Mack.'

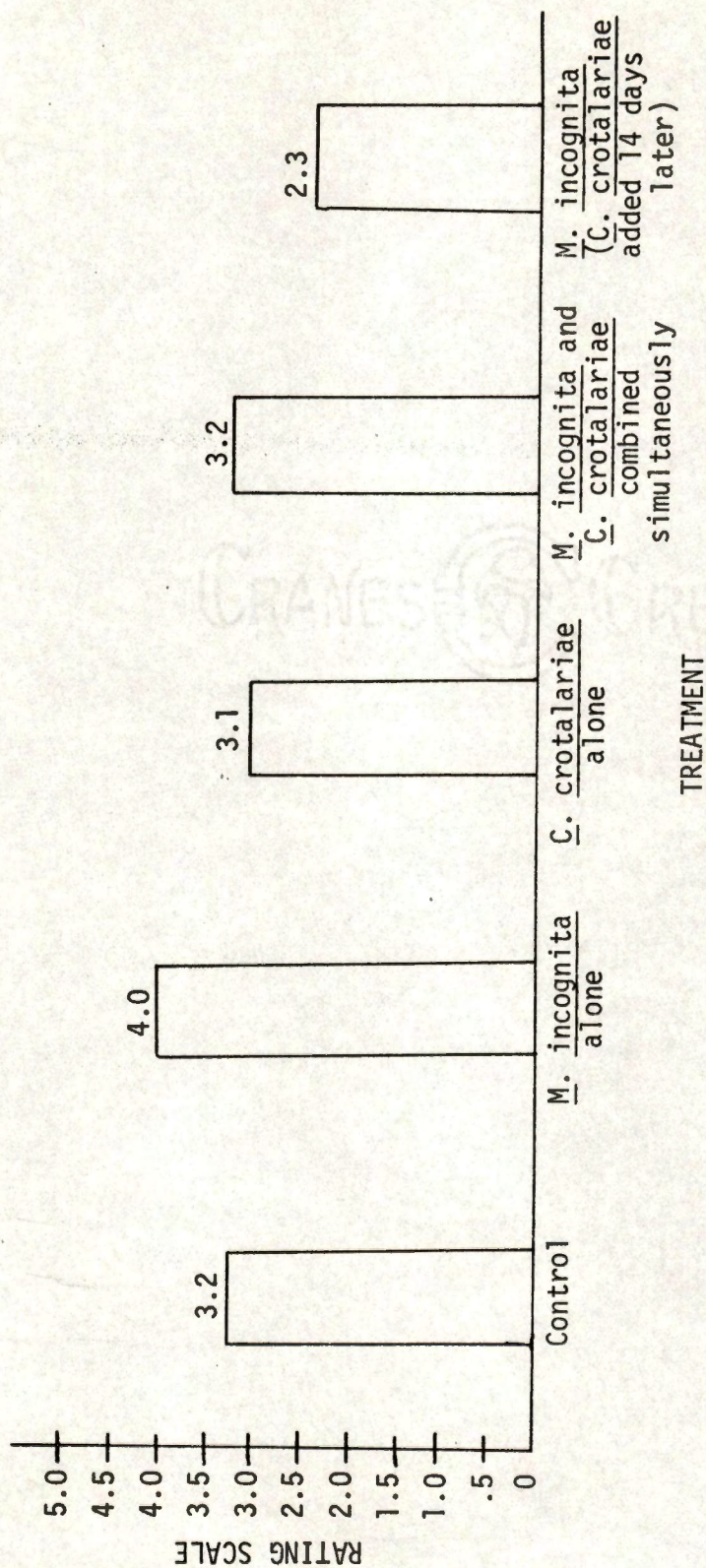


Figure 13. Treatment comparisons of root nodulation ratings for plants treated with C. crotalariae and M. incognita. Data expressed as nodule index (number of nodules present): 0 = none, $\bar{1} = 1-5$ nodules, $\bar{2} = 6-12$ nodules, $\bar{3} = 13-25$ nodules, $\bar{4} = 26-100$ nodules, $\bar{5} = \text{over } 100$ nodules.

TABLE I

VARIETY COMPARISONS BY TREATMENTS FOR NEMATODE GALL
FORMATION RATINGS ON THOSE PLANTS TREATED
WITH M. INCOGNITA AND C. CROTALARIAE^a

Treatment	Varieties ^b			Treatment Means ^c
	'Hill'	'Lee'	'Mack'	
Control	0.0	0.0	0.0	0.0 b
<u>M. incognita</u>	2.9	3.4	1.9	2.0 a
<u>M. incognita</u> and <u>C. crotalariae</u> simultaneously	1.1	2.8	3.5	1.8 a
<u>M. incognita</u> added 14 days before <u>C. crotalariae</u>	1.7	3.2	3.7	2.2 a
Variety means	1.4 b	2.3 a	2.2 a	

^aData based on root-knot index (number of galls and egg masses present): 0 = none, 1 = 1-5 galls and egg masses, 2 = 6-12 galls and egg masses, 3 = 13-25 galls and egg masses, 4 = 26-100 galls and egg masses, 5 = obviously over 100 galls and egg masses.

^b'Hill' is resistant to M. incognita; 'Lee' and 'Mack' are susceptible to M. incognita.

^cDifference in letters denotes significant difference at the 5 percent level of probability (Duncan's Multiple Range Test).

TABLE II

NECROSIS RATINGS BY TREATMENT AND VARIETY FOR PLANTS TREATED
WITH M. INCOGNITA AND C. CROTALARIAE^a

Treatment	Varieties ^{b,c}		
	'Hill'	'Lee'	'Mack'
Control	.90 d	1.6 bcd	.97 cd
<u>M. incognita</u> alone	1.7 bcd	2.6 abc	1.7 bcd
<u>C. crotalariae</u> alone	2.10 bcd	2.6 abc	1.7 bcd
<u>M. incognita</u> and <u>C. crotalariae</u> simultaneously added	2.0 bcd	2.2 bcd	2.14 bcd
<u>M. incognita</u> added 14 days before <u>C. crotalariae</u>	3.8 a	3.0 ab	2.9 ab

^aData expressed as necrosis index (percent visibly necrotic): 0 = none, 1 = less than 10 percent, 2 = 11-25 percent, 3 = 26-50 percent, 4 = 51-75 percent, 5 = 76-100 percent.

^b'Hill' is resistant to M. incognita; 'Lee' and 'Mack' are susceptible to M. incognita.

^cDifference in letters denotes significant difference at the 5 percent level of probability (Duncan's Multiple Range Test).

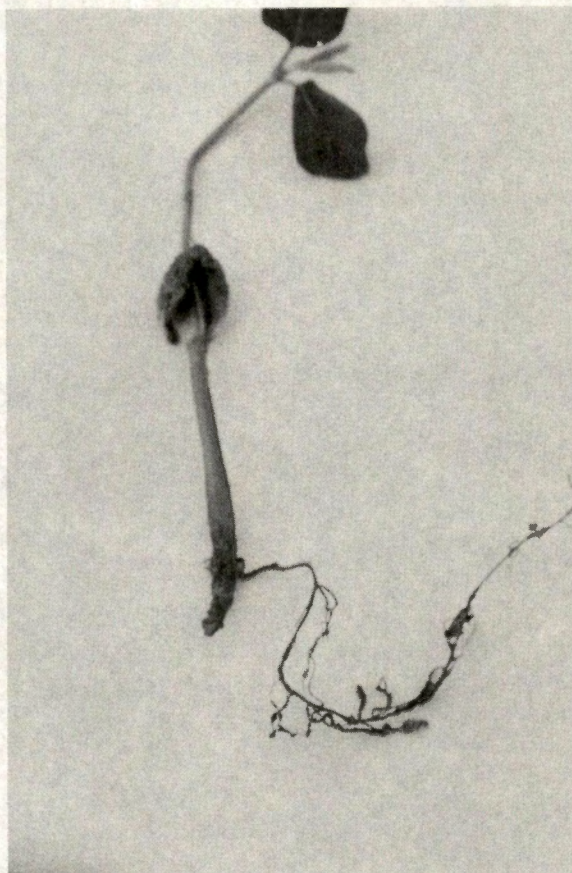


Figure 14. Root necrosis on soybean plant resulting from inoculation with Cylindrocladium crotalariae.

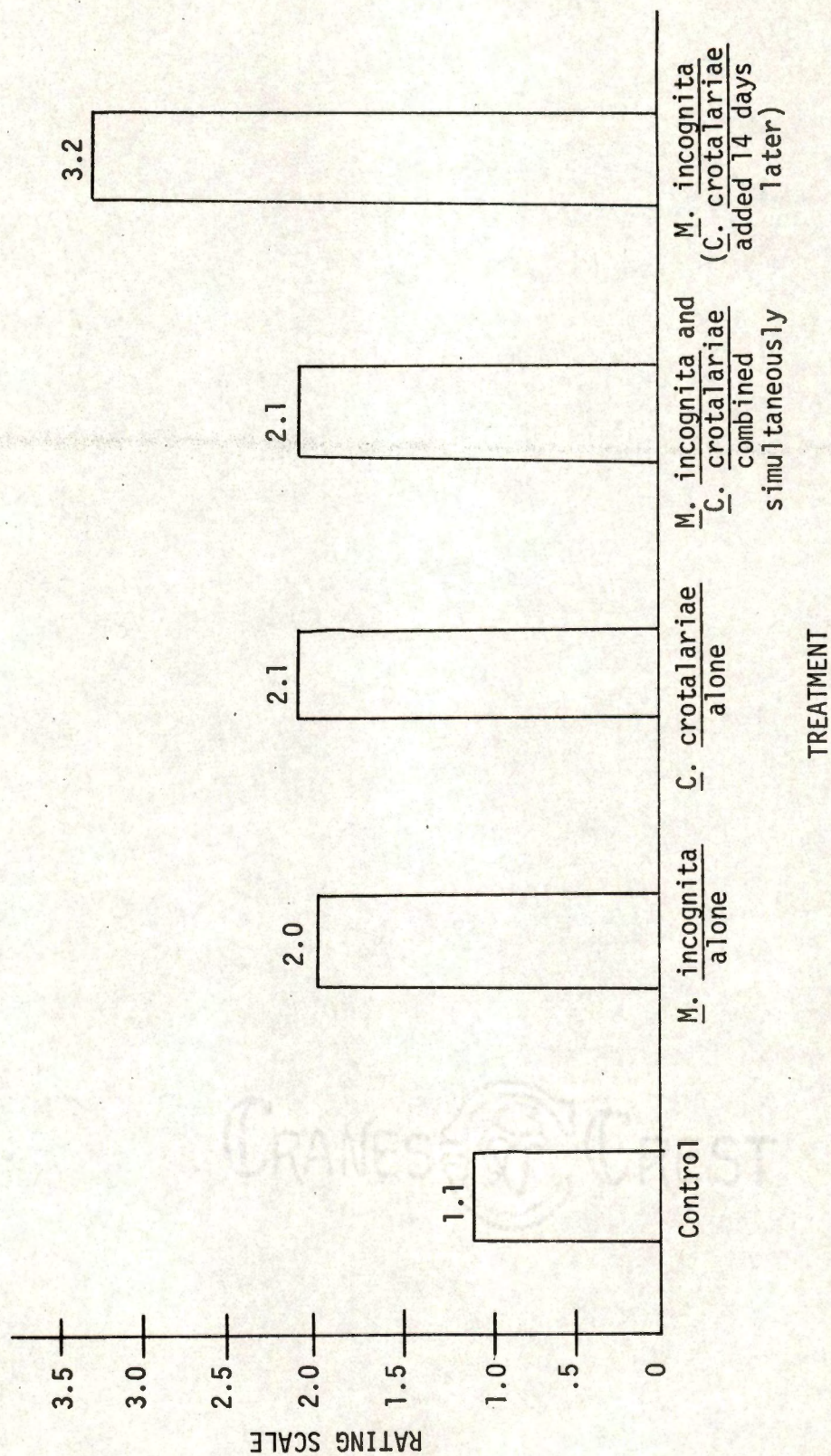


Figure 15. Treatment comparisons of root necrosis ratings for plants treated with *C. crotalariae* and *M. incognita*. Root necrosis index or disease index (percent visibly necrotic): 0 = none, 1 = less than 10 percent, 2 = 11-25 percent, 3 = 26-50 percent, 4 = 51-75 percent, 5 = 76-100 percent.

CHAPTER IV

DISCUSSION

Analysis of plant mortality, plant height, root nodulation, nematode gall and egg mass formation, and root necrosis indicates a significant interaction between M. incognita and S. rolfsii and between M. incognita and C. crotonariae. The time at which the nematode and fungus pathogens were combined also is significant.

Fungus-nematode combinations involving S. rolfsii and C. crotonariae caused higher mortality than any pathogen alone. Highest mortality for both fungus treatments was evident when the nematode and fungus were combined simultaneously. Soybean seeds were inoculated and planted at the same time. Germinating seeds are more susceptible to fungus pathogens than 14 day old plants (6), which may explain the difference in mortality between simultaneous and 14 day combination treatments. Susceptibility in soybeans to fungus pathogens declines after the six leaf stage of plant growth (17). Plants of the 'Mack' variety exhibited the highest mortality in treatments where S. rolfsii and C. crotonariae were combined with M. incognita. This was expected as 'Mack' variety is susceptible to M. incognita. Reactions of 'Lee' and 'Hill' varieties to the treatments were also as expected since 'Lee' is susceptible to M. incognita and 'Hill' is resistant. Although S. rolfsii is specifically a stem pathogen while C. crotonariae is generally a root pathogen, when they are combined with nematodes the same mortality patterns occur. Similarity in data between the two

pathogens is attributed to the fact that in both cases they are combined with nematodes and not to their individual pathogenicity to the host.

Analysis of plant height on the 'Lee' variety indicated greater height for the control plants than plants where M. incognita and C. crotalariae were combined after 14 days. Sasser, Lucas, and Powers (27) working on M. incognita and Phytophthora parasitica (Dast.) var. nicotianae (B. deHaan) (Tucker) on tobacco also found height reductions as a result of the nematode-fungus combination. They noted that plants growing in soil infested with both pathogens were severely stunted. Plants inoculated with either pathogen alone grew almost as tall as the control. Cylindrocladium crotalariae on peanuts resulted in stunted and chlorotic plants (25) which is also symptomatic of root-knot nematode invasion and gall formation (4). However, it was found that the greatest height reduction occurred when these two pathogens were combined and not when they were inoculated alone. This was probably due to increased disease development on plants where pathogens were combined. Goswami et al. (11) studied the association of M. incognita and S. rolfsii on eggplant. Maximum height reduction was observed where the pathogens together were inoculated over all other treatments.

Studies concerning root nodulation showed that the greatest nodulation occurred where plants were inoculated with root-knot nematodes alone. Nematode galls are believed to be closely associated with Rhizobium nodules. Taha and Raski (33) found that an average of 15 nodules per gall developed on Meloidogyne javanica (Treub) galls.

Gall formation was also found to be present on nodules. They found that nematodes appeared to show a preference for gall formation associated with nodules than where nodules were not found. Southards and Priest (30), testing the variation of pathogenicity of M. incognita isolates, found that only certain isolates produced galls on nematode resistant cowpeas. Galls produced, however, were found mainly associated with or on Rhizobium nodules. Our study shows that plants treated with nematodes alone had the greatest number of galls and the greatest nodulation. Thus, it appears that there is a definite association between root-knot nematodes and soybean nodules. Least nodulation occurred in S. rolfsii treatments where M. incognita and S. rolfsii were combined simultaneously. Root necrosis associated with S. rolfsii may be responsible for the reduction in nodulation. In treatments where C. crotonariae was used, the least nodulation occurred where plants were inoculated with M. incognita at planting and C. crotonariae was added after 14 days. Plants with this treatment also had the greatest necrosis of all treatments involving C. crotonariae. Reduced nodulation, again, may be the result of extensive necrosis.

The root-knot index, based primarily on the extent of gall formation and reproduction, was determined for roots inoculated with nematodes. Treatments of root-knot nematode alone and root-knot plus S. rolfsii combined after 14 days had significantly more galls than when the fungus and nematode were combined simultaneously. Root necrosis may again be the reason that fewer galls were found when the fungus and nematode were applied simultaneously. In treatments of root-knot nematode alone, the nematodes had no other pathogen with

which to compete. Plants to which the fungus was added 14 days after the nematode also had more extensive gall formation. This may again be a result of a lack of competition as for 14 days nematodes were alone on the plant. These results on plants treated with S. rolfsii indicate that the combination of M. incognita and S. rolfsii may have a negative effect on gall formation. In plants which are infected with both nematode and fungus, giant cells on which nematodes feed are often invaded by fungi. This may disrupt nematode feeding and they fail to develop to maturity (16). This is in contrast to findings of Mountain and McKeen (21) that a synergistic relationship occurs between the fungus and the nematode in the etiology of eggplant wilt. At low and intermediate levels of fungus inoculum, the number of nematodes within the root was increased by the fungus. In a later work (22) they again found nematode populations to be higher on eggplant and tomato hosts in the presence of the fungus than in its absence. The pathogens they used here were Pratylenchus penetrans (deMan) and Verticillium spp. Without further study, reasons for the reduction of gall formation in association with fungi cannot be known. Variety differences in gall formation for plants treated with a fungus appeared to be consistent whether treated with S. rolfsii or C. crotalariae.

For all treatments the 'Hill' variety had significantly less galls than 'Mack' or 'Lee' varieties. Crittenden (5) showed that soybean varieties 'Hill' and 'Anderson' were more resistant than other varieties to M. incognita. He also found that susceptible varieties of soybeans infected with M. incognita had more giant cells and enlarged nuclei than resistant varieties. Dropkin (6) showed a

positive correlation in giant cell size, gall formation, and reproduction with susceptible varieties. He noted that the size and numbers of galls was a function of the stimulation of each larvae found in the gall. In this study the presence of either of the fungus pathogens had no effect on the resistance or susceptibility of the soybean plant to the nematodes.

Percentage root necrosis was estimated for each pot of plants. Measurements of root necrosis expressed the extent to which plants were diseased. Treatments of S. rolfsii were all significantly more necrotic than the uninoculated control. Sclerotium rolfsii, although primarily a stem pathogen, may invade roots as well. Garren (9) observed no root rot on peanut plants affected by S. rolfsii. Ezekiel and Taubenhaus (8) showed that roots of cotton plants remained unaffected even after the death of aboveground parts caused by S. rolfsii. Necrosis ratings as a disease index were not as accurate for treatments of S. rolfsii as for C. crotalariae in this study. Sclerotium rolfsii caused a high rate of mortality in infected plants. This percentage mortality more accurately measures disease effects of S. rolfsii than does necrosis. Those roots on which necrosis ratings were made did not include roots of dead plants. Necrosis measurements on C. crotalariae are much more significant as this is a root pathogen (Figure 14, page 25). When first observed on peanuts by Bell and Sobers (3), symptoms of C. crotalariae included extensive necrosis of the plant's root system (3). This disease has been referred to on peanuts as *Cylindrocladium* black rot. Symptoms observed on soybean showed that in all cases root systems were severely rotted (25).

Meloidogyne incognita-C. crotalariae combined, with the fungus added after 14 days, caused significantly more necrosis than treatments where either pathogen was inoculated alone or was combined simultaneously. Not only does this show that a distinct interaction occurs, but it also indicates that the time of inoculation is significant.

Wyllie and Taylor (34) showed that *Phytophthora* root rot on soybeans was more severe if nematodes were present along with the fungus than when the fungus was alone. Cylindrocarpon radicicola (Wollenweber), a fungus of the same family as Cylindrocladium, caused more extensive root rot when in combination with the nematode Pratylenchus pratensis (deMan) than when alone. The inhibition of growth by the fungus and nematode as a mixed culture was usually greater than the sum of either pathogen alone (12). The association of the root-knot nematode and various fungus pathogens has been known to break resistance in plants. *Fusarium* wilt resistant varieties became affected by the disease only in cases where root-knot nematodes were present (14).

Work by Powell and Nusbaum (24) confirmed that black shank symptoms on tobacco were severe in highly resistant varieties if M. incognita acrita (Chitwood) was present. In this test, necrosis ratings were the same for the 'Hill' variety, which is resistant to the root-knot nematode, as the 'Mack' variety, which is susceptible to the root-knot nematode. The 'Lee' variety, however, had the highest necrosis and is susceptible to root-knot nematodes. Plants of the 'Hill' variety, when treated with fungus-nematode combinations, were less resistant to infection than when inoculated with nematodes alone.

Time at which pathogens are combined also appears to have a significant effect on disease development. This study shows that on plants where the fungus was inoculated 14 days after the nematodes, significantly greater necrosis occurred. Batten and Powell (2) using *Fusarium wilt* in combination with the root-knot nematode, showed that plants inoculated with the fungus two to four weeks after being exposed to nematodes were more severely diseased than where there had been simultaneous inoculation of nematodes and fungi.

Powell (23) found that species of Curvularia, Botrytis, Aspergillus, and Penicillium were plant pathogens only when roots had been attacked two to four weeks previously by M. incognita. These fungi showed no pathogenicity when inoculated alone. Melendez and Powell (20) cite that the maximum effects exercised by nematodes in host plants occur three to four weeks after plants are infected and maximum fungal development also occurs when inoculated at this time. In instances where the fungus is inoculated following nematodes, nematode predisposition of plant tissue to the fungus is thought to occur.

Predisposition has been defined as the tendency of nongenetic conditions acting before infection to affect the susceptibility of plants to disease (35). Nematode predisposition is believed to be more complex than a simple wounding of the roots. Host physiology altered by nematode infection provides an ideal site for fungus colonization. Phytophthora parasitica on tobacco was found to thrive on tissue surrounding the site of nematode infection. Mycelium were observed inside gelatinous egg masses and in hyperplastic regions of

nematode infection (20). Thus it is believed that the basis of the interaction is not a direct consequence of one pathogen on the other. It is rather an effect of the nematode on the host and of the fungus on the modified host tissue (24). This research on M. incognita and C. crotonariae further supports the view that fungal colonization is aided by prior nematode infection. Histopathological studies would be necessary to determine to what extent nematode galls and fungal mycelium were interrelated. Studies to determine the exact time necessary for adequate nematode predisposition of the host tissue would also be beneficial.

CHAPTER V

SUMMARY AND CONCLUSION

This work indicates that an interaction does exist between M. incognita and C. crotalariae. Evidence pointing to this fact includes: (1) the greatest plant mortality for treatments involving C. crotalariae occurred where M. incognita and C. crotalariae were combined, (2) the lowest height among plants treated with C. crotalariae occurred where M. incognita was placed in pots at planting and C. crotalariae was added after 14 days, and (3) the least nodulation occurred and the most extensive necrosis was evident on those plant roots where M. incognita and C. crotalariae were combined with the fungus added after 14 days. It is obvious that treatments where roots were pre-exposed to nematodes prior to fungus infection showed more evidence of disease than where nematodes and fungi were combined simultaneously.

Evidence of an interaction between M. incognita and S. rolfsii is established by the fact that the greatest plant mortality as well as the least nodulation occurred where M. incognita and S. rolfsii were combined simultaneously.

This study suggests that an interaction among these pathogens does exist and that further research involving these interactions is necessary. Histopathological studies are necessary to determine how the nematode and the fungus are associated.

Re-isolation of each pathogen should be undertaken to determine

if virulence has been altered. A reassessment of the means of disease control on soybeans for plants with symptoms of S. rolfsii and C. crotonariae is necessary. Perhaps the use of a nematicide for disease control in lieu of a fungicide is feasible. Disease development when plants were inoculated with fungus alone was less than were a combination of pathogens occurred. This was evident, especially where nematodes were allowed to predispose the host.

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