THE EFFECT OF CROTALARIA SPECTABILIS ROTH ON ROOT-KNOT NEMATODE

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I. INTRODUCTION

Many factors both positive and negative, influence the growth of plants. One of the more serious negative factors in plant growth is the root parasite. Until very recently there has been little or no recognition of the economic importance of these parasites. According to Steiner (35) the possible annual saving in the United States with effective nematode control would amount to several hundred million dollars. It is immediately obvious that root parasites, especially the root-knot nematode, present a very serious problem to the producer of susceptible crops. This problem, however, is not confined to the United States but presents a serious hazard to susceptible crops in the major food producing areas of the world.

The study of plant parasitic nematodes and crop production has become an increasingly important field of agricultural research. In the attempts to control the root-knot nematode many methods have been investigated, but most of them that have been proposed are difficult and in most cases impractical under field condition.

The objective of this investigation was to gain a better understanding of the factors that might contribute to the control of the root-knot nematode by growing Crotalaria spectabilis Roth in infested soil. The problems involved in this study are of primary interest to the agronomist, nematologist, plant pathologist, and plant chemist. It is hoped by the author that the necessary amalgation of these several research fields will not be considered a fault.

II. LITERATURE REVIEW

It is well known that Heterodera species are all obligate parasites of roots (24). Heterodera marioni (Cornu, 1879) Goodey, 1932 (24) is a non-cyst forming root-knot nematode. The work of Christie and Havis (13) demonstrated that Heterodera marioni comprised several races. Chitwood (10) studied the morphological comparison of several populations of H. marioni and concluded that they should be separate from Heterodera. He suggested the genus name Meloidogyne, as the earliest, valid, generic name and divided the genus into five species and one variety.

The life cycle of H. marioni has been clarified by Christie and Cobb (12). The life cycle is given here since it must be kept in mind for a logical evaluation of host-parasite relations. According to Christie and Cobb, the first stage, the ovic larva, molts before it has attained its maximum length and while it is still relatively inactive. After the first molt of the ovic larva, it continues to grow before it is hatched from the egg. The second growth stage or molt occurs soon after the organism enters a root and at this time it changes from a cylindrical to saccate form. There is no third stage of either the male or female since two cuticles are loosened simultaneously, representing the second and third molts. The fourth stage of the female is very short, as it molts a fourth time almost immediately following the third molt, while the life cycle of the male shows a longer interval between the third and fourth molts. In this interval between the third and fourth molts, metamorphosis of the male occurs. The male is transformed from a relatively short and broad form, similar to that of the female before the second and third molts,

to the slender elongate form of the mature male. Cobb found that a male molts a fourth time immediately on completing its metamorphosis and while it is still within the sac formed by the second and third cuticles. Tyler (38) studied the reproduction of root-knot nematodes asexually in aseptic root culture, and he concluded that the root-knot nematode could reproduce without mating.

According to Cunningham (15) the larvae are capable of living in the soil for several months without food, but unless they come in contact with some host plant upon which to feed there will be no further development. He also carefully examined the infested roots of potatoes through three years, and reached the conclusion that not more than one generation was produced on the root.

The gall formation induced by the feeding of root-knot nematodes on tomato roots has been described by Christie (11). He found that the entering larva tends to pass between the cells. When permanently located, the head of the larva usually is in the plerome near the beginning of the region of cell elongation. During the first 48 to 60 hours, cells of the central cylinder lying in the region of parasite's head remain undifferentiated. After about 3 days these undifferenciated host cells enlarge slightly, the neuclei swells, and the cell walls disintegrate. Next, the protoplasmic content of adjacent cells coalesce to form a giant cell. The giant cell invades adjacent areas and other cells are absorbed after dissolution of cell walls. Christie thought that these morphological developments in the roots were due to the stimulating action of same secretion expelled through the mouth of the nematode.

The length of the life cycle of the root-knot nematodes varies greatly with temperature and the host plant (15). Eighty-seven days are

required for the complete life cycle on tomato roots at 16.5°C., while at 27°C. only 25 days are necessary. Full development of the females at 27°C. was reached at 16 days, and an additional nine days were required for the development and hatching of eggs. In experiments where the temperature was 14.3°C. or above 31.5°C. it was found that root-knot nematodes could not complete their life cycle. The nematode is quite resistant to long continued low soil temperatures. Cunningham (15) did not find any apparent difference in the amount of infection on plants grown on soil which had been frozen intermittently. He kept soil temperature at 25°F. throughout the period of the experiment. Even the long continued low temperatures of 1933-34 in Long Island, New York, had no noticeable effect in reducing the infection. Cunningham also studied the depth which the root-knot nematode would penetrate a silt loam soil with a gravelly subsoil. From November 25, to April 25, nematode population was severe in the first 12 inches, medium to slight in the next 12 inches, and usually free of nematodes below 24 inches.

Watson (39, 40, 41, 42) planted 17 species of crotalaria on soil heavily infested with nematode, and found that nematodes did not enter the roots of any species. Later he found that Crotalaria spectabilis can be substituted for velvet beans, a nematode resistant crop. He found that Crotalaria spectabilis yields were considerably higher than velvet beans grown on the same soil. Further work was done on a method of starving out root-knot nematodes by the use of Crotalaria spectabilis. His recommended procedure was, to plant the crotalaria in rows and cultivate it frequently to eliminate other plants and to aerate the soil. It was found that, with a pure stand of crotalaria, control was fully as good where it was sown broadcast without cultivation as row planting.

However, where other plants were allowed to grow with the crotalaria control was much less complete. The work of Beckenbach (7) demonstrated that of four cover crops tested in the summer of 1945 on their experiment farm, Crotalaria spectabilis produced a satisfactory cover. He obtained 14 tons of green weight per acre, and 3.7 tons dry weight per acre (6). As a result of this experiment, Crotalaria spectabilis (early strain), was recommended as the best cover crop. The Georgia Coastal Plain Experiment Station (20) tried chemicals to control nematodes. They used 100 to 200 pounds per acre of chloropicrin on an infested field, heavy applications of carbon bisulphide and its emulsion, ammonium thiocyanate and formalin. They found that, chloropicrin, carbon bisulphide and emulsion all gave complete or nearly complete nematode control, when applied in sufficient amounts, while ammonium thiocyanate and formalin had little apparent effect on the nematodes. However, their conclusion was, that chemicals for nematode control could not be recommended because most were ineffective, and the crops grown would not justify the expense. They (21) suggested that by growing crotalaria as a crop highly resistant to nematodes, that a garden plot could be kept clean for a period of 3 years. They (22) also grew Crotalaria spectabilis, as one of treatments, until mature and plowed it under as green manure, with oats used as a winter crop. They found that this treatment gave highly significant positive differences in growth of trees compared to plots which had grown nematode susceptible cover crop.

Barrons (5) made a microscopical study of the rootlets of a number of susceptible and resistant varieties and species of plants; he found that there were no significant differences in the rate of larval entry either in the seedling or adult stage. It had previously been assumed

by a number of workers that resistant plants actually resisted the entry of nematode larvae. He also used Crotalaria spectabilis in his experiment, and proposed a hypothesis that root-knot resistance is due to substances synthesized by the plant which conteract the giant-cell-inducing effect of the salivary secretion of nematode larvae.

The toxicity of crotalaria alkaloids was reported as early as 1884. Bessay (9) showed Crotalaria sagitallis Linn. to be the cause of death among horses suffering from "Missouri river bottom disease." In 1909, Robertson[1] in Africa, described the hepatic damage to horses fed senecio. It has been reported (4) that when one-fourth pound of Crotalaria juncea L. fed to a healthy mature sheep daily for a period of 14 days and one-half pound daily for 12 additional days, the animal developed a weakened "tucked-up" appearance, and catarrh developed shortly after the 14th day. Death occurred on the 26th day.

Becker, Neal, Arnold and Shealy (8) studied the palatability of 11 species of crotalaria, especially of C. spectabilis Roth. They found that at least 8 out of 10 species of crotalaria were probably not toxic to cattle, but Crotalaria spectabilis was definitely toxic to cattle. An 8 month old steer was grazed artificially on crotalaria hay for 4 days. On the third day blood was found in the feces, and again on the morning of the fourth day, the animal died in the afternoon of the fourth day. The total weight of hay fed was 9.5 pounds. Typical lesions of Crotalaria spectabilis poisoning were observed in the heart, liver, gall bladder, inner surface of the trachea, and mesentery of the yearling steer which was sacrificed just prior to death from crotalaria poisoning.

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[1] Robertson. Cape Agricultural J. May, 1906. Quoted by Ratnoff and Mitrick (29).

[2] C. spectabilis and Crotalaria spectabilis are used interchangeably in this report.

Thomas (36) force-fed C. spectabilis seeds to several groups of chickens. Death occurred 35 and 64 days after feeding 80 crotalaria seeds, and the post mortem of the birds all showed lesions due to chronic poisoning. Other groups of experimental birds also showed similar lesions except those force-fed less than 40 seeds. He also found that smaller amounts of seeds killed the birds if fed daily. Quail and dove also died by force-feeding of the seeds, but turkeys were not poisoned. He found that there were two types of crotalaria seed poisoning, acute and chronic, depending on the amount of seed consumed and the resistance of the individual. Emmel, Sanders and Henley (18) fed ground Crotalaria spectabilis Roth to hogs. They found that crotalaria poisoning in swine showed such clinical symptoms as anemia and accumulation of fluid in the abdominal and thoracic cavities etc. Their later report (19) showed that swine under field conditions were more likely to be poisoned by the green foliage of C. spectabilis than by the seeds of this plant. Sanders, Shealy and Emmel (31) also studied clinical symptoms, gross and microscopic lesions in a herd of cattle under natural conditions, and by daily oral administration of pulverized Crotalaria spectabilis seed.

Emmel (16) (17) studied the toxicity of Crotalaria retusa, and spectabilis seed on the domestic fowl. The toxity of C. retusa L. seeds was almost as great as C. spectabilis Roth seed which were considered highly toxic.

Adams and Rogers (1) demonstrated that monocrotaline, an alkaloid derived from crotalaria, contained active retronocine alkanolamine also present in senecio alkaloid. Several investigators have used this pure material to investigate its effects on animals. Harris, Anderson and

Chen[1] observed that mice injected intravenously with monocrotaline died with pulmonary edema, ascites, hydrothorax, and thymic neocrosis. There was a central hepatic necrosis, hemorrhage and sinusoidal congestion, with little leukocytic reaction. Ratnoff and Mitrick (29) tested rats grown on a balanced diet, a moderately low protein diet, and a low protein diet. All rats were then injected intraperitoneally every second day with 46 mg. of 1% monocrotaline per kilogram. The results showed the pathologic effects of monocrotaline to be: apathy, anorexia, weight loss, dark urine, epistaxes, purpuric paws, and occasional terminal paraphlegia and others. Male rats fed on a moderately low protein diet were more susceptible than females fed on a moderately low protein diet. Both sexes fed on a moderately low protein diet were more susceptible to poisoning than rats fed on a normal diet. Rats of both sexes on a deficient protein diet were as resistant as rats fed a normal diet. Injection of testosterone propionate increased the susceptibility to monocrotaline of male and female rats fed low protein diets. Rats injected with this hormone show no difference in susceptibility between sexes.

The toxic material monocrotaline was first isolated from Crotalaria spectabilis by Neal, Rusoff and Ahmann (28). They extracted monocrotaline from crotalaria stems, leaves, and seeds with 1.25% sulfuric acid and then extracted it with 0.1 N Sodium hydroxide and it was finally removed by extracting with chloroform. The compound slowly decolorizes potassium permanganate, and gives a yellowish color with sulfomolybdic acid reagent. It yields a yellowish precipitate with picric acid, a reddish brown precipitate with Wagner's iodide reagent, a white precipitate with Mayer's

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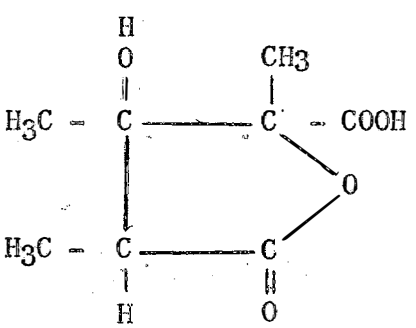
[1] Harris, P. N., R. C. Anderson and K. K. Chen. Jour. Pharmacology and Expl. Therapy. 45:78. 1942. Quoted by Ratnoff (29).

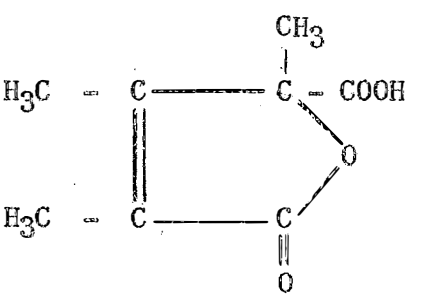
reagent, and a yellow-white precipitate with Sonnenschein's phosphomolybdic acid reagent. It forms precipitates with salts of heavy metals. All these tests used were characteristic of alkaloids. These investigators suggested the name monocrotaline for this alkaloid, and reported a tentative formula of $C_{16}H_{26}O_6N$.

Succeeding alkaloids from Crotalariae have been named monocrotaline, dicrotaline, tricrotaline etc., following Couch's hexalupine (14), which had been isolated from lupine.

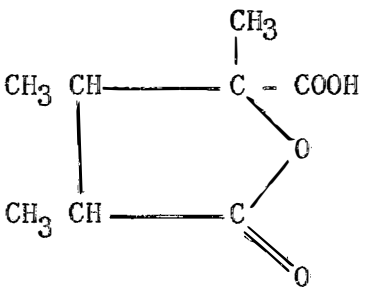
Adams and Rogers and their coworkers (1, 2, 3) reported the results of careful analysis of pure monocrotaline, and suggested $C_{16}H_{23}O_6N$ as the empirical formula. It has also been shown that monocrotalic acid is optically active, monobasic, and upon treatment with alkali gives the optically inactive acid, monocrotic acid, $C_7H_{12}O_3$, which is obtained by alkaline hydrolysis of the alkaloid. As the result of their continuous study (1a), the structure of monocrotaline, monocrotalic acid, anhydromonocrotalic acid, and dihydroanhydromonocrotalic acid were established as follows: by reducing the methyl esters with lithium aluminum hydride, followed by an oxidative degradation of the products. The structure of the compounds were established.



monocrotalic acid

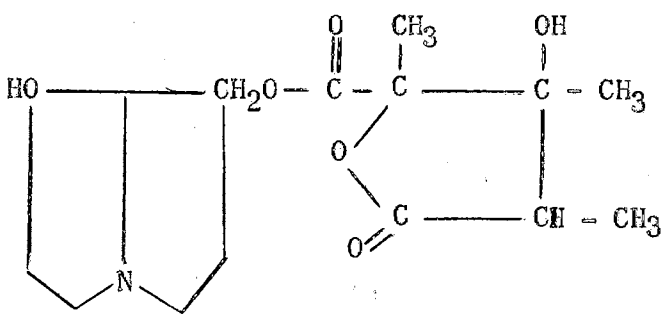


anhydromonocrotalic acid



dihydroanhydromonocrotalic acid

All products were shown to be optically active. The hydrolysis of the methyl ester of dihydroanhydromonocrotalic acid showed this isomer to have a different melting point and different optical rotation. Hydrolysis with HCl, NaOH, and treatment of the ester with KCN at high temperature followed by boiling with HCl, each gave a different ester. The constitution of the alkaloid monocrotaline must be



Henry (25) published a study of this alkaloid in his 4th edition of "The Plant Alkaloid." He found that monocrotaline decomposes at the melting point of 197-198°C., has an optical rotation of $[\alpha]_D^{26^\circ}$ -55.7° ($CHCl_3$), and forms a hydrochloride, with an M. P. of 184°C. (decompose). It has an optical rotation of $[\alpha]_D^{28^\circ}$ - 38.4° (H_2O), and the methiodide, B. MeI, 3MeOH, has an M. P. of 205°C. (decomposes). In boiling aqueous barium hydroxide solution the alkaloid is hydrolysed to retronecin and monocrotic acid, and on hydrogenation in presence of platinic oxide in acetic acid, the scission products are retronecanol and monocrotalic acid.

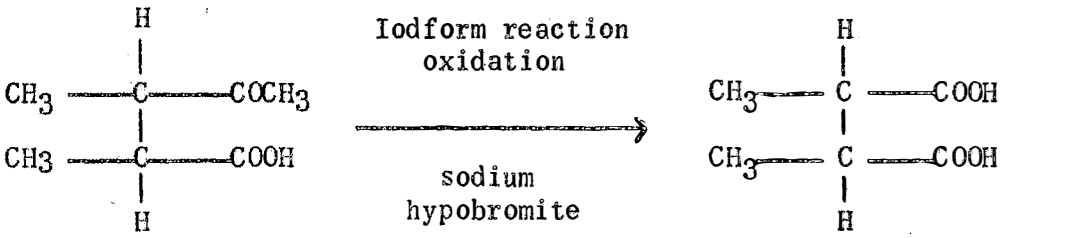
(a) $C_{16}H_{23}O_6N \xrightarrow[Ba(OH)_2]{\text{hydrolysis}} C_8H_{13}O_2N + C_7H_{12}O_3$

retronecine M. P. 121-2° monocrotic acid B.P. 145-6/18 mm.

(b) $C_8H_{13}O_2N \xrightarrow[\text{platinic oxide}]{H_2} C_8H_{15}ON$

retronecine retronecanol B.P. 140°/30mm.

(c)



monocrotic acid dimethyllaevalic acid

dl-, and meso-αα'- dimethyl succinic acid

(d) Monocrotaline $\xrightarrow{\text{hydrogenation}}$ monocrotalic acid + rectronecanol

III. EXPERIMENTAL METHODS AND RESULTS

Experiment I

To find the relation between root-knot nematode and *Crotalaria spectabilis*, soil was taken from the Department of Horticulture[1] experimental plots at Perkins Farm which was known to be heavily infested with root-knot nematode.

The soil was sieved and mixed and 34 pounds was weighed into glazed non-porous pots. Twenty-two pots were set on the greenhouse bench, and 25 seeds of *Crotalaria spectabilis*[2] (early strain) were sown in 20 pots in October 1954. The other two pots were planted to *Sioux* variety of tomato, which was developed by the Nebraska Agricultural Experiment Station from a cross between *Allred* and *Stokesdale*. The pots were irrigated with distilled water throughout the experiment to insure favorable soil moisture for seed germination and growth, crotalaria was rather slow growing during the first 2 months.

On January 3, 1955, the indicating crop *Sioux* tomatoes were removed from the pots and examined for nematode infestation. Unfortunately no nematode was found in the roots. This might have been due to the extremely hot and dry summer of 1954, and the relatively low greenhouse temperatures as previously discussed by Cunningham (15).

In order to infect the soils artificially, 10 two gallon pots of

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[1] Soil furnished by courtesy of Dr. Frank Cross, Head, Department of Horticulture.

[2] Seed furnished by courtesy Dr. F. Gray, Assoc. Prof. of

Figure 1. Crotaralia spectabilis on greenhouse bench.

Figure 2. Vigorous growth of crotalaria, just before the composting.

the same soil were prepared and infested by about 2 pounds of sweet potato roots heavily infested with Meloidogyne incognita[1]. The roots with galls were cut in about 0.5 cm. pieces and mixed into the upper 2 inches of soil, and kept moist to prevent undue dryness. After 7 days the pots were planted to the All Gold sweet potato which is known as a very susceptible variety to nematode.

On February 12 and 13, the pot which had been previously planted to crotalaria were treated as follows:

Treatment 1. 4 pots crotalaria roots and tops removed from soil.

Treatment 2. 4 pots crotalaria tops removed and roots cut into 1 inch length and returned to the soil.

Treatment 3. 4 pots whole crotalaria plant chopped and returned to the soil.

Treatment 4. 4 pots crotalaria roots removed from soil and tops cut into 1 inch length and returned to the soil.

Treatment 5. Check. Obtained fresh soil from Perkins Farm and added oats straw and nitrogen as ammonium sulfate equivalent to the analysis and growth of crotalaria roots and tops.

Crotalaria grew vigorously after the first two months and showed dark green color and no visual deficiency symptoms were observed. The root had many pink colored nodules showing that the plant was in a good condition for nitrogen fixation. After the treatments in regard to the composting of crotalaria were carried out about 200 grams of heavily nematode infested soil and roots from the 10 pots on which All Gold

[1] Sweet potato roots furnished by courtesy Dr. B. Struble, Assoc. Prof. of Botany and Plant Pathology.

sweet potato had grown, were added to all pots except to Treatment 5. Then four Sioux tomatoes seedlings were transplanted into each pot.

On March 27, after one month of growth, the tomato roots were removed and examined for root-knot nematode galls. A system of indexing was set up in order to classify the degree of nematode infection, as shown in Figures 3, 4, 5, 6, and 7.

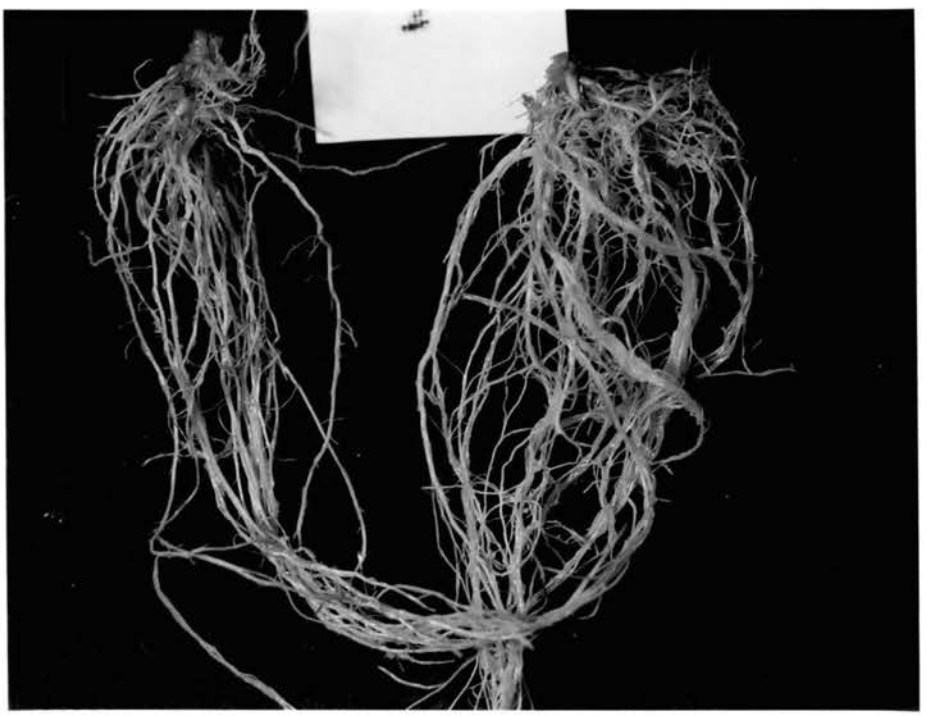

Figure 3. Index 0. Absolutely no galls can be found in tomato roots.

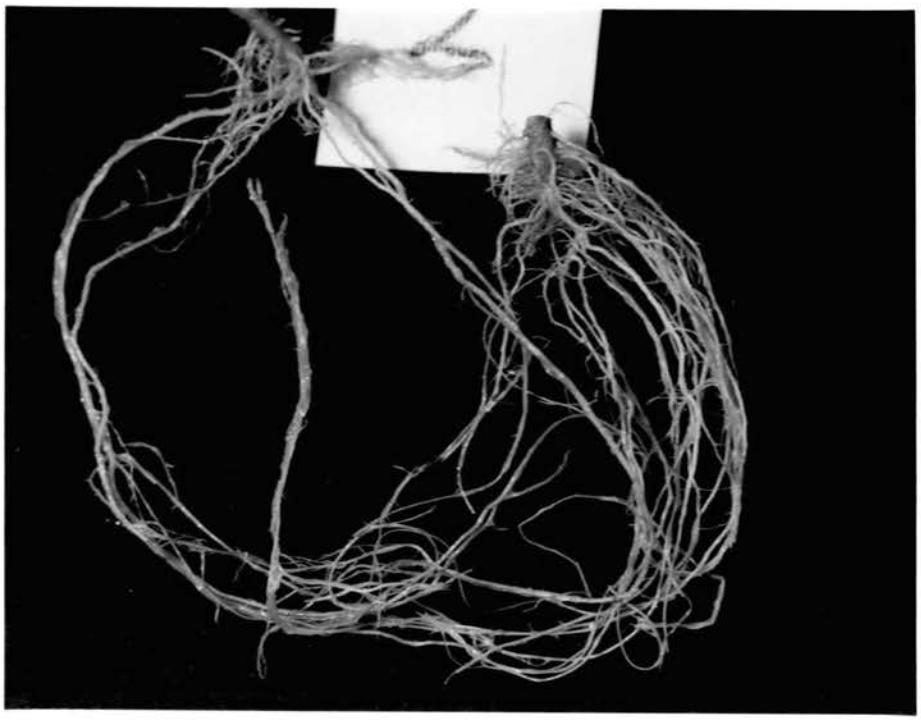

Figure 4. Index 1. Slight, only little galls on tomato roots.

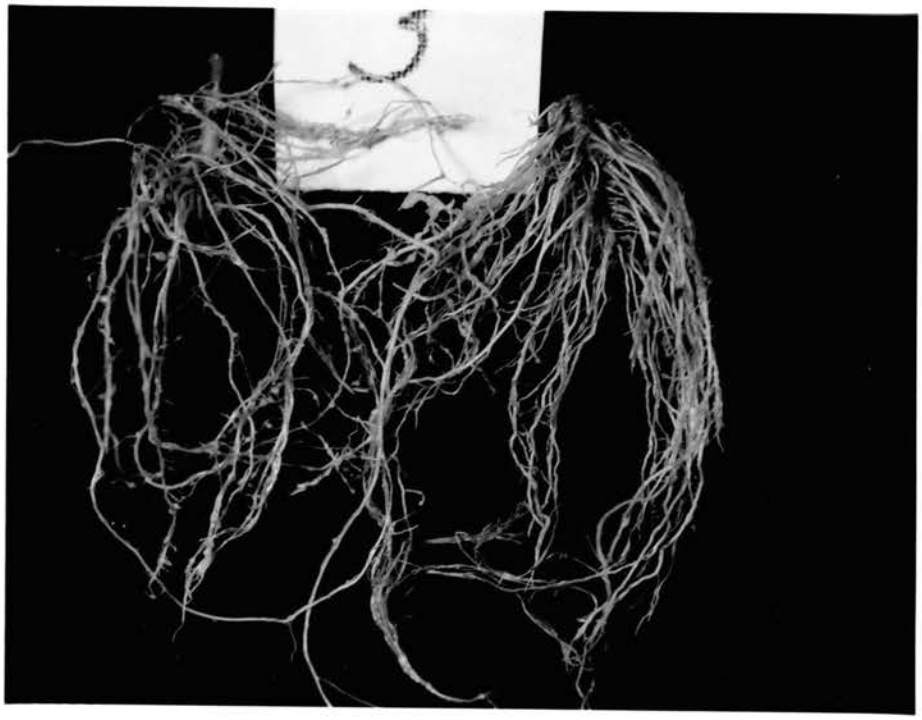

Figure 5. Index 2. Middle, a few nematode galls on roots.

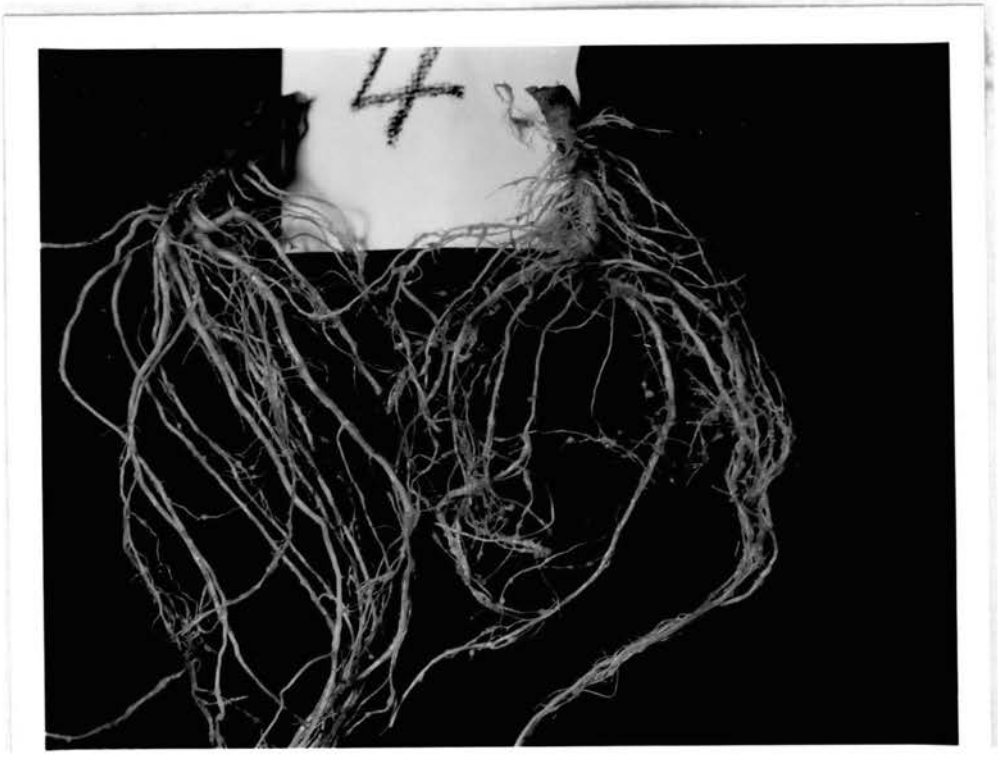

Figure 6. Index 3. Severe, many galls on roots.

Figure 7. Index 4. Very severe, quite many galls on roots.

The results of the indexing of nematode infection is shown in Table 1.

Table 1. The index values of root-knot nematode infection of the roots of the tomato, Sioux variety.

Replication	Treatment					Total
	1	2	3	4	5	
Rep. 1	3	4	2	4	1	
	3	4	3	3	0	
	3	4	3	3	0	
	4	3	3	4	1	
Total	13	15	11	18	2	
Average	3.25	3.75	2.75	4.50	0.5	
Rep. 2	4	4	2	1	0	
	4	4	3	1	0	
	4	3	3	2	0	
	3	3	2	2	0	
Total	15	14	10	6	0	
Average	3.75	3.50	2.50	1.50	0	
Rep. 3	4	4	1	4	1	
	4	4	2	3	0	
	4	2	2	3	1	
	4	2	3	3	0	
Total	16	12	8	13	2	
Average	4.0	3.0	2.0	3.25	0.5	
Rep. 4	4	3	1	2	0	
	4	3	1	4	0	
	4	3	2	3	0	
	3	3	2	2	0	
Total	15	12	6	11	0	
Average	3.75	3	1.5	2.75	0	
Total of Average	14.75	13.25	8.75	12.00	1.0	49.75

Table 2. Statistical analysis of the severity of the root-knot nematode infection in the roots of the tomato, Sioux variety.

Computation:

For total		For treatment	
SX_2	49.75	SX^2	614.6875
SX^2	160.1875	Sx^2	29.918
C. F.	123.753		
Sx^2	36.4345		

Analysis of Variance

Source of Variation	Degree of Freedom	Sum of Squares	Mean Squares
Total	19	36.4345	
Treatment	4	29.918	7.479
Error	15	6.5165	0.4344

$$F = \frac{7.479}{0.4344} = 17.216^{**}$$

According to the distribution of F value, when $n_1 = 4$ and $n_2 = 15$, at 5%, $F = 3.06$ and at the 1% level, $F = 4.89$. The computed F value is considerable larger than tabulated value even at 1% level. These results can be interpreted to show that the treatments were quite significant.

However, treatment 5 (check) was a fresh farm soil, and even though it was taken from a plot of soil heavily infested by nematode, the others were very heavily inoculated by sweet potato root galls. Probably treatments 1, 2, 3, and 4 cannot be directly compared with the check for these reasons. When treatment 5 was eliminated and treatments 1, 2, 3 and 4 were statistically analyzed by the same method, it was felt that the relation between treatments could be shown more clearly. Shown in Tables 3 and 4.

Table 3. Index values of root-knot nematode infection of the roots of the tomato, Sioux variety, grown on soil previously treated with Crotalaria spectabilis Roth.

	Treatment				
	1	2	3	4	Total
Average of indexes for each pot	3.25	3.75	2.75	4.50	
	3.75	3.50	2.50	1.50	
	4.00	3.00	2.00	3.25	
	3.75	3.00	1.50	2.75	
Total	14.75	13.25	8.75	12.00	48.75

Table 4. Statistical analysis for root-knot nematode infection in the roots of the tomato, Sioux variety, grown on soil previously treated with Crotalaria spectabilis Roth

Computation:

For total:

$SX_2 = 48.75$

$SX^2 = 159.6875$

C.F. = 148.5315

$Sx^2 = 11.15235$

For treatment;

$SX^2 = 613.6875$

$Sx^2 = 4.88665$

Analysis of Variance

Source of variation	Degrees of Freedom	Sum of Squares	Mean Squares
Total	15	11.15235	
Treatment	3	4.88665	1.6288
Error	12	6.2657	0.522
Treatment 3 vs other treatment	1	3.9388	3.9388
Treatment 1 vs other treatment	1	2.1888	2.1888

		Significant F value at 5% level
F value for treatment	3.118	3.75
F value for treatment 3 vs other treatment	7.5455*	4.75
F value for treatment 1 vs other treatment	4.1931	4.75

The results as presented by the statistical analysis show that the four treatments did not differ appreciably, however, treatment 3, or a compost of the whole crotalaria plant could significantly control the formation of galls. This might have been due to the effect of fresh organic material added to the soil which caused the nematode larvae to seek a new source of food. The toxic material in the plant residue, however, might have caused their death. Evidently the greater the amount of fresh crotalaria residue the soil contains the more efficient is the control of the nematode.

Experiment II

After examining the tomato roots, the soils were returned to original pots, and again the soil was planted to crotalaria on March 28, 1955. The plants were managed by the same procedure as in the first experiment. The plants grew very slowly and some pots had to be reseeded in order to maintain six good plants per pot. On June 17, 1955 the crotalaria was composted as in the first experiment. In other words the soil by this time had received the same treatment twice. This time, however, the soil was poured out of the pot onto a clean unbleached muslin cloth for separation of crotalaria from the soil. The pots had previously been severely infested with nematodes, so they were not infested again by the artificial method.

On June 18, 1955 four sweet potato plants, the All Gold variety, which had been previously rooted in water were planted in the pots. The sweet potato seedling attained 2 to 3 inches of healthy roots and was growing vigorously. The fresh soil for the check was taken from the check plots of a nematode experiment with sweet potatoes on Perkins Farm (Horticulture Department).[1] The sweet potato plants in those plots were showing galls on their roots.

After about one month, on July 19, 1955, the sweet potato tops were harvested and then the roots were removed from each pot. The soil was sieved in order to obtain as many of the small roots as possible. As soon as the roots were washed in tap water, they were put in a paper sack, all of the roots wrapped with a plastic sheet, and then they were stored in the refrigerator. The roots were then photographed and the number of galls counted. In the first experiment with tomatoes most of the galls tended to be in clusters, because of the extremely high population of nematodes. However, in the second experiment the galls were not clustered together on the roots, and the work of counting was much easier.

[1] This soil was supplied through the courtesy of Dr. H. B. Cordner, Professor of Horticulture.

Table 6. Statistical analysis of the number of nematode galls in the roots of the sweet potato, All Gold variety, grown on soil treated with Crotalaria spectabilis Roth.

Computation for the statistical analysis:

For total		For treatment	
SX	486.15	SX^2	150,526.8475
SX^2	39,422.7725	Sx^2	26,814.62
C.F.	11,817.09		
Sx^2	27,605.68		

Analysis of Variance

Source of variation	Degrees of freedom	Sum of squares	Mean Squares	F value
Total	19	27,605.68		
Treatment	4	25,814.04	6,453.51	50.420**
Error	15	1,791.64	127.97	
Check vs treatments	1	25,564.82	25,564.82	199.77**
Treatment 1 vs other treatments	1	224.03	224.03	1.75
Treatment 2 vs other treatments	1	0.175	0.175	0.0013
Treatment 3 vs other treatments	1	36.575	36.575	0.2858
Treatment 4 vs other treatment	1	72.275	72.275	0.5647
Treatment 1 vs check	1	13,744.82	13,744.82	107.40**
Treatment 2 vs check	1	16,042.88	16,042.88	125.36**
Treatment 3 vs check	1	16,928.00	16,928.00	132.281**
Treatment 4 vs check	1	17,321.20	17,321.20	135.352**

** Significance at 1% level. (33)
1% significant value for treatment. 5.03
1% significant value for the others. 8.68

Figure 9. Sweet potato root after Experiment 2, from Treatment 1.

Figure 10. Sweet potato roots after Experiment 2, from Treatment 2.

Table 7. The green weight of sweet potato tops (All Gold variety) grown on a nematode infested soil treated with Crotalaria spectabilis Roth.

	Treatment					
	1	2	3	4	Check	Total
Green weight of sweet potato tops (grams)	132	98.5	146	63	66	
	108	113	160	130	114	
	122	96	122	135.5	49	
	114	114	128	170	75	
Total	476	421.5	546	498.5	304	2246

Table 8. Statistical analysis of the green weight of sweet potato (All Gold variety) foliage grown on a nematode infested soils treated with Crotalaria spectabilis Roth.

Computation for the statistical analysis:

For total		For treatment	
SX	2,246	SX^2	1,043,272.5
SX^2	270,522.5	Sx^2	8,592.32
C.F.	252,225.08		
Sx^2	18,296.70		

Analysis of Variance

Source of variation	Degrees of freedom	Sum of squares	Mean Squares	F value
Total	19	18,296.70		
Treatment	4	8,592.32	2,148.08	3.32*
Error	15	9,704.38	646.958	
Treatment vs. check	1	13,261.25	13,261.25	20.49**
Treatment 1 vs other treatments	1	30.08	30.08	0.04
Treatment 2 vs other treatments	1	1,365.33	1,365.33	2.11
Treatment 3 vs other treatments	1	1,220.0	1,220.0	1.88
Treatment 4 vs other treatments	1	56.33	56.33	0.08
Treatment 1 vs check	1	3,698.00	3,698.00	5.71*
Treatment 2 vs check	1	1,725.78	1,725.78	2.66
Treatment 3 vs check	1	7,320.50	7,320.50	11.31**
Treatment 4 vs check	1	4,728.78	4,728.78	7.30*

** Significance at 1% level. (33)
* Significance at 5% level.
1% significant value for treatment. 4.89
5% significant value for treatment. 3.06
1% significant value for others 8.68
5% significant value for others. 4.54

Figure 11. Sweet potato roots after Experiment 2, from Treatment 3.

Figure 12. Sweet potato roots after Experiment 2, from Treatment 4.

Figure 13. Sweet potato roots after Experiment 2, from Treatment 5. Check.

Experiment III.

In order to ascertain the monocrotaline content of Crotalaria spectabilis Roth and its relation to the incidence of root-knot nematode, seeds of the crotalaria plant were used as a source of the alkaloid and the nematode larvae were treated with a monocrotaline solution.

1. Isolation of monocrotaline.

According to Adam's method (1), 600 grams of Crotalaria spectabilis seed ground to 20 mesh was extracted for seventy-two hours with 95% ethanol. The greenish black solution then was acidified sufficient to change the color of congo red paper with citric acid, the solvent removed in vacuo, and the residue taken up in 400 cc. of water. The suspended fat was extracted with successive portions of ether until the ether extracts were colorless, and the extract was extracted with two 100 cc. portions

of chloroform. The aquaeous solution was then treated with 200 cc. of chloroform and a saturated solution of sodium carbonate solution was added with shaking, until the solution was distinctly alkaline to litmus. The color of the solution during this operation changed from a dull yellow-brown to golden-yellow. The alkaloid was then extracted with an additional portion of 200 cc. and three portions of 100 cc. of chloroform. The chloroform extracts were evaporated to dryness in vacuo and the crude alkaloid obtained as a yellowish white crystalline mass. The crystalline material was then purified by two crystallizations from absolute ethanol. The white prisms had a M. P. of 195-197°C. (uncorr.) with decomposition. The identity of the material was established by comparing the melting point results against Adams' (1) data.

2. Effect of monocrotaline solution on nematode larvae.

A group of galls were collected from sweet potato roots and crushed in a mortar. Ten ml. of different concentrations of the monocrotaline solution, 1/200, 1/400, 1/800 and distilled water for check were added to 0.3 grams of the sweet potato root-gall material. Samples of the root-gall tissue were then examined under the microscope at intervals of twelve hours.

Before this experiment could be carried out, however, it was necessary to examine three different dyes in order to find a suitable staining method (26). The dyes examined were methylene blue (0.05%) water solution, and methylene blue (0.05%) distilled water solution and an equal volume of neutral red (0.05%) distilled water solution. These two staining solutions could distinguish the dead nematode larvae. However, the live larvae could not be stained, and not all of the dead cells would take on a dark blue tone. When the live larvae were killed by steam heating for 7-10 minutes, some of the dead cells still could not be satisfactorily stained. It was

found that a neutral red (0.05% distilled water) solution was suitable for this purpose. The neutral red solution would leave the living larvae apparently free of dye and nearly transparent and it stained successively all of the larvae which were killed by the heat of the steam plate. The samples for microscopic examination were taken from the solution while vigorously stirring and then two drops were taken for examination. At the beginning of the experiment most larvae could move in the solution quite vigorously, however, most of them stopped this vigorous activity after 24 hours, so that the only means for distinguishing the live and dead larvae was by the help of neutral red staining.

Table 9. Number of dead and living larvae in different concentrations of monocrotaline. (From two drops of solution examined)

Time of Observation	Treatment							
	Monocrotaline Solution						Check	
	1/200		1/400		1/800		Water	
	Dead larvae	Living larvae	Dead larvae	Living larvae	Dead larvae	Living larvae	Dead larvae	Living larvae
After 12 hours	1	3	1	3	0	4	0	2
24 hours	5	0	1	1	1	1	1	2
36 hours	3	1	3	1	2	0	3	1
48 hours	2	1	1	0	2	1	1	1
60 hours	1	1	2	0	1	0	1	0

3. Effect of monocrotaline on larvae in galls.

Sweet potato roots which contained galls were soaked in distilled water and one two hundredth solution of monocrotaline. The galls were then crushed on a glass slide and then stained by a neutral red (0.05%) solution. These galls in treatment were examined every 12 hours.

It was assumed as a working hypothesis that the nematode in the gall would hardly be affected by the monocrotaline. However, the results seem to show that the monocrotaline was effective. Up to about 60 hours the monocrotaline solution killed more larvae than were killed in the check in distilled water. But, after that the results seemed to indicate that there were more living nematode larvae in the monocrotaline solution than dead larvae. The dead larvae in monocrotaline solution might have decomposed more rapidly than those in distilled water, however, this is merely conjecture.

The materials were kept on test after changing to new solutions. After one week from beginning of the experiment it was found that about one larvae per gall was still living in the monocrotaline solution, while about two were still living in distilled water. It might be possible to conclude from these data that monocrotaline is not a quick acting poison for nematode larvae. Further work could be suggested at this point, that is, that larvae treated with a monocrotaline solution could be added to a sterilized soil and the subsequent population examined by growing indicator plants.

Table 10. Number of living and dead larvae per sweet potato (All Gold variety) root-gall in 1/200 solution of monocrotaline and distilled water.

Time of Observation	Distilled Water			1/200 Monocrotaline Solution		
	Living larvae	Dead larvae	% of dead larvae	Living larvae	Dead larvae	% of dead larvae
After 6 hours	11	0	0	14	2	12.5
12 hours	35.3	21	37.3	19	52	73.9
24 hours	39	20	29.5	20	25	55.5
36 hours	31	18.6	37.5	20	24	54.5
48 hours	44	52	54.1	13.3	66	90.0
60 hours	24	32	57.1	12	36	75.0
72 hours	6	22	78.5	23.6	32.3	57.7
84 hours	11	16	59.0	21	26	55.3
96 hours	17	35	67.3	4	29	87.8

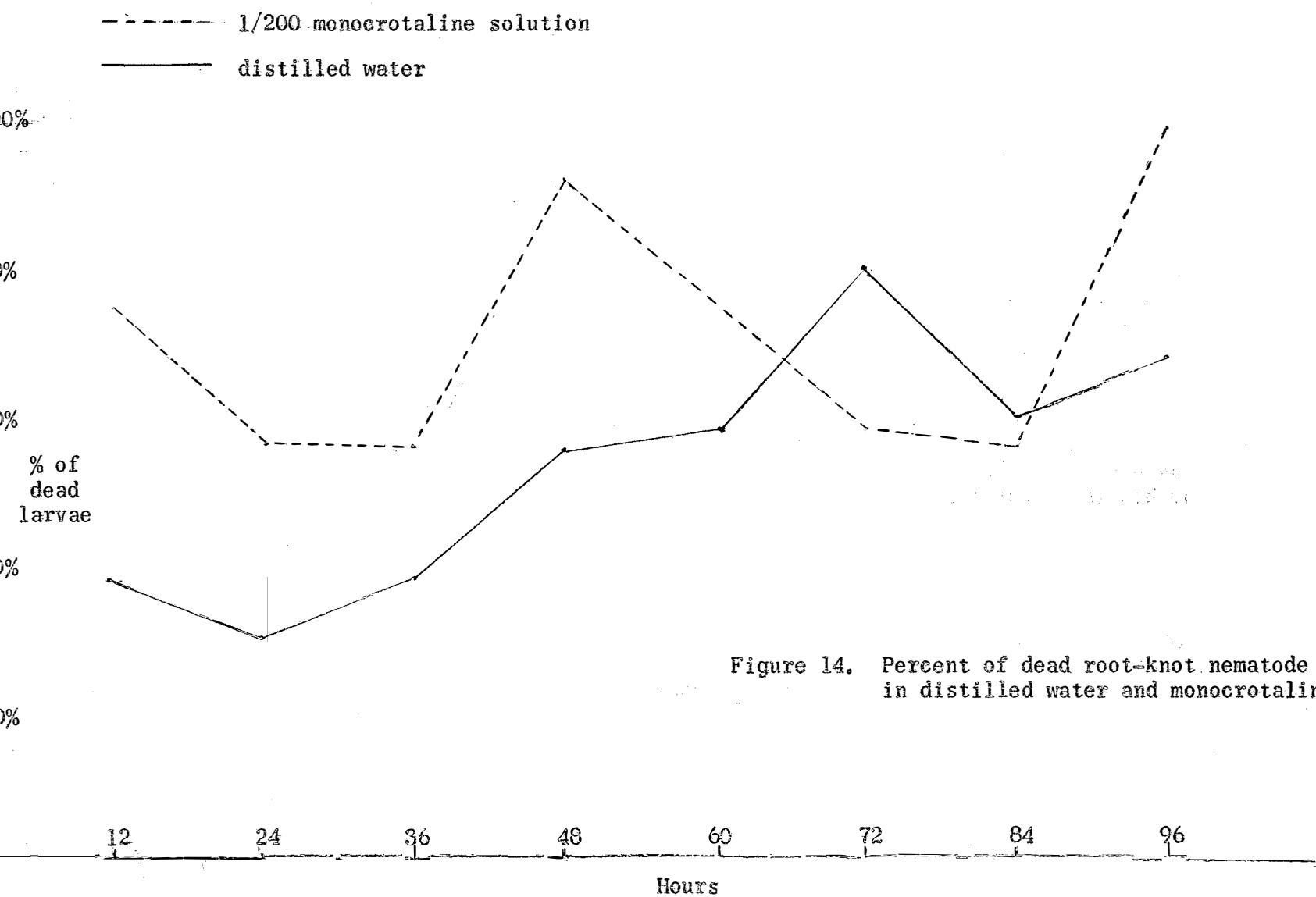

Figure 14. Percent of dead root-knot nematode larvae presen in distilled water and monocrotaline with time.

DISCUSSION AND CONCLUSIONS

The root-knot nematode problem is present in all parts of the United States especially in sandy soils and particularly on the soils growing the intensive type of horticulture crops. The damage to crops is not only to reduce the yields but also to affect the quality of crops, for example, the effect of nematodes on Long Island potato tubers (15). External evidence of the presence of the nematode on the potato will not be received at the warehouse without question, and the marketing of such tubers will create a very unfavorable impression of the quality. Although the yield and quality are not heavily affected on infested soil, in dry weather the potato plant will wilt more quickly than the healthy potato plant (15).

The root-knot nematodes are readily spread by means of infested roots, tubers, irrigation water, wind, seeds and other plant refuse. When the soil is wet, they may be spread from field to field by means of infested soil which adheres to the boots of workers, tractor wheels, other tools, and feet of wild animals. Heavy rains may cause considerable soil erosion on light soils, and soil and water movement of this kind is a very important function in the spreading of the root-knot nematode parasite. By these means, the nematode is easily carried from field to field and in some instances it may even be transported long distances.

The research reported here is thought to be a good suggestion for root-knot nematode control in the southern part of the United States, because Crotalaria spectabilis Roth is a tropical or subtropical type of plant. From the data presented as a result of the first experiment, it was shown that crotalaria compost lessened the nematode infection. This

might have been due to the effect of the monocrotaline content compost of whole roots and tops. The whole plant contains more monocrotaline and more organic material than the plant parts, so the nematode might have been controlled by the effect of whole plant rather than only one factor. Tyler (37) reported on the effect of plowing down a heavy stand of a cover crop or green manure crop on the nematode population of a soil. He allowed the organic material to decay under conditions of warmth and moisture. The heat and gases formed by the decomposition of this material was sufficient to kill from 5 to 25 percent of the nematode population. Monocrotaline and larger amounts of organic matter would seem to be a more efficient method of control of the root-knot nematode. Experiment 1 is good information on the influence of monocrotaline and organic material on the nematode, as the nematode was added at the same time as the compost.

In Experiment 2, all treatments except the check were shown to reduce greatly the number of galls, as shown by the photographs, Figures 9, 10, 11, 12 and 13. As the root-knot nematode cannot reproduce without living host, any susceptible crop which follows a highly root-knot resistant crop, such as Crotalaria spectabilis, should starve out most of the parasite population.

As shown before (5), the larvae of the root-knot nematode can enter the roots of Crotalaria spectabilis, but they do not develop to maturity or reproduce in this root. It is logical to assume that the nematode in the root dies when the plant dies, if not before. There is little doubt that every nematode entering the root of the crotalaria plant was permanently removed from the soil population. Quite a few galls were found on sweet potato plants, these larvae probably did not enter the crotalaria roots from the previous treatment. Also the green weight of sweet potatoes

showed a significant difference between check and treatments. This might have been due to the function of organic material decomposition products from crotalaria and a different degree of root-knot infection.

Treatment 1 where the whole crotalaria plant was removed showed a significant difference in green weight yield between check. It is evident that this treatment reduced the nematode infection of the susceptible plants and increased the yield of crops. In other words, starvation of the nematode by growing crotalaria was sufficient to reduce the nematode population and this reduction of population will apparently affect the yields.

As shown by these experiments, Crotalaria spectabilis can starve out the root-knot nematode and monocrotaline seems to be toxic to the nematode. In combating any kind of parasite, it is necessary to understand its life cycle and habits. The foregoing review of the life cycle of the nematode and its behavior is exceedingly important as a background for practical application. The control measures of rotation, fallow, and flooding are based on starving out the larvae in the soil. It is necessary to keep in mind that not only must the producer grow resistant crops and destroy available host plants, but at the same time should provide a favorable condition for keeping the larvae active, as a means of hastening their exhaustion. The principle is to apply a control method during the summer time and grow some green cover crop in the winter. The success of any starvation program depends on weed control, because weeds are possible reservoirs of infestation.

Since root-knot nematode cannot grow or reproduce in the Crotalaria spectabilis root, this plant could be used to starve out most of the nematode larvae during the warm season that is most favorable for the develop-

ment of their life cycle. This legume can also form an ideal cover during the hot summer, and large amounts of organic material are thought to reduce the population of this parasite. An alternation of crotalaria and resistant grains is recommended in every case where it is at all practical. Control will be more complete with highly resistant crops planted in rows, so that weeds can be destroyed and the soil can be frequently cultivated. Crops for rotation should be carefully selected by farmers. Rye for winter crop is a desirable practice. Corn, peanuts, velvet beans, oats, and other grains are generally not badly damaged by the nematode, and have a tendency to decrease the nematode population, so that these crops can be planted as rotation crops. It was found at the Georgia Coastal Plain (23) that Meloidogyne Arenaria caused a considerable loss to tested peanut varieties, but that the other two species did not. Cotton varieties and species of Meloidogyne also have the same kind of relationship. Meloidogyne arenaria could not damage the three varieties of cotton tested, but M. incognita and M. javanica could easily attack 1 or 2 varieties of cotton tested. Thus attention should be kept on species, or variety of crop and nematode. The control of the nematode on the peanut is thought to be a simple task where the nut is harvested, although an immune variety should be used. After the peanut is harvested, the root, and soil are exposed to air and sunlight, and the adult, larvae, and nematode eggs can be killed by desiccation and heat.

Crotalaria spectabilis grows very slowly during its' early stages of development. In order to keep it as long as possible on the field, seed may be sown on young oats during April at the rate of 20 to 25 pounds of scarified seed per acre. The oats can be harvested when mature, and the young crotalaria should then grow to a stand, and should be an excellent summer green manure crop.

SUMMARY

The effect of Crotalaria spectabilis Roth on root-knot nematode is reported in this study. Crotalaria was grown on the greenhouse bench and different parts of the plant were composted. The soil was infested artificially with Meloidogyne incognita, and at the same time the susceptible tomato, Sioux variety, was planted as the indicating crop. After one month of growth the tomato roots were removed and the degree of infestation was determined. Next, the pots were again planted to crotalaria and the sweet potato was used as the indicator plant the second time. It was found that the number of galls were apparently reduced by two crops of crotalaria. The green weight of sweet potato grown on pots treated with crotalaria was significantly increased when compared with the check which did not receive the treatment. An alkaloid known to be a toxic material, monocrotaline, was isolated from crotalaria seeds and used to ascertain the toxicity toward the nematode. Crushed root-knot galls or uncrushed roots which had been placed in a monocrataline water solution were observed frequently under the microscope. It was observed that monocrotaline could apparently increase the number of dead larvae when compared with those in water solution alone. From the results described above, Crotalaria spectabilis is recommended as a green manure crop on infested soil. It can make a very ideal summer cover and starve out the nematode during the most active season of the larvae. Rye, oats or other grains or some other resistant crop could enter this system to control nematode. However, the species or varieties should be carefully selected by farmers.

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APPENDIX

Soil Characteristics as Determined by Laboratory Analysis

Test	Results	Remarks
Mechanical Composition	Sand 81% Silt 13% Clay 6%	Loamy sand
Soil reaction	pH 6.5	Slightly acid
Percent total nitrogen	0.061%	
Cation exchange capacity	5.18 m. e. per 100 grams.	
Exchangeable calcium	1.25 m.e. per 100 grams.	
Exchangeable potassium	0.56 m.e. per 100 grams.	
0.1 N acetic acid Soluble phosphorus	77.20 p.p.m. or 155.4 lbs./A	

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TYPIST PAGE

THESIS TITLE: THE EFFECT OF CROTALARIA SPECTABILIS ROTH ON ROOT-KNOT NEMATODE

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