A STUDY OF THE ROOT-NEMATODE (HETERODERA RADICICOLA) AND ITS CONTROL

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INTRODUCTION

The root-nematode, eel-worm or root-gallworm, has long been known in the United States, particularly in soils of the South and occasionally in soils of the northern states. It is also widely distributed in greenhouses and the direct or indirect damage done by the nematode is very great. Up to date, an entirely satisfactory remedy has not been found, consequently this aspect of the problem offers a rich field for investigation. At the outset, then, it was necessary to consider all possible phases, and as a result certain points are revealed in this study which are of general interest, but these do not directly affect or concern the problem of practical control.

Since the discovery of the root-nematode in 1855 by Rev. M. J. Berkeley, it has been reported as occurring on 480 different species of plants, some of which are the tomato, celery, beet, lettuce, lima bean, radish, spinach, egg-plant, gardenia, carnation, sweet-pea, rose, violet, ginseng and other plants which are not so common.

Nematode infestation becomes noticeable when the plants show dwarfing and have a general unhealthy appearance. In some cases, however, infestation is not apparent until the roots are examined. An infested root is usually enlarged at the tip as a result of the irritation produced by the nematode. In this respect it differs from the round and lateral tubercles of bacteria formation, and furthermore, the swellings due to the root-nematode are elongated and pear-shaped.

The larva, or early stage in the animal's development, is a slender transparent eel-like organism which moves about by a whipping process. In a liquid containing no sediment this thrashing about does not produce a change of location while in a solution containing small particles the thrashing results in locomotion due to friction against the particles. The larva is about 400μ or approximately 0.5 mm. in length and nearly 15μ in width. It is colorless, but the body-wall and particles of food within the animal serve to differentiate it from the medium in which it is located. The larva, on hatching from the egg, searches for a growing root and upon finding it, enters at the growing tip and a gall is subsequently formed. Many larvæ may enter a single root but

the size of the gall and the number of larvæ within are not necessarily correlated.

While in the root, the larvæ undergo several molts and become true males and females. The mature male is similar to the larva in form and appearance. It is, however, much larger, being about 1400μ in length and 30μ in thickness (fig. 1). The female becomes pear-shaped and is from 400 to 1000μ in length, of a pearly white color. After being fertilized by the male the eggs begin to form.

Brown, oval eggs are forced out by the female. These eggs measure about 90μ in length and 40μ in thickness. Repeatedly, larvæ were observed coiled within the thick shell of the egg. (Fig. 1.)

Under average greenhouse temperatures, the eggs hatch in 5 days and the newly-hatched larvæ may immediately attack susceptible plants. The entire life-cycle in a greenhouse is completed in about 30 days.

The larvæ are capable of encysting and thereby capable of resisting adverse conditions, such as drought and certain chemicals. The eggs are likewise very resistant and this stage is able to withstand many strong chemicals.

Before 1898 (10) very little attention had been given to the study of nematode control; the limited amount of investigation before this period dealt mainly with the morphology and physiology (1). From the standpoint of control, steam sterilization and some chemicals have been tested (2, 3, 7, 8). Satisfactory control is claimed by steam sterilization of the soil but this method is in many cases impossible or at least impracticable. A large number of chemicals (8, 10) has been tried but so far as known none has given satisfactory results. In view of the above facts, it was thought necessary to consider all phases of this problem that time would permit.

EXPERIMENTS

Moisture

The first experiment tried was to determine the effect of moisture on root-nematode activity.

In the preliminary experiments with moisture it was seen that galls, taken from infested gardenia plants and placed in water over a source of mild heat, produced very active nematodes. For further study galls of approximately the same size (3.5 x 3 mm.) were thoroughly washed in distilled water and placed singly in stender dishes and each was treated, in a duplicate series, with different quantities of distilled water and maintained at a constant temperature for 72 hours and examined under the low power of a microscope at different intervals of time. All observations were from 1 hour to 72 hours. The results were the same at the end of 1 hour as at the end of 72 hours. The observations are given in table 1. In the table, Series A and B are duplicates and have the same treatment.

Another moisture experiment was made to confirm the preliminary one. This was run in triplicate series, each series having the same treatment. Results are given in table 2.

It can be seen from table 2 that the activity of the root-nematode is increased, the temperature remaining constant, with an increase in moisture

TABLE 1
Effect of moisture on root-nematode activity (at 21°C.)

WATER	SERIES A	SERIES B
cc.		
0.0	Inactive	Inactive
0.1	None out	None out
0.2	Very few out and slightly active	None out
0.3	Very few out and active	Few out and slightly active
0.4	Very few out and active	Few out and slightly active
0.5	Many out and active	Many out and very active
0.6	Many out and not active	Many out and few active
0.7	Immerged, few slightly active	Immerged but inactive
0.8	Immerged inactive	None seen
0.9	None seen	None seen
1.0	None seen	None seen
2.0	None seen	None seen
5.0	None seen	None seen
10.0	None seen	None seen

TABLE 2
Effect of moisture on root-nematode activity (at 19°C.)

WATER	SERIES A—LARVÆ	SERIES B—LARVÆ	SERIES C—LARVÆ
cc.			
0.0	Inactive	Inactive	Inactive
0.2	One out and active	One out and active	None seen
0.3	Slightly active	Slightly active	Slightly active
0.4	Some very active	Slightly active	Some very active
0.5	Many very active	Many active	Some very active
0.6	Many very active	Many active	Some very active
0.7	Many very active	Many active	Slightly active
0.8	Few active	Slightly active	None seen
0.9	None seen	Slightly active	None seen
1.0	Slightly active	Slightly active	Inactive

until an optimum moisture content is reached, and when continued above that point the activity is decreased.

In the above experiments, sterile water was used in order to eliminate the toxic substances, such as chlorine, copper salt, etc., found in tap water. This introduced another factor—that of plasmolysis. It was suggested that the concentration of sterile water was below the concentration of the liquid in the

body of the nematode and therefore plasmolysis might occur. This theory was tested by placing gardenia galls in a series of stender dishes containing sterile water and a similar series using water extracted from the soil—the natural solution in which the nematode normally lives. It was observed that the results from both solutions (0.2 to 1.0 cc.) compared very closely.

This proved that sterile water had no plasmolyzing effect used in rates up to 1 cc., and that the experiments were therefore sufficiently accurate.

Also, in connection with this experiment it was thought that because a definite number of larvæ were not used in each stender dish results would not be accurate. This possibility of error was dismissed on the ground that sufficient numbers (200 to 400) of larvæ were present in each dish to give safe averages. In every test the gall was crushed in order to reveal the dormant larvæ in observations recorded as "none seen." The fact that some larvæ emerged and others did not may be due to their location in the gall, that is, some of them were not near enough to the surface of the root to be affected by the moisture. The galls became submerged with 0.7 cc. of water.

If in the above tables on moisture, "inactivity" means death, then flooding the soil might offer a remedy for nematode infestation. Furthermore, it has previously been observed in the South that the root-nematode does not occur in bogs or water-soaked lands. Many observers have noticed that the nematode occurs mostly in sandy soils and not in clays. This may be due to the fact that sand does not retain water for any considerable length of time and thus allows sufficient aeration for the nematode. On the other hand, clay soils retain moisture and thus reduce the amount of air held. For the majority of cases, therefore, flooding was considered impracticable, and an unsatisfactory control, except perhaps under special conditions, and was dismissed from further consideration.

Temperature

The next test made was to determine the effect of temperature on nematode activity.

As in the experiment on moisture, gardenia galls of approximately the same size (3 mm. x 3.5 mm.) were immersed in 0.4 to 1.0 cc. of distilled water and triplicate series placed under three different temperatures. To keep the moisture constant the stender dishes were placed in tin trays containing water, and were covered with cheese-cloth which rested in the water and was kept moist by capillary attraction.

Series I was placed out-of-doors, sheltered from rain. The temperature averaged about 8.8°C., or 48°F.

Series II was placed in an electric incubator at a temperature from 36.6° to 38.8°C., or 98° to 102°F.

Series III was placed in a greenhouse at a temperature of 26.6°C., or 80°F.

The contents of all dishes were examined at the end of 24 hours under the

low power of a microscope. The results are given in table 3. Here again Series A, B and C have the same treatment.

In the above table "none out" and "none seen" indicate that no larvæ had emerged, but at the end of this experiment each gall was crushed and inactive larvæ were found.

The observations recorded in table 3 seem to warrant the conclusion that the optimum temperature for the nematode is between 65° and 85°F. Generally speaking, temperatures above 65°F. increase nematode activity, and temperatures below 65°F. and above 85°F. decrease nematode activity. The animal may be killed by freezing (32°F.), and by heating to 101°F. the larvæ and eggs are killed.

TABLE 3
Effect of temperature on root-nematode activity

	WATER	I (AT 8.8°C.)	II (AT 36.6° TO 38.8°C.)	III (AT 26.6°C.)
	cc.			
Series A ...	0.4	Out and active	Out and inactive	Out and inactive
	0.6	Out and active	Out and inactive	None out
	0.8	None out	Out and inactive	Out and slightly active
	1.0	Out and active	Out and slightly active	Out and slightly active
Series B ...	0.4	Out and active	Out and inactive	Out and very active
	0.6	Out and active	Out and inactive	Out and very active
	0.8	Out and active	Out and inactive	Out and very active
	1.0	Out and active	None out	Out and very active
Series C ...	0.4	Out and active	None out	Out and very active
	0.6	Out and active	Out and active	Out and very active
	0.8	Out and active	Out and inactive	Out and very active
	1.0	Out and active	None seen	Out and very active

The above results suggested that raising the temperature of the greenhouse soil to 101°F. would kill the larvæ and eggs. This however did not appear to be practicable for most greenhouses. High temperature may be applied with some degree of success at least, by exposing infested soil to the hot rays of the sun and turning it over to insure thorough sterilization.

Chemicals

Failing to discover a satisfactory remedy for nematode attack in the study of moisture and temperature conditions, attention was next directed to the study of chemicals. At the outset it was seen that a great many of the favorite chemicals used in soil sterilization could be omitted, for J. A. McClintock (8) has shown that the nematode could not be satisfactorily combated by any of the following: carbon disulfide, corrosive sublimate, calcium carbide, ammo-

nium sulfate, formalin, nicotine, benzine or kerosene. Furthermore, Stone and Smith, in Massachusetts (10), demonstrated that the following chemicals are ineffective: manganese sulfate, common salt, potassium nitrate, magnesium sulfate, calcium sulfate, kainit, sodium nitrate and lime.

The recent success with sodium cyanide (NaCN) obtained by Dr. T. J. Headlee of the New Jersey Agricultural Experiment Station, as a soil sterilizer in the control of wire-worms suggested that the same treatment might be used in combating the root-nematode.

Gardenia galls were placed in stender dishes as in experiments with moisture and temperature and treated with amounts of cyanide, ranging from 0.7071 gm. to 0.0707 gm. dissolved in 10 cc. of water, giving solutions from 0.7 to 7 per cent concentration. On standing 48 hours the larvæ had completely disappeared. This disappearance may have been due to disintegration. The eggs also were attacked and made colorless.

The above experiment was repeated in duplicate, with 2 mg. NaCN dissolved in 0.5 cc. of water (shown by the first experiment to be the approximate optimum moisture). The results are given in table 4.

TABLE 4
Sodium cyanide treatment (at 18°C.)

	A—LARVÆ	B—LARVÆ
1	None out	Dead
2	Dead	Dead
3	Dead	Dead
Check (no NaCN)	Very active	Slightly active

On crushing the gall in the cylinder 1A, the larvæ were found to be dead. This test shows conclusively the high killing effect of cyanide on the larvæ. This strength of cyanide (25 per cent) did not affect the eggs.

Sphagnum Moss

It has been noticed that roots of plants growing in sphagnum moss did not become infested with nematodes while roots on the plants not in sphagnum moss became infested.

This interesting point was summarily tested. Dry sphagnum moss was placed in a beaker of distilled water and boiled for 10 minutes. The mixture was then filtered and the moss extract applied at the rate of 0.5 cc. to gardenia galls in stender dishes as in previous experiments. The observations showed that the moss extract killed the larvæ (table 5).

TABLE 5

Effect of sphagnum moss extract treatment (at 18°C.)

	CC.	A	B
1	0.5	Dead	Dead
2	0.5	Dead	Dead
3	0.5	Dead	Dead
Check		Very active	Very active

Formaldehyde

A similar experiment was also tried with 40 per cent formaldehyde and the results are given in table 6.

This demonstrates the high killing effect of formaldehyde.

TABLE 6

Effects of formaldehyde (40 per cent) treatment (at 18°C.)

	CC.	A—LARVÆ	B—LARVÆ
1	0.5	Dead	Dead
2	0.5	Dead	Dead
3	0.5	Dead	Dead
Check		Very active	Very active

DISCUSSION OF RESULTS

The root-nematode (*Heterodera radicicola*) has been a serious pest to plants growing in certain soils and particularly in greenhouse soils. The investigations concerning this pest have been mainly morphological and the attempts to control it have thus far not given entirely satisfactory results. Steam sterilization of the soil does give complete control where it can be practically applied and with thoroughness, but the average conditions do not offer a way for the application. For example, greenhouses fitted for hot-water heating are not adapted to the use of steam. It was for such a condition that the attempt was made to find another method.

The first experiment was to determine the effect of moisture upon the nematode and it was observed that moisture is a necessity for activity but if the water content is in excess, then the animal becomes inactive. So there is an optimum moisture for the nematode.

An experiment to test the effect of temperature demonstrated that the nematode is inactive at comparatively low temperatures and at high temperatures, and its greatest activity is at a temperature from 65° to 85° F. So there is also an optimum temperature.

The recent success with sodium cyanide suggested this treatment as a possible remedy and it was found to be very effective in killing the larvæ and in a moderately concentrated solution to destroy the egg stage.

Similarly sphagnum moss extract and formaldehyde were found to be toxic to the nematode.

GREENHOUSE EXPERIMENTS

The satisfactory results of different treatments in the laboratory led to experiments with soil in the greenhouse. Fortunately, a very thoroughly infested greenhouse bench offered itself for the experiment. This bench had just produced tomato plants which were thoroughly infested with nematodes.

The tomato plants were removed and the soil raked clean of the roots and other objectionable materials and turned over thoroughly. Next the bench was divided into 7 plots, each separated by 5 inches of air space to prevent migration of nematodes from one plot to another.

Plots 2, 4 and 7 were used as check plots. Plots 1 and 3 were treated with sodium cyanide (NaCN) at the rates of 100 pounds and 200 pounds per acre (or 0.036 ounce and 0.072 ounce, respectively). The cyanide was dissolved in water at the rate of 1 gallon of water to $\frac{1}{2}$ ounce and $\frac{1}{4}$ ounce of cyanide, respectively.

The solution was applied to the soil with a sprinkling can at the rate of $\frac{1}{3}$ gallon per square foot of soil, and at the same time the soil was turned back and then thrown over to cover the solution. In this way every particle of soil received the same strength of the solution. After 4 days, the soil was broken up and aerated.

Another similar application was made to these two plots 5 days after the first application. This was done on the theory that the eggs of the animal might have resisted the first application and the larvæ would have hatched at the end of 5 days under the greenhouse temperature (70° to 80° F.) and would be killed by the second application of cyanide.

At the end of 3 days these soils were turned over and completely aerated to remove all traces of cyanide gas. The soil in these and check plots was all the time kept moist and in good tilth. At the end of another 3 days (February 7, 1917) these two plots and the check plots, No. 2 and 4, were planted to cucumbers, radishes and tomatoes. Mice attacked the seeds and necessitated replanting the cucumbers and radish seeds.

At this time it was thought that the mice might have carried some particles of soil from the infested check plots, so note was made of this partly to explain any error that might occur.

Plots 5 and 6 were treated with sodium cyanide at the rates of 50 and 100 pounds, respectively, to ascertain whether a weaker application would control the nematode, as laboratory experiments seemed to show, and whether or not one treatment would be sufficient. The same method of cyanide application was used as with plots 1 and 3. Plot 7 was reserved as a check plot. These plots were aerated 3 days after and planted 6 days after the application to lettuce, radishes, and tomatoes.

The percentage of seed germination was good and the seedlings grew well for about 3 weeks, when it became evident that they were not making average growth. Then it was realized that the soil was low in available nitrogen and tankage was subsequently applied at the rate of 10 gm. per square foot (or approximately 800 pounds per acre). In a few days the plants began to show the effect and made a rapid growth.

While waiting for the plants in these plots to make sufficient growth to observe results, several other experiments were performed.

The question of the effect of the application of sodium cyanide on plants growing in soil was tested by potting up 4 infested tomato plants in infested soil and allowing them to grow under normal conditions for 2 weeks. One of these plants was treated with cyanide dissolved in water and applied at the rate of 50 pounds per acre and another at 100 pounds per acre, which were amounts found to kill the nematode in the laboratory. The remaining two plots were used as checks. The plants in the treated pots wilted at the crown at the end of one day, and at the end of 5 days were completely dead, while the check plants grew as normally. Galls were examined from all plants and the nematode larvæ in them were found to be alive and very active.

The results of the greenhouse experiments were recorded from the time the plants had made a growth of 6 inches up to the time when they were 10 inches high (April 21, 1917). The plants were 18 weeks old at the time the final observations were recorded (table 7).

TABLE 7
Greenhouse experiments with cyanide treatment

DATE	NUMBER OF PLANTS EXAMINED	Plot 1			Plot 2			Plot 3			Plot 4			Plot 5			Plot 6			Plot 7		
		Galls			Galls			Galls			Galls			Galls			Galls			Galls		
		S*	M	L	S	M	L	S	M	L	S	M	L	S	M	L	S	M	L	S	M	L
March 31, 1917..	20	56	9	0	148	10	0	0	0	0	101	15	0	8	3	0	17	0	0	125	7	0
April 11, 1917....	18	30	9	1	130	9	7	2	0	0	40	0	0	15	0	0	60	2	0	37	0	0
April 18, 1917....	15	35	7	0	100	3	4	1	0	0	36	0	0	19	0	0	130	3	0	110	0	0
April 21, 1917....	27	30	3	10	120	7	13	0	0	0	57	2	2	30	0	0	110	3	2	130	10	15

* S = small M = medium L = large

This experiment demonstrated that cyanide to be effective must be applied in larger quantities than 100 pounds per acre to kill the larvæ, and under such applications the soil must be free from growing plants. As shown by the previous experiment, seed will germinate and plants will grow well in soil which has been treated with sodium cyanide used as high as 200 pounds per acre in two applications, and which has been well aerated after the application.

The table of observations shows that the treatment of 200 pounds of cyanide per acre followed by a second treatment 5 days later gives nearly a perfect control. Out of 80 plants examined only 3 galls were found. This is plainly

within experimental error and the results warrant the recommendation of this method of sterilization.

Due to the fact that the treated and untreated plots were so close together, some of the particles of soil and water carrying the nematodes could easily have been transmitted from one plot to the other.

There was no apparent difference in growth of plants in the various plots. They all were very healthy-looking at 18 weeks after the seed was planted. The experiment was then discontinued.

SUMMARY

1. A serious pest to plants, growing in warm soils and especially in greenhouse soils, is the root-nematode (*Heterodera radicicola*) sometimes called the eel-worm or root-gallworm.

2. This parasite is a minute organism which penetrates the roots of plants causing them to become enlarged and deformed, and resulting in a hindrance to growth.

3. The nematodes may be distributed by their own motion, by water, in soil clinging to implements, by infested plants transferred to an uninfested soil, by seed, by cuttings, and by manure.

4. The following conditions are necessary for their growth and reproduction: (a) an optimum moisture, (b) an optimum temperature, (c) food, and (d) oxygen.

5. The following were found to decrease nematode activity and may be applied as methods of control: (a) excess of moisture, (b) high temperature (101°F.), (c) formaldehyde, (d) sphagnum moss extract, and (e) sodium cyanide (NaCN) dissolved in water.

6. In experiments with greenhouse soils the following treatment gave a satisfactory and practical remedy for nematode infestation:

Sodium cyanide dissolved in water is applied in the proportion of 200 pounds per acre, one-third gallon per square foot of soil. One week after the first treatment a second similar treatment is given. This method is for soils free from plants. The soil should be aerated and leached well before seed or plants are put into it in order to remove the greater amount of cyanide gas.

7. The cost of materials for this treatment is \$112 per acre. For greenhouse soils where steam sterilization is impracticable this is a remedy which is to be recommended.

CONCLUSIONS

The object of our study is thus accomplished. A satisfactory and practical control for the soil nematode is found in sodium cyanide.

In treating nematode-infested soil, the number of square feet of soil should first be determined. The number of pounds of cyanide should then be calculated on the basis of 200 pounds per acre. Next, the amount of water,

figured on the basis of $\frac{1}{3}$ gallon per square foot of soil, should be determined. Dissolve the cyanide in the proper amount of water and apply the solution to the soil with a sprinkler. The soil should be well mixed and kept slightly moist and warm for 5 or 6 days before the treatment in order to bring the larvæ from their cysts and therefore render them more susceptible to the treatment. The third day after the first treatment the soil should be stirred and aerated slightly. The same favorable conditions for nematode activity should be maintained until after the second treatment. About one week after the first treatment the second application determined in the same manner should be given.

Of course, all plants should be removed from the soil before the application of cyanide, for most plants succumb to such a treatment. Before any seeds are planted or plants set in the sterilized soil, the soil should be thoroughly aerated and leached somewhat to remove the traces of cyanide gas.

Using this method of soil sterilization, the cost of the cyanide used would be 400 pounds at \$.28 per pound, or \$112. For greenhouse soil sterilization, this is an economical and entirely satisfactory investment.

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PLATE 1

FIGS. 1 to 6. DEVELOPMENT OF FREE-LIVING NEMATODE
FIG. 7. YOUNG LARVA
FIG. 8. IMMATURE FEMALE
FIG. 9. MATURE FEMALE
FIG. 10. MATURE MALE