NUTRITION OF LIMA BEAN *PHASEOLUS LUNATUS* L. IN RELATION TO DISEASE DEVELOPMENT INCITED BY A ROOT-KNOT NEMATODE, *MELOIDOGYNE INCOGNITA* AND TO DEVELOPMENT OF THE PATHOGEN

By

Bakir Abbas Oteifa

Thesis submitted to the Faculty of the Graduate School
of the University of Maryland in partial
fulfillment of the requirements for the
degree of Doctor of Philosophy

1953

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF FIGURES CONT'D.

ACKNOWLEDGMENT

The author wishes to express to Dr. Walter F. Jeffers his sincere appreciation for the assistance and council offered during the course of work and preparation of the manuscript. Thanks and credit are due to Dr. Gotthold Steiner, of the U. S. Department of Agriculture, for his valuable suggestions and guidance during the course of the experimental work involved and making available the facilities of the Division of Nematology. Sincere thanks are extended to Mr. Albert L. Taylor and Dr. Benjamin G. Chitwood, of the Division of Nematology, for their helpful criticism, advice and interest. Grateful acknowledgment is expressed to Dr. L. E. Scott, Department of Horticulture, for his supervision of the chemical determinations.

INTRODUCTION

Plant nematology is a comparatively recent science. To date, most investigations in this field have been concerned primarily with a study of pathogenic nematodes, the diseases they cause and possible means of control of these diseases. However, little attention has been given to the fundamental problem of host nutrition in relation to development of diseases incited by these organisms. As most plant parasitic nematodes are obligate parasites, direct nutritional studies on them are impossible, since they generally cannot be grown on artificial media, and only their behavior as influenced by nutrition of the host can be observed. It is then apparent that the relation of these pathogens to nutrition of their hosts represents such a wide range of interactions that no simple physiological pattern can be anticipated. Plant nutrition is one of the environmental factors which, along with others such as temperature, humidity, soil moisture and soil reaction may have a measurable effect upon the course of disease development.

The relation of host plant nutrition to nematode disease development can be ascertained in two major ways: first, by determining effects of the parasites on growth response, mineral content and physiology of the host plant and secondly, by determining influences of host nutrition on development of the parasite. These latter effects can be approached in various ways, e.g., by the rate at which the nematodes develop, by the extent to which they develop and finally by the rate of their reproductivity.

The objectives of this study are to ascertain the effects of these two major factors; to determine whether degree of infection could be altered through changes in nutrient balance and whether such changes would explain some of the interactions between host nutrition and disease development.

LITERATURE REVIEW

There are many plant diseases in which nutrition of the host has an effect upon the rate and degree of disease development. Many field experiments and controlled nutritional studies have been carried out with diseases caused by bacteria and fungi as well as virus diseases to determine the effects of various nutritional levels upon the course of disease development. Spencer (17), Thomas (19), Walker (23) and many other workers have observed that the reaction of certain plants to pathogenic bacteria and fungi may be altered by varying the mineral composition of nutrient solutions supplied to the host. It is also well established that nutritional conditions of the host plants have a marked influence on growth and development of various bacteria and fungi that parasitize them. As far as can be determined, literature on the influence of nutrition on the reaction of host plants to parasitic nematodes is quite limited. Although the beneficial action of fertilizers in reducing nematode injury has been demonstrated in infested soil, the nature of this action has not been explained. Bessey (2) in 1906 conducted several fertilizer experiments with various plants infected with the root-knot nematode. He showed that fertilizers containing nitrogen, when not in too great excess, benefited the plants somewhat while phosphatic fertilizers showed no benefit at all. On the other hand, potash exerted a remarkable beneficial effect upon the plants and enabled them to make a good crop in spite of infection by nematodes. Bessey postulated that potassium might increase the ability of plants to prevent the entrance of nematodes into the roots.

A study of the mineral content of plants infected with nematodes was initiated by Vanha (22) and Wilfarth and Wimmer (24) in the case of diseased sugar beets. These workers found that diseased beets were characterized by low calcium, phosphorous, magnesium, and nitrogen and a very low potassium content. They concluded that the plants were deprived of nutritional substances due to presence of nematodes and stipulated that an abundant potassium fertilization would maintain the proper sugar content within the beets but would not prevent a lowering of the yield. Roemer (15), however, was able to increase yields of nematode-infected sugar beets from 18,000 to 19,000 Kg per 2.47 acres by applying only a potassium fertilizer. Ellenby (8) tested various elements in an attempt to correct "potato sick" soils and found that boric acid, zinc sulfate and magnesium chloride gave increased yields. Parris and Jehle (13) reported root-knot infected Fordhook lima beans deficient in phosphorous despite an adequate supply of this substance in the soil. Chlorosis has long been a recognized symptom of diseases due to various root-parasitic nematodes. Tarjan (18) analysed boxwood plants infected with meadow nematodes Pratylenchus spp., and found that infected plants suffered from deficiences of essential elements. Chitwood, Specht and Havis (5) found changes in potassium, calcium and magnesium concentrations of peach leaves on plants infected with root-knot nematodes.

Development of root-knot nematodes has been reported to be influenced mainly by two factors: temperature and type of host plant. Temperature was investigated by Godfrey (9) and Jones (12). Tyler (21) in an extensive study on development of root-knot nematodes as affected by temperature indicated that the minimum time required for the life cycle of this nematode from larvae to larvae in tomato roots was 25 days at 27° C., and increased to 87 days at 16.5° C. Development from gall formation to egg-laying required 15 days at 27° C., and 79 days at 14.3° C. Demonstrating the effect of the host on parasite development, Godfrey and Oliveira (10) found that in cowpea grown at a temperature permitting rapid development, the interval between penetration of larvae and first deposition of eggs was 19 days, but in pineapple, grown under identical conditions, it was 35 days. Chitwood (4) and Christie (7) have also indicated that a given species of nematode may have considerably diverse rates of development in different host plant species, however no experimental data were given to indicate that nutritional differences were a factor. Tyler (20) in studies on parthenogenic reproduction of root-knot nematodes in aseptic root cultures postulated that a healthy condition of the host is an important factor for development of its parasite.

From the preceeding review, it therefore can be seen that in the case of certain nematode diseases of plants there is a marked reduction in certain of the nutritional elements contained in the plants. Because a more adequate explanation of the relation of host plant nutrition to infection by root-knot nematodes was desirable, further investigation of this question was undertaken.

MATERIALS AND METHODS

Inoculum Preparation

The root-knot nematode species used in these experiments was *Meloidogyne incognita* (Kofoid and White, 1919) Chitwood, 1949. Identification of this species was determined according to Chitwood's criteria (3). For obtaining a pure population of this nematode, egg masses of the identified females were allowed to propagate on the Rutgers variety of tomato *Lycopersicon esculentum* Mill. on which production of egg masses readily occurs. In the first experiment concerning studies of different levels of nitrogen, phosphorous and potassium in deficient and excess concentrations, three levels of nematode inoculum (0, 10 and 50 egg masses) were employed. Egg masses were carefully dissected from infected roots and immediately used for inoculum. In all other experiments higher inoculum levels of 0, 50, and 200 egg masses were used. As it was necessary to dissect a considerable number of egg masses from infected roots, another method for obtaining the representative amounts of inoculum was employed. Heavily infected tomato roots bearing numerous egg masses were finely chopped and thoroughly mixed. Egg masses contained in a portion of this tissue were counted under a binocular microscope and the corresponding weights of roots containing approximately 50 and 200 egg masses were then computed. Before any inoculum was selected, several representative egg masses were examined microscopically to be certain that either first or second stage larvae were present.

Sand Culture Preparation

Glazed, three-gallon crocks provided with drainage were used as containers. Washed, white quartz sand was used as the growing medium. Crocks and sand were steam sterilized at 20 pounds pressure and 259° F. for a minimum of three hours. Crocks were filled to one quarter of their capacity with a coarse sand of eight mesh, on top of which a finer sand of approximately 40 mesh was added so that they were about half full. The required amount of inoculum was then distributed evenly over the surface, after which an additional layer of the fine sand was added thus bringing the surface to about two inches from the top of the crock.

Seeds of lima bean *Phaseolus lunatus* L. variety Henderson were treated with tetrachloro parabenzo-quinone (Spergon) and were sown into germination boxes containing sterilized, white, coarse sand. The germination boxes were covered with glass sheets for three days and were held on the greenhouse bench at 80° to 90° F. When the seedlings had reached the third or fourth leaf stage, selected plants of uniform length and growth were transplanted using one per each crock. Nutrient treatments were begun immediately after transplanting.

Nutrient solutions in distilled water were prepared from molar stock solutions of the following C.P. salts: $Ca(NO_3)_2 \cdot 4H_2O$, KNO_3, $CaCl_2$, KCl, $NaCl$, $MgSO_4$, KH_2PO_4, and NaH_2PO_4. The nutrient solution used throughout was a modification of that formulated by Hoagland and Snyder (11). In addition to the complete solution (NPK), solutions containing extra quantities of one of the elements N, P and K and solutions lacking one of these elements were used. Composition of these various nutrient solutions is given in Table I. The reaction of all solutions was adjusted to a pH range of 5.8 to 6.4. The same amount of minor

TABLE I. Composition of nutrient solutions used in studying the effect of nitrogen, phosphorous and potassium nutrition on reaction of lima bean plants to infection by a root-knot nematode *Meloidogyne incognita*.

Stock solutions	Ml. of molar stock solutions added to distilled water to make 1 liter of nutrient.						
	Complete	-N	+N	-P	+P	-K	+K
M/1 $Ca(NO_3)_2.4H_2O$	5.0	...	5.0	5.0	5.0	5.0	5.0
M/1 KNO_3	5.0	...	5.0	5.0	5.0	...	5.0
M/1 $NaNO_3$	...	...	2.0	...	...	5.0	...
M/1 $CaCl_2$	...	5.0	...	...	...	...	...
M/1 KCl	...	5.0	...	1.0	...	...	2.0
M/1 NaCl	2.5	2.5	0.5	2.5	1.5	2.5	0.5
M/1 $MgSO_4$	2.0	2.0	2.0	2.0	2.0	2.0	2.0
M/1 KH_2PO_4	1.0	1.0	1.0	...	1.0	...	1.0
M/1 NaH_2PO_4	...	...	...	...	1.0	1.0	...
A - Z*	1.0	1.0	1.0	1.0	1.0	1.0	1.0

*Micronutrient solution prepared according to Hoagland and Snyder (11).

element stock solutions was added to each of the various solutions. In treatments involving higher concentrations of the nutrients, NaCl was added so that the proportions of the major elements could be varied without changing the total salt concentration.

On the assumption that the form of nitrogen might have an influence on disease development, one experiment was conducted to determine the effect of NH_4 ions as compared with NO_3 ions. The NO_3 solution was the same as the complete solution (NPK) used in the first experiment. In the NH_4 solution $Ca(NO_3)_2$ and KNO_3 were replaced by $CaCl_2$ and KCl, respectively, and an equal number of nitrogen atoms was added in the form of NH_4Cl.

For further studies regarding the effect of potassium host nutrition, it was desirable to place the mineral elements on a chemically active equivalent basis to insure accuracy of the analysis. For this reason the nutrient solutions employed in the potassium experiment series were prepared on a milliequivalent basis. Composition of these nutrients is shown in Table II. Potassium concentrations were selected on the basis of a preliminary test so that treatment K_1 was expected to result in potassium deficiency, treatment K_2 to result in optimal growth and treatment K_3 to provide excessive potassium.

Throughout the period of experimentation, nutrient solutions were supplied at the rate of 250 ml. daily for the first week, after which an application of 400 ml. was added three times a week. All crocks were flushed with tap water once a week in order to prevent any accumulation of salts.

The experimental design was a randomized complete block with four replications. Throughout the period of experiments continuous greenhouse temperature records were obtained which showed a daily temperature varying from 65 to 85° F. with an average of 75° F.

TABLE II. Composition of nutrient solutions used in the potassium experiment series.*

Treatment designation	Milliequivalents per liter							
	Ca	Mg	K	Na	NO_3	H_2PO_4	SO_4	Cl
K_1	10.0	4.0	0.5	10.0	15.0	1.0	4.0	4.5
K_2	10.0	4.0	6.0	4.5	15.0	1.0	4.0	4.5
K_3	10.0	4.0	10.0	0.5	15.0	1.0	4.0	4.5

*In addition a micronutrient solution as described by Hoagland and Snyder (11) was added to each treatment.

Chemical Determinations

In harvesting, the plants were cut at the sand surface and fresh weights of tops and roots were determined. All the leaves were removed from each plant and kept separately in a paper bag and remains of the plant were kept in another bag. The plants were then dried in an oven for 24 hours at approximately 125° F. and total dry weight of each was then recorded. Leaves were ground in a Wiley mill equipped with a 60 mesh wire screen. Ash solutions were prepared and analysed for phosphorous, magnesium, calcium and potassium. Total nitrogen was determined by the micro-Kjeldahl apparatus according to the method described by Ranker (14), and was modified by using 2% boric acid to receive the distillate. Potassium and calcium were determined by use of the Beckman flame spectrophotometer. Magnesium readings were obtained with a Leitz photoelectrometer. Phosphorous was determined essentially by the official methods (1). Leaves of each plant were analysed separately with two replicates of each plant.

Manner of Recording Results and Disease Criteria

When plants had reached maturity, roots were washed free of sand and rinsed in tap water. After drainage for a few seconds remaining free water was blotted from them with toweling. Fresh weights and dry weights of tops and roots were then determined.

Indexing of infected roots was accomplished by using a system similar to that described by Smith and Taylor (16). Infected root systems were examined visually and grouped according to the following criteria:

0. No evidence of galling.
1. Trace of galling; occasional individual galls.
2. Moderate gall formation.
3. Extensive production of galls.
4. Severe galling with most of root system infected.

Roots of infected plants were finely chopped and one gram from each plant was preserved in 5% formalin for future counts of females and egg masses. Values for the rate of nematode reproduction were obtained by dividing the number of egg masses by the number of mature females per plant.

Analysis of variance was applied to the analytical data, growth data, and nematode counts.

Demonstration of Nematode Developmental Stages within the Root Tissue

The final experiment was designed in an attempt to demonstrate the effect of potassium host nutrition on developmental stages of the parasite. Lima bean seedlings were transplanted to small pots each containing 10 egg masses of *M. incognita*. After three days such seedlings were removed and the roots were washed free of sand and rinsed in tap water. Such cleaned seedlings were then retransplanted to glazed one gallon crocks filled with sterilized pure quartz sand. Nutrient treatments were followed immediately after transplanting. Effects of three levels of potassium representing low, optimum and excessive concentrations on development of the parasite were studied. Composition of the nutrient solutions used is given in Table II. Application of 250 ml. was made to each crock every other day and they were also flushed weekly with tap water. All plants were subjected to the same temperature range. Every other day one plant from each treatment was removed from the crock, the sand washed free from the roots, and the entire root system was fixed with a modification of Flemming's formula.[1] In preparing the solution, six cc of distilled water, 10 cc of 1% chromic acid, 10 cc of 10% acetic acid and two cc of 2% osmic acid were combined and heated to approximately 55° C. Roots were immersed for about ½ hour after which they were washed in running water for at least four hours; they were then transferred to 15% ethyl alcohol for 15 minutes, 30% for ½ hour, 50% for one hour and absolute alcohol for two hours.

---

[1]The technique employed was a slight modification of a method suggested by H. W. Reynolds, Division of Nematology, U. S. Cotton Field Station, Sacaton, Arizona.

The dehydrated roots were then cleared in methyl salicylate. This technique was very satisfactory for staining the parasites and clearing the root tissues. A small portion of the root system was clipped off and examined under a dissecting microscope and every nematode found was classified and recorded according to Christie's groups of stages of development (6). This system is illustrated in Figures 11, 12, 13, 14, and 15. Examination continued until 100 parasites had been counted from each plant.

EXPERIMENTAL RESULTS

Influence of Nitrogen, Phosphorous and Potassium on the Reaction of Lima Bean Plants to *Meloidogyne incognita*

In this experiment, the effects of a complete balanced nutrient solution, deficient concentrations of nitrogen, phosphorous and potassium as well as excessive concentrations of these three elements were studied. The analysis of variance given in Table III reveals that the different nutritional treatments and the amounts of nematode inoculum exerted a significant effect on plant growth. In Table IV and Figure 1 are presented the mean values of fresh weights of lima bean plants as influenced by variations in nutrient supply and nematode inoculum. Data indicate that uninoculated plants subjected to deficient treatments of nitrogen, phosphorous and potassium made significantly poor growth as compared to plants supplied with complete nutrient solution. On the other hand, when these uninoculated plants were supplied with excessive amounts of nitrogen, phosphorous and potassium they did not increase significantly in fresh weight as compared to plants supplied with the complete nutrient solution. This indicates that composition of the solution used as a complete nutrient treatment was an optimum nutrient level for growth of lima bean plants under conditions of this experiment. For this reason, plants grown in this complete nutrient solution will be regarded as control plants for the purpose of comparing with other nutrient series.

Effects of nematode infection with respect to each individual nutrient treatment can be illustrated as follows:

1. Nitrogen treatment. Two tests were carried out with nitrogen

TABLE III. Analysis of variance of the effects of different nutritional levels and amounts of nematode inoculum on fresh weights of lima bean plants.

Source of variation	D.F.	Sum of squares	Variance	F value
Nematode treatment	2	20,548.36	10,274.18	320.57**
Nutrient treatment	6	486,809.26	81,134.88	2531.5**
Nematode X nutrient interaction	12	9,352.16	779.35	24.32**
Error	63	2,018.97	32.05	....
Total	83	518,738.75	....	....

**Significant at the 1% point.

nutrition. The first had three levels of nitrogen representing deficient, optimum and excessive levels. Plants at the deficient level made significantly less growth and were very yellow and stunted in appearance. Nematode inoculum produced a marked effect on plants subjected to a deficient supply of nitrogen. While the 10 egg masses inoculum level slightly reduced fresh weights of the plants, the 50 egg masses inoculum level had a highly significant effect in decreasing their fresh weights. With the excessive nitrogen treatment, both levels of inoculum exerted a highly significant decrease in fresh weights. Data also indicate that plants which received excessive amounts of nitrogen and were inoculated with few egg masses did not differ significantly in their fresh weights from plants which received complete nutrient solution. However, with an inoculum of 50 egg masses, fresh weights of plants which received excessive amounts were greatly reduced as

TABLE IV. Fresh weights of lima bean plants grown in different nutrient solutions and inoculated with three levels of nematode egg masses.

Nutrient treatments	Fresh weights* of plants under three levels of nematode egg masses inoculum		
	0	10	50
Complete nutrient sol.	197.7	177.7	145.7
Deficient nitrogen sol.	48.2	40.6	24.9
Excessive nitrogen sol.	201.5	179.2	125.8
Deficient phosphorous sol.	11.8	11.2	10.5
Excessive phosphorous sol.	201.4	177.3	143.7
Deficient potassium sol.	53.9	27.8	9.4
Excessive potassium sol.	200.3	203.1	189.7

Least significant difference

0.05 .... 8.0

0.01 .... 10.6

*Basic figures represent average fresh weights in grams of four replications.

compared to fresh weights of plants which received the complete nutrient solution. Such findings indicate that deficiency of nitrogen as well as an excessive supply of this element had a marked influence in increasing susceptibility of lima bean plants to nematode infection.

In the second test of this nitrogen experiment series, a comparison of nitrogen as ammonia and as nitrate in relation to nematode infection was studied. Table V shows response of the host plant under two nitrogen sources when subjected to nematode infection. Studies on the rate of nematode reproduction as influenced by the nitrogen ion are also shownin Table V. Data reveal that differences in fresh weights between the uninoculated plants supplied with NO_3 ions and those supplied with NH_4 ions were not significant. On the other hand, there were significant differences in the case of inoculated plants. The difference between NO_3 and NH_4 sources of nitrogen was highly significant when plants were inoculated with 200 egg masses. The number of mature females as well as the number of egg masses produced per gram of root was found to be greater in plants which received nitrate nitrogen as compared to those which received ammonia nitrogen. Root gall index was also found to be higher in the nitrate treatment than in the ammonia treatment. Despite the differences in number of females and egg masses produced under the two treatments, rates of nematode reproduction were equal in both NO_3 and NH_4 solutions indicating that at the nutrient concentration used, neither form of nitrogen had any effect on this phase of nematode development. The decrease in number of females as well as number of egg masses obtained under the ammonia nitrogen treatment was possibly due to the inhibitory effect of the NH_4 ions on hatching of eggs used for inoculum. Once

TABLE V. Effects of nitrogen ions on growth of lima beans and rate of reproduction of *Meloidogyne incognita*.

Types of nitrogen ions	Unit of inoculum (egg mass)	Root gall index	Plant fresh weight (gm.)	No. females per gram root	No. egg masses per gram root	Rate of reproduction*
	0	0	207.1	0	0	0
NO_3	50	2.8	160.4	201	94	0.468
	200	4.0	104.7	468	211	0.450
	0	0	200.3	0	0	0
NH_4	50	2.0	177.8	149	68	0.456
	200	3.0	140.4	236	112	0.474
Least significant difference						
0.05			17.9	55.7	14.8	0.119
0.01			24.6	78.2	20.8	0.167

*Number of egg masses divided by number of mature females.

nematodes were able to hatch and infect the host plant they apparently were able to develop normally within the host tissue.

2. Phosphorous treatment. Phosphorous deficient plants made greatly reduced growth when compared to plants growing on other nutrient treatments. Nematode inoculum had no apparent effect on fresh weights of plants suffering from lack of this element. However, when phosphorous was supplied at an excessive level, nematode inoculum showed a marked effect in reducing fresh weight of plants. No significant difference was obtained between the excessive phosphorous treatment and the complete nutrient treatment under the three levels of nematode inoculum.

3. Potassium treatment. Under deficient potassium treatment, both levels of nematode inoculum caused highly significant reductions in plant growth. On the other hand, under excessive potassium treatment nematodes produced only a slight response. Apparently the excessive amount of potassium exerted a beneficial effect upon the plants and enable them to make good growth despite nematode infection. From the data in Table IV, it is evident that 10 egg masses inoculum had no significant effect on plants supplied with excessive potassium. With 50 egg masses inoculum, growth of the plants was reduced to some extent but they were able to maintain growth comparable to that of uninoculated plants supplied with complete nutrient solution.

Results of this experiment reveal that with deficient nutrient treatments, degree of plant response to nematode infection was greater under deficient potassium, deficient nitrogen and deficient phosphorous treatments respectively. On the other hand, with excessive nutrient treatments, severity of infection was greater under excessive nitrogen, somewhat less with excessive phosphorous, and least at the excessive potassium treatment. It is evident then, that nematode injury was greatest under potassium deficiency and least when potassium was abundant. The striking effect of potassium nutrition of the host in relation to infection by this nematode, indicated the desirability to further investigate this phenomenon.

Potassium Experiment Series

Effects of Potassium Host Nutrition and Nematode Inoculum on Plant Growth

From the previous experiments on host nutrition it was obvious that deficient and excessive nutrients supplied to host plants affected the rate and degree of disease development incited by a root-knot nematode. Studies of potassium host nutrition and amount of nematode inoculum were carried out to determine their effects on the degree of visible symptoms and rate of growth of lima bean plants.

Foliage symptoms. Foliage symptoms resulting from deficient, optimum and excessive potassium treatments combined with nematode infection are shown in Figures 2, 3, and 4. Two weeks after planting, symptoms of potassium deficiency in treatment K_1 were beginning to appear. Plants were very stunted, dark green in color and older leaves showed yellow-green circular areas. Potassium deficient plants inoculated with 50 egg masses as well as 200 egg masses did not differ in their degree of symptoms. They were both very stunted in appearance; older leaves showed signs of scorching then became yellowish and finally fell off. Nearly all mature leaves were lost, but the plants continued to grow and formed new leaves. Pods in general were small in size and exhibited chlorotic areas which became brown and dry (Fig. 5). Plants treated with K_2 solution appeared healthy and showed a normal green color. In this treatment, plants inoculated with 50 egg masses were reduced in size and their lower leaves became gradually yellow and finally dropped. Those inoculated with 200 egg masses were stunted and older leaves died prematurely. Pods also showed chlorotic dry areas. With treatment K_3, although the plants appeared very similar to those in treatment K_2, they were somewhat taller. Inoculum of 50 egg masses did not appear to have an effect on plants receiving an excessive potassium supply and those inoculated with 200 egg masses made good growth despite the higher inoculum level. However, some basal leaves became yellow and later dropped. No evidence of chlorotic, dry areas was observed on the pods.

Growth response. As measures of total growth, the fresh weight per plant and total dry weight per plant were used. These data as well as those for percentage dry matter for the whole plants are shown in Table VI. Data reveal that growth of uninoculated plants increased as the potassium concentration increased from K_1 to K_2 while treatment K_3 did not cause a significant increase in either fresh weight or dry weight as compared to treatment K_2. Growth of inoculated plants was significantly reduced as compared to uninoculated plants when the level of potassium nutrition was similar but growth of inoculated plants increased significantly when potassium supply was increased from K_1 to K_2 to K_3. From dry weight data it is also evident that plants infected with 50 egg masses and supplied with excessive potassium made

TABLE VI. Total fresh weight, total dry weight and percentage dry matter of lima bean plants grown at various potassium levels and inoculated with *Meloidogyne incognita*.

Treatment	Total fresh weight (gm. per plant)	Total dry weight (gm. per plant)	Percentage dry matter
K_1**	113.2*	22.6	19.9
K_2	203.3	38.6	19.0
K_3	208.5	39.5	19.0
50*** EMK_1	99.5	19.9	20.0
50 EMK_2	147.4	28.0	19.0
50 EMK_3	180.5	36.1	20.0
200 EMK_1	89.1	16.0	19.0
200 EMK_2	100.9	19.1	19.0
200 EMK_3	113.0	22.5	20.0
Least significant difference			
0.05	9.67	1.82	n.s.
0.01	13.06	2.46	n.s.

*Figures represent average weights of four plants.

**Three levels of potassium nutrition.

***EM = Egg masses.

growth almost equal to the plants in treatment K_2. Percentage dry matter for the whole plants did not differ significantly among treatments. Figure 6 shows growth response of the plants on a dry weight basis under conditions of this experiment.

Effects of Potassium Host Nutrition and Nematode Inoculum on Mineral Content of the Host Plant

Chlorosis as a result of nematode infection of plants is now a matter of common knowledge, but the nature of this symptom is not well understood. Since it seems likely that such chlorosis could be due to mineral deficiency the present study was carried out in an attempt to ascertain the type of such deficiency under controlled plant nutritional conditions. The total amounts of certain mineral elements of uninoculated and inoculated plants are recorded in Table VII. In general uninoculated plants contained a higher amount of these elements than infected plants. In the growth data (Table VI) it is shown that between treatments K_2 and K_3 there were no significant differences in total dry weight or fresh weight. A similar situation as far as the amount of minerals contained in the previous two treatments also existed, except that potassium content in treatment K_3 was significantly higher. This condition indicates what is known as "luxury absorption" when the content of potassium in the tissue increases without increase in growth. On the other hand, the amount of mineral elements of infected plants usually increased when potassium concentrations of the nutrient solution increased. Data indicate that infected plants with a relatively moderate amount of inoculum (50 egg masses) and supplied with excessive potassium maintained mineral levels comparable with that of control plants in treatment K_2. However, with a heavy amount of inoculum (200 egg masses)

TABLE VII. Mineral contents of lima bean plants grown in nutrient solution with variations in potassium concentrations and nematode inoculum levels.

Treatment	Grams per plant*				
	K	Ca	Mg	N**	P***
K_1	0.091	2.07	0.330	0.617	0.321
K_2	1.118	2.87	0.600	0.881	0.365
K_3	1.733	2.91	0.602	0.925	0.392
50 EMK_1	0.065	1.82	0.283	0.415	0.214
50 EMK_2	1.039	2.25	0.463	0.540	0.222
50 EMK_3	1.250	2.67	0.639	0.752	0.311
200 EMK_1	0.054	1.22	0.224	0.280	0.125
200 EMK_2	0.586	1.32	0.250	0.381	0.133
200 EMK_3	0.725	1.93	0.432	0.652	0.223
Least significant difference					
0.05	0.052	0.26	0.048	0.050	0.028
0.01	0.071	0.35	0.065	0.070	0.038

*Based on total grams dry weight as shown in Table VI.

**As total nitrogen.

***As P_2O_5.

the excessive amount of potassium supplied apparently was not enough to maintain a proper mineral level as compared to control plants.

Differences in absorption of certain mineral elements in the infected plants when potassium concentration of the nutrient solution was increased from optimum to excessive levels are shown in Table VIII and are illustrated in Figure 7. It will be noted that as the number of egg masses used for inoculum was increased these differences became progressively less for potassium and greater for the other elements.

Rate of Nematode Reproduction as Affected by Potassium Nutrition of the Host Plant

Little or no information is available regarding influence of nutrition on reproduction of parasitic nematodes. The present study was undertaken to investigate the effect of various concentrations of potassium on the production of females and egg masses of the root-knot nematode, *Meloidogyne incognita*. Results of this experiment are given in Table IX. Examination of the data reveals that the average increase in number of females and egg masses was significant with each increase in concentration of potassium. The mean effect of increase of inoculum increment was likewise highly significant in increasing the number of females and egg masses produced. Chitwood (4) has shown that with certain root-attacking nematodes the mean number of females and the mean number of egg masses produced per unit of inoculum were inversely proportional to the amount of inoculum. The present results tend to confirm this. For each of the optimum and excessive potassium levels it was found that an increase of egg masses in the inoculum usually resulted in a corresponding significant decrease in number of mature females as well as a decrease in number of egg masses produced on the test plants per

TABLE VIII. Differences in absorption of certain mineral elements by plants infected with <u>Meloidogyne incognita</u> when potassium supply of the host increased from optimum to excessive levels.

Units of nematode inoculum (egg masses)	Difference in absorption* of mineral elements between treatments K_3 and K_2				
	K	Ca	Mg	N	P
0	0.615	0.004	0.002	0.044	0.027
50	0.211	0.042	0.172	0.212	0.089
200	0.139	0.061	0.180	0.271	0.090

*Grams per plant.

unit of inoculum. However, in the case of deficient potassium level this relationship was only slightly evident. This indicates that the number of egg masses produced per unit of inoculum may be influenced by the amount of potassium available.

Reproductive activities of the nematodes may be limited more by availability of potassium than by amount of root space available to the nematode. When potassium was low an average of five egg masses was produced per unit of inoculum and the mean root weight per plant was 12 gm. When the level of potassium was optimum the mean number of egg masses produced per unit of inoculum was 30 or six times as great as that of the low potsssium level, while the average root weight was 27 gm. or only slightly more than double the root weight for the low potassium level. When potassium level was excessive the mean number of egg masses produced per unit of inoculum was 32 and the root weight was 21 gm., indicating that an increase in potassium from optimum to excessive had little effect on the corresponding root weight and number

TABLE IX. Relationship of potassium nutrition and amount of inoculum to rate of reproduction of *Meloidogyne incognita* in infected roots of lima bean plants.

Potassium level	Units of inoculum (egg masses)	Mean root-gall index	Mean root weight (gm.)	Mean no. females per gm. root	Mean no. females per plant	Mean no. females per unit* inoculum	Mean no. egg masses per gm. root	Mean no. egg masses per plant	Mean no. egg masses per unit** inoculum	Mean rate*** of reproduction
K_1	50	3.2	9.2	117	1084	22	20	200	4	0.168
	200	3.8	15.0	246	3675	18	83	1229	6	0.334
K_2	50	2.7	32.5	126	4079	82	54	1665	32	0.428
	200	4.0	23.1	346	8034	40	196	4564	28	0.568
K_3	50	3.0	21.2	158	3330	67	108	2281	45	0.688
	200	4.0	21.1	361	7668	39	186	3964	20	0.514
Least significant difference										
.05			5.8	16.9	589.0	10.6	13.7	305.8	5.7	0.173
.01			7.8	23.2	807.8	14.6	18.8	419.3	7.9	0.236

*Females per plant divided by egg masses in inoculum.

**Egg masses per plant divided by egg masses in inoculum.

***Number of egg masses divided by number of mature females.

of egg masses produced per unit of inoculum. Thus while root weight increases by a factor of two from K_1 to K_2 and K_3 levels the number of egg masses produced per unit of inoculum increases by the factor of six. These data indicate that the maximum number of females which the roots supported under the optimum and excessive potassium levels of this experiment was in the vicinity of 350 females per gram of root. When roots were infected to that extent the rate of reproduction was about 0.5. Plants inoculated with 200 egg masses and given the excessive level of potassium did not support a greater number of females and egg masses than such plants receiving the optimum level of potassium. Similar results were evident in the comparison of root gall indices of these plants. Such failure was attributed to the effects of overcrowding. When roots were not heavily infected, as in the case of plants receiving 50 units of inoculum, the number of egg masses per gram of root increased with the increase in potassium level. Rate of reproduction also increased significantly as the levels of potassium increased. Among plants receiving a relatively low amount of inoculum, rates of reproduction were apparently limited by the amount of potassium available, as indicated by the highly significant differences between treatments. On the other hand, with higher amounts of nematode inoculum an increase in potassium correspondingly increased the rate of reproduction up to a certain point between that produced by the K_2 and K_3 levels. Thus it seems that rates of reproduction of nematodes among plants receiving higher inoculum and treated with higher potassium concentrations are correlated with the amount of root available and with competition between nematodes for root space rather than with the amount of potassium available.

Development Time of the Nematode within Root Tissue of the Host Plant as Affected by Variations in the Amount of Potassium Supplied to Host

Host-parasite relationships of *M. incognita* were determined primarily by effects of the parasite on the host and effects of the host on the parasite. Effects of the host on the parasite become manifest in various ways, e.g., by the rate at which the nematodes develop, by the extent to which they develop, by the percentage of females that reach maturity and by the number of eggs deposited by these females. However, host-parasite interactions cannot be separated from the interplay of the environmental factors upon them. Temperature in this respect seems to be the most important factor. On the other hand, plant nutrition, as a major environmental factor, has not been studied extensively but it is one which deserves much more consideration. For this reason, an experiment to determine influence of various levels of potassium host nutrition on development of a root-knot nematode was studied. Results are shown in Table X. Development of the parasite was slower in lima bean plants receiving a deficient potassium nutrition level than in plants receiving either optimum or excessive potassium levels. While the period that elapsed from the time of inoculation until the parasites developed through stages A, B and C (Figs. 11, 12, and 13) was approximately identical in the three nutrient treatments, the period required for further development was considerably influenced by nutrition of the host plant. Nematodes reached full growth (stage D) after 18, 16, and 12 days from the time of inoculation under deficient, optimum and excessive potassium treatments respectively. The period required for egg deposition was also considerably affected by variations in the levels of potassium supplied to host. In this respect the time

TABLE X. Development time of the root-knot nematode, *Meloidogyne incognita*, in lima bean plants supplied with deficient, optimum and excessive potassium nutrient levels.

Number of days after inoculation	Number of parasites in each group														
	Deficient K					Optimum K					Excessive K				
	A	B	C	D	E*	A	B	C	D	E	A	B	C	D	E
4	100	0	0	0	0	100	0	0	0	0	100	0	0	0	0
6	97	3	0	0	0	93	7	0	0	0	87	13	0	0	0
8	83	17	0	0	0	82	18	0	0	0	77	23	0	0	0
10	77	23	0	0	0	35	45	0	0	0	35	52	13	0	0
12	66	20	4	0	0	17	75	8	0	0	7	68	15	10	0
14	41	47	12	0	0	15	63	22	0	0	3	41	40	16	0
16	29	47	24	0	0	11	31	52	6	0	0	22	24	23	31
18	16	42	38	4	0	8	21	64	7	0	0	0	39	17	44
20	16	33	52	9	0	5	16	66	13	0	0	0	3	27	70
22	12	19	55	14	0	0	12	69	19	0	0	0	0	6	94
24	9	21	50	19	0	0	5	47	28	20					
26	4	13	45	28	0	0	0	17	35	48					
28	1	19	54	27	0	0	0	3	13	84					
30	0	9	48	33	0	0	0	0	8	92					
32	0	5	65	30	0	0	0	0	2	98					
34	0	2	54	42	0										
36	0	0	49	61	0										
38	0	0	32	68	0										
40	0	0	28	70	2										
42	0	0	28	67	5										
44	0	0	19	70	11										
46	0	0	11	54	35										
48	0	0	9	55	46										
50	0	0	5	44	51										
52	0	0	4	37	59										
54	0	0	0	12	88										

*Letters refer to developmental stages of the nematode as illustrated in Figures 11, 12, 13, 14, and 15.

that elapsed from stage D in which the females are fully grown (Fig. 14) to stage E in which eggs are deposited (Fig. 15), was 22, 8 and 4 days under deficient, optimum and excessive levels of potassium respectively.

It is evident from the preceeding data that the number of parasites which reached each stage of development was greater in plants receiving excessive and optimum potassium treatments than the number in plants receiving the deficient potassium treatment. However, no differences were noticed in the sizes of the parasites under these conditions. Table XI is a condensed comparison of lengths of time required for developmental stages of the nematode in lima bean plants supplied with three different levels of potassium concentrations. It is evident that the greatest difference between the three nutritional treatments lies in their influence on length of time required for development from the mature female (stage D) to egg-laying (stage E). The first eggs were not observed until about 40 days from the time of inoculation in deficient potassium series, 24 days in the optimum potassium series, and 16 days in the excessive potassium series. Such findings indicate that potassium host nutrition is of considerable importance in determining development time of this root-knot nematode.

TABLE XI. Comparative development times of the root-knot nematode, _Meloidogyne incognita_, as influenced by variations in the amount of potassium supplied to the host plant.

Stages of development	Period of development in days		
	Deficient K	Optimum K	Excessive K
From stage A to stage B	6	6	6
From stage B to stage C	6	6	4
From stage C to stage D	6	4	2
From stage D to stage E	22	8	4
Egg production from time of inoculation			
First females depositing eggs	40	24	16
50% of females depositing eggs	50	26	20
85% of females depositing eggs	54	30	22

DISCUSSION

The subject of plant nutrition in relation to diseases incited by nematodes has received little attention but is one which deserves much more consideration. Root-knot nematodes, Meloidogyne spp. are obligate parasites and their mode of parasitism requires a living tissue to secure an adequate food supply in order to complete their life cycle. The nature of substances necessary for their growth and development are not known since it has not been possible to cultivate them artificially on nutrient media. However, their behavior in this respect can be observed as influenced by nutrition of their hosts. Since field fertilizer experiments are subjected to many microclimatic factors, it is difficult therefore to distinguish between such factors and the nutritional effects on the course of disease development. By growing infected plants in nutrient solutions, an approach to basic nutrient requirements of the host as well as the pathogen was provided.

The information presented in these studies indicates clearly that the types and amounts of nutrients supplied to host plants are important factors in predisposing plants to nematode injury and in determining the degree of disease development. There was a wide difference in the degree of susceptibility of plants to nematode infection in the deficient nitrogen, phosphorous and potassium series even though all of the plants showed reduced vigor. Nematode injury was pronounced particularly in the potassium deficient plants. In contrast to these, it was noticed that enhanced growth which resulted from an extra supply of nitrogen also promoted development of root knot. This is probably due to the fact that oversupply of nitrogen will result in feeding the parasites on more succulent roots so they are well nourished and reproduce better. In this connection a question was raised as to whether nitrogen as ammonia or as nitrate might affect the course of disease development. In general ammonia decreased the amount of nematode injury as well as the number of females and egg masses produced on infected roots. The rate of nematode reproduction, however, was equal in both nitrate and ammonia treatments indicating that development of the parasite within root tissues was independent of the two types of nitrogen ions. Excessive application of phosphorous showed no beneficial effect.

Potassium apparently plays a very important role in the response of lima bean plants to infection by Meloidogyne incognita. Infected plants were found to be lower in nitrogen, phosphorous, calcium and magnesium and considerably lower in potassium when compared to healthy plants. When potassium was supplied at an excessive level, infected plants made almost normal growth and maintained mineral levels comparable to uninoculated plants. Rate of mineral absorption was affected by presence of nematodes in the roots. Differences in relative absorption of these elements when potassium supply of the host was increased from an optimum to an excessive level reveal that when there was no nematode infection potassium absorption increased while absorption of the other elements did not significantly increase. This condition which is known as "luxury absorption" occurs when the amount of potassium in the tissue increases without increase in total growth. This condition was altered when nematodes were introduced to plants, as an increase in nematodes inoculum correspondingly decreased potassium absorption while the absorption of other elements was relatively increased.

Since *Meloidogyne incognita* has not been grown in pure culture it is not easy to determine whether potassium affected the parasite directly, but in view of the host chemical analysis and the fact that potassium deficiency markedly increased nematode injury, it may be possible to postulate that this element is necessary for growth and development of the parasite as well as for the host.

It has been reported that development of root-knot nematodes is influenced mainly by temperature and type of host plant. Upon the basis of the data obtained in the present investigation it was possible to demonstrate that the nutritional status of the host also plays an important role in influencing the length of nematode life cycle. The life cycle is usually considered to be the time that has elapsed from a newly deposited egg through maturity of the adult to the first deposition of eggs. But as is well known larvae of root-knot nematodes are capable of remaining in soil in their second stage for an indefinite period, and do not develop further until they enter a root. The actual length of life cycle, therefore, may vary from a few days to several months under field conditions. It is of more practical significance to consider the time from entry of the root to egg production. This might be called the "development time" of root-knot nematodes. In this respect, potassium nutrition of the host showed that the length of time required for development of this nematode from the fully grown female stage to the egg-laying stage and the time required for egg deposition decreased as amount of potassium increased. The rate of nematode reproduction was correspondingly increased when the potassium supply increased. Increased injury will result when the time required for nematode development increases since the parasite will continue to feed to secure its necessary supply of potassium from the host tissue. Thus it seems likely that plants deficient in potassium may be more severely injured by root-knot than plants growing on higher levels of this element.

The beneficial effect of potassium fertilizers is still in dispute. It has been claimed by Bessey (2) that potassium may increase the resisting power of the plant against the entrance of the nematode into the roots. According to the present findings, nematodes were able to enter plants regardless of the nutritional status. As a matter of fact they were able to increase in number and reproduce faster under high potassium levels. Thus, heavy application of potassium is likely to promote the building up and maintenance of nematode populations. At the same time, such excessive application protects the plant to some extent from nematode injury. A possible explanation to this condition, is that a considerable amount of this element is required for nutrition and egg production of this nematode, as well as being an important mineral element for growth of the host plant.

It seems apparent that presence of nematodes affect nutrition of the host plant. These effects may be due to the fact that: (1) Root systems of plants infected by root-knot nematodes are much reduced and therefore absorb nutrients from a smaller volume of soil than the roots of healthy plants. (2) The galls produced by nematodes involve vascular tissue of the roots and thus interfere with translocation of nutrients. (3) Because of the interference of the nematodes with the root system the metabolic activities of the plants could conceivably be altered. (4) Possibly the differential absorption of potassium shown by the plants was due in some degree to the use of this element by nematodes for their nutrition and egg production. As a result any marked disturbance of host metabolism by mineral deficiency might affect the relationship with the parasite in such a way as to increase the competition for elaborated food and perhaps for mineral nutrients. Explanation then of the disturbing changes in plant nutrition as a result of nematode infection can result in much controversy. This subject of plant nutrition in relation to nematode diseases is much too embryonic to permit generalizations. Until some time when complete nutrition of the plant and the nematode are both better understood no single interrelationship can be fully understood.

SUMMARY

Henderson lima bean plants were grown in sand culture inoculated with the root-knot nematode, Meloidogyne incognita. Effects of a complete nutrient solution, deficient solutions of nitrogen, phosphorous and potassium as well as excessive solutions of these three elements were studied. Results showed a wide difference in the degree of susceptibility of plants to nematode infection in the deficient nitrogen, phosphorous and potassium series even though all the plants showed reduced vigor. Nematode injury was pronounced particularly in plants deficient in potassium. Excessive nitrogen promoted development of root-knot symptoms. Excessive phosphorous showed no effect. Excessive potassium apparently plays an important role in the response of lima bean plants to nematode infection. Chemical analysis showed that infected plants had lower N, P, Ca, Mg, and considerably lower K contents as compared to healthy plants. When potassium was supplied in an excessive level infected plants made almost normal growth and maintained mineral levels comparable to control plants. Studies on the effect of potassium nutrition of the host on development of the parasite indicate that the length of time elapsed from inoculation to egg deposition was 40, 24 and 16 days in the deficient, optimum and excessive potassium series respectively. Rate of nematode reproduction was also increased correspondingly with potassium supply. Thus it appears, that heavy application of potassium favors rapid development and reproduction of the nematode, and at the same time protects the plants to some extent from nematode injury. Thus it appears that a considerable

amount of potassium is required for nutrition and egg production of this nematode, as well as for the growth of the plant. Hence, potassium applications to plants growing in nematode infested soils would increase plant growth, provided the soil is deficient in this element. Unfortunately, such treatment will also serve to promote increase of the root-knot nematode population.

LITERATURE CITED

1. Association of Official Agricultural Chemists. 1940. Methods of analysis. Ed. 5. Washington, D. C.

2. Bessey, E. A. 1911. Root-knot and its control. U. S. Dept. Agr., Bur. Pl. Ind. Bul. 217.

3. Chitwood, B. G. 1949. Root-knot nematodes. Part 1. A revision of the genus *Meloidogyne* *Goeldi*, 1887. Proc. Helm. Soc. Wash. 16: 90-104.

4. Chitwood, B. G. 1951. Root-knot nematodes. II. Quantitative relations of the root-knot nematode, *Meloidogyne* *hapla* Chitwood, 1949 with tomatoes, onions and lima beans. Plant and Science 3: 47-50.

5. Chitwood, B. G., A. W. Specht, and L. Havis. 1952. Root-knot nematodes. III. Effects of *Meloidogyne* *incognita* and *M.* *javanica* on some peach rootstocks. Plant and Science 4: 77-95.

6. Christie, J. R. 1946. Host-parasite relationships of the root-knot nematode, *Heterodera* *marioni*. II. Some effects of the host on the parasite. Phytopathology 36: 340-352.

7. Christie, J. R. 1949. Host-parasite relationships of the root-knot nematodes, *Meloidogyne* spp. III. The nature of resistance in plants to root-knot. Proc. Helm. Soc. Wash. 16: 106-108.

8. Ellenby, C. 1942. Trace elements and potato sickness. Nature 149: 50.

9. Godfrey, G. H. 1926. Effects of temperature and moisture on nematode root-knot. Jour. Agr. Res. 33: 223-254.

10. Godfrey, G. H., and J. Oliveira. 1932. The development of the root-knot nematode in relation to root tissues of pineapple and cowpea. Phytopathology 22: 325-348.

11. Hoagland, D. R., and W. R. Snyder. 1933. Nutrition of the strawberry plant under controlled conditions. Proc. Amer. Soc. Hort. Sci. 30: 288-294.

12. Jones, L. H. 1932. The effect of environment on nematode of the tomato gall. Jour. Agr. Res. 44: 275-285.

13. Parris, G. K., and R. A. Jehle. 1943. Root-knot on lima beans in Maryland. U. S. Dept. Agr. Plant Dis. Reptr. 27: 235.

14. Ranker, E. R. 1927. Determination of total nitrogen in plants by the semi-micro Kjeldahl method. Jour. Assoc. Off. Agr. Chemists 10: 230-251.

15. Roemer, T. 1927. Handbuch des Zuckerrüben Baues. Berlin. 366 p.

16. Smith, A. L., and A. L. Taylor. 1947. Field methods of testing for root-knot infestation. Phytopathology 37: 85-93.

17. Spencer, E. L., and G. L. McNew. 1938. The influence of mineral nutrition on the reaction of sweet corn seedlings to _Phytomonas stewarti_. Phytopathology 28: ~~221~~ 213-223.

18. Tarjan, A. C. 1950. A consideration of mineral nutrition of boxwood in relation to infection by meadow nematodes, _Pratylenchus_ spp. Jour. Wash. Acad. Sci. 40: 157-160.

19. Thomas, H. R. 1948. Effect of nitrogen, phosphorous and potassium on susceptibility of tomatoes to _Alternaria solani_. Jour. Agr. Res. 76: 289-306.

20. Tyler, J. 1933. Reproduction without males in aseptic root cultures of the root-knot nematode. Hilgardia 7: 373-388.

21. Tyler, J. 1933. Development of the root-knot nematode as affected by temperature. Hilgardia 7: 392-415.

22. Vanha, L. 1893. Die Enchytraeiden als neue Feinde der Zuckerrüben, der Kartoffeln, und andere landwirtschaftlichen Kulturpflanzen. Zeitschr. Zuckerindustrie Böhmen. 17: 157-182.

23. Walker, J. C. 1946. Soil management and plant nutrition in relation to disease development. Soil Sci. 61: 47-54.

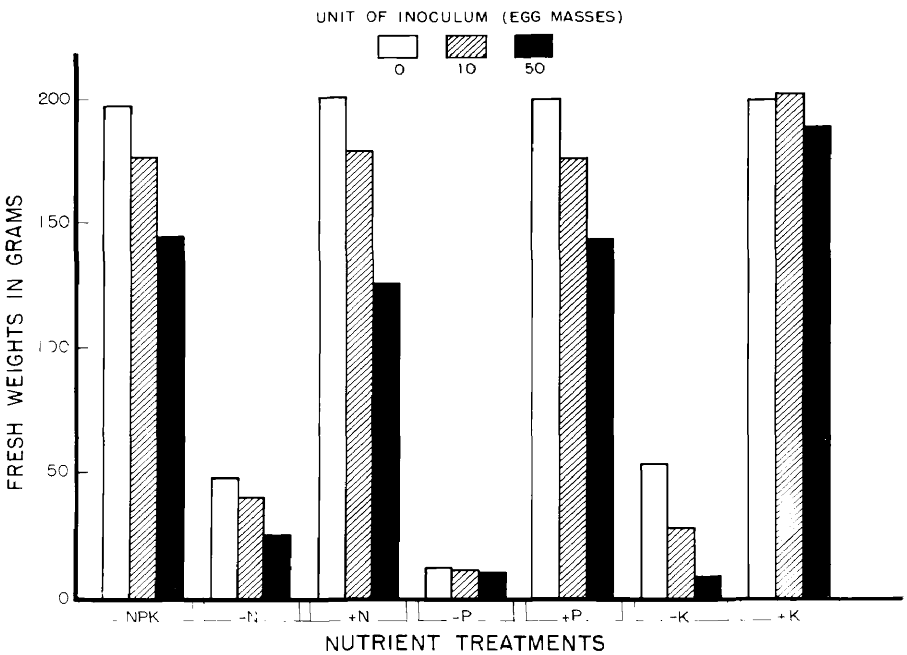
24. Wilfarth, H., and G. Wimmer. 1903. Untersuchungen über die Wirkung der Nematoden auf Ertrag und Zusammensetzung der Zuckerrüben. Ztschr. d. Ver. d. Deutsch. Zuckerindustrie. 53: 1-41.

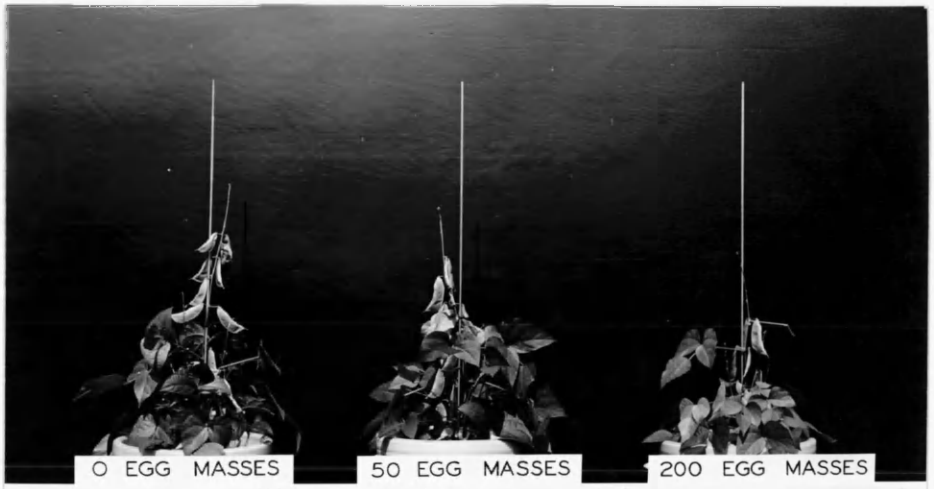
Figure 1. Effects of various levels of nutrient treatments on fresh weights of lima bean plants infected with *Meloidogyne incognita*.

Figure 2. Lima bean plants grown in a deficient potassium solution and inoculated with three levels of egg masses of *M. incognita*.

Figure 3. Lima bean plants grown in a complete nutrient solution and inoculated with three levels of egg masses of *M. incognita*.

Figure 4. Lima bean plants grown in an excessive potassium solution and inoculated with three levels of egg masses of *M. incognita*.


UNIT OF INOCULUM (EGG MASSES)
0
10
50
200
150
100
50
0
FRESH WEIGHTS IN GRAMS
NPK
-N
+N
-P
+P
-K
+K
NUTRIENT TREATMENTS



DEFICIENT K

COMPLETE

EXCESS K

Figure 5. Healthy pods of lima bean plants as compared to those exhibiting potassium deficiency as a result of heavy nematode inoculum. Notice the chlorotic, dry discoloration areas along the pods.

Figure 6. Effects of deficient, optimum and excessive potassium levels on dry weights of lima bean plants inoculated with three levels of egg masses of *M. incognita*. K_1= deficient potassium level; K_2= approximately optimum potassium level; K_3= excessive level of potassium.

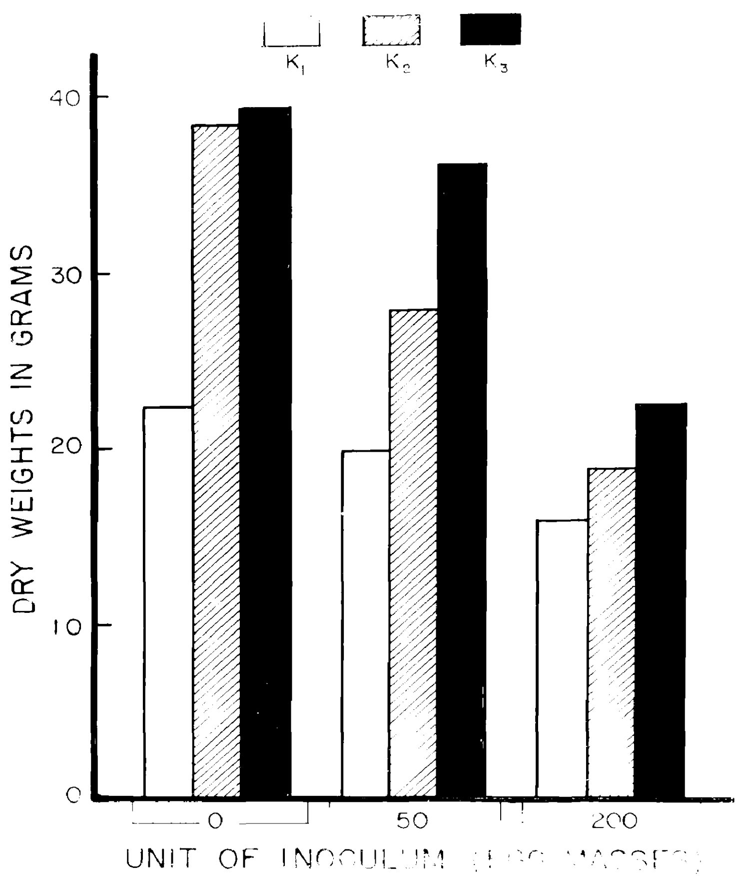

K1
K2
K3
DRY WEIGHTS IN GRAMS
0
10
20
30
40
0
50
200
UNIT OF INOCULUM

Figure 7. Differential absorption of certain mineral elements by lima bean plants infected with three levels of egg masses of *M. incognita* when potassium supply of the host increased from optimum to excessive levels.

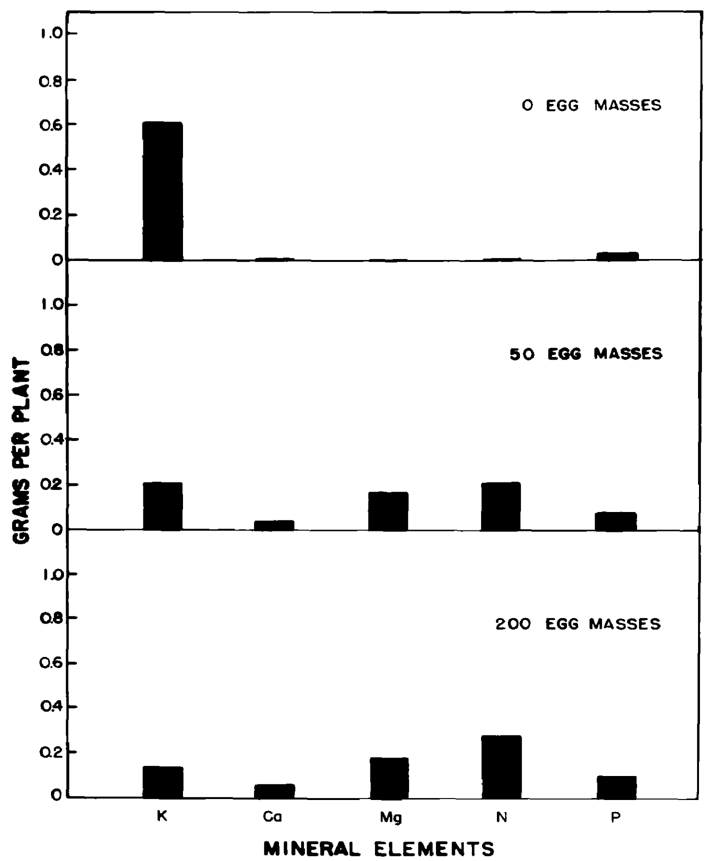

0 EGG MASSES
50 EGG MASSES
200 EGG MASSES
GRAMS PER PLANT
1.0
0.8
0.6
0.4
0.2
0
K
Ca
Mg
N
P
MINERAL ELEMENTS

Figure 8. Effect of potassium supply on the number of females per gram of root of infected lima bean plants under two units of inoculum.

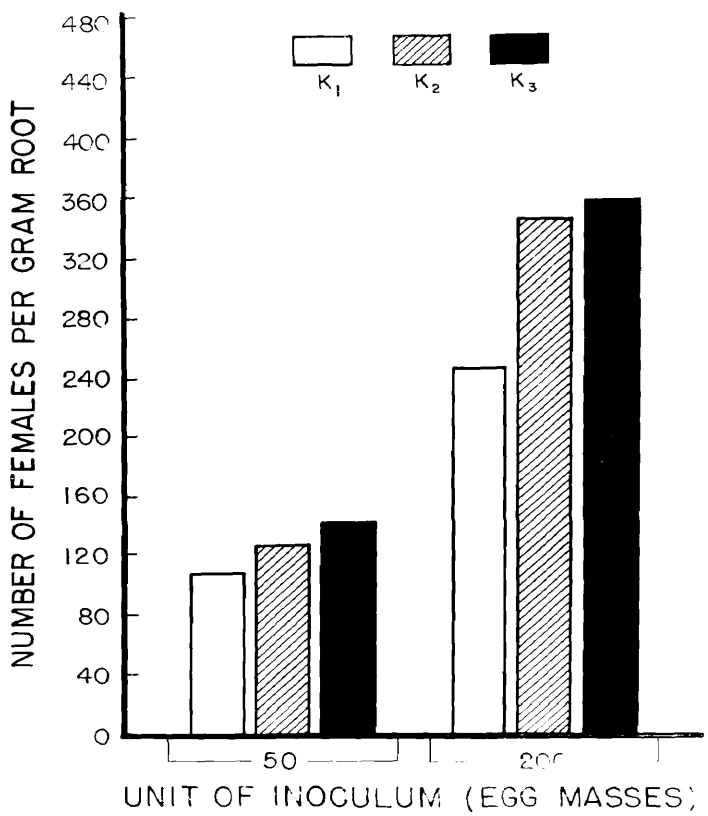

K1
K2
K3
NUMBER OF FEMALES PER GRAM ROOT
0
40
80
120
160
200
240
280
320
360
400
440
480
50
200
UNIT OF INOCULUM (EGG MASSES)

Figure 9. Effect of potassium supply on the number of egg masses per gram of root of infected lima bean plants under two units of inoculum.

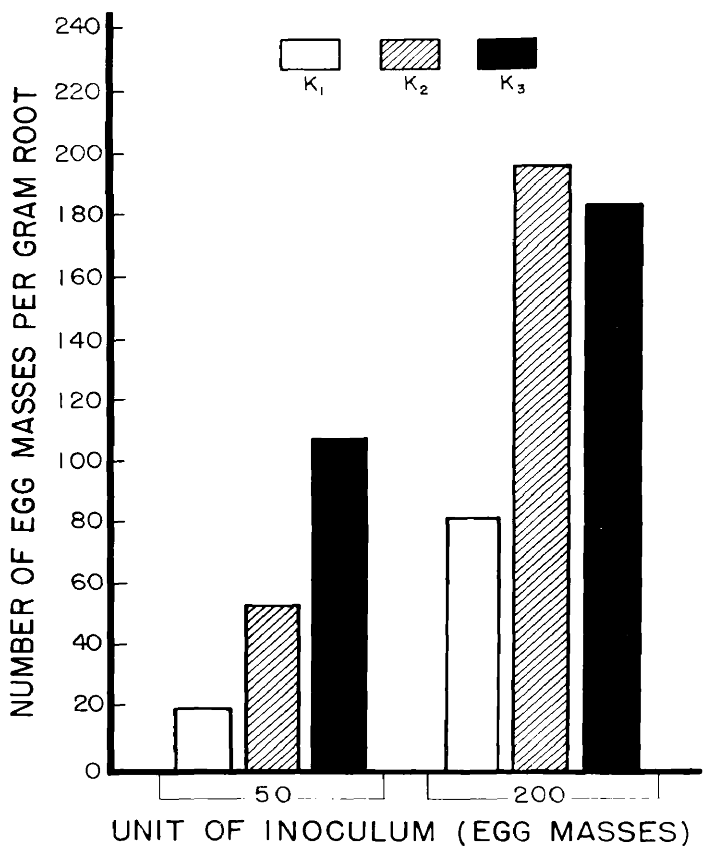

K1
K2
K3
240
220
200
180
160
140
120
100
80
60
40
20
0
NUMBER OF EGG MASSES PER GRAM ROOT
50
200
UNIT OF INOCULUM (EGG MASSES)

Figure 10. Effect of potassium supply on the rate of reproduction of the nematode *M. incognita*. Rate of reproduction is determined by dividing the number of egg masses by the number of mature females.

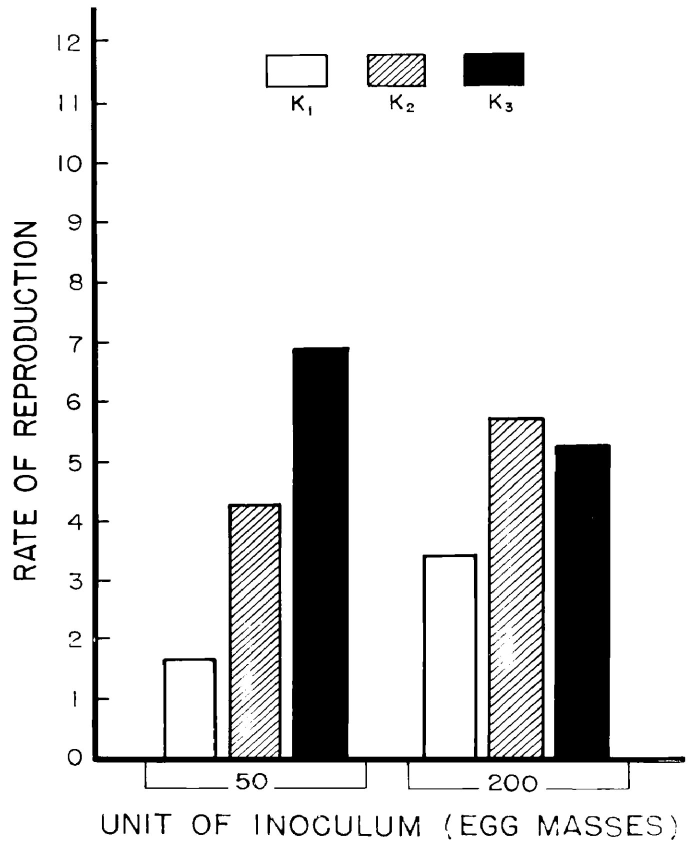

12
11
10
9
8
7
6
5
4
3
2
1
0
K1
K2
K3
RATE OF REPRODUCTION
50
200
UNIT OF INOCULUM (EGG MASSES)

Figure 11. Development of the root-knot nematode *M. incognita* within the root tissue of lima bean plants.

Stage A -- Includes larvae from the stage where they have begun to grow to the stage where they still possess a more or less conical tail.

Figure 12. Development of the root-knot nematode _M. incognita_ within the root tissue of lima bean plants.

Stage B -- Includes larvae from the stage where they have acquired a more or less hemispherical posterior end, terminated by a spike, to the stage where they are about to complete the final molts.

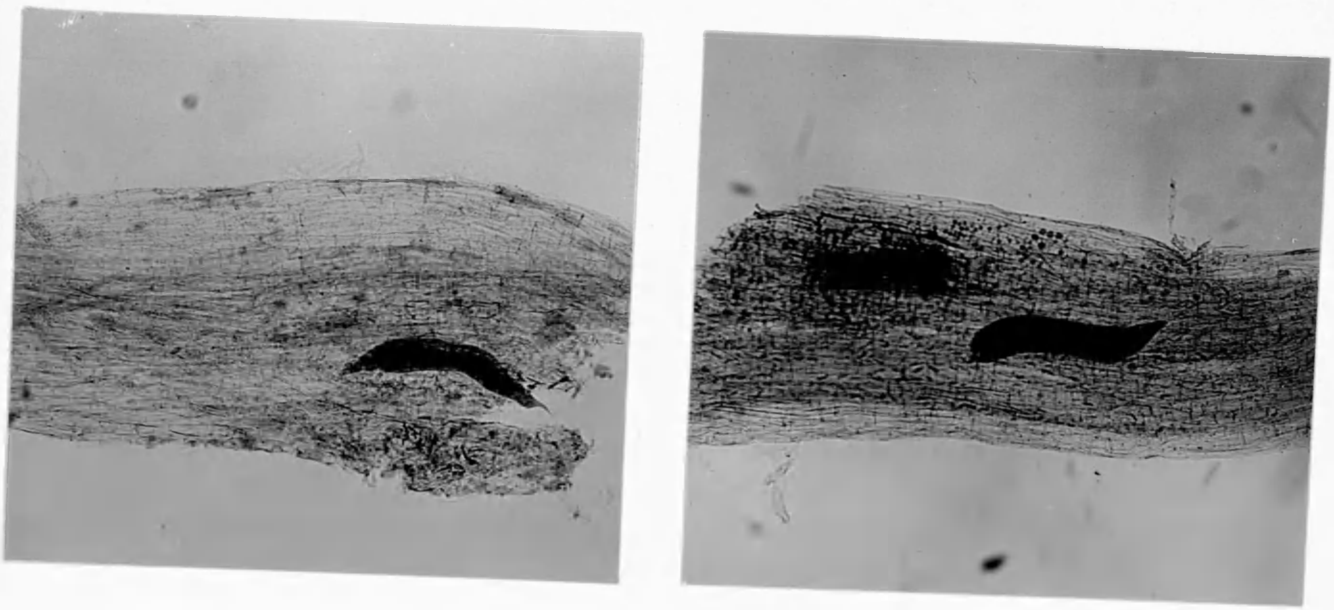
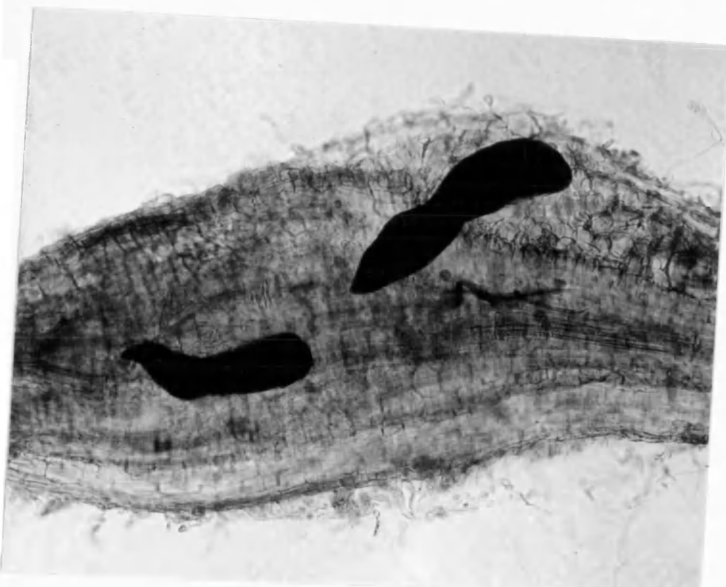


Figure 13. Development of the root-knot nematode _M. incognita_ within the root tissue of lima bean plants.

Stage C — Includes females from the stage where they have completed the molts to the stage where they are almost, though obviously not quite, fully grown.



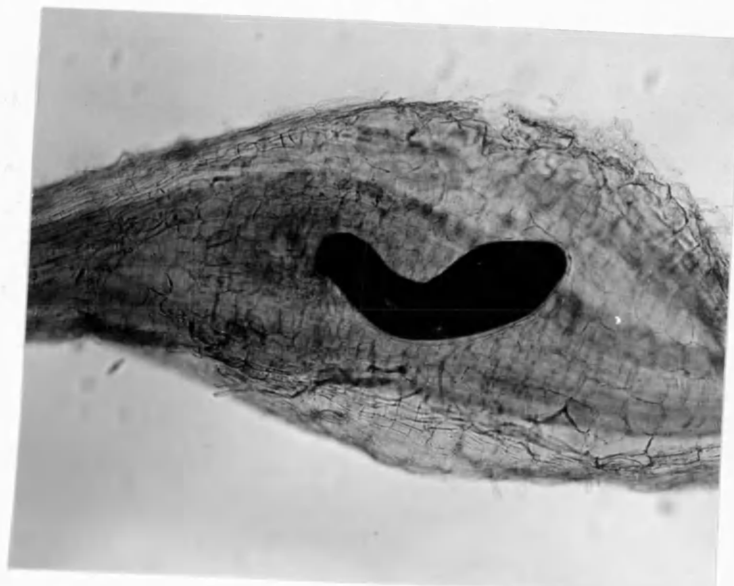


Figure 14. Development of the root-knot nematode *M. incognita* within the root tissue of lima bean plants.

Stage D -- Includes females that are fully grown or almost fully grown but have not yet laid eggs.

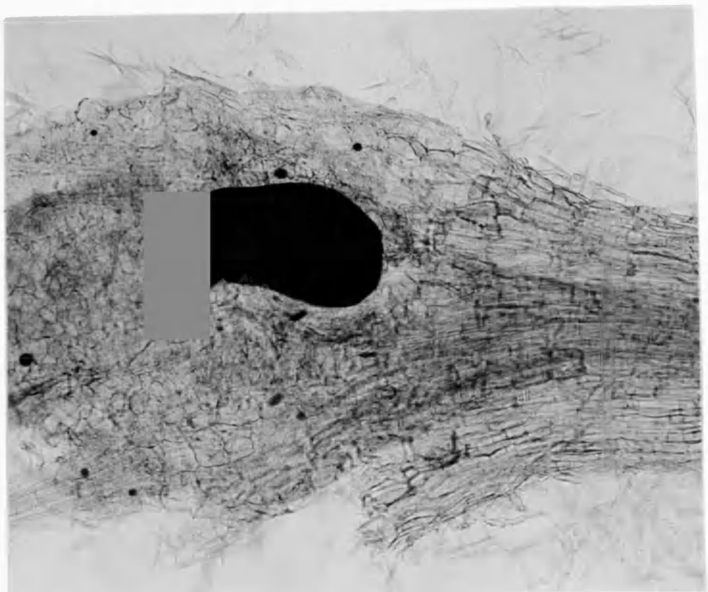
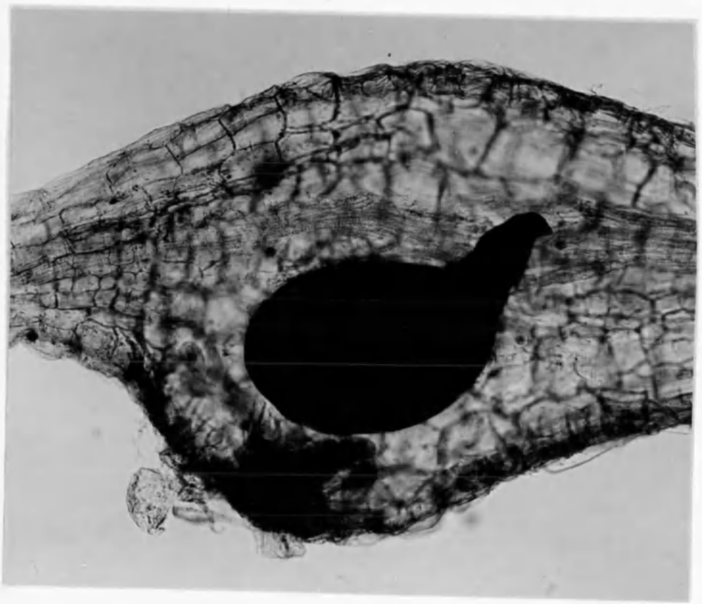
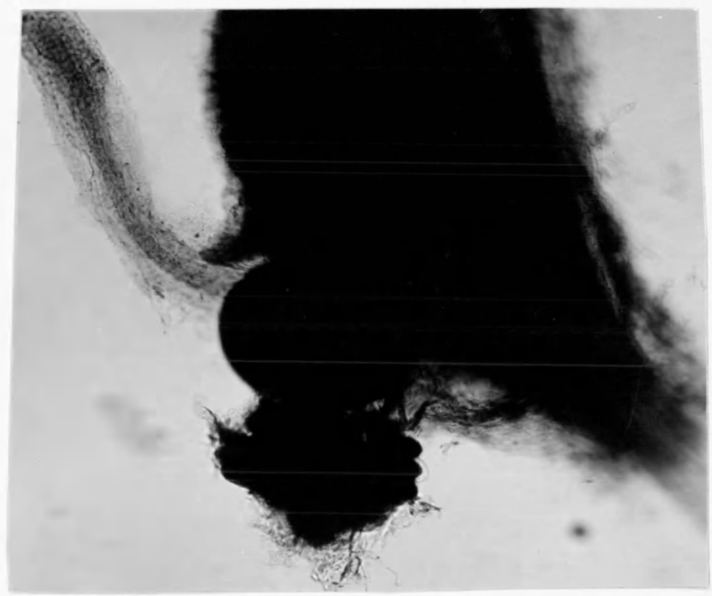


Figure 15. Development of the root-knot nematode *M. incognita* within the root tissue of lima bean plants.

Stage E -- Includes egg-laying females.





VITA

Name in full: Bakir Abbas Oteifa

Permanent address: Cairo, Egypt

Degree to be conferred: Doctor of Philosophy -- 1953

Date of birth: August 21, 1921

Place of birth: Tanta, Egypt

Secondary education: Tawfikia High School, Cairo, Egypt

Collegiate institutions attended:	Dates	Degrees	Date of Degree
Fouad 1st University Giza, Egypt	1941-45	B.S.	May 1945
Kansas State College	1948-49	M.S.	Aug. 1949

Publications:

1. 1951. Effects of potassium nutrition and amount of inoculum on rate of reproduction of Meloidogyne incognita (Kofoid and White, 1919) Chitwood, 1949. Jour. Wash. Acad. Sci. 41: 393-395.

2. 1952. Potassium nutrition of the host in relation to infection by a root-knot nematode, Meloidogyne incognita. Proc. Helm. Soc. Wash. 19: 99-104.

3. 1952. Nematodes parasitic on plants. Ann. Rev. Microbiology 6: 151-184. (Junior author with B. G. Chitwood)

4. 1953. Development of the root-knot nematode, Meloidogyne incognita as affected by host nutrition. Phytopathology (in press).

Positions held:

1. September 1945 to August 1948 -- Instructor in Agriculture Zoology, College of Agriculture, Fouad 1st University, Giza, Egypt.

2. September 1948 to August 1949 -- Egyptian Government Official Student, Kansas State College.

3. September 1949 to present time -- Egyptian Government Official Student, University of Maryland.

4. June 1952 to present time -- Fellowship in Mycology Division, American Type Culture Collection, Washington, D. C.