THE PLANT-PARASITIC NEMATODES ASSOCIATED WITH CULTIVATED BLUEBERRIES IN MICHIGAN

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ABSTRACT

THE PLANT-PARASITIC NEMATODES ASSOCIATED WITH CULTIVATED BLUEBERRIES IN MICHIGAN

by James Peter Tjepkema

Two hundred and seventy soil samples from 30 blueberry farms were processed to determine the frequency of occurrence of plant-parasitic nematodes in Michigan cultivated blueberries. Soil from beneath blueberry bushes with shoestring and necrotic ringspot virus symptoms was processed to learn if any nematode was associated with the spread of these viruses. Studies on the vertical distribution and seasonal variation of populations of nematodes were conducted to evaluate the sampling procedures. Attempts were made to rear several species of nematodes in the greenhouse on blueberries.

Fourteen species or genera of plant-parasitic nematodes occurred in soil samples from blueberry fields but only *Pratylenchus crenatus* (Loof) and *Trichodorus christiei* (Allen) occurred frequently. *Xiphinema americanum* (Cobb) was associated with bushes which had necrotic ringspot virus symptoms. The low number of nematodes in samples was partly accounted for by a seasonal reduction in nematode populations and was not due to the practice of sampling only the top 6 inches of soil. *T. christiei* and *Tetylenchus joctus* (Thorne) increased on blueberries in the greenhouse; 6 other species failed to increase.

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By

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INTRODUCTION

Michigan shares leadership with New Jersey in the production of cultivated blueberries. There are 9000 acres of blueberries under cultivation in Michigan. Most of the acreage is in Berrien, Van Buren, Allegan, Ottawa, and Muskegon counties. These counties occur along the southern half of the eastern shore of Lake Michigan.

Little is known about the effect of nematodes on the production of blueberries. Nematodes may influence blueberry production by [1] spreading plant viruses, and [2] acting as plant pathogens. Since information on either of these two possibilities is inadequate, the following studies were undertaken: [1] a general nematode survey, [2] a survey of nematodes associated with plants with necrotic ringspot and shoestring virus symptoms, [3] a study on seasonal variation in numbers of nematodes, [4] a study on the vertical distribution of nematodes, and [5] a study on the increase of nematodes in potted blueberry plants.

The purpose of the general survey was to determine which nematodes are associated with blueberries and the frequency of occurrence of these nematodes. The survey of fields which exhibited virus symptoms was designed to learn whether there were any particular nematodes associated with these virus symptoms. Studies on seasonal variation in numbers and vertical distribution of nematodes were included to help evaluate the accuracy of the survey data. Research on the increase of nematodes on potted plants was included to give some indication of the ability of nematodes found in the surveys to act as parasites of blueberries.

LITERATURE REVIEW

There are only a few papers which deal with nematodes associated with blueberries in Michigan. Knierim (9) listed several genera of nematodes found in soil samples from Michigan blueberry fields. Xiphinema americanum (Cobb)from Michigan was used to transmit necrotic ringspot virus (NRSV) from blueberries to cucumbers (5). Attempts to use nematodes to transmit shoestring virus of blueberries were frustrated by difficulties in rearing nematodes, (12).

A number of surveys for nematodes in blueberry fields have been conducted in eastern United States. Blueberries were included in surveys of nematodes associated with crops in both New Jersey (7) and Massachusetts (18). The occurrence of nematodes associated with blueberries in five states was recorded in a paper titled "Distribution of Stylet-Bearing Nematodes in the Northeastern United States" (11). In another paper the occurrence and relative abundance of some nematodes from blueberries in four eastern States were presented (4). Information from these surveys will be presented under the section listing plant parasitic nematodes associated with Michigan blueberries.

Race and Hutchinson reported that blueberry roots are unattractive to Pratylenchus penetrans (Cobb) (13). This statement was based on observations of the behavior of P. penetrans in relation to roots of blueberries and other plants grown in petri dishes. Hutchinson et al. (6) observed large numbers of Trichodorus christiei (Allen) associated with stunted blueberry cuttings.

Zuckerman innoculated blueberry plants grown in pots with nematodes and observed nematodes feeding on the roots of blueberry seedlings grown in agar (16, 17). He found that Trichodorus christiei, Hemicycliophora similis (Thorne) and Tetylenchus joctus (Thorne) reproduced in pots and feed on the roots grown in agar. Stubby roots were associated with the feeding of T. christiei and terminal root galls were associated with H. similis feeding. There were no symptoms associated with Tetylenchus joctus feeding. Root growth of cuttings set into soil innoculated with T. christiei and with H. similis was significantly reduced. Root growth of cuttings set into soil innoculated with Tetylenchus joctus was not significantly reduced. Cuttings innoculated with T. christiei after root growth had started showed a variable amount of root reduction.

Lister et al. (10) presented evidence that NRSV is similar to tobacco ringspot virus which is known to be transmitted by Xiphinema americanum. Also, they reported that X. americanum was associated with NRSV in several different geographical locations.

GENERAL PROCEDURES

Soil Sampling Procedures

Soil samples were taken within 2 ft. of the bases of blueberry bushes with a soil sampling tube. Four or more probes were taken with the sampling tube to accumulate one half pint of soil in each sample. Only the top 6 inches of soil were sampled except in the vertical distribution study where 12 inch probes were taken and divided into two 6 inch sections. Samples were processed shortly after they were collected or were refrigerated at 40 to 50° F. and kept in plastic bags to prevent them from losing moisture.

Nematode Extraction Procedures

The following methods were used to extract nematodes from soil: [1] the Christie-Perry technique (3), [2] a modification of the inverted flask technique (1) combined with a paper cup Baermann funnel, and [3] Jenkins' modification of the sugar flotation technique (8).

The Christie-Perry Technique.--A soil sample was mixed throughly with water in a bucket. Then the mixture was allowed to stand for a few seconds so that heavy particles in the soil could settle to the bottom of the bucket. The particles which remained suspended in the water were decanted through a 25-mesh screen into a second bucket. The washed particles remaining in the first bucket were washed two additional times by the process just described. This procedure seperated the nematodes in the sample from heavy particles such as sand which remained in the first bucket and from large particles, such as leaves and sticks, which were caught on the 25-mesh screen.

The contents of the second bucket were stirred, allowed to stand for a few seconds, and decanted onto a 325-mesh screen. Particles which remained in the bucket were washed by the process just described two additional times. Some heavy particles remained in the bucket. Nematodes were caught on the 325-mesh screen along with other small organic and mineral particles. Very small particles such as silt and clay passed through the 325-mesh screen.

The nematodes were seperated from the residue on the 325-mesh screen by means of a Baermann funnel. The nematodes and residue on the screen were washed into a 250 ml beaker. A piece of muslin cloth was fasted over the mouth of the beaker and the beaker was inverted and partly submerged in a water filled funnel. The nematodes seperated themselves from the residue by wiggling through the cloth; they settled into a test tube which was fastened to the bottom of the funnel with a piece of rubber tubing. The extraction was completed when the test tube was removed after four days.

The Inverted Flask Technique.--A 2000 ml flask was filled with water and one half pint of soil which had been screened through one quarter inch mesh hardware cloth. An adapter fitted with a rubber stopper (Plate II) was attached to the mouth of the flask. The flask was inverted several times to suspend the nematodes in the water. Then, the flask was placed upside-down in a ring stand above another 2000 ml water filled flask with the adapter submerged in the water in the neck of the lower flask (Plate III). The stopper was removed while submerged with a wire designed for this purpose. After 2 to 3 minutes, when the larger particles had settled into the lower flask, the stopper was replaced. The contents remaining in the upper flask were poured onto a 325-mesh screen; nematodes and small particles were caught on the screen and washed into a paper cup Baermann funnel. The particles which had settled into the lower flask were processed by repeating the extraction technique just described two additional times to remove nematodes which may not have been recovered.

The Paper Cup Baermann Funnel.--The body of the funnel (Plate I) was constructed from a 12 oz. paraffin coated, conical paper cup. The tip of the cup was cut off to make a hole one quarter inch diameter. A short glass tube was cemented to tip of the cup with paraffin and connected to a test tube with a short length of rubber tubing.

Plate I.--Filter cup and body of paper cup Baerman funnel.

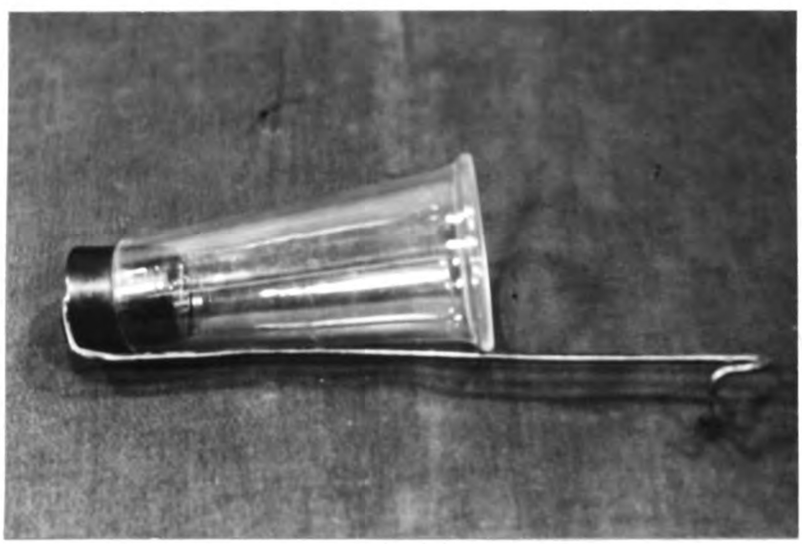


Plate II.--Adapter and stopper with handle.

Plate III.--Inverted flask
apparatus ready for operation.

Plate IV.--Inverted flask
apparatus in operation.

A filter cup (Plate I) was designed to fit inside the body of the funnel. It was constructed from the upper part of two conical paper cups and a piece of muslin cloth. The cloth was cemented between the two telescoped pieces of paper cup with paraffin. The cloth formed the bottom of the filter cup. The diameter of the bottom was 2/3's the diameter of the top of the paper cup.

The filter cup was partly submerged in water inside the body of the funnel; then the exract from the inverted flask technique was placed in the filter cup. The nematodes separated themselves from the small particles by wiggling through the cloth and settled to the bottom of the test tube. They were collected after four days by removing the test tube.

<u>The Sugar Flotation Technique</u>.--Nematodes and small particles were exracted from 50 cc of soil by the sieving-decanting process described in the Christie-Perry technique. The nematodes and particles were washed into two 50 ml tubes and spun for 3 to 4 min. at full speed in a clinical centrifuge. The nematodes were retained in the sediment in the bottom of the tube and the supernatant was discarded. The sediment was mixed with a solution containing one pound of sugar per liter of water. The tubes were centrifuged again at full speed for 1 min. The supernatant, which then contained the nematodes, was poured onto a 325-mesh screen, rinsed with water, and washed into a test tube. Nematodes

which did not remain in suspension during centrifugation were extracted by centrifuging a second time after suspending them again in the sugar solution.

Discussion of Nematode Extraction Techniques

Each of the three extraction techniques described above was used for one season of sampling. Since the efficiency of the three techniques was unknown, the records of nematodes extracted during one season could not be accurately compared with the records of any other season. However, exact comparison between nematodes extracted in different seasons was not essential to this study. The advantage of the changes from one extraction technique to another was in conservation of time. The inverted flask technique combined with the paper cup Baermann funnel which was used in 1964 was faster than the Christie-Perry technique which was used in 1963, and the sugar flotation technique which was used in 1965 was faster than the other two techniques.

Procedures for Identifying and Counting Nematodes

To count and identify nematodes to genus a small counting-chamber was scanned at a magnification of 100 diameters with a compound microscope by manipulating the microscope's mechanical stage. All nematodes which were known, or suspected, to be parasitic on higher plants were recorded with a few exceptions. *Aphelenchoides* spp., *Aphelenchus* spp., and *Tylenchus* spp., though present in some samples were not recorded. These genera are small and hard to identify and contain species which may not be parasites of higher plants. Most of the species determinations were made from specimens mounted in glycerin, although temporary water mounts were used on occasion. The nematodes which were mounted in glycerin were killed with heat, fixed in F. A. A., and processed by Seinhorst's method (15).

THE GENERAL SURVEY

Procedures

From July through August 1964, soil samples were taken from 10 farms in each of three regions; (1) Grand Junction in Allegan county, (2) Holland in Ottawa county, and (3) Fruitport in Muskegon county. There were three sample sites on each farm; one site for each of the blueberry varieties: Rubel, Jersey, and Stanley. On a few occasions when only two of the three varieties could be found on one farm, the other variety was sampled on a nearby farm. At each site three one cup samples of soil were taken within a block of 100 bushes. Each sample was composed of soil cores which were taken from the bases of several bushes. Nematodes were extracted by the inverted flask method combined with the paper cup Baermann funnel, and were identified and counted.

Results and Discussion

Pratylenchus spp. and *Trichodorus christiei* (Allen) occurred on 2/3's and 1/3 of the farms sampled respectively; other genera of nematodes were found infrequently (Table 1). Three-fourths of the *Pratylenchus* spp. were *P.* crenatus; the remaining were *P. penetrans* (Cobb). *Pratylenchus* spp. and *T. christiei* were found in samples from all three regions and all three varieties that were sampled (Tables 1 and 2).

The occurrence of various genera of nematodes in soil samples from Michigan blueberry fields which were processed by Knierim (9) corresponded roughly to the occurrence of the same genera of nematodes reported here. *Pratylenchus* spp. were also the most common nematodes found in samples examined by Knierim. More direct comparison between Knierims findings and the findings in this study was not possible because samples on which Knierim's report was based were collected in an irregular manner by growers and agricultural agents for various purposes.

In the eastern United States *Tetylenchus* sp. and *Hemicycliophors* spp. are the most common nematodes from blueberry fields; *Pratylenchus* spp. is relatively uncommon there (7, 11, 18). *Tetylenchus* sp. and *Hemicycliophora* spp. also occur in wild blueberries in the east (6). Wild blueberries were not sampled extensively in Michigan; however, they are not generally found near commerical blueberry fields here. This suggests that the principle nematodes found in eastern blueberry fields may have come from wild blueberries and that this may not be the case here.

The largest number of nematodes per cup of soil recorded in the survey was 43. Several hundred nematodes per cup of soil have been recorded from blueberries in New

TABLE 1.--Incidence of several genera and species of nematodes on blueberry farms from 3 regions in Michigan.

Region	Total Number of Locations*	Number of Locations per Genera of Nematode**						
		P	T	X	H	Ty	Pa	L
Grand Junction	10	7	3	2	1	1	2	1
Holland	10	6	4	0	0	0	0	0
Fruitport	10	6	2	0	1	1	0	0
Total	30	19	9	2	2	2	2	1

*Nine samples per location.
**Key to the genera and species at bottom of this page.

TABLE 2.--Incidence of several genera and species of nematodes in 3 varieties of Michigan blueberries.

Region	Total Number of Locations*	Number of Locations per Genera of Nematode**						
		P	T	X	H	Ty	Pa	L
Jersey	30	11	3	0	1	0	1	1
Rubel	30	7	6	0	1	1	0	0
Stanley	30	12	2	2	0	1	1	0

*Three samples per location.
**Key to genera and species below.

Key to genus and species of nematodes:

A -- *Atylenchus decalineatus* (Cobb)
C -- *Criconemoides* sp.
H -- *Hemicycliophora* spp.
Ho -- *Hoplolaimus galeatus* (Cobb)
L -- *Longidorus* sp.
P -- *Pratylenchus crenatus* (Loff) and *P. penetrans* (Cobb)
Pa -- *Paratylenchus* sp.
T -- *Trichodorus christiei* (Allen)
Ty -- *Tylenchorhynchus claytoni* (Steiner)
X -- *Xiphinema americanum* (Cobb)

Jersey (7). Either the season when fields were sampled or a more efficient extraction technique may account for larger numbers recorded there. (The effect of season will be discussed in another section).

SURVEY FOR NEMATODES ASSOCIATED WITH NECROTIC RINGSPOT AND SHOESTRING VIRUSES

Procedures

Soil samples were taken from the bases of bushes which had symptoms of necrotic ringspot (NRSV) or shoestring virus (SSV) on several farms during the summers of 1963, 1964, and 1965. Approximately equal numbers of samples were taken from plants with each virus symptom each year. A sample consisted of soil from beneath a single bush. Soil samples were extracted on the first, second, and third summers by the Christie-Perry technique, the inverted flask technique combined with the paper cup Baermann funnel, and the sugar flotation technique, respectively; the extracted nematodes were identified and counted.

Results and Discussion

Xiphinems americanum (Cobb) was associated with NRSV symptoms on 8 to 12 farms; the same nematode was not associated with SSV symptoms of 14 farms. Other nematodes were associated about as often with NRSV as they were with SSV. *Pratylenchus* spp. and *Trichodorus christiei* (Allen) were common near bushes with either SSV or NRSV symptoms (Table 3).

The frequent association of *X. americanum* with NRSV symptoms indicates that it may be the primary vector of NRSV. *Pratylenchus* spp., primarily represented by *P. crenatus* according to the general survey, was as frequently associated with NRSV as was *X. americanum,* but it was also common in fields with SSV symptoms and in the general survey. Therefore, its prevalence near NRSV symptoms may only reflect its overall prevalence in blueberries. The repeated association of *Pratylenchus* spp. with SSV symptoms and *T.* christiei with both SSV and NRSV symptoms may be explained the same way. However, *X. americanum* seems to occur infrequently in blueberries, except in those with NRSV symptoms, since it was not found in any fields with SSV symptoms and was found on only 2 of 30 farms in the general survey.

X. americanum may have originally acquired NRSV from weeds and spread it to blueberries or it may have originally acquired NRSV from diseased cultivated blueberries and spread it to other blueberry plants. If *X. americanum* originally acquired NRSV from weeds, it could have become a vector of the virus in blueberries when blueberries were planted where the nematodes and weeds infected with NRSV occurred. If *X. americanum* originally acquired NRSV from cultivated blueberries, it could have become the

TABLE 3.--Incidence of several genera and species of nematodes near blueberries with necrotic ringspot virus (NRSV) or shoestring virus (SSV) symptoms.

Type of Virus Symptom	Total Number Of Locations	Number of Locations Per Genera or Species of Nematodes*									
		P	T	X	H	Ty	C	C	Ho	L	Pa
NRSV	12	8	6	8	2	0	3	0	2	1	1
SSV	14	4	4	0	3	2	2	2	1	1	1

* Key to genera and species of nematodes on p. 15.

vector of NRSV in blueberries when both blueberries with the virus and healthy blueberries were planted where the nematode occurred.

Since X. americanum was found on only 2 of 30 farms in the general survey, it may have occurred infrequently where blueberries were planted. If X. americanum originally acquired NRSV from cultivated blueberries and the nematode occurred infrequently where blueberries were planted, it would very rarely become a vector of NRSV unless many of the planted blueberries were virus infected. If X. americanum originally acquired NRSV from weeds and spread it to blueberries it could have become a vector of the virus in blueberries even though only healthy blueberries were planted where the nematode occurred. Cases of X. americanum spreading NRSV in blueberries would not be extremely rare even if the nematode occurred infrequently where blueberries were planted if weeds with NRSV were always present. However, nothing is known about the weed hosts of NRSV since no attempts to find weed hosts of NRSV have been reported.

TABLE 4.--The occurrence of several genera and species of nematodes on blueberry farms where necrotic ringspot virus symptoms appeared.

Farm	Year	Number of Sites	Number of Samples	Genera and Species of Nematodes Recovered *							
				X	P	T	H	C	Ho	L	Pa
Benkie	63	2	2	+	-	-	-	-	+	+	-
AA #1	63	3	5	-	+	+	-	-	-	-	-
	64	1	3	-	+	-	-	-	-	-	-
AA #2	65	1	1	+	+	-	+	-	-	-	-
Pollard	63	1	1	+	+	+	-	-	+	-	+
	64	1	many**	+	+	-	-	-	-	-	+
	65	1	1	+	+	-	-	+	-	-	-
Foretsch	63	2	2	-	+	+	-	-	-	-	-
	64	2	2	+	+	-	-	-	-	-	-
Hartman	63	1	1	-	-	-	-	-	-	-	-
Woods	63	1	1	-	-	-	-	-	-	-	-
	64	1	3	-	+	+	-	-	-	-	-
Johnson	63	1	1	-	+	-	+	-	-	-	-
	64	1	3	-	-	-	-	-	-	-	-
Decker	64	2	2	+	+	-	-	-	-	-	-
Krohn	65	1	1	+	-	-	-	+	-	-	-
N. Lake	65	1	1	+	+	+	-	-	-	-	-
Bush	65	1	1	+	-	+	-	+	-	-	-

* Key to genera and Species of nematodes on p. 15.

** Sampled through out the year for study on seasonal variation in numbers.

TABLE 5.--The occurrence of several genera and species of nematodes on blueberry farms where shoestring virus symptoms appeared.

Farm	Year	Number of Sites	Number of Samples	Genera and Species of Nematodes Recovered *								
				P	T	H	Ty	A	C	Pa	L	Ho
Isreal	63	3	5	-	-	-	-	-	-	-	-	-
	64	1	1	-	-	-	-	-	-	-	-	-
	65	1	1	-	-	-	-	-	-	-	-	-
Wiepert	63	3	5	-	-	+	-	-	-	-	-	-
Hartman	63	2	3	+	-	-	+	-	-	+	+	+
	64	1	1	+	+	-	-	-	-	-	-	-
	65	1	1	+	+	-	-	-	+	-	-	-
Depree	63	3	6	+	-	-	-	-	-	-	-	-
	64	1	1	-	-	-	-	-	-	-	-	-
	65	1	1	-	-	-	-	+	-	-	-	-
Elhart	63	1	2	-	-	+	-	-	-	-	-	-
	64	1	1	-	-	-	+	-	-	-	-	-
Behm	63	2	2	-	-	-	-	-	-	-	-	-
Peterson	63	1	1	-	-	-	-	-	-	-	-	-
G. Kiel	63	1	1	-	+	-	-	-	-	-	-	-
R. Kiel	63	1	1	+	-	-	-	-	-	-	-	-
Aussicer	63	1	1	-	+	-	-	-	-	-	-	-
	64	1	1	+	-	-	-	-	-	-	-	-
Lippenga	63	1	1	-	-	-	-	-	-	-	-	-
	64	1	1	-	-	-	-	-	-	-	-	-
Diepen-	63	1	1	-	-	-	-	-	-	-	-	-
horst	64	1	1	-	-	-	-	-	-	-	-	-
Patzloff	64	2	2	-	-	-	-	-	-	-	-	-
Fischer	65	1	1	-	+	+	-	+	+	-	-	-

*Key to genera and species on p. 15.

SEASONAL VARIATION IN NUMBERS OF NEMATODES

Procedures

Samples from each of 3 farms near Grand Junction were taken at 2 week intervals in the summer and at 1 month intervals in the winter, except in January and February when the ground was frozen and could not be sampled. Three samples were taken on each sampling date from each site (the area beneath a single blueberry bush). Nematodes were extracted from the samples by the inverted flask combined with the paper cup Baermann funnel and were identified and counted.

Results and Discussion

Pratylenchus crenatus (Loof) (Figure 3) and *Xiphinema americanum* (Cobb) (Figure 4) at Hartman's and Pollard's farms, respectively, and *Trichodorus christiei* (Allen) (Figures 1 and 2) at Hartman's and Jones' farms were highest in numbers in the fall or early winter and lowest in numbers in the summer. *T. christiei* showed a strong population reduction from late July until the middle of September. This reduction occurred at a time when there was little rain fall and at the time when the blueberry

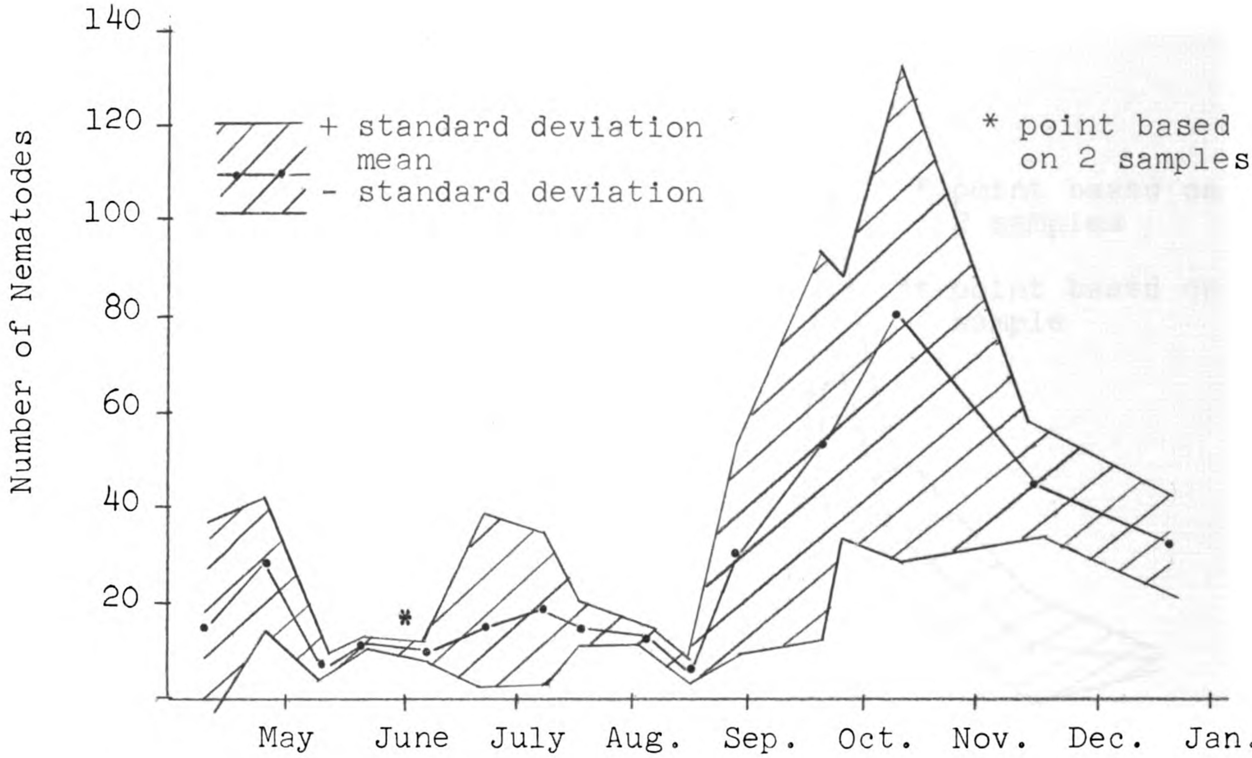


Figure 3.--Seasonal variation in numbers of <u>Pratylenchus crenatus</u> (Loff) on the Hartman blueberry farm.

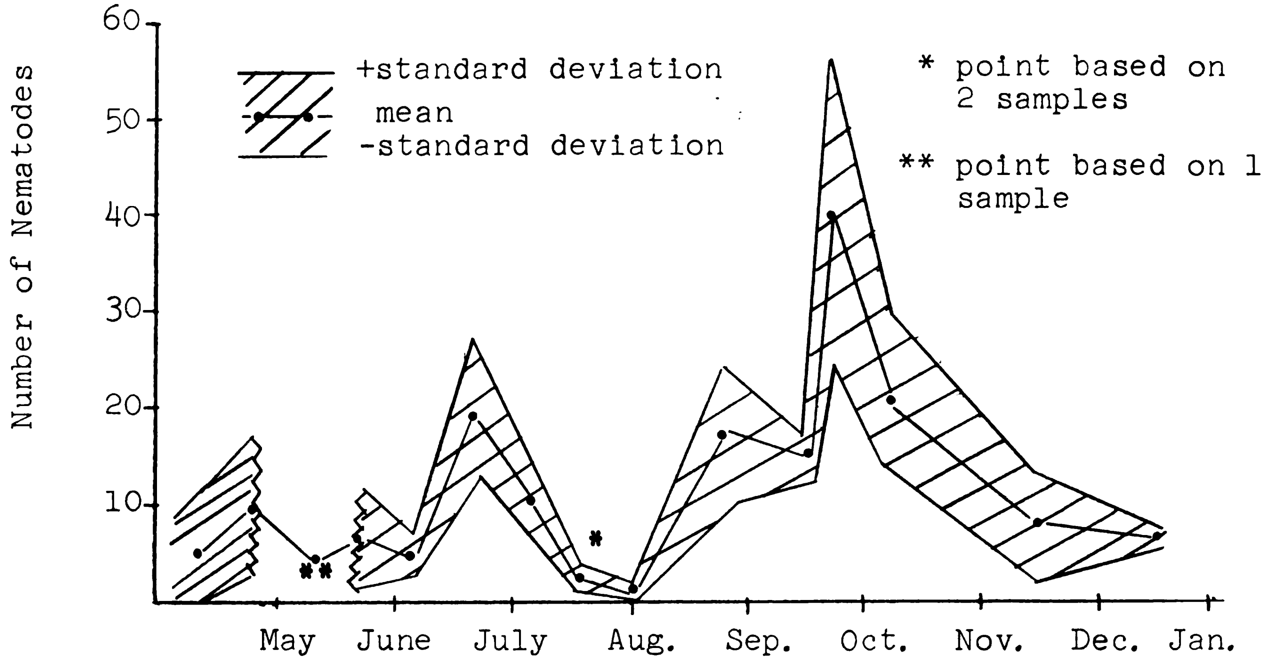


Figure 4.--Seasonal variation in numbers of Xiphinems americanum (Cobb) the Pollard blueberry farm.

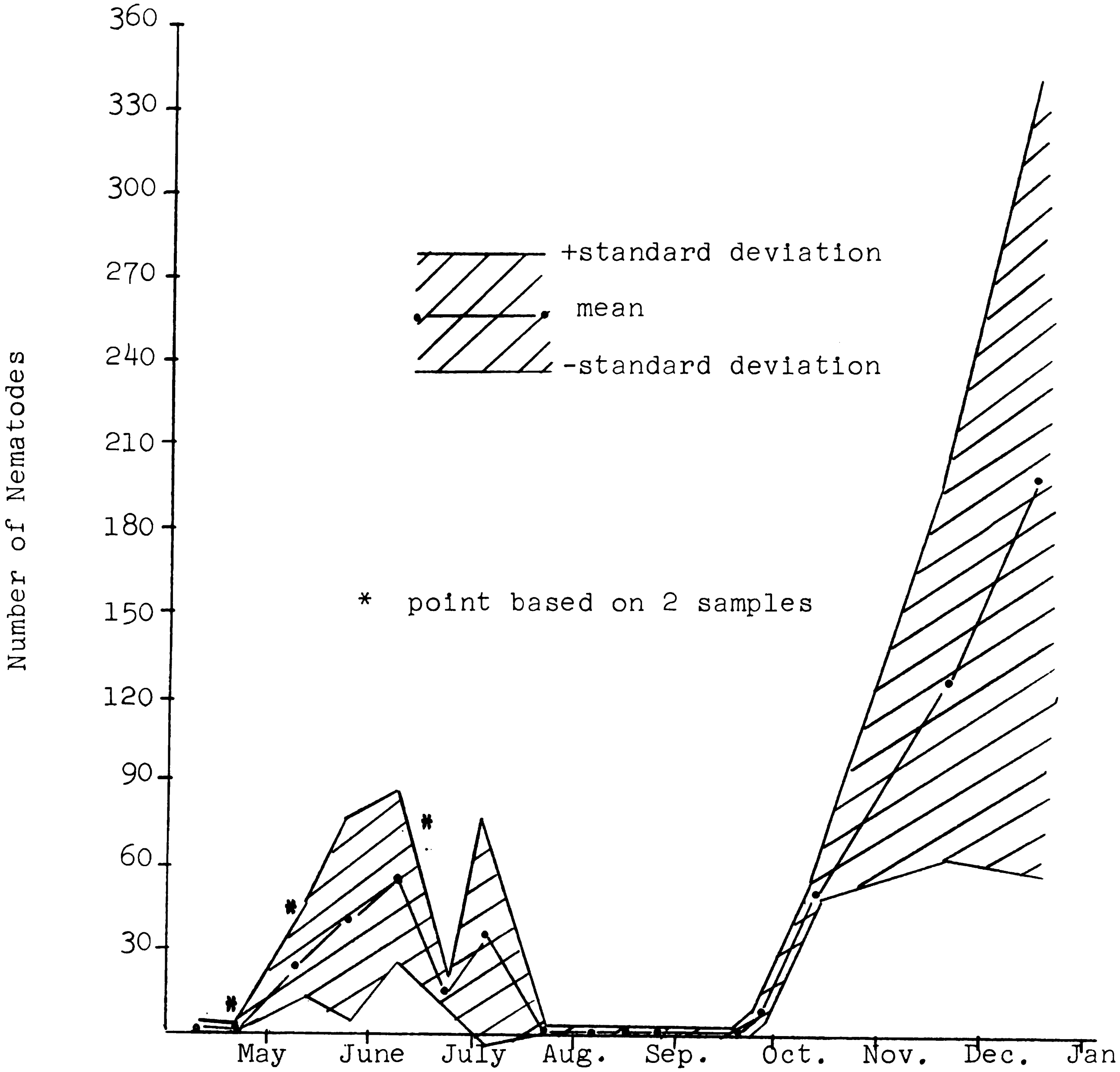


Figure 1.--Seasonal variation in numbers of <u>Trichodorus christiei</u> (Allen) on the Jones blueberry farm.

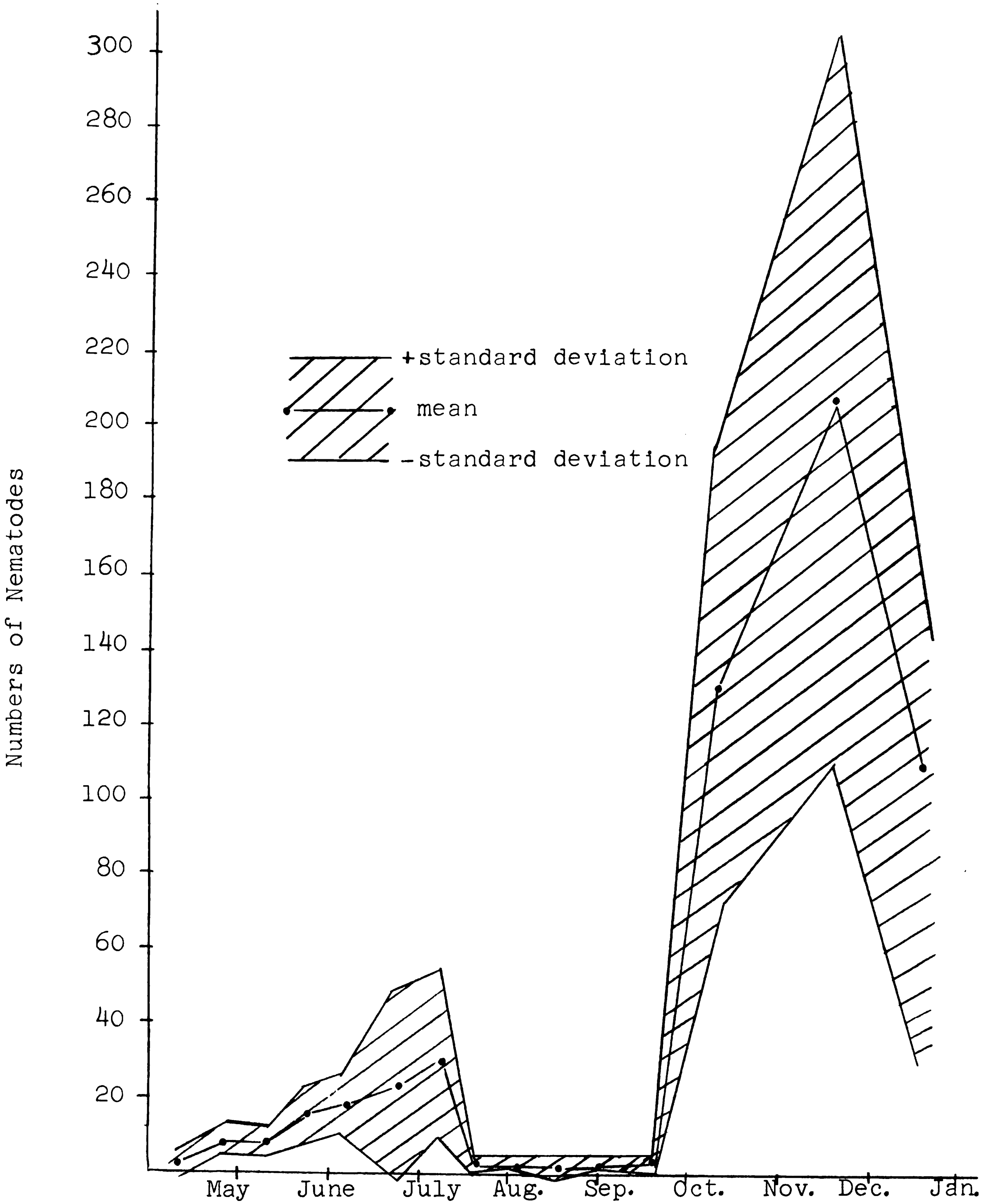


Figure 2.--Seasonal variation in numbers of *Trichodorus christiei* (Allen) on the Hartman blueberry farm.

fruit matured. The peak number of nematodes recorded for P. crenatus and X. americanum were 81 per half pint of soil in late September and 41 per half pint of soil in early October respectively. T. christiei had a peak of 200 per half pint of soil in the middle of November at one location and 209 per half pint of soil in late December at the other location.

Although the sampling procedure could not be followed in January and February because the ground was frozen, some chunks of frozen soil were extracted but only a few nematodes were recovered. On March 16 normal sampling was resumed and the average number of nematodes per half pint of soil was 92 T. christiei at Jones', 94 T. christiei at Hartman's, 11 X. americanum at Pollard's, and 77 P. crenatus at Hartman's.

The seasonal variation in numbers of nematodes suggests that the low number of nematodes found in the general survey may be partly accounted for by seasonal factors and may not be characteristic of plant parasitic nematodes in blueberry fields in Michigan throughout the year. The reduction in numbers of T. christiei which was found to occur in the summer, which is the time when most of the samples were taken, indicates that T. christiei may be even more wide spread than was reported. The probability of recovering T. christiei would have been greater if the samples had not been taken in the season when its population was low.

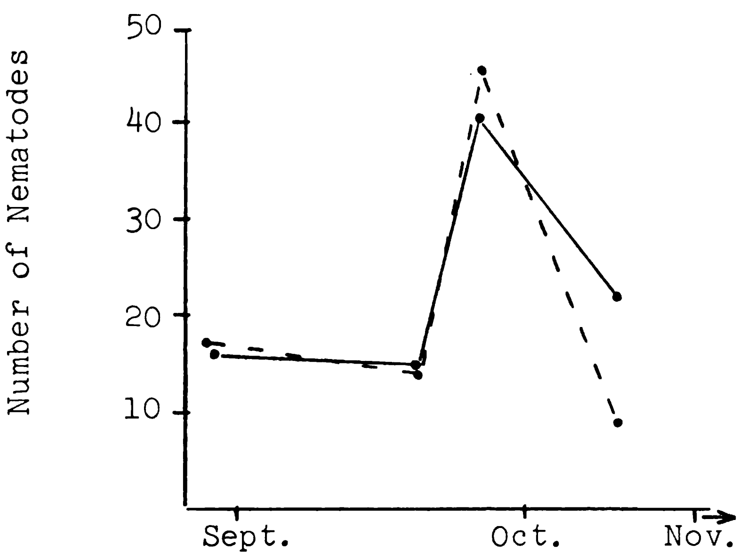
THE VERTICAL DISTRIBUTION OF NEMATODES

Procedures

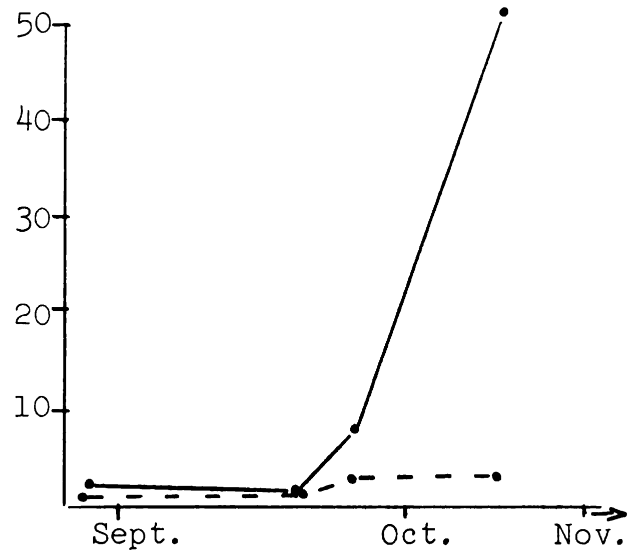
The procedures used in this study are the same as those used in the study of seasonal variation in numbers except that the soil was sampled to a depth of 12 inches instead of 6 inches. The samples were taken at 2 week intervals from August 28 to November 10. To determine vertical distribution, the 12-inch core of soil was evaluated in two 6 inch sections.

Results and Discussion

Pratylenchus crenatus (Loof) and *Trichodorus christiei* (Allen) at Hartman's farm, *T. christiei* at Jones' farm, and *Xiphinema americanum* (Cobb) at Pollard's farm were as prevalent or more prevalent at the first 6 inches of soil as they were in the second 6 inches of soil (Figure 5). Soil samples from the top 6 inches of soil contained numbers of these nematodes that at least equaled the numbers in a sample of the same size taken from the top 12 inches of soil. Therefore, the procedure used throughout this investigation of sampling only the top 6 inches of soil rather than sampling to a greater



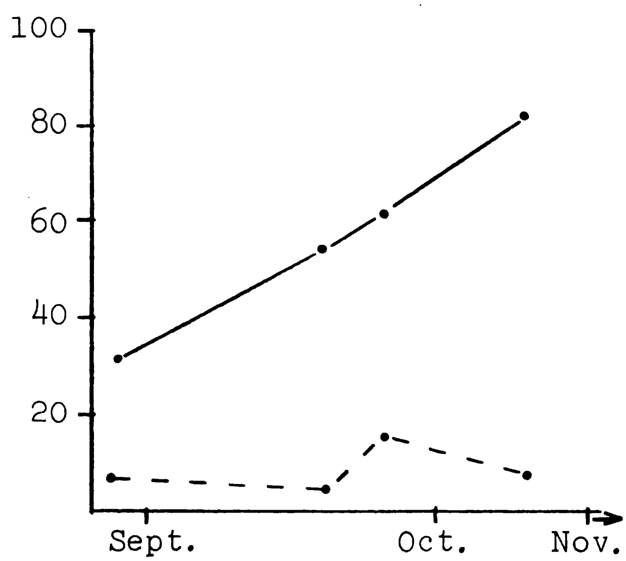
Xiphinema americanum (Cobb) on Pollard's farm.



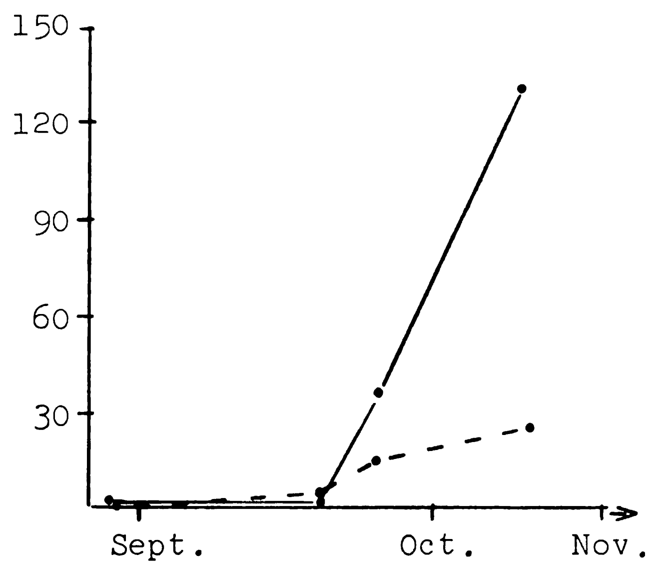
Trichodorus christiei (Allen) on Jones' farm.

•——• first 6 inches

•– –• second 6 inches



Pratylenchus crenatus (Loof) on Hartman's farm.



Trichodorus christiei (Allen) on Hartman's farm.

Figure 5.--Distribution of several nematodes between the first 6 inches of soil and the second 6 inches of soil on 3 blueberry farms.

depth, probably does not account for the small number of nematodes that were usually recovered. However, the recovery of small numbers of nematodes was partly accounted for by a seasonal reduction in nematode populations. Information on nematodes found at a depth greater than 12 inches would be desirable but it is likely that most nematodes that are associated with blueberries can be found within the top 12 inches of soil. Blueberries are shallow rooted and the distribution of nematodes associated with them would be expected to coincide with the distribution of their roots.

THE GREENHOUSE STUDY

Procedures

Thirty to 150 nematodes of several species were introduced into pots containing young blueberry plants to study their ability to survive and increase on blueberry roots. The nematodes were extracted by the sugar flotation technique from soil collected from blueberry fields; no attempt was made to separate them from small numbers of unidentified plant parasitic nematodes and other soil microflora and microfauna which were found in the soil extract. Two or three pots were used for each species of nematodes; three pots which were not treated with nematodes were kept as controls. Usually a pot was treated with one species, however, in two cases where two species were prevalent in one soil sample both species were added to the same pot.

The plants used in the study were rooted cuttings, variety Jersey, which were taken from cutting beds in the fall and kept in cold storage for a month, then potted in one quart cans on soil made up of 50% peat and 50% potting soil. The plants lost their leaves in cold storage but developed new growth shortly after they were potted. Three months after the nematodes were added to the pots, five 50 cc samples from each pot were processed using the sugar flotation extraction technique to determine the degree of increase or survival of the nematodes.

Results and Discussion

Nematodes of all the species which were introduced into the pots were recovered after 3 months. Only *Tetylenchus joctus* (Thorne) and *Trichodorus christiei* (Allen) increased in numbers. There were a few more *Xiphinema americanum* (Cobb) extracted than were added, however, extraction of the controls indicated that there may have been some *X. americanum* in the pots before any nematodes were added. The slight increase in numbers of *X. americanum* may be due to survival but not increase of added nematodes, plus the survival of nematodes that were there before *X. americanum* was added. There were also a small number of *T. christiei* extracted from the control pots. The number of *T. christiei* extracted from the treated pots was so much larger than the number of those in the control pots and the number of those that were originally added to the treated pots that the increase can not be explained the same way the small increase of *X. americanum* was explained.

The increase in *T. christiei* and *Tetylenchus joctus* is taken as evidence that they established themselves as parasites of blueberries. The failure of the other species to increase may or may not indicate that they are not parasites of blueberries. The conditions of this study may have prevented nematodes that can increase on blueberries in the field from increasing in the greenhouse. The increase of *T. christiei* and *Tetylenchus joctus* supports similar findings of Zuckerman (16, 17). Greenhouse studies using blueberry plants as potential hosts for the nematodes which failed to increase in this study have not been previously reported. *Hemicycliophora similis* (Thorne), which was not included in this study because sufficient numbers of it could not be found, has been shown to increase on blueberries in the greenhouse by Zuckerman (17).

TABLE 6.--The increase over a 3 month period of nematodes on potted blueberries.

Nematode	No. of Pots	Mean No. Added	Mean No. Extracted After 6 Months	Increase	Mean No. Extracted From Control
Trichodorus christiei	2	128	873	7 X	4
Helicotylenchus sp.	3	111	24	0.2 X	0
Pratylenchus crenatus	3	50	36	0.7 X	40*
Tylenchorynchus sp.	3	98	26	0.3 X	0
Pratylenchus penetrans	3	110	26	0.2 X	40*
Tests in which two species were added to each pot:					
Trichodorus christiei	2	98	1293	13 X	4
and					
Tetylenchus jostus	2	156	357	2 X	0
Xiphinema americanum	3	128	149	1 X	138
and					
Criconemoides sp.	3	29	2	0.1 X	0

*Only the genus of *Pratylenchus* found in controls was determined.

A LIST OF PLANT PARASITIC NEMATODES ASSOCIATED WITH CULTIVATED BLUEBERRIES IN MICHIGAN

Atylenchus decalineatus (Cobb)

This nematode has been found on a few blueberry farms in Michigan as well as in blueberry fields in New Jersey (7) and Massachusetts (18). It is found in cranberry bogs as well as blueberries fields (11). The high water table necessary for these two crops favors *A. decalineatus* since it is known to have affinity for wet soil (2). Another nematode which has an affinity for west soil is *Dolichodorus heterocephalus* (Cobb) which has been reported from blueberries in New Jersey but not in Michigan.

Criconemoides sp.

This genus, of which some specimens from Michigan were identified as *C. sphaerocephalus* (Taylor) has been found only occasionally in blueberries here and in New Jersey (7). This may have been due to the inability of *Criconemoides* sp. to wiggle through the filter in Baerman funnels which were used to extract it. When the sugar flotation technique of extraction was used *Criconemoides* sp. was found in samples from 5 of 9 farms.

Helicotylenchus sp.

In both Michigan and New Jersey (7) *Helicotylenchus* sp. has been recovered a few times from blueberries.

Hemicycliophora spp.

Nematodes from this genus were occasionally found in Michigan blueberry fields; most of those that were determined to species were *H. similis* (Thorne) although *H. vaccinium* (Reed and Jenkins) was identified once. *Hemicycliophora* spp., including *H. gracilis*, (Thorne) *H. similis*, and *H. uniformis* (Thorne) were among the most common nematodes found on blueberry farms in the eastern United States and were also common in various species of wild blueberries and cranberries in those states (17, 6). Zuckerman found galls and reduced root growth associated with the parasitism of blueberry cuttings by *H. similis* (17).

Hoplolaimus galeatus (Cobb)

H. galeatus was recovered from a few blueberry fields in Michigan and has been reported from blueberry fields in New Jersey (7).

Longidorus sp.

In Michigan a few specimens of this genus have been recovered from blueberry fields.

Paratylenchus sp.

Nematodes of this genus were recorded a few times from blueberry fields both in Michigan and eastern United States (11).

Pratylenchus spp.

Members of this genus were the most common nematodes that were found in Michigan blueberries. About 3/4's of those that were identified were _P. crenatus_ (Loof); the rest were _P. penetrans_ (Cobb). _Pratylenchus_ spp. were infrequently recovered from blueberry fields in New Jersey (7) and Massachusetts (18). Hutchinson and Race rated blueberries as a poor host for _P. penetrans_ (13). Both _P. penetrans_ and _P. crenatus_ failed to become established as parasites of blueberries in the greenhouse.

Rotylenchus sp.

This nematode was not found by the author but has been recorded from Michigan blueberries by Knierim (9) and was found in blueberries in New Jersey and Maryland (11).

Tetylenchus joctus (Thorne)

T. joctus was recovered once by the author while collecting nematodes for the greenhouse study. The only other record of this genus in Michigan blueberries is from a soil sample sent to Knierim by a grower. In New Jersey and Massachusetts, _T. joctus_ is the most common nematode from blueberry farms and is also common in wild blueberries and cranberries (7, 17). Greenhouse pot studies described in this thesis and studies made by Zuckerman (17) indicate that it is a parasite of blueberries. Zuckerman's tests, however, indicate that it may not be destructive to blueberries.

Trichodorus christiei (Allen)

The only species of *Trichodorus* that was identified from Michigan blueberries is *T. christiei*. Because of difficulties in determining species in this genus there is a possibility that other species were over looked. *T. christiei* was found to be wide spread in Michigan but was apparently not so wide spread in New Jersey (7) and Massachusetts (18). Zuckerman's research (16) and research reported here indicates that *T. christiei* is a parasite of blueberries. Zuckerman also has shown that *T. christiei* is associated with reduced growth of blueberry cuttings and with a stubby root condition in blueberries.

Tylenchorynchus claytoni (Steiner)

T. claytoni was found infrequently in Michigan blueberry fields. *T. claytoni* and *Tylenchorynchus* sp. have been reported from cultivated blueberries in several eastern states (11).

Xiphinema americanum (Cobb)

X. americanum has been implicated as the vector of necrotic ringspot virus of blueberries by the research of Griffin et al. (5) and Lister et al. (10). It was found in several fields in which symptoms of necrotic ringspot virus were present. It was not recovered from several fields in which shoestring virus symptoms were evident. Generally it was found infrequently in blueberries in both Michigan and the eastern United States (18, 11).

SUMMARY AND CONCLUSIONS

Plant parasitic nematodes from 11 genera were found in Michigan blueberry fields. Only the genus *Pratylenchus*, represented primarily by *P. crenatus* (Loof), and *Trichodorus christei* (Allen) were found frequently. They were found on three blueberry varieties and in three blueberry regions. Study on the relation of *P. crenatus* and *T. christiei* to blueberry production is merited on the basis of their wide spread occurrence. Their effect on blueberry production could be important due to such frequent occurrence.

The procedure of sampling the top 6 inches of soil proved to be suitable, at least for *P. crenatus*, *T. christiei*, and *Xiphinema americanum* (Cobb). There were as many or more of these nematodes in the top 6 inches of soil as there were in the second 6 inches of soil. The numbers of *T. christiei* and to a lesser extent *P. crenatus* and *X. americanum* were lowest in the summer. The probability of recovering these nematodes was reduced by sampling during the summer.

X. americanum was found frequently in fields with necrotic ringspot virus (NRSV) symptoms and was found infrequently in other fields. It is likely that *X. americanum* is a vector of NRSV because; [1] it is frequently associated with NRSV symptoms, [2] it has been used to transmit NRSV to cucumbers (5), and [3] it can transmit tobacco rings-pot virus which is closely related to NRSV (10). There was no particular nematode associated with bushes showing shoe-string virus (SSV) symptoms. However, *T. christiei* should be considered as a possible vector of SSV since it is common in Michigan blueberry fields and since members of the genus *Trichodorus* can transmit other plant viruses(14).

X. americanum may have spread NRSV from weeds that had the virus to blueberries. If this is the case infestations of NRSV in blueberries could start even if NRSV was eliminated from existing blueberries and only NRSV free blueberries were planted. Since there is not information available on the weed hosts of NRSV, it would be desireable to determine if any weeds in areas where blueberries are grown harbor NRSV.

T. christiei and *Tetylenchus joctus* (Thorne) increased in numbers on potted blueberry plants in the greenhouse over a three month period; species which did not increase were *P. crenatus*, *P. penetrans* (Cobb) *Tylenchorhynchus claytoni* (Steiner) *Helicotylenchus* sp., *Criconemoides* sp., and *Xiphinema americanum*. The nematodes that did not increase may not be parasites of blueberries or they may have been prevented from increasing by abnormal environmental conditions imposed on them in the greenhouse.

The ability of *T. christiei* and *Tetylenchus joctus* to increase on blueberries confirms similar findings by Zuckerman (16, 17). These greenhouse tests support the recommendation that work should be done on the relation of *T. christiei* to blueberry production even though *T. christiei* did not consistently reduce growth of young blueberry plants in a test conducted by Zuckerman (16). Although Zuckerman's test suggests that, except in cutting beds, *T. christiei* may not damage blueberries, more extensive proof of this is needed due to the wide occurrence of *T. christiei* in Michigan blueberry fields and its ability to increase on blueberry roots.

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