To the Graduate Council:

I am submitting herewith a thesis written by W. Ray Stockdale entitled "Relationship of Sampling Date and Soil Type to Southern Root-Knot Nematode (Meloidogyne incognita) Populations in Selected Fields in Cocke County, Tennessee." I have examined the final copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Agricultural Extension.

Robert S. Dotson, Major Professor

We have read this thesis
and recommend its acceptance:

Charles Haddock

Ernest C Bernard

Cecil E. Easley Jr.

Accepted for the Council:

Vice Provost
and Dean of the Graduate School

RELATIONSHIP OF SAMPLING DATE AND SOIL TYPE TO SOUTHERN ROOT-KNOT NEMATODE (*MELOIDOGYNE INCOGNITA*) POPULATIONS IN SELECTED FIELDS IN COCKE COUNTY, TENNESSEE

A Thesis

Presented for the

Master of Science

Degree

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W. Ray Stockdale

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I especially thank Dr. Ernest Bernard for helping to plan and coordinate the research project and working with the author until completion.

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ABSTRACT

In Cocke County, Tennessee, tomato (Lycopersicon esculentum) is the third most important cash crop grown. Most fields have an infestation of the southern root-knot nematode (Meloidogyne incognita). Past studies have shown that M. incognita reduces yields, predisposes plants to other diseases, and causes otherwise productive land to be of little value for tomato production. This study was undertaken to: (a) determine the best time of year for farmers to sample for the presence of root-knot nematode in tomatoes; (b) determine the effect of some soils on nematode population increase; (c) determine the behavior of root-knot nematode larvae in several soil types during the fall and winter following the growing season.

Five fields, with known nematode infestation, were selected for study. Samples were taken monthly from a ten meter long and six rows wide plot in each field for fourteen months. Sampling began in February 1983 and continued through March 1984. Samples were taken in accordance with procedures outlined in "Nematode Sampling Analysis," Form 664, revised May 1980. A centrifugal-flotation method was used for extracting nematode larvae from the soil.

The study found that the best time to sample was immediately after the production season. Some soils appeared to promote root-knot nematode larvae population increase, whereas others seemed

to suppress increases of the larvae. Larval counts dropped rapidly after the production season, leveled off in early winter, then dropped again in late winter.

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CHAPTER I

INTRODUCTION

In Cocke County, Tennessee, tomato (Lycopersicon esculentum L.) is the third most important cash crop grown, with an annual gross value of about $4.5 million.

Root-knot nematode (Meloidogyne incognita [Kofoid & White] Chitwood) has been shown to reduce tomato yields by as much as one half when compared with treated plots (6,7).*

Much research has been done to determine yield loss caused by root-knot nematodes in various densities and in various soil types (6,7,10,20). From a pest management perspective, it is important to know the potential for controlling nematodes by all available means. The objectives of this study were to:

1. Determine the best time of year for farmers to sample for the presence of M. incognita in tomato;
2. Determine the effect of some soils on nematode population increases;
3. Determine the behavior of M. incognita larvae in several soil types during the fall and winter following the growing season.

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*Numbers in parentheses are alphabetically listed references in the bibliography; numbers after colons are page numbers.

CHAPTER II

LITERATURE REVIEW

Much research has been done on the southern root-knot nematode, Meloidogyne incognita, and its effect on tomato. However, most research has been concerned with the effects of the root-knot nematode on yields, or on ways of controlling it. Very little research has been done to determine proper times for growers to sample for the presence of M. incognita.

Work done by Bernard and Hadden (6,7), Harris and Wall (13), and Eguiguren (12) show that the nematode can be effectively controlled by the use of various chemicals. Chemical control methods, however, have been shown to be effective for only a single growing season.

Acosta and Negron (1), and Narayana and Reddy (17) studied M. incognita resistance in some tomato cultivars. Although resistance to M. incognita was found in some cultivars, none of those with resistance to the root-knot nematode were satisfactory packing tomatoes. Araujo et al. (2) and Bost et al. (8) suggested that resistance is caused by one simple dominant gene, and that when cross-breeding occurs, the F1 hybrid will segregate at the standard 3:1 ratio.

Wergin and Orion (26) studied the life cycle of M. incognita with the scanning electron microscope. They observed that females begin egg production within four weeks after inoculation.

Research done by DiVito et al. (10), Khan et al. (15), and Sidhu et al. (22) demonstrated that the presence of Meloidogyne spp. in tomato plants will reduce yields and may predispose plants to invasion by other disease organisms.

Araujo et al. (3) and Edongali et al. (11) demonstrated that the optimum temperature for growing tomatoes is the same as that for reproduction of M. incognita. Also, Taylor and Sasser (25) found that hatch rates declined as soil became drier. Prot and Van Gundy (20) found that soil texture was an important factor in the migration of root-knot juveniles. More migration by a larger percentage of the larvae occurred in the 95% sand-5% clay mixture than in mixtures with a higher percentage clay.

The effect of ascorbic acid on root penetration by M. incognita was studied by Arrigoni et al. (4), who found that at higher concentrations of ascorbic acid in the roots, a susceptible plant becomes resistant. However, at levels high enough to achieve resistance, the plant was stunted, thus reducing yields.

Research was conducted by Spiegel et al. (23,24) on nutritional levels in the roots and in soil with regard to nitrogen and potassium levels. They showed that neither root nutritional level nor nitrogen source had any effect on penetration of root-knot nematodes. However, higher levels of N and K in the soil were correlated with a decrease in the rate of increase of root-knot nematodes. Also, roots infected with root-knot nematodes had a higher K content, apparently due to inhibition of K transportation from roots to leaves.

From a review of all pertinent literature, it appears that no research has been done to determine normal fluctuation of larval counts as they relate to sampling time.

CHAPTER III

MATERIALS AND METHODS

Five tomato fields in Cocke County, known to have infestayion of Meloidogyne incognita, were chosen for the study of population fluctuations of the parasite over time.

A ten meter long, six-row wide plot was marked by flags in each field so the same area would be sampled each time. Each plot was sampled monthly for fourteen months. Samples were taken as close to the same time of the month as weather conditions and time would permit.

Recommended sampling procedures, as outlined in "Nematode Sampling Analysis," Form 664, revised May 1980 (see Appendix A), were used. Sampling was done in the row, between each plant, with a soil sampling tube to a depth of 25 to 30 cm. The soil cores were collected in a plastic bucket, mixed thoroughly, placed in a plastic bag and mailed to The University of Tennessee Nematology Laboratory immediately for assay.

A centrifugal-flotation method (14) was used to extract juvenile root-knot nematodes from each sample.

The soil samples were mixed thoroughly and three 100 cm^3 samples were taken from each field sample for nematode extraction. Water was added to bring the contents up to 800 ml. After stirring, the slurry was decanted through an 80-mesh sieve over a 400-mesh sieve.

Residue from the 400-mesh sieve was rinsed into a centrifuge tube and centrifuged at 1550 rpm for four minutes. The supernatant was discarded and the pellet was resuspended with a sucrose solution (454 grams/liter H_2O), then centrifuged again at 1550 rpm for one minute. The sucrose supernatant was decanted onto a 500-mesh screen and rinsed into a counting dish.

Counts obtained from each of three subsamples were averaged to get the mean density for each sample. Raw data appears in Appendices B, C, D, E, and F.

For plotting on graphs, all data were transformed to $\log_{10}(x + 1)$.

CHAPTER IV

RESULTS AND DISCUSSION

Sampling Time

Root-knot nematodes in tomato take many dollars of profit from farmers each year. This loss can be reduced by changing certain grower practices.

Farmers have habitually waited until spring to take samples for nematode density determination. There are several reasons for this spring sampling. Farmers are often uncertain whether they will grow tomatoes the next year at the completion of a current year's crop. In addition, much of the land used for tomato production is leased from land owners by other growers, who may or may not know until winter or spring what land will be available.

One objective of this study was to determine the best time for soil sampling for root-knot nematode.

Normally, tomato plants are transplanted to the field from mid-April to mid-May. These tomatoes will go to market from early July until mid to late August. The fields sampled in this study would fall into these categories.

The population dynamics of M. incognita over a fourteen month period in the five fields used in this study are shown in Table 1. The population curve for the larvae in each field follows the same basic pattern. A relatively high density is present in late

Table 1. Relationship of Sampling Date to Density of Meloidogyne incognita in Tomato Fields.

Field		1983 Feb	Mar	Apr	May	June	July	Aug	Sept	Oct	Nov	Dec	1984 Jan	Feb	Mar
A	Mean	373*	55	7	1	27	3681	4307	2396	2465	2396	2903	2374	120	44
	Range	272-	44-	0-	0-	20-	3150-	2674-	2088-	2370-	2283-	2534-	2052	60-	20-
		480**	76	12	4	32	4200	5728	2630	2582	2477	3384	2614	176	88
B	Mean	3667	573	260	132	5	2087	2635	4839	3939	3226	2266	1681	888	504
	Range	2912-	512-	188-	4-	0-	1874-	2230-	4448-	3870-	3102-	2036-	1486-	800-	408-
		4112-	632	392	276	8	2236	2976	5320	4012	3392	2472	1888	1048	608
C	Mean	976	291	8	128	4	883	1795	3702	2123	1764	1255	1827	1221	917
	Range	864-	248-	0-	96-	0-	496-	1432-	3120-	1990-	1496-	1032-	1408-	944-	400-
		1072	360	20	144	8	1504	2128	4026	2270	1925	1372	2168	1464	1824
D	Mean	3597	2033	281	995	84	3333	1893	3773	3036	1972	1377	215	1227	133
	Range	3520-	1776-	96-	512-	56-	1988-	1552-	3470-	2934-	1805-	568-	120-	696-	72-
		3696	2284	492	1424	136	5210	2384	4238	3114	2213	1804	280	1824	216
E	Mean	10	23	0	1	12	27	411	438	317	124	85	23-	16	45
	Range	6-	12-	0-	0-	4-	16-	320-	144-	218-	102-	32-	18-	12-	24-
		12	36	0	4	24	48	544	772	430	146	168	32	20	80

*Each number represents the number of Meloidogyne incognita larvae/100 cm^3 soil and is the mean of three replications.

**Each set of numbers represents the range of Meloidogyne incognita larvae/100 cm^3 subsample.

winter with a reduction in numbers about the time spring plowing is done. These numbers remain low through transplating time and during the initial growth of the tomato plant. A dramatic increase occurs during the harvesting period of July and August and remains high throughout September, October, and November. After the occurrence of the colder weather of winter, a reduction in number of larvae began to appear.

Soil Suitability

Meloidogyne incognita increased during the growing season in all soil types. Some variation in the rate of increase and in the total increase occurred.

Fields A, B, and D were sandy loam soils and were very similar in texture. Soil from Field C contained considerably more clay than Fields A, B, and D. Field E was a sandy loam soil that was much more cohesive in structure and was not as well drained than the other fields.

In Fields A. B, and D, a rapid increase in root-knot nematode larvae populations during the harvest season of July and August (Figures 1, 2, 4) was observed. The rate of increase was not as rapid in Field C (Figure 3).

Nematode population changes in Field E (Figure 5) differed greatly from the other fields. Even though root-knot nematode larvae were found throughout the period from February to June, the rate of population increase was much slower and never reached the high levels of any of the other fields. This finding suggests that some

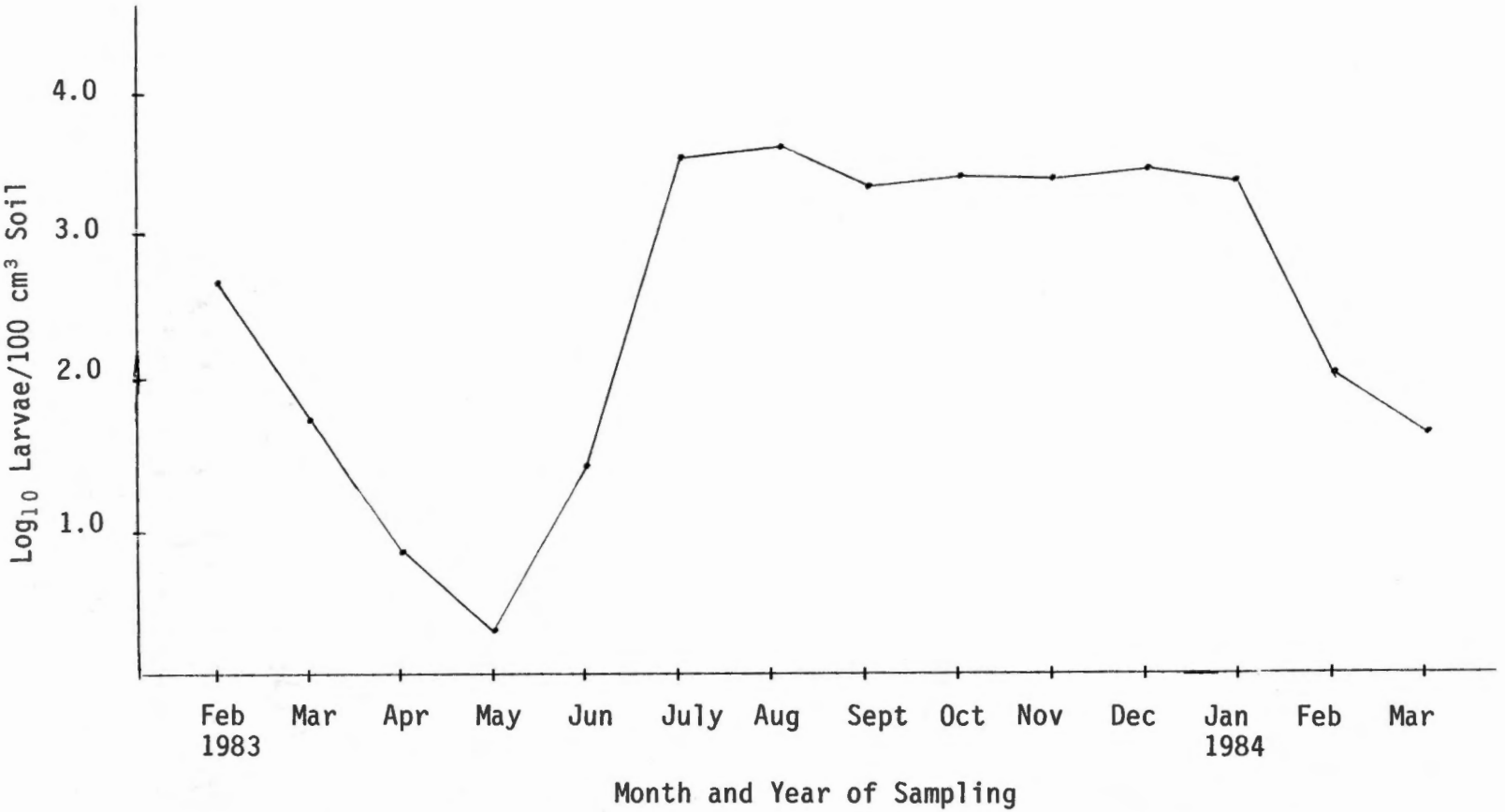

Figure 1. Relationship of Sampling Date to Density on Meloidogyne incognita in Tomato Field A.

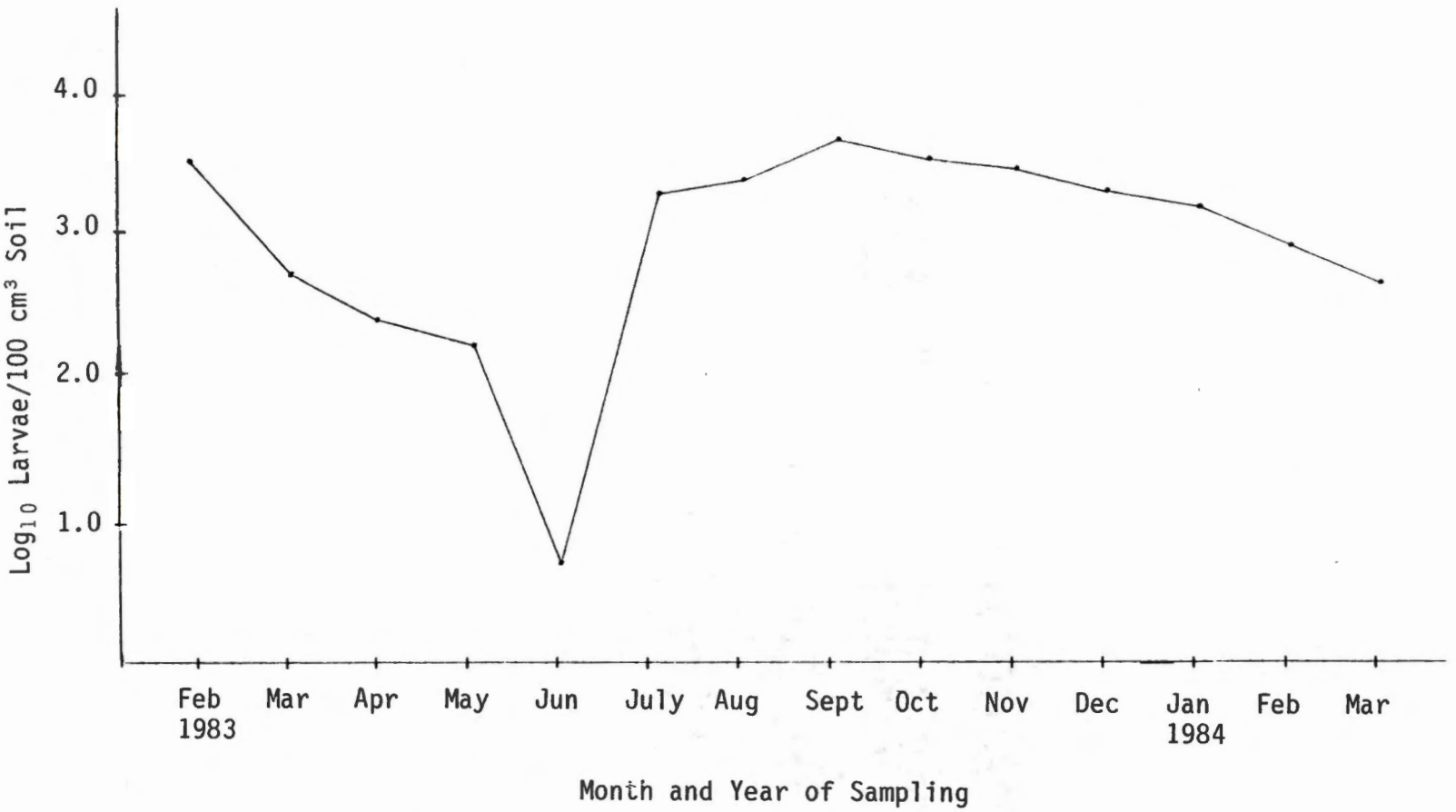

Figure 2. Relationship of Sampling Date to Density of Meloidogyne incognita in Tomato Field B.

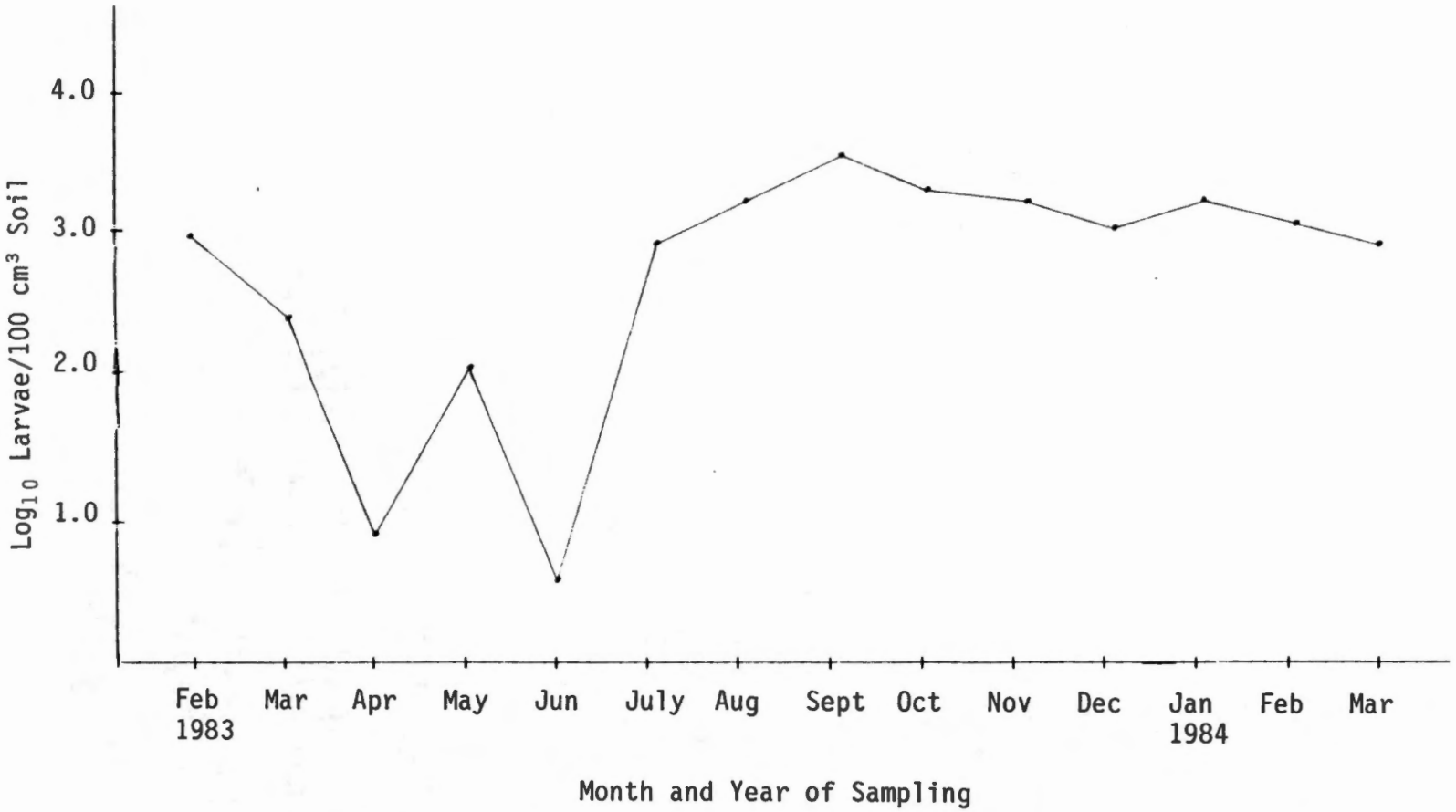

Figure 3. Relationship of Sampling Date to Density of Meloidogyne incognita in Tomato Field C.

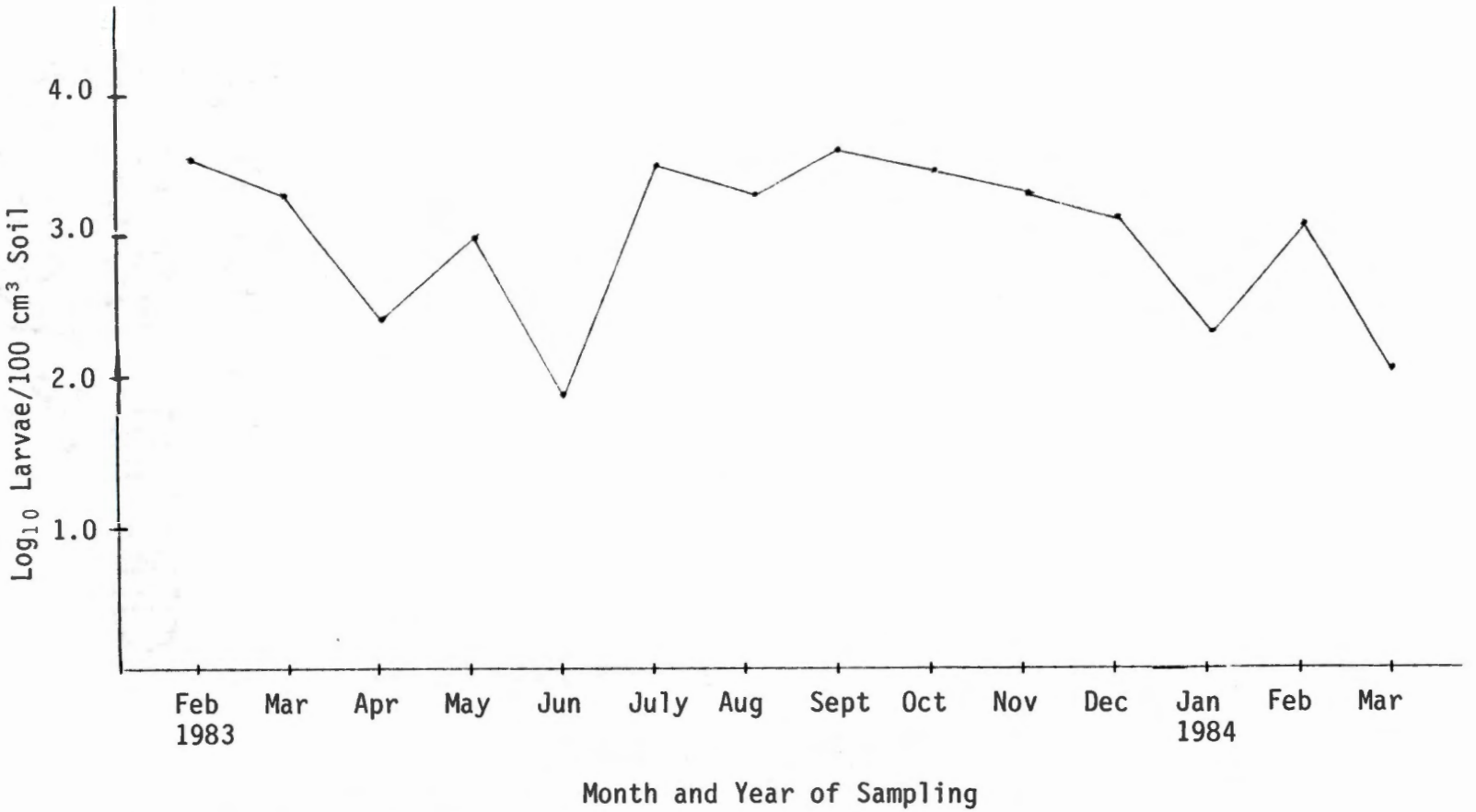

Figure 4. Relationship of Sampling Date to Density of Meloidogyne incognita in Tomato Field D.

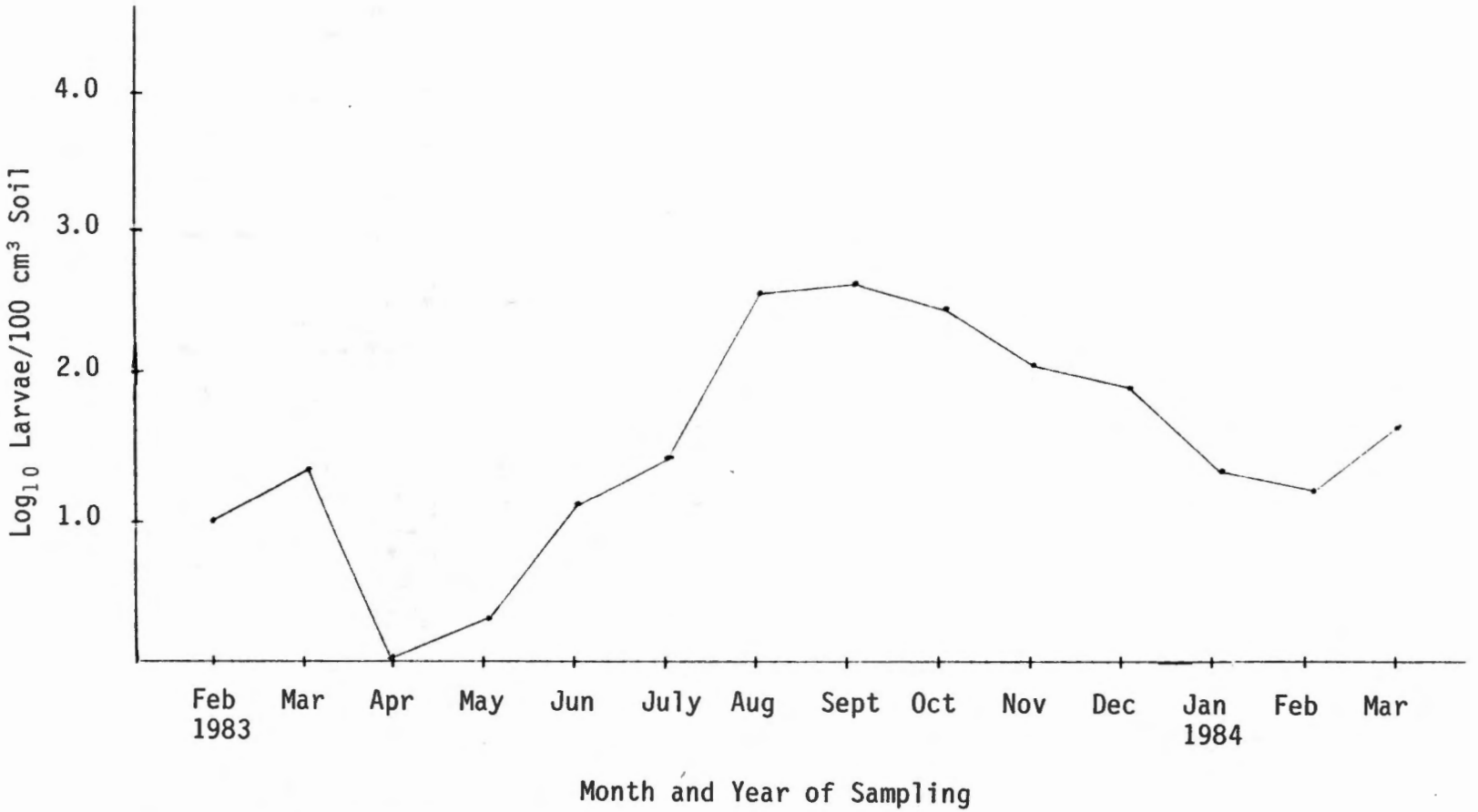

Figure 5. Relationship of Sampling Date to Density of Meloidogyne incognita in Tomato Field E.

soil factor was present that lessened the ability of the nematodes to reproduce and increase rapidly.

All of the fields had densities of root-knot nematodes that would cause economic losses to tomato growers if control measures were not taken.

Declining Populations of Root-knot Nematode

Decline in the populations of Meloidogyne incognita in tomato soils appeared to be a two-stage decline. In most cases (Table 1, Figures 1, 3, and 5) there was a definite decline immediately following the growing season, a leveling off in late fall and early winter, then another sharp decline in late winter. In Field B (Figure 2), the decline was rather steady from the end of the growing season through the winter.

CHAPTER V

SUMMARY AND CONCLUSIONS

Root-knot nematode (Meloidogyne incognita) infects tomato (Lycopersicon esculentum) root systems in large numbers. The soil remains infested with larvae the entire year, with the largest number of larvae present during the growing season.

To determine the potential for nematode reproduction in a crop the following year, fields should be sampled immediately after the harvest or during the latter part of the production season.

This study demonstrates that a dramatic increase in populations of larvae occurs during July and August, indicating that samples taken at times other than these could well give a false estimate of potential nematode populations, because many larvae have been killed over winter.

Spring samples had a much reduced larval count. It may be that much of the spring inoculum comes from overwintered eggs. Additional research is needed to correlate fall larval counts with spring egg counts before a reliable determination can be made concerning potential root-knot nematode populations from samples taken in the spring.

This study found that one soil appeared to repress the reproduction and increase of nematodes over the growing season. Even though root-knot nematode larvae counts were very similar in

spring samples, the increase in populations over the growing season was much less in Field E (Figure 5) than in the other fields. More research needs to be done to find whether this is due to soil type or whether some other factor is present in certain soils that prevent or depress the reproduction of the root-knot nematode. A study done by Prot and Van Gundy (29) found that soil texture may play an important role in the ability of nematodes to reproduce and migrate to plant roots. In addition, nematodes decreased rapidly following the growing season, usually in two separate stages, and densities remained low until fields were replanted in the spring of the year.

In conclusion, nematode counts in this study increased through the growing season, and diminished thereafter. The greatest decrease was noted in mid-winter. Therefore, it is recommended that samples be taken near the end of the growing season and into the middle of winter in Cocke County and in other similar situations. It also suggests that some soils in Cocke County, Tennessee, are conducive to root-knot nematode larval increase while one soil was suppressive.

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APPENDICES

APPENDIX A

TAKING SOIL SAMPLES FOR NEMATODE ANALYSIS

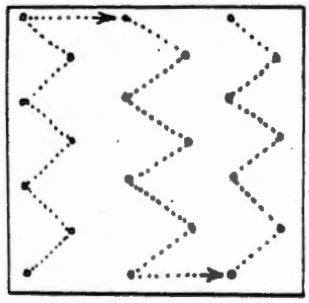
When to Sample: Soil and root samples may be taken whenever the soil is not frozen. In Tennessee, samples should be taken at least 45 days after root growth begins, up to the time of the first frost.

How to Sample: Soil should be sampled to a depth of 10-12 inches, and should contain as many feeder roots as possible. Take the sample when soil is moist. It should not be too wet or too dry.

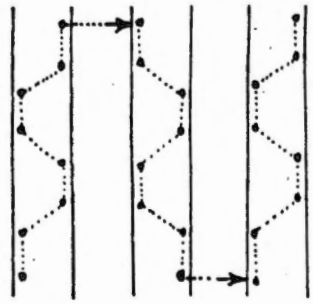
Each sample should consist of a pint to a quart of soil taken from a larger sample composed of 10 or more subsamples. The number of subsamples depends on the size of the area to be sampled; but always take at least 10 subsamples. For fields of an acre or more in size, 15 or more subsamples are desirable.

Since plant-parasitic nematodes feed only on living tissues, soil and root samples should be taken from *margins* of a *problem area*. Other situations should be sampled according to the following diagrams:

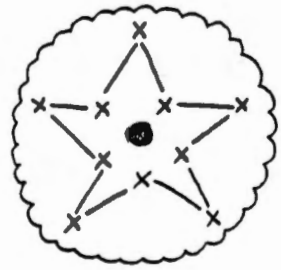
FALLOW FIELDS
COVER CROPS



ROW CROPS



TREES



Samples should be placed in a *plastic bag* (double bagging is best) and sealed with a rubber band, twistum, or by tying; they should be sent immediately to the Extension Service office. If you send more than one sample, mark the sample number and field on the bag. They should not be allowed to dry out, nor be exposed to direct sunlight or stored in automobile trunks. Temperatures greater than 40° C. (100° F.) will kill nematodes.

Fill out the nematode analysis form completely and place in a separate envelope to prevent moisture from destroying the form, and mail to:

Dr. Melvin Newman
Entomology & Plant Pathology
Agricultural Extension Service
605 Airways Boulevard
Jackson, Tennessee 38301

Dr. Charles Hadden
Entomology & Plant Pathology
Agricultural Extension Service
Post Office Box 1071
Knoxville, Tennessee 37901

APPENDIX B

Table 2. *Meloidogyne incognita* Larvae/100 cm^3 Soil in Field A.

	1983 Feb	Mar	Apr	May	June	July	Aug	Sept	Oct	Nov	Dec	1984 Jan	Feb	Mar
Subsample 1	272	44	0	0	20	3150	2674	2088	2370	2283	2534	2052	60	20
Subsample 2	366	44	8	0	28	3692	4520	2472	2444	2427	2790	2456	124	24
Subsample 3	480	76	12	4	32	4200	5728	2630	2582	2477	3384	2614	176	88
Mean	373	55	7	1	27	3681	4307	2397	2465	2396	2903	2374	120	44

APPENDIX C

Table 3. Meloidogyne incognita Larvae/100 cm^3 Soil in Field B.

	1983 Feb	Mar	Apr	May	Jun	July	Aug	Sept	Oct	Nov	Dec	1984 Jan	Feb	Mar
Subsample 1	2912	512	188	4	0	1874	2230	4448	3870	3102	2036	1486	800	408
Subsample 2	3976	576	200	16	8	2150	2700	4750	3936	3183	2290	1670	816	496
Subsample 3	4112	632	391	276	8	2236	2976	5320	4012	3392	2472	1888	1048	608
Mean	3667	573	260	132	5	2087	2635	4839	3939	3226	2266	1681	888	504

APPENDIX D

Table 4. *Meloidogyne incognita* Larvae/100 cm^3 Soil in Field C.

	1983 Feb	Mar	Apr	May	Jun	July	Aug	Sept	Oct	Nov	Dec	1984 Jan	Feb	Mar
Subsample 1	864	248	0	96	0	496	1432	3120	1990	1496	1032	1408	944	400
Subsample 2	988	264	4	144	4	648	1824	3960	2110	1872	1360	1904	1256	528
Subsample 3	1072	360	20	144	8	1504	2128	4026	2270	1925	1372	2168	1464	1824
Mean	975	291	8	128	4	883	1795	3702	2123	1764	1255	1827	1221	917

APPENDIX E

Table 5. *Meloidogyne incognita* Larvae/100 cm^3 Soil in Field D.

	1983 Feb	Mar	Apr	May	Jun	July	Aug	Sept	Oct	Nov	Dec	1984 Jan	Feb	Mar
Subsample 1	3520	1776	96	512	56	1988	1552	3470	2934	1805	568	120	696	72
Subsample 2	3576	2040	256	1048	60	2800	1744	3612	3060	1897	1760	244	1160	112
Subsample 3	3696	2284	492	1424	136	5210	2384	4238	3114	2213	1894	280	1824	216
Mean	3597	2033	281	995	84	3333	1893	3773	3036	1972	1377	215	1227	133

APPENDIX F

Table 6. *Meloidogyne incognita* Larvae/100 cm^3 Soil in Field E.

	1983 Feb	Mar	Apr	May	Jun	July	Aug	Sept	Oct	Nov	Dec	1984 Jan	Feb	Mar
Subsample 1	6	12	0	0	4	16	320	144	218	102	32	18	12	24
Subsample 2	12	20	0	0	8	16	368	398	302	124	56	20	16	32
Subsample 3	12	36	0	4	24	48	544	772	430	146	168	32	20	80
Mean	10	23	0	1	12	27	411	438	317	124	85	23	16	45

VITA

William Ray Stockdale was born in Faxon, Tennessee on March 2, 1937. He attended Lick Creek Elementary School and Big Sandy High School and graduated in May 1955. The following September he enrolled at The University of Tennessee, Martin. In June 1959 he received his Bachelor of Science degree in General Agriculture. He worked with the U.S. Department of Agriculture from June 1959 until December 1960. He was in the U.S. Army from December 1960 to December 1962. He returned to the U.S. Department of Agriculture upon completion of military service, and worked until April 1967. In May 1967 he began working in Cocke County, Tennessee with the Cooperative Extension Service as an assistant extension agent, and has worked there until the present time. He became a graduate student in February 1980 at The University of Tennessee, Knoxville and is presently a candidate for the Master of Science degree in Agricultural Extension.