STUDIES ON THE SURVIVAL OF TYLENCHID NEMATODES ASSOCIATED WITH ORGANIC SOD

Thesis for the Degree of M. S.
MICHIGAN STATE UNIVERSITY
Robert Akpan Itam
1968

THESIS

ABSTRACT

STUDIES ON THE SURVIVAL OF TYLENCHID NEMATODES
ASSOCIATED WITH ORGANIC SOD

by Robert Akpan Itam

Fourteen genera of plant nematodes were observed for occurrence and vertical distribution in Merion grass grown on organic soil. The effect of sod heating on 6 of these was studied at 3 ranges of temperature namely: 60-104°F for 37 hours; 60-114°F for 50 hours; 68-117°F for 36 hours. Comparisons of extraction techniques and nematode occurrence in organic and mineral sod were also studied.

Merion Kentucky bluegrass sod grown on mineral soil contained larger numbers of genera and individual tylenchid nematodes than in sod raised on organic soil. Heat accumulation in merion sod stacks to 115°F or higher signified the maturation within the stacks of conditions lethal to most of the associated tylenchid nematodes, including *Aphelenchoides* sp., *Aphelenchus* sp., *Ditylenchus* sp., and *Tylenchus* sp. Populations of Pratylenchus and pre-adult Paratylenchus survived merion sod heating beyond 115°F.

These findings indicate that practically all tylenchid nematodes shipped in organic merion sod would survive the heat development in the sod stacks during the first day of shipment. As the stack period exceeded 24 hours and the temperature rose above 104°F, the effect of "sod-heating" would become increasingly harmful to both the nematodes

and the grass. A more precise knowledge of nematode species involved and heat development within sod stacks is needed before the necessity of control measures can be assessed.

STUDIES ON THE SURVIVAL OF TYLENCHID NEMATODES

ASSOCIATED WITH ORGANIC SOD

By

Robert Akpan Itam

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Entomology

1968

ACKNOWLEDGEMENTS

My thanks are due to the Michigan State Department of Agriculture for suggesting this project; and to Dr. G. E. Guyer, Chairman of the Department of Entomology, Michigan State University, for providing the opportunity, facilities, and support for the research. Special thanks are also due to the Proprietor and Staff of the Halmich Sod Nurseries, East Lansing, Michigan, for supplying the sod samples utilized in the investigation.

I am indebted also to all the Professors on my Thesis Committee for their interest and close supervision. My gratitude goes to Dr. E. C. Martin, Chairman of the Committee, for many helpful hints, and for the photographs; and to Dr. J. B. Beard, who made available all relevant information on turf science, research, and industry; to Dr. J. A. Knierim, my Major Professor, for guidance and technical assistance; and to both Dr. J. Bath and Dr. P. H. Wooley, for useful comments on the purview of the investigation, and for information on the principles of scientific rhetoric.

I also owe my gratitude to Mrs. Natalie Knobloch, Technician in Nematology, for help in the identification of specimens; to Mr. John King, for his preliminary data on sod-heating; and to Dr. D. L. Haynes and Mr. W. G. Ruesink, for help in the statistical analysis of results.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

The importance of the Turfgrass industry to American economy, as measured in terms of maintenance expenditures, has been estimated recently at $4.3 billion per annum (Nutter, 1965). An estimate of the farm value on a national scale has not been made; but the total acreage under commercial sod production for the year 1965 was 48,000, and 95,000 for 1967 (Beard, 1967). The corresponding 1966 figures for the State of Michigan were: $149,133,200 for the annual maintenance cost (Beard & Hoglund, 1966), with 19,546 acres and 22,000 acres under cultivation in 1966 and 1967 respectively. The farm value to Michigan sod growers in 1966 was $26,200,000 (Beard, 1967). These figures are the highest for any single State in the United States. Sixty-five percent of Michigan's sod in 1966 was raised on organic soils and 35 percent on mineral soils; the total value of the industry, including growing, shipping, and laying, being $73,360,000 (Beard, 1967). The industry is currently ranked as the fastest growing industry, and the fifth leading agricultural industry, in this State (Beard, 1967).

The foregoing statistical evaluation of the American turf industry, impressive though it is, does not reveal the probable role of sod in the dissemination of harmful nematodes that destroy our food and ornamental crops. Several species of pathogenic nematodes are already known to possess host ranges that include most of the turfgrass species grown on mineral soils. For example the stubby-root nematode, *Trichodorus christei* Allen has over 100 potential host plants, including grains, legumes, vegetables, ornamentals, and turfgrasses (Rhode *et al.*, 1957; Coursen *et al.*, 1958). It is therefore presumable that mineral sod is involved in the dispersal of these destructive nematodes. Such a role cannot, however, be readily inferred for organic sod, owing to the lack of information on the identity and biology of the nematodes that are supported by sodgrasses produced on organic soils. Moreover, for organic as well as mineral sod, it is yet to be experimentally established that associated sod nematodes survive the metabolic heat which develops within the stacks of harvested sod rolls.

The present studies are therefore an attempt to elucidate some of these unknowns as they relate to organic sod. The objectives were twofold: (i) to identify some of the tylenchid nematodes associated with muck sod; and (ii) to determine, by means of laboratory experiments, which of these nematodes would likely survive the heat build-up in sod-stacks. Certain parallel studies were also conducted with mineral sod samples, for purposes of comparison. In addition, a study of the vertical distribution of muck sod nematodes was also carried out to help in establishing the validity of the results and conclusions.

LITERATURE REVIEW

Existing knowledge of the occurrence and biology of turf nematodes pertains particularly to nematodes associated with turfgrasses grown on mineral soils. Similar information on nematodes associated with turf grown on highly organic soils, like muck, is not available. In relation to nematode occurrence in mineral sod, Goodey (1933) studied gall formation on the leaves of fine bentgrass, *Agrostis tenuis* Sibth., by the seed-gall nematode, *Anuguina graminophila*. In the United States, the first published work, which documented the parasitic association of nematodes with turfgrass, appeared in 1951. Tarjan *et al*. (1951) investigated the parasitic involvement of *Panagrolaimus rigidus* and *Eucephalobus oxyuroides* in the yellow tuft disease of bentgrass, *Agrostis* sp. Eleven years later, Rhoades (1962) listed 21 genera and species of nematodes known to be pests of turf in the United States; but Taylor's (1962) critical analysis of the situation showed that valid experimental evidence for pathogenicity existed for only seven of these. A tentative estimate of the figures today indicates that more than twice as many parasites and pathogens have been established. The newly added pathogenic species include: *Hypsoperine graminis*, (Dickerson, 1966; Minton *et al*., 1967); *Meloidogyne incognita acrita*, (Gaskin, 1965; Hodges *et al*., 1963); *Pratylenchus scribneri*, (Minton, 1965); *Panagrolaimus* sp., (Pepper, 1965); *Heterodera leuceilyma*, (Perry, 1965); *Belonolaimus longicaudatus*, (Thoades, 1962); *Trichodorus* christei, (Rhoades, 1962); Trichodorus proximus, (Rhoades, 1965); and Ditylenchus radicicola, (Smithson et al., 1963).

Rhoades' (1962) list of turfgrass nematodes shows that parasitic mineral sod nematodes belong to two taxonomic orders: Tylenchida and Dorylaimida; the greater majority of them being tylenchids. Couch's (1962) account of turf diseases caused by nematodes shows that each turfgrass species is usually associated with more than one of these worms. For instance, Kentucky bluegrass (Poa pratensis L.), the species selected for the present investigation, is associated with no less than seventeen parasitic tylenchids. Five of these have been proven to be pathogens: -- viz., three species of Helicotylenchus which cause the 'summer dormancy' disease of Kentucky bluegrass, H. digonicus, H. microlobus, and H. pumilus (Perry, 1959); an unidentified species of Panagrolaimus which has been connected with the 'melting-out' disease of the bluegrass (Pepper, 1965); and the grass root-knot nematode, Meloidogyne incognita acrita Chitwood (Gaskin, 1965). Other parasitic species which have been associated with this turfgrass include, Tylenchorhynchus maximus Allen, T. nudus Allen, T. dubius Butschli, Helicotylenchus platyurus Perry, Pratylenpratensis (de Man) Filipjev, Heterodera trifolii Goffart, Xiphinema americanum Cobb, Hoplolaimus coronatus Cobb, and Meloidogyne hapla Chitwood, (Perry, 1959); Trichodorus christei Allen, (Rhode et al., 1957); Paratylenchus projectus Jenkins, (Coursen et al., 1958b); and a species of Aphelenchus (Pepper, 1965).

The ecological life of plant parasitic nematodes, as recently reviewed by Wallace (1963), is under the influence of a complex interplay of various environmental factors: -- including, the biotic, physical, and chemical factors of the soil environment; the host-plant factor; cropping practice and history; the original native vegetation of the soil; climatic factors; and factors due to the inherent specific differences of the nematodes themselves. For this reason, Wallace points out, it is usually difficult to relate cause and effect in many field and laboratory studies of the ecology and biology of plant nematodes. *Heterodera schachtii*, for example, is absent from the very acid peaty soils in the Fen district of England (Petherbridge & Jones, 1944). *Heterodera rostochiensis*, on the other hand, is particularly abundant and active in these same areas of the Fen district (Wallace, 1960). Some authors find no relationship between soil type and nematode occurrence; while others suggest that there is an association between soil type and the distribution of some nematode species (Wallace, 1963). For example, Caveness (1957) studied the nematodes associated with sugar beet production in some northwest and north central States of the U.S.A. He found no evidence for an association between any genus or species of parasitic nematodes and any particular soil type. Sasser (1954), on the other hand, found that infestations of *Meloidogyne incognita*, *M. incognita acrita*, and *M. hapla* were more severe in the sandy loam soils than in the heavy clay soils of Eastern Maryland.

Laboratory studies of the temperature relations of plant nematodes have also been shown to present similar complications, because the host plant itself is affected by the temperature treatment. For example, Krusberg (1959) found that the optimum temperature for reproduction of *Tylenchorhynchus claytoni* was 21 to 27°C on wheat and 29 to to 35°C on tobacco. Blake (1962) showed that reproduction of *Ditylenchus dipsaci* in oats was greater at 8°C than at 15°C.

Other factors which have been shown to influence the temperature relations of plant parasitic nematodes are those introduced by the nematodes themselves; -- e.g. developmental stage, age, and physiological state. Thus, Ditylenchus dipsaci is more resistant to low temperatures (Bosher & McKeen, 1954) and to high temperatures (Courtney & Latta, 1934) when the preadult stage -- i.e. the fourth stage larva -- is in the dry quiescent state. Sherman (1934) and Cairns (1953) also showed that this stage is more resistant to heat than other stages of the nematode. Similarly, Rhoades and Linford (1961) found that the preadult stage of Paratylenchus projectus could survive sudden exposure to low temperatures (about $-19^{\circ}C$) better than other stages.

The thermal death curves of different plant nematodes also exhibit a wide variation between species of the same genus (Wallace, 1963). Apart from factors stemming from intrinsic differences between species, much of this variation has been traced to one essential factor: differences in the heat penetrability of the medium or tissue in which the tested nematode is enclosed (Staniland, 1950; Blake, 1961). This variation notwithstanding, Wallace (1963) tentatively concludes that plant nematodes are probably killed instantly at $52^{\circ}C$ ($129^{\circ}F$), and that long exposures to temperatures above $40^{\circ}C$ ($104^{\circ}F$) may prove lethal to the nematodes. A recent exemplification of this conclusion by Heald and Wells (1967) showed that an infestation of Criconemoides sp., Hypsoperine sp., and Tylenchorhynchus sp. in cores of bermudagrass turf could be completely eradicated by hot water treatment at $55^{\circ}C$ ($131^{\circ}F$) for 15 minutes. In contrast with this, the optimum temperatures for population increase of five nematode species maintained on Tall Fescue host fall 10 or more degrees below the lethal temperature range of 104 to

129^{o}F, as follows: Hoplolaimus tylenchiformis, 77 to 84^{o}F; Helicotylenchus mannus, 70 to 94^{o}F; Tylenchorhynchus claytoni, 70 to 94^{o}F; Trichodorus christei, 63 to 94^{o}F; and Paratylenchus projectus, 63 to 77^{o}F (McGlohon et al., 1962).

There are at present no published observations on the subject of sod-heating per se, nor on its effects on any of the associated macroorganisms. Unconfirmed observations, however, seem to indicate that the lethal temperature to the turfgrass lies between 104^{o}F and 115^{o}F, (King and Beard's preliminary data, 1967). Sod farmers claim the first 24 hours after harvesting to be safe from harmful heating in the stack but the safe period probably varies widely, depending on the size of the sod-stack, the temperature and relative humidity of the atmosphere, and the metabolic rate of the microflora in the thatch and root zones.

MATERIALS AND METHODS

Sampling Procedures

Two forms of sod samples were employed in these investigations: i) freshly cut commercial sod strips of standard dimensions, 52 in. by 24 in. by 1 in., ii) 6-in. deep cores cut with a 4-in. diameter corer. Yates and Finney (1942) recommended the use of the 4-in. diameter corer as best for soil animals, since it maintains a relatively uniform sampling efficiency at low as well as at high animal population densities. Both types of samples were obtained from healthy merion Kentucky bluegrass turf farms within the State of Michigan. Five farms, maintained on muck soils, served as sources for the samples. One of these, the Halmich Sod Nurseries in East Lansing, provided all the samples for the stack-heating tests. Samples used for the preliminary temperature tests were obtained from the Green Acres Turf farm, Mason. The three remaining farms which provided samples for the survey investigations were Baldwin Sod Farms, Stockbridge, Emerald Valley Nurseries, Gregory and Reding's Sod Farms, Livonia. For a comparative study of nematode occurrence in mineral sod, samples were obtained from two other sources -- viz., Hiram F. Godwin & Son mineral sod farms, South Lyon; and the Horticultural garden at Michigan State University, East Lansing. Also, samples from the Battle Creek Golf Course, taken exclusively from the putting greens, supplied the inoculum of *Tylenchorhynchus* sp. used in one of the heating tests.

Samplings were made throughout most of Spring, Summer, and Autumn of 1967. The collection of samples was, as a rule, done in the evenings, when the average soil temperature, measured at a depth of one inch, varied from 44°F in May to 71°F in mid-summer, and down again to 43°F in October. Samples were collected in polythene bags; and were preserved in a cold chamber at 40°F when it was not possible to utilize them immediately. However, all samples were usually put to use within 48 hours after collection; otherwise they were discarded.

The pattern of collecting samples from the field was as follows: The sod strips were picked up from the field at random. The 6-inch deep cores were cut from various casually selected spots on the field, simply by walking across the farm diagonally and removing cores at intervals along the line. Similar methods of sampling were used by Tunstall and Matthews (1961) who estimated the level of occurrence of red bollworm eggs (*Diparopsis castanea*) by taking two diagonal traverses the field and counting the eggs at regular intervals.

A more systematic pattern of sampling was adopted in taking core samples from the harvested strips of sod. In the preliminary temperature tests, five adjacent rows of sod cores were sequentially removed from the entire surface area of a piece of sod strip. Thus, the results of each row served as a check on those of contiguous rows. For the sod-heating tests, two adjoining pairs of cores were drawn from the centre of each experimental piece of sod; one of each pair of cores serving as the control. Each experiment was repeated four times in the preliminary tests, and eight times in the sod-heating tests.

Extraction of the Nematodes

(i) The Christie-Perry Technique --

Nematodes present in sod samples used for the preliminary temperature tests were recovered by a modification of Christie-Perry's (1951) technique. Two operations were involved: wet sieving, and funnel filtration. Each core of sod was teased and washed into a plastic bucket of about 2.5 gallons in capacity. A jet of cold water, directed from a sprinkler, ensured a thorough rinsing of the grass shoots and roots which, after squeezing to expel the water, were discarded. Large clods of soil were broken, and more water added to fill about half the capacity of the bucket. The mixture was then thoroughly stirred; allowed to stand for about 30 seconds, and decanted through a 25-mesh sieve into a second bucket. In this way, sand and other heavy particles were retained in the bucket, while the lighter and larger organic debris were caught on the sieve. The filtrate in the second bucket was also stirred thoroughly, allowed to stand for 30 seconds, and then decanted through a 325-mesh sieve. The 325-mesh sieve, together with the residues, was set aside, and the whole screening process repeated, beginning with the 25-mesh sieve. Three such repetitions, making a total of four screening operations for each core of sod, were carried out. At the start of each repeat operation, the residues on the 25-mesh sieve were returned to the first bucket, half a bucket of water added, and the operation carried through to the 325-mesh screen stage. The total amount of residues on the 325-mesh sieve was then rinsed with water to eliminate all fine and silty matter.

The separation of nematodes from unwanted components of the residues was achieved by the funnel dynamic filtration technique. The residues were washed into a filtering apparatus made from a short length of thin walled metal tubing three inches in diameter and having a piece of muslin cloth fastened to the bottom with a rubber band. The filtering can, together with its contents, was then placed in a 6-inch Baermann funnel, and the latter filled with water so that only the lower part of the filtering can was submerged. The nematodes left the residues, filtered through the cloth, and fell to the bottom of the funnel to collect in a small test-tube attached to the stem of the funnel. The filtration process was continued for four days.

(ii) Centrifugal-Flotation Technique --

Jenkin's (1964) modification of the centrifugal-flotation technique, in combination with Christie-Perry's filtering technique, was used in extracting nematodes present in sod cores employed for the sod-heating tests. This procedure included a sieving operation exactly like that already described in the preceding section; differential flotation of the nematodes by the use of a solution of specific gravity greater than that of the nematodes and funnel filtration of the products of flotation, as a means of separating the nematodes from other matter of similar specific gravity. This last step was carried out in a manner similar to that already described under the Christie-Perry technique.

Differential flotation of the nematodes was achieved as follows: The products of the sieving operation were first shared equally between four 100-ml centrifuge tubes. Equal amounts of water were then added to the tubes to nearly fill them; after which, the tubes with their contents were centrifuged for five minutes at full speed. This threw down all solid matter, leaving a clear supernatant liquid in each tube. The liquid was carefully poured off and discarded, and a solution of sucrose sugar (1 lb. per litre of water) was added in its place. After thoroughly mixing the sugar solution and sediment the tubes were again centrifuged at full speed for 30 seconds. The supernatant suspension, containing the nematodes as well as other matter of similar density, was then filtered through a 325-mesh sieve. The residues were thoroughly rinsed with water to wash away the sugar, and then transferred to the muslin cloth filter. The nematodes wriggled through the cloth, leaving the debris behind, and collected below in a small test-tube connected to the stem of the funnel. Most of the nematodes were recovered within the first day but filtration was not terminated until after four days.

Evaluation of the Extraction Methods

The low numbers of tylenchid nematodes extracted from the sod cores, using Christie and Perry's modification of Baermann's technique (Table 3), made necessary a critical examination of the effectiveness of this extraction method. Christie-Perry's funnel residues were processed for unrecovered nematodes by the centrifugal-flotation method of Jenkins. Nematode counts showed that the degree of efficiency of the Funnel method varied with different genera (Table 1). The more active nematodes e.g. *Aphelenchoides* sp. and *Aphelenchus* sp., filtered through more successfully than sluggish ones such as *Paratylenchus* sp. and *Ditylenchus* sp.

Fig. 1.--Plastic cylinders as used for the dynamic filtration technique.

TABLE 1.--An evaluation of Baermann funnel extraction of muck-sod nematodes

Genera Observed	No. of Nematodes in Funnel Residues					Avg. in Fun. Extr.	Funnel Extract %
	Test 1	Test 2	Test 3	Total	Avg.		
Aphelenchoides	0	12	12	24	8	38	83
Aphelenchus	16	0	48	64	21	54	72
Ditylenchus	16	0	448	464	155	104	40
Heterodera larva	4	0	0	4	2	1	33
Paratylenchus	12	0	0	12	4	2	33

Jenkin's centrifugal-flotation technique gave generally higher yields of all nematodes (Table 2) but its usefulness was limited by the presence in the extract of large amounts of suspended debris. This hampered visibility, making nematode identification under the microscope difficult. The problem was, however, overcome by what amounted to a combination of the centrifugal-flotation method with Christie-Perry's funnel technique. As shown on Tables 2 and 9, 99 percent recovery was obtained using a paper filter and over 97 percent with muslin cloth filter. Furthermore no plasmolytic distortion of any of the nematodes was observed. On the basis of this high level of efficiency, the combined technique was considered reliable for determining the effects of the different treatments used in the present studies.

TABLE 2.--A combination of funnel filtration with the centrifugal flotation method, as a means of separating the nematodes from plant matter

Centrifugal Flotation Extracts	Funnel Filtrate[a] # Nematodes/Day 1	2	3	4	Total	Funnel Residues # Nematodes Live	Dead	Grand Total	% Funnel Filtrate
1st	850	260	250	119	1479	2	3	1484	99.7
2nd	220	25	26	20	291	0	1	292	99.7
3rd	68	17	5	7	97	0	1	98	99

[a]Paper filter (Kimwipes tissue paper, #1300).

Identification and Enumeration of Nematodes

Identification and enumeration were confined to Tylenchid genera only. Identification to the species level was done only in the case of the population of Tylenchorhynchus used in the inoculation tests. For this, permanent slide mounts of the nematode were prepared as outlined below.

In preparation for counting, the aqueous suspension of nematodes extracted from each core of sod was placed on a square counting tray made of glass, 1.75 in. by 1.75 in. The effective counting surface, measuring 1.5 in. by 1.5 in., was divided into four quarters by three engraved lines. The suspension was then uniformly dispersed over all four quarters of the tray and counts were taken from two proximate quarters -- an outer one and the next inner one. Both live and dead specimens were enumerated. A marginal embankment of solidified "Duragloss" finger-nail paint prevented spilling of the suspension. Estimated totals were obtained by doubling the actual counts.

Permanent slides of Tylenchorhynchus sp. for identification to the species were prepared according to the glycerol-ethanol method of Seinhorst (1959). The nematodes were first killed and relaxed by gentle warming over an alcohol flame and then fixed in Formol-Acetic (F.A. 4:10). Following this, the specimens were dehydrated through a mixture of glycerol and ethanol to pure glycerin. Mounting was then done in dehydrated glycerin in Cobb aluminium slides.

Statistical Evaluation of Data

Tabulation of the data obtained in terms of samplings and treatments were made for all experiments. Data from studies of occurrence and vertical distribution were analyzed simply as measures of the mean number of nematodes per core of sod for each observed genus. The six tylenchid genera which exhibited continuous occurrence were selected for a study of their temperature relations and detailed analyses of data were made for these. The six genera included Aphelenchoides, Aphelenchus, Ditylenchus, Paratylenchus, Pratylenchus, and Tylenchus. These genera obviously represented mixed populations.

For the purposes of this study, a population of nematodes must be defined as an individual species or a mixture of known or unknown species belonging to a single genus. Designations such as Tylenchorhynthus sp. or Aphelenchoides sp. indicate the complement of a population or mixture of populations in individual experiments. Except in the case of Tylenchorhynchus sp., mixed populations of the genera were involved in the temperature studies.

Numbers of nematodes in the pure and mixed populations varied widely between experiments and in the different samplings and treatments of individual experiments. As such, in estimating the number of Tylenchorhynchus sp. contained in subsamplings of the mass inoculum, a coefficient of variation (V) of about 17 percent was considered sufficiently accurate for these studies (Table 7). In a comparable situation, Church and Strickland (1954) accepted a coefficient (V) of about 25 percent as sufficiently accurate for estimates of insect population densities in which the insect species exhibited wide ranges of population fluctuations.

Two objectives were aimed at in the analysis of the data from temperature studies: i) to determine the significance of the differences in population counts between the controls and the treatments; ii) to bring out the comparative heat endurance of the six tylenchid associates of muck sod.

In testing the significance of the data, the arithmetic means of the controls and the treatments were compared in pairs for each of the four sets of experiments. This procedure freed the analysis of any dependence on the effect of differences in positions of the heated sod cores within the sod-stack. The t-statistic test for difference between two means was applied at the 0.05 level to test the null hypothesis (H_o) that no real difference in nematode numbers results from subjection to sod-heating and that the observed mean differences may be accounted for by mere sampling variability. Or, symbolically expressed, $H_o : (\bar{x}_1 - \bar{x}_2) = 0$.

EXPERIMENTS AND RESULTS

A. Preliminary Temperature Tests

The preliminary temperature tests were carried out to indicate the range of non-lethal temperatures above which the sod-heating experiments could be meaningfully performed.

Procedure -

Sixteen cores of sod, in four sets of four per set, were subjected to constant temperature treatment in a thermostatic oven. The first set was maintained at 70°F for 24 hours; the second set at 70°F for 48 hours; the third at 80°F for 24 hours; and the fourth at 80°F for 48 hours. A fifth set of four cores was processed immediately for nematodes, to serve as the control.

Each treated core of sod was suspended in a covered bottle. A small quantity of water in each bottle maintained maximum relative humidity around the sod core. The internal temperature of each core of sod was measured with a portable Rubicon potentiometer connected to the sod by a copper-tungsten thermocouple wire. Isolation of nematodes from the treated cores was done by the Christie-Perry funnel method.

Results and Conclusion -

The average nematode counts at both 70°F and 80°F, for the 24 hours' or the 48 hours' duration, exhibited no trend which could be related to the influence of temperature treatment (Table 3). Variations

TABLE 3.--Effect of heat on the survival of muck-sod nematodes

Genera	Number of Nematodes per Core of Sod																	
	Controls						Trmt. 1, 70°F; 24 hrs.						Trmt. 2, 70°F; 48 hrs.					
	I	II	III	IV	Total	Avg.	I	II	III	IV	Total	Avg.	I	II	III	IV	Total	Avg.
Aphelenchoides	2	0	2	5	9	2	3	0	0	0	3	1	0	0	0	0	0	0
Aphelenchus	16	0	0	0	16	4	0	0	0	0	0	0	0	0	0	0	0	0
Ditylenchus	0	0	0	0	0	0	3	0	0	0	3	1	0	0	0	0	0	0
Helicotylenchus	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0
Heterodera larva	22	14	11	1	48	12	1	0	0	4	5	1	1	0	0	0	1	0
Paratylenchus	4	0	0	2	6	2	0	0	2	0	2	1	0	2	0	0	2	1
Pratylenchus	6	0	1	3	10	3	2	0	0	0	2	1	0	1	1	0	2	1
Tylenchorhynchus	2	0	0	0	2	1	0	0	0	0	0	0	0	0	0	0	0	0
Tylenchus	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0

in counts between the control samples and the treated samples were such as could be readily attributed to sampling variability. It was therefore concluded that the range of non-lethal temperatures for these nematodes extended much beyond 80°F. Some support for this conclusion was found in the work of Courtney and Latta (1934) which showed that the non-lethal temperatures for inhibition of activity of *Ditylenchus dipsaci* and of certain species of *Heterodera* were 100.4°F and 95°F respectively. On the strength of these findings, it was decided to conduct the sod-heating tests at temperatures over 100°F.

B. Sod-heating Tests

The sod-heating tests were performed to observe the effect of sod-heating on the survival of the associated tylenchid nematodes. Altogether, four sets of experiments were carried out. In three of these, use was made of the indigenous population of tylenchid nematodes present in the cores of sod. With the fourth set, an introduced population of mineral sod tylenchids from bentgrass putting greens was observed instead.

Procedures -

A stack of 13 pieces of merion sod strips, each approximately 12 in. by 12 in., was set up within a styrofoam box 13 in. by 13 in. by 19 in. inside dimensions. A lid, also of styrofoam material, kept a loose closure of the opening at the top during the course of an experiment. Thermocouple wires, one to each piece of sod, were admitted through two side holes in the box. The fifth to the eighth strip of sod, numbering from the top, were selected for use as the experimental strips. At those levels in the stack, the strips of sod maintained a very narrow range of temperature difference between them, differing by only one or two degrees Fahrenheit. The entire structure of insulated sod-stack was then placed in an oven adjusted to maintain an air temperature of 100°F around the box. The temperature of each strip of sod in the stack was measured at 6-hourly intervals with the help of a potentiometer connected to the corresponding thermocouple wire.

In one set of tests, the stack of sod was allowed to heat up in 37 hours from a starting temperature of about 60°F to a finishing temperature of 104°F. A pair of control cores of sod was removed from each of the four experimental strips prior to stacking. In their places, four other pairs of spare cores of sod were put, so as to fill the gaps. At the termination of heating, four corresponding pairs of heated cores of sod were cut out of the experimental strips. All eight pairs of cores -- four controls and four treated -- were processed for nematodes by the combined centrifugal-flotation and funnel filtration technique.

In the second set of tests, the experimental strips of sod were incubated from about 60°F to 114°F; and the duration of heating was 50 hours. Other than these, the procedural details were the same as for the first set of tests.

In a third set of tests, the heating range was from about 68°F to 117°F and the duration of heating was 36 hours. This set of tests was significantly different from the first two, in that the surrounding oven air was preheated to 100°F before introducing the box of sod strips. This was a variation in experimental procedure meant to shorten the duration of sod-heating while maintaining a range of heating

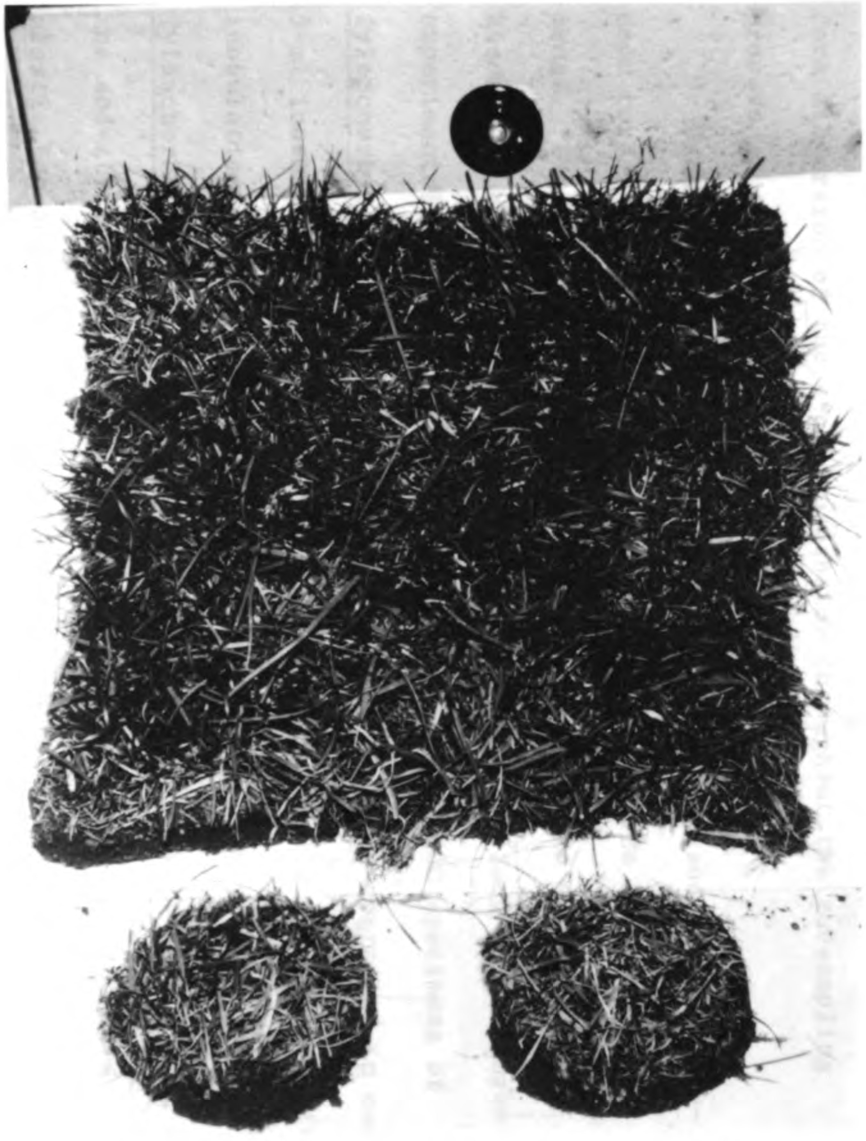

Fig. 2.--Four-inch diameter cores and standard dimension sod strip.

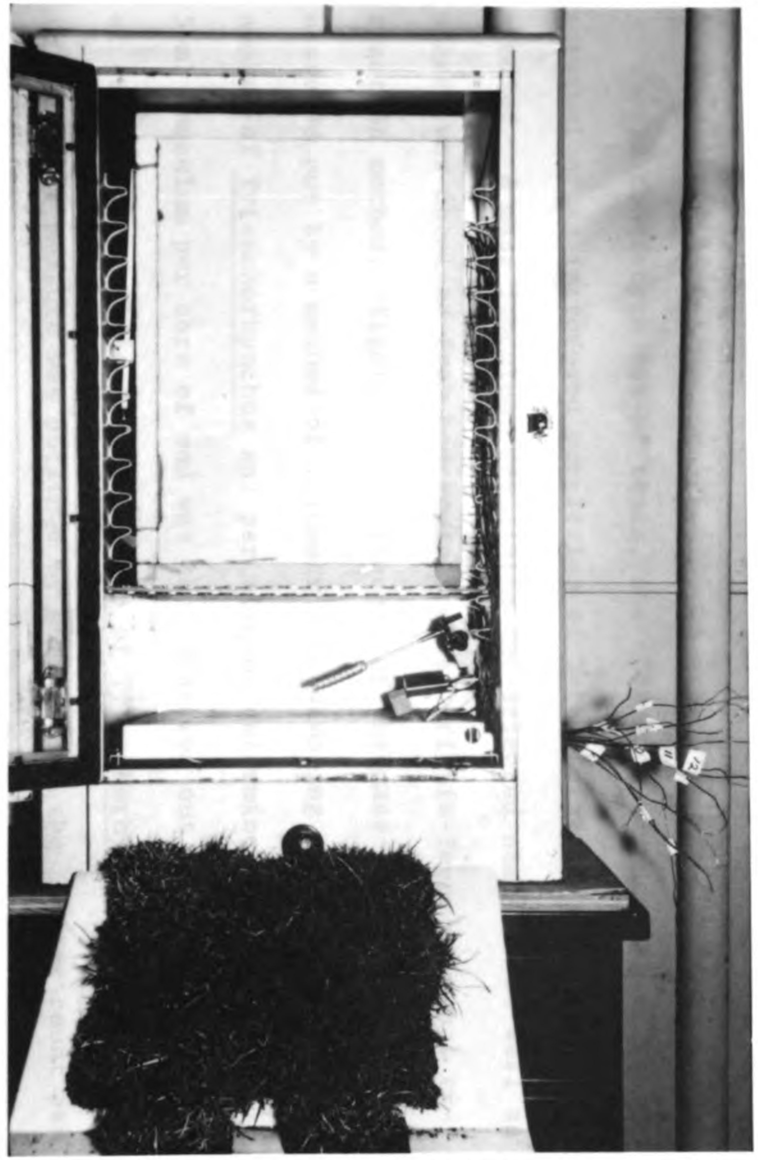

Fig. 3.--Heating oven and thermostatic controls.

temperatures comparable to that in the second set of tests. Other procedural details remained the same as for the first set of tests.

In the fourth set of tests, the effect of sod-heating on the survival of an introduced population of Tylenchorhynchus sp. was studied. A mass inoculum, containing the selected nematode as well as others was obtained from bentgrass sod by Christie-Perry's funnel extraction method. Eight 0.5 ml aliquots of the mass inoculum were measured out by a method of volumetric subsampling and the average number of Tylenchorhynchus sp. per aliquot determined (Table 7). A 5-ml inoculum per core of sod was then measured out by the same method of subsampling. The estimated number of Tylenchorhynchus sp. contained in each 5-ml measure was obtained by multiplying the average count per 0.5 ml aliquot tenfold. A magnetic stirrer was used to maintain uniform dispersion of the stock suspension throughout the subsampling process.

From each of the four experimental strips of sod, four contiguous cores were drawn -- one as the control and three for the treatments. The control core was processed immediately to determine the kinds and numbers of indigenous nematodes present. Each of the three experimental cores was mass inoculated with about 1500 specimens of Tylenchorhynchus sp., this being the estimated number contained in each 5-ml inoculum. One of these three cores was processed immediately after inoculation so as to give an estimate of the expected yield of Tylenchorhynchus sp. (Treatment 1); another was subjected to heating in the sod-stack, (Treatment 3); and the third core was enclosed in a plastic bag and set aside at 77^{o}F for the duration of the stack heating operation, (Treatment 2). These last two treated cores were processed for nematodes soon after treatment; the former expressing the effect of sod-heating on the nematodes and the latter indicating the influence of the new environment on them. The heating in the sod-stack lasted for 50 hours and increased from 76°F to 114°F.

Results of the Sod-heating Tests -

The results of the sod-heating tests showed, on analysis, that only *Tylenchus* sp. suffered a significant reduction in population numbers under the heat treatment of 60 to 104°F in 37 hours (Tables 4, 4A). *Aphelenchoides* sp., *Aphelenchus* sp., and *Ditylenchus* sp. were definitely not adversely affected by this treatment. Nor were *Paratylenchus* sp. and *Pratylenchus* sp., despite their low numbers in the different samplings.

When heated from 60 to 114°F in 50 hours, *Tylenchus* sp., *Aphelenchoides* sp., *Aphelenchus* sp., and *Ditylenchus* sp., in that order, suffered significant numerical losses. *Paratylenchus* sp. and *Pratylenchus* sp. were still unaffected by this degree of treatment (Tables 5, 5A). *Pratylenchus* sp. also survived the more intensive heat treatment of 68 to 117°F in 36 hours and although scanty, the data for *Paratylenchus* sp. indicated a similar survival ability for this nematode under the same conditions (Tables 6, 6A).

As regards the inoculation tests with *Tylenchorhynchus* sp., the results indicated a conclusive lethal effect of the tested level of sod-heating on this nematode (Table 8). The data also bore out the results obtained with indigenous sod nematodes under a similar heat treatment (Table 5). However, when the indispensable losses in numbers of *Tylenchorhynchus* sp. were taken into account, the significance of

TABLE 4.--Effect of sod-heating on the survival of muck-sod nematodes

		No. of Nematodes per Core of Sod[a]								
		Control				Treated				
Test	Genera	I	II	Total	Avg.	I	II	Total	Avg.	Heat Treatment
1	Aphelenchoides	284	346	630	315	530	296	826	413	62 - 104°F
	Aphelenchus	2	14	16	8	0	0	0	0	(37 hrs.)
	Ditylenchus	12	10	22	11	12	12	24	12	
	Paratylenchus	0	0	0	0	6	6	12	6	
	Pratylenchus	2	12	14	7	10	6	16	8	
	Tylenchus	46	44	90	45	0	6	6	3	
2	Aphelenchoides	508	156	664	332	322	166	488	244	61 - 104°F
	Aphelenchus	8	10	18	9	0	4	4	2	(37 hrs.)
	Ditylenchus	128	70	198	99	60	28	88	44	
	Paratylenchus	0	6	6	3	0	10	10	5	
	Pratylenchus	4	4	8	4	2	6	8	4	
	Tylenchus	32	38	70	35	2	2	4	2	

3	Aphelenchoides	1106	172	1278	639	240	1004	1244	622	61 - 104°F
	Aphelenchus	2	2	4	2	2	0	2	1	(37 hrs.)
	Ditylenchus	30	12	42	21	8	0	8	4	
	Paratylenchus	2	2	4	2	18	2	20	10	
	Pratylenchus	4	4	8	4	6	12	18	9	
	Tylenchus	18	14	32	16	2	2	4	2	
4	Aphelenchoides	110	580	690	345	332	338	670	335	60 - 104°F
	Aphelenchus	0	2	2	1	6	2	8	4	(37 hrs.)
	Ditylenchus	12	34	46	23	38	18	56	28	
	Paratylenchus	0	6	6	3	2	8	10	5	
	Pratylenchus	2	0	2	1	6	2	8	4	
	Tylenchus	24	64	88	44	2	0	2	1	

[a]Source: Halmich Sod Nurseries, E. Lansing, Mich.

TABLE 4A.--Analysis of the data of Table 4

	# Aphelenchoides			# Aphelenchus			# Ditylenchus			# Paratylenchus			# Pratylenchus			# Tylenchus		
Test	Contl	Trtd	d[a]	Contl	Trtd	d	Contl	Trtd	d	Contl	Trtd	d	Contl	Trtd	d	Contl	Trtd	d
1	315	413	-98	8	0	8	11	12	-1	0	6	-6	7	8	-1	45	3	42
2	332	244	88	9	2	7	99	44	55	3	5	-2	4	4	0	35	2	33
3	639	622	17	2	1	1	21	4	17	2	10	-8	4	9	-5	16	2	14
4	345	335	10	1	4	-3	23	28	-5	3	5	-2	1	4	-3	44	1	43
$S_{(d)}$	17			13			66			-18			-9			132		
$\overline{d}$	4.25			3.25			16.5			-4.5			-2.25			33		
V_d	5888.25			26.92			750.3			9			4.92			180.7		
$V\overline{d}$	1472.06			6.73			187.58			2.25			1.23			45.18		
$s\overline{d}$	38.32			2.59			13.71			1.5			1.11			6.72		
t	0.1109			1.25			1.2			3			2.03			4.9		
N	3			3			3			3			3			3		
$P_{(0.05)}$	>0.9			0.2-0.3			0.3-0.5			0.05-0.1			0.1-0.2			.01-.02		
H_o	Valid			Valid			Valid			Valid			Valid			Reject		

[a]d = difference between means; $S_{(d)}$ = sum of the differences; $\overline{d}$ = mean difference; Vd = variance of the difference; $V\overline{d}$ = variance of mean difference; $s\overline{d}$ = standard deviation of the mean; t = student's statistic; N = degrees of freedom; P = probability at .05 level of significance; H_o = null hypothesis.

TABLE 5.--Effect of sod-heating on the survival of muck-sod nematodes

		No. of Nematodes per Core of Sod[a]								
		Control				Treated				
Test	Genera	I	II	Total	Avg.	I	II	Total	Avg.	Heat Treatment
1	Aphelenchoides	1040	314	1354	677	8	0	8	4	60 - 113°F
	Aphelenchus	8	20	28	14	8	0	8	4	
	Ditylenchus	20	18	38	19	0	0	0	0	
	Paratylenchus	114	96	210	105	146	110	256	128	
	Pratylenchus	2	2	4	2	0	2	2	1	
	Tylenchus	84	24	108	54	0	0	0	0	
2	Aphelenchoides	242	286	528	264	0	0	0	0	61 - 113°F
	Aphelenchus	10	2	12	6	2	0	2	1	(50 hrs.)
	Ditylenchus	16	12	28	14	0	0	0	0	
	Paratylenchus	16	38	54	27	88	94	182	91	
	Pratylenchus	0	2	2	1	0	0	0	0	
	Tylenchus	62	58	120	60	0	4	4	2	

3	Aphelenchoides	568	1006	1574	787	0	2	2	1	62 - 113.6°F
	Aphelenchus	20	2	22	11	6	2	8	4	(50 hrs.)
	Ditylenchus	14	6	20	10	0	0	0	0	
	Paratylenchus	108	28	136	68	90	70	160	80	
	Pratylenchus	10	0	10	5	0	2	2	1	
	Tylenchus	30	56	86	43	0	0	0	0	
4	Aphelenchoides	460	1420	1880	940	0	8	8	4	62 - 113.6°F
	Aphelenchus	12	20	32	16	10	8	18	9	(50 hrs.)
	Ditylenchus	56	14	70	35	0	0	0	0	
	Paratylenchus	14	70	84	42	106	72	178	89	
	Pratylenchus	14	12	26	13	0	0	0	0	
	Tylenchus	28	60	88	44	2	0	2	1	

[a]Source: Halmich Sod Nurseries, E. Lansing, Mich.

TABLE 5A.--Analysis of the data of Table 5.

	# Aphelenchoides			# Aphelenchus			# Ditylenchus			# Paratylenchus			# Pratylenchus			# Tylenchus		
Test	Contl	Trtd	d*	Contl	Trtd	d	Contl	Trtd	d	Contl	Trtd	d	Contl	Trtd	d	Contl	Trtd	d
1	677	4	673	14	4	10	19	0	19	105	128	-23	2	1	1	54	0	54
2	264	0	264	6	1	5	14	0	14	27	91	-64	1	0	1	60	2	58
3	787	1	786	11	4	7	10	0	10	68	80	-12	5	1	4	43	0	43
4	940	4	936	16	9	7	35	0	35	42	89	-47	13	0	13	44	1	43
$S_{(d)}$	2659			29			78			-146			19			198		
$\bar{d}$	664.75			7.25			19.5			-36.5			4.75			49.5		
Vd	82539			4.25			120.3			549.7			32.25			59		
$V\bar{d}$	20634.75			1.06			30.08			137.43			8.06			14.75		
$s\bar{d}$	143.53			1.03			5.49			11.71			2.84			3.85		
t	4.63			7.04			3.55			3.12			1.67			12.86		
N	3			3			3			3			3			3		
P	0.01-0.02			.001-.01			.02-.05			.05-.10			.10-.20			0.001-0.001		
H_o	Reject			Reject			Reject			Valid			Valid			Reject		

*See footnotes of Table 2A for explanation of symbols.

TABLE 6.--Effect of sod-heating on the survival of muck-sod nematodes

		No. of Nematodes per Core of Sod[a]								
		Control				Treated				
Test	Genera	I	II	Total	Avg.	I	II	Total	Avg.	Heat Treatment
1	Aphelenchoides	92	52	144	72	0	2	2	1	68 - 115°F
	Aphelenchus	2	0	2	1	0	0	0	0	(36 hrs.)
	Ditylenchus	60	36	96	48	8	4	12	6	
	Heterodera larva	4	0	4	2	0	0	0	0	
	Pratylenchus	2	2	4	2	0	4	4	2	
	Tylenchorhynchus	0	0	0	0	2	0	2	1	
2	Aphelenchoides	30	50	80	40	0	10	10	5	69 - 116°F
	Ditylenchus	70	48	118	59	20	12	32	16	(36 hrs.)
	Pratylenchus	10	0	10	5	2	0	2	1	
3	Aphelenchoides	120	10	130	65	4	0	4	2	68 - 117°F
	Ditylenchus	38	20	58	49	2	2	4	2	(36 hrs.)
	Pratylenchus	16	2	18	9	0	0	0	0	

4	Aphelenchoides	146	38	184	92	4	2	6	3	69 - 117°F
	Ditylenchus	98	34	132	66	4	2	6	3	(36 hrs.)
	Paratylenchus	0	0	0	0	2	0	2	1	
	Pratylenchus	12	12	24	12	0	0	0	0	
	Tylenchorhynchus	0	2	2	1	0	0	0	0	
	Tylenchus	4	48	52	26	0	0	0	0	

[a]Source: Halmich Sod Nurseries, E. Lansing, Mich.

TABLE 6A.--Analysis of the data of Table 6

	# Aphelenchoides			# Ditylenchus			# Pratylenchus		
Test	Contl	Trtd	d*	Contl	Trtd	d	Contl	Trtd	d
1	72	1	71	48	6	42	2	2	0
2	40	5	35	59	16	43	5	1	4
3	65	2	63	49	2	47	9	0	9
4	92	3	89	66	3	63	12	0	12

$S_{(d)}$	258	195	25
$\overline{d}$	64.5	48.75	6.25
Vd	505	94.92	28.25
$V\overline{d}$	126.25	23.73	7.06
$s\overline{d}$	11.23	4.87	2.66
t	5.74	10.01	2.35
N	3	3	3
P	.01-.02	.001-.01	0.1
H_o	Reject	Reject	Valid

*See footnotes of Table 2A for explanation of symbols.

TABLE 7.--Distribution of *Tylenchorhynchus* sp. in 0.5 ml subsamples of a mass inoculum of turfgrass nematodes[a]

0.5 ml aliquots	I	II	III	IV	V	VI	VII	VIII	Total
# Tylenchorhynchus sp.	130	148	164	154	112	152	196	132	1188

[a]Mineral sod nematodes from bentgrass putting greens.

Description of the distribution--

Arithmetic mean ($\bar{x}$) = 148.5

Standard deviation (S) = 25.3

Standard error ($S\bar{x}$) = 9.0

Coefficient of variation (V) = 17%

TABLE 8.--Effect of sod-heating on the survival of *Tylenchorhynchus* sp.[a]

Test	# Tylenchorhynchus sp. per sod core[b]				Temperature Range for Treatment 3
	Control	Treatment 1	Treatment 2	Treatment 3	
1	0	706	178	0	76 - 113°F (50 hrs)
2	0	412	230	0	- do -
3	0	576	252	0	76 - 114°F (50 hrs)
4	0	580	286	0	- do -
Total	0	2274	948	0	
Avg.	0	568.5	237	0	

[a]Mineral sod nematodes from bentgrass putting greens.

[b]Organic sod from Halmich Farms, E. Lansing, Mich.

the data became less apparent. The indispensable loss, that is, that part of the reduction in number which was not a concomitant of sod-heating, was measured as the difference in counts between the first and second treatments (Table 8). It amounted to nearly 60 percent of the expected numbers shown under Treatment 1 (Table 8). Only 40 percent of the nematodes, therefore, had a chance of going through the sod-heating treatment and none of them survived it.

C. Vertical Distribution Studies

Four tests were conducted with 6-inch organic sod cores to find the nature of the zonal distribution of nematodes between the upper stratum of rhizomes, roots and humus and the remaining lump of muck soil containing a few straggling roots. The top one inch of each 6-inch core was separated and the two resulting segments processed for nematodes separately, by the centrifugal-flotation technique followed by funnel filtration.

The results (Table 10) showed that *Aphelenchoides* sp., *Aphelenchus* sp., *Ditylenchus* sp., and *Tylenchus* sp. were absent from the lower portion of the core. Most of the other parasitic genera were present in both sections of the core. *Paratylenchus* sp. and *Pratylenchus* sp. were better represented in the lower portion and *Tylenchorhynchus* sp. was absent from the upper portion.

D. Occurrence of Nematodes in Organic Sod Strips

The identifications and enumerations of the various stylet-bearing nematodes extracted from all untreated cores of muck sod were pooled so as to manifest the occurrence trends of these nematodes

TABLE 9.--A check on the effectiveness of the recovery of *Tylenchorhynchus* sp. from the funnel filters[a]

Test	# Tylenchorhynchus sp. in filtrate	# Tylenchorhynchus sp. in residues
1	706	76
2	412	0
3	576	0
4	580	0
Total	2274	76
Average	568.5	19

Effectiveness of cloth filter = 97 - 100%

[a]Muslin cloth filter.

TABLE 10.--Vertical distribution of stylet-bearing nematodes in organic-sod soil[a]

	No. of Nematodes per 6-in. Core											
	Top 1 in. (Thatch & Root Zone)						Next Lower 4-5 in. of Soil					
Genera Observed	I	II	III	IV	Total	Avg.	I	II	III	IV	Total	Avg.
Aphelenchoides	-	28	112	3	143	35.8	-	-	-	-	-	-
Aphelenchus	45	-	48	68	161	40.3	-	-	-	-	-	-
Criconemoides	-	-	-	-	-	-	-	-	-	-	-	-
Ditylenchus	15	54	2	293	364	91	-	-	-	-	-	-
Helicotylenchus	1	-	-	-	-	0.3	6	10	-	-	16	4
Heterodera larva	2	0	0	-	2	0.5	1	-	-	-	1	0.3
Longidorus	-	-	-	-	-	-	-	-	-	-	-	-
Meloidogyne larva	-	-	-	-	-	-	-	-	-	-	-	-
Paratylenchus	5	1	1	-	7	1.8	38	7	8	-	53	13.3
Pratylenchus	1	4	-	-	5	1.3	3	12	-	-	15	3.8
Trichodorus	1	-	-	2	3	0.8	-	1	-	3	4	1
Tylenchorhynchus	-	-	-	-	-	-	-	-	-	12	12	3
Tylenchus	-	-	2	-	2	0.5	-	-	-	-	-	-
Xiphinema	-	-	-	-	-	-	-	-	-	-	-	-

[a]Survey conducted in June, 1967 using samples from four sod farms in Michigan.

(Table 11). The figures were tabulated as monthly estimates of each nematode genus per core of sod. However, as the total number of sod cores processed per month was different for different months, these data may not represent the whole picture. In any case, Aphelenchoides sp., Ditylenchus sp., Paratylenchus sp., Tylenchus sp.., Aphelenchus sp., and Pratylenchus sp., in that order, showed up as the prevalent nematodes associated with muck sod. Heterodera cysts were constantly encountered too, although in small numbers.

A somewhat reversed trend of occurrence was shown for mineral sod nematodes (Table 12). The less abundant nematodes occurring in organic sod were the predominant ones in mineral sod. Helicotylenchus sp., Tylenchorhynchus sp., Criconemoides sp., Tylenchus sp., Ditylenchus sp., Aphelenchoides sp., Paratylenchus sp., and Pratylenchus sp. were represented in descending order of numerical abundance.

The data presented on Table 11 were also suggestive of the occurrence of population fluctuations in the nematode fauna. Different genera attained maximum numbers at different times: Aphelenchoides sp., Paratylenchus sp., and Tylenchus sp. in October; Aphelenchus sp. in July; and Ditylenchus sp. and Pratylenchus sp. in August. As a whole, numbers were highest in autumn (October), and lowest in spring (May).

TABLE 11.--Occurrence of stylet-bearing nematodes in organic sod[a]

1967	Mean No. of Nematodes							
Genera Observed	May	June	July	Aug.	Sept.	Oct.	Total	Avg.
Aphelenchoides	0.54	11	92.8	134.5	226	537.4	1002.2	167
Aphelenchus	1.35	12.4	34.8	0.5	0	8.4	57.5	9.6
Criconemoides	0	0	0	0	0	0	0	0
Ditylenchus	4.2	28	24.1	98.5	0.25	29	184.1	30.7
Helicotylenchus	0.03	0.08	0	0	0	0	0.11	0.02
Heterodera[b] larva	2.3	0.15	0	1	0	0	3.45	0.6
Longidorus	0	0	0	0	0	0	0	0
Meloidogyne larva	0	0	0	0	0	0	0	0
Paratylenchus	0.56	0.54	0.1	0.5	30	41.5	73.2	12.2
Pratylenchus	0.5	0.4	0	14	3	5.2	23.1	3.9
Trichodorus	0.31	0.23	0	0	0	0	0.54	0.09
Tylenchorhynchus	0.05	0.15	0	1	0	0	1.2	0.2
Tylenchus	0.82	0.15	0	21.5	1.5	42.6	66.6	11.1
Xiphinema	0	0	0	0	0	0	0	0

[a]Only the associated soil was examined.

[b]Heterodera cysts were well represented.

TABLE 12.--An estimate[a] of stylet-bearing nematodes associated with mineral sod

Genera observed	# Nematodes per Core of Sod[b]					# Nematodes per Core of Sod[c]				
	I	II	III	Total	Avg. per Core	I	II	III	Total	Avg. per Core
Aphelenchoides	+	+	+		50	+	+	+		10
Aphelenchus	0	0	0	0	0	+	+	+		5
Criconemoides	766	290	1574	2630	876.7	0	0	0	0	0
Ditylenchus	1	8	4	13	4.3	54	4	0	58	19.3
Helicotylenchus	2792	68	1318	4178	1392.6	716	2114	426	3256	1085.3
Heterodera[d] larva	0	0	0	0	0	0	0	0	0	0
Longidorus	0	0	0	0	0	0	0	0	0	0
Meloidogyne larva	0	0	0	0	0	0	0	0	0	0
Paratylenchus	0	8	4	12	4	0	0	0	0	0
Pratylenchus	8	0	0	8	2.7	0	0	0	0	0
Trichodorus	0	0	0	0	0	0	0	0	0	0
Tylenchorhynchus	76	192	1414	1682	560.7	164	96	336	596	198.7
Tylenchus	71	20	380	471	157	464	58	70	592	197.3
Xiphinema	0	0	0	0	0	0	0	0	0	0

[a]Conducted in Aug.-Sept. 1967; examining associated soil only.

[b]Sod from the Horticultural garden at Michigan State University.

[c]Commercial sod from South Lyon, Michigan.

[d]Heterodera cysts were fairly well represented.

+ = Few.

DISCUSSION AND CONCLUSIONS

The results of the sod-heating experiments clearly confirm the generalization, arrived at by Wallace (1963), that the thermal death points of plant nematodes lie between 104 and 129°F. At 104°F all the associated tylenchids, except _Tylenchus_ sp., were well represented numerically; while at 115°F all but two, _Paratylenchus_ sp., and _Pratylenchus_ sp., were reduced in number. However, these results cannot be interpreted on the basis of heat effects only. Experiments such as these, which utilize intact portions of the terrestrial habitat, often present the difficulty of isolating the particular factor under study from the many others that are associated with the ecosystem. "Sod-heating" is really a comprehensive term which describes by means of the temperature effects what must be a complex phenomenon of various factors. The moisture factor, the poor aeration within the sod stacks, the increased microbial activity, and the accumulation of toxic metabolites, are among the factors that may contribute to the effect of "sod-heating". It is therefore difficult to explain off the observed effect in terms of a simple causal relationship.

Practically all the individuals of _Paratylenchus_ sp. which survived the higher sod-heating treatment were at the preadult larval stage. This observation bears some relation to that of Rhoades and Linford (1961) on the ability of the preadult stage of _Paratylenchus projectus_ to endure low extremes of temperature. It is also probable that the preadult stage of *Ditylenchus* sp. would exhibit a similar degree of endurance of sod-heating, as indicated by the work of Courtney and Latta (1934).

The possible involvement of unknown habitat factors in the inoculation tests precludes any attempt to make generalizations concerning the effect of sod-heating on the introduced population of *Tylenchorhynchus*. The high percentage of indispensable reduction in numbers suggests the operation of some potent factor or factors other than sod-heating. Anyhow, as none of the survivors (40%) of these unknown environmental factors could further survive the sod-heating treatment, it may be concluded that sod-heating played a major role in their elimination.

Observations made from the studies of occurrence and vertical distribution of nematodes in muck sod led to the following conclusions: (i) Compared to mineral sod, only a small number of tylenchid genera were of common occurrence in muck sod. (ii) The majority of these tylenchids were those known to contain parasitic as well as free-living, microphagous, or mycophagous species: namely, *Aphelenchoides* sp., *Aphelenchus* sp., *Ditylenchus* sp., and *Tylenchus* sp. This would explain the more superficial distribution of these populations among the grass roots, rhizomes, and decaying vegetable matter. (iii) Interseasonal as well as intraseasonal fluctuations in population numbers appeared to be a common characteristic of the nematodes; an observation which offers one possible explanation of the low numbers of parasitic genera like *Helicotylenchus*, *Trichodorus*, and *Tylenchorhynchus*. Some support for this deduction was found in the work of Hollis and Fielding (1958), which showed that the most violent intraseasonal fluctuations of plant nematodes in Louisiana occurred among ecto-parasitic populations, and that these fluctuations tended to obscure population increases.

As a whole, the findings of this research may be summarized as follows. Firstly, the tylenchid nematodes that occurred in merion Kentucky bluegrass sod grown on organic soil were relatively fewer in number and kind than those occurring in merion sod raised on mineral soil. Secondly, heat accumulation in merion sod stacks to 115°F or higher signified the maturation within the stacks of conditions lethal to most of the associated tylenchid nematodes, including _Aphelenchoides_ sp., _Aphelenchus_ sp., _Ditylenchus_ sp., and _Tylenchus_ sp. Lastly, populations of _Pratylenchus_ showed an outstanding capability of surviving merion sod heating beyond 115°F. So too did populations of _Paratylenchus_, by virtue of its possession of a resistant preadult stage.

These findings indicate that practically all the tylenchid nematodes that are shipped along with organic merion sod would survive the heat development in the sod stacks during the first day of shipment, when, as has been suggested (King and Beard's preliminary data, 1967), the heat level does not usually exceed 104°F. As the exposure period exceeds 24 hours and the temperature rises above 104°F, so will the effect of "sod-heating" become increasingly harmful to both the nematodes and the grass. If it could be shown that the temperature-time-mortality curve for the nematodes lay below that for the turfgrass, then this would hold out a possibility of eliminating the nematodes through stacking, with little or no harm to the grass. However, the practical importance of these observations is difficult to assess until a detailed faunal survey for muck sod nematodes and a more precise determination of the heat development within sod stacks, have been carried out.

LITERATURE CITED

Beard, J. B. 1967. Extent and summary of value of the Turfgrass Industry in Michigan and the U.S.A. Unpublished data. Crop Sc. Dept., Mich. State Univ.

Beard, J. B., and R. C. Hoglund. 1966. Extend and cost of maintaining turfgrass area in Michigan. (To be published in Quarterly Bull. of the Mich. Agr. Exp. Sta.)

Bell, A. A., and L. R. Krusberg. 1964. Occurrence and control of a nematode of the genus Hypsoperine on Zoysia and Bermuda grasses in Maryland. Plant Disease Reptr. 48(9):721-722.

Blake, C. D. 1961. Root rot of bananas caused by Rhadopholus similis (Cobb) and its control in New South Wales. Nematologica 6:295-310.

Blake, C. D. 1962. Some observations on the orientation of Ditylenchus dipsaci and invasion of oat seedlings. Nematologica 8:177-192.

Bosher, J. E., and W. E. McKeen. 1954. Lyophilisation and low temperature studies with the bulb and stem nematode Ditylenchus dipsaci (Kuhn, 1858) Filipjev. Proc. helm. Soc. Wash. 21(2): 113-117.

Cairns, E. J. 1953. Relationship of the environmental moisture conditions of the mushroom-spawn nematode, Ditylenchus sp. to its control by heat. Mushroom Science 11, Gembloux 1953: 161-164.

Caveness, F. E. 1957. A study of nematodes associated with sugar beet production in selected Northwest and North Central States. Beet Sugar Development Foundation, Fort Collins, Colorado.

Christie, J. R., and V. G. Perry. 1951. Removing nematodes from soil. Proc. Helminth. Soc. Wash. D.C. 18:106-108.

Church, B. M., and A. H. Strickland. 1954. Sampling cabbage aphid populations on brussels sprouts. Plant Pathol. 3:76-80.

Couch, H. B. 1962. Diseases of Turfgrasses. Reinhold Publishing Corporation, N. Y.

Coursen, B. W., and W. R. Jenkins. 1958a. Host-parasite relationships of the pin nematode, Paratylenchus projectus on tobacco and tall-fescue. (Abstr.) Phytopathology 48:460.

Coursen, B. W., R. W. Rhode, and W. R. Jenkins. 1958b. Additions to the host lists of the nematodes Paratylenchus projectus and Trichodorus christei. Plant Disease Reptr. 42:456-460.

Courtney, W. D., and R. Latta. 1934. Some experiments concerning the revival of quiescent Anguillulina dipsaci. Proc. helm. Soc. Wash. 1(1):20.

Dickerson, O. J. 1966. Some observations on Hypsoperine graminis Sledge and Golden, in Kansas. Plant Disease Reptr. 50(6).

Gaskin, J. A. 1965. Susceptibility of bluegrass to root-knot nematodes. Plant Disease Reptr. 49(1).

Goodey, T. 1933. Anguillulina graminophila n. sp., a nematode causing galls on the leaves of fine bentgrass. Journ. Helminth. 11:45-56.

Heald, C. M., and H. D. Wells. 1967. A "Good Hot Bath" for nematode control in sod. Agric. Res. p. 5., (Dec.)

Hodges, C. F., D. P. Taylor, and M. P. Britton. 1963. Root-knot nematode on creeping bentgrass. Plant Disease Reptr. 47(12): 1102.

Hollis, J. P., and Max J. Fielding. 1958. Population Behaviour of Plant Parasitic nematodes in soil fumigation experiments. Louisiana Agr. Exp. Sta. Bull. 515.

Jenkins, W. R. 1964. A rapid centrifugal-flotation technique for separating nematodes from soil. Plant Disease Reptr. 38: 807-808.

Krusberg, L. R. 1959. Investigations on the life cycle, reproduction, feeding habits and host range of Tylenchorhynchus claytoni Steiner. Nematologica 4:187-197.

Minton, N. A. 1965. Reaction of white clover and five other crops to Pratylenchus scribneri. Plant Disease Reptr. 49(10).

Minton, N. A., and H. Ivey. 1967. The pseudo-root-knot nematode on Bermudagrass in Alabama. Plant Disease Reptr. 51(2):148.

Minton, N. A., E. J. Cairns, E. B. Minton, and B. E. Hopper. 1963. Occurrence of plant parasitic nematodes in Alabama. Plant Disease Reptr. 47(8):743-745.

McGlohon, N. E., J. E. Sasser, and R. T. Sherwood. 1962. Effect of fallowing, desiccation, and soil temperature on certain plant parasitic nematodes. Phytopathology 52(1):20-21.

Nutter, G. C. 1965. Turfgrass Is a $4 Billion Dollar Industry. Turfgrass Times 1(1):1-22.

Pepper, E. H. 1965. Nematodes associated with melting-out of turf-grass. Plant Disease Reptr. 49(6).

Perry, V. G. 1965. Turf and Nematode research: Gainsville, Florida. Proc. Univ. Fla. Turfgrass Mngt. Conf. vol. 13.

Perry, V. G., H. M. Darling, and G. Thorne. 1959. Anatomy, Taxonomy, and control of certain spiral nematodes attacking bluegrass in Wisconsin. Univ. of Wisconsin Res. Bull. 207.

Petherbridge, F. R., and F. G. W. Jones. 1944. Beet eelworm (Heterodera schachtii Schm.) in East Anglia. Ann. Appl. Biol. 31(4):320-332.

Rhoades, H. L. 1962. Effects of sting and stubby-root nematodes on St. Augustine grass. Plant Disease Reptr. 46:424-427.

Rhoades, H. L. 1965. Parasitism and pathogenicity of Trichodorus proximus Allen 1957 to St. Augustine grass. Plant Disease Reptr. 49(3).

Rhode, R. A., and W. R. Jenkins. 1957. Host range of a species of Trichodorus and its host-parasite relationship on tomato. Phytopathology 47:295-298.

Rhoades, H. L., and M. B. Linford. 1961. Biological studies on some members of the genus Paratylenchus. Proc. helm. Soc. Wash. 28(1):51-59.

Sasser, J. N. 1954. Identification and host-parasite relationships of certain root-knot nematodes (Meloidogyne sp.). Univ. Md. agric. Expt. Sta. Bull. A-77.

Seinhorst, J. W. 1959. A rapid method for transfer of nematodes from fixative to anhydrous glycerin. Nematologica 4:67-69.

Sherman, G. W. 1934. Survival and revival of Anguillulina dipsaci from narcissus material. Proc. helm. Soc. Wash. 1(1):19-20.

Smithson, H., L. B. Loring, and H. J. Jensen. 1963. The Grass Root-gall nematode found on annual bluegrass roots in Oregon. Plant Disease Reptr. 47(5):440.

Staniland, L. N. 1950. Experiments on the control of chrysanthemum eelworm (Aphelenchoides ritzema-bosi, Schwartz) by hot water treatment. Ann. appl. Biol. 37:11-18.

Tarjan, A. C., and M. H. Ferguson. 1951. Observations of nematodes in yellow turf of bentgrass. U.S. Golf Assn. J. and Turf Mngt. 4:28-30.

Tarjan, A. D., and M. H. Ferguson. 1951. Association of certain nematodes with yellow tuft of bentgrass. (Abstr.) Phytopathology 41(6):566.

Taylor, D. P. 1962. Nematodes in grasses. Proc. Illinois Turfgrass Conf. No. 3.

Taylor, D. P., M. P. Britton, and H. C. Hechler. 1963. Occurrence of plant-parasitic nematodes in Illinois golf greens. Plant Disease Reptr. 47(2):134-135.

Tunstall, J. P., and G. A. Matthews. 1961. Cotton insect control recommendations for 1961-2 in the Federation of Rhodesia and Nyasaland. Rhodesia Agric. J. 58(5):289-299.

Wallace, H. R. 1960. Movement of eelworms. 6. The influence of soil type, moisture gradients and host plant roots on migration of the potato root eelworm, Heterodera rostochiensis Wollenweber. Ann. Appl. Biol. 48:107-120.

Wallace, H. R. 1963. The Biology of Plant Parasitic Nematodes. Edward Arnold (Pubs) Ltd. London.

Yates, F., and D. J. Finney. 1942. Statistical problems in field sampling for wireworms. Ann. appl. Biol. 29:156-167.