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PATHOGENICITY OF PRATYLENCHUS HEXINCISUS ON
CORN, SOYBEAN, AND TOMATO AND POPULATION
CHANGES AS INFLUENCED BY HOSTS, TEMPERATURE,
AND SOIL TYPE.

IOWA STATE UNIVERSITY, PH.D., 1979

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Pathogenicity of Pratylenchus hexincisus on corn, soybean,
and tomato and population changes as influenced by hosts,
temperature, and soil type

by

Mohammad Esmail Zirakparvar

A Dissertation Submitted to the
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INTRODUCTION

Corn and soybean production are important to Iowa's economy. Several plant-parasitic nematodes have been associated with these crops in the state, and some are known to reduce yields. Pratylenchus hexincisus Jenkins and Taylor is the most common endoparasitic nematode attacking corn in the upper Midwest. High populations of this nematode have been observed in association with stunted and sometimes lodged corn in Iowa. In addition to corn, this species has been associated with different plant species in other parts of the United States. The pathogenicity of P. hexincisus on corn and other field crops and its host range have never been demonstrated under controlled environments.

Soil type and temperature are important factors for nematode reproduction and survival. Few ecological studies with P. hexincisus have been made, and these have not included comparative studies involving soil properties and temperatures. The purposes of this study were: 1) to determine the pathogenicity of P. hexincisus on corn, soybean, and tomato, 2) to determine the host range of P. hexincisus on selected plant species, and 3) to study the effect of different temperatures on reproduction of P. hexincisus in different soil types planted to corn under controlled conditions.

LITERATURE REVIEW

Several species of plant-parasitic nematodes have been associated with corn in the United States (6, 17, 20, 31) and in other parts of the world (37, 41). Pratylenchus hexincisus was first described as a new species from specimens associated with sweet corn in Kent County, Maryland (46). Since then this species has been associated with grasses, legumes, and weeds in the field (14, 15, 25, 32, 33, 40, 48, 50). Pratylenchus hexincisus has been associated with corn in the upper Midwest (14, 15, 25, 35, 40, 47, 48, 49, 56), but reports of its effect on corn yield in the area are few. Pratylenchus spp. have been associated with increases in the amount and incidence of root rot in corn (27). Plants damaged by Pratylenchus spp. have reduced root and stalk weight, and root discoloration (9, 26, 44, 45, 55). Severe parasitism affects nutrient and water uptake (6), resulting in a significant negative correlation between the Pratylenchus spp. density and top dry weight of the plant (9, 26, 45, 56).

The relationship of a nematode to its environment is important to its successful establishment. Soil properties are important relative to occurrence and population dynamics of nematodes (53). Some of these are pH (4, 22, 29), soil texture and type (11, 23, 30, 34, 51), and organic matter (28, 36). The interactions within soil characteristics, and between soil characteristics and types of vegetation and nematode species are complex. Many of these factors are interrelated (34, 36). For example, populations of Pratylenchus zaeae and P. brachyurus were greater in sandy loam or loam than in sand and clay loam on strawberry. On tobacco, the populations were much greater in sandy loam than

in any other soil texture (11). Large numbers of P. hexincisus have been found in heavy (33, 50) as well as sandy soils (35, 56).

Temperature is important in governing nematode reproduction and survival (2, 8, 34). The optimum temperature for reproduction of P. penetrans was higher in corn than potatoes, but population increases were not necessarily correlated with root damage (9). Fewer P. penetrans were required to damage onion seedlings at 7-13 C than at 16-26 C (12). Host susceptibility to nematodes also can be altered by high temperatures (10, 18, 54). The number of heat units needed for a nematode to complete its life cycle and/or produce a certain number of individuals may differ with environmental factors, such as nutrients or host and soil type (13, 21, 43, 52). Reports of temperature effects on Pratylenchus hexincisus are few (42).

MATERIALS AND METHODS

Pathogenicity of Pratylenchus hexincisusTreatments

The Pratylenchus hexincisus used was originally obtained from field corn in Iowa and was increased on corn in the greenhouse. Pathogenicity tests of this nematode on corn, Zea mays L., 'Pioneer 216x238'; soybean, Glycine max (L.) Merr., 'Corsoy'; and tomato, Lycopersicum esculentum P. Mill, 'Bonny Best', were conducted for three months in the greenhouse. The corn test was a randomized block design with four treatments and four replications with three samplings. The four treatments were 1) 25 ml of water containing $5,000 \pm 200$ nematodes were pipetted into the soil above the planted seed and covered with soil, 2) nematode-free wash water collected during the nematode extraction was added to the pots to monitor effects of microbial contaminants on plant growth, 3) corn plants (checks) without nematodes or wash water, and 4) $5,000 \pm 200$ nematodes added at 2.5 cm depth in pots without corn to measure the survival of P. hexincisus.

In a second test, the same procedures were used for soybean, tomato, and corn with the following differences: 1) inoculation occurred three days after seed germination, 2) an additional treatment consisted of corn seedlings inoculated with $20,000 \pm 400$ nematodes, and 3) no survival test.

Nematode recovery

Samples were obtained monthly for three months in all tests. The entire root system was removed from one pot from each replication at each sampling. The soil was shaken from the roots and mixed thoroughly

and 100 cm³ of mixed soil along with the root system from each pot were transported to the laboratory in polyethylene bags. Adhering soil particles were washed from the roots and 1-2 grams of roots from each root system were randomly selected and cut into 1.5 cm segments for nematode extraction by the shaker method (3). This procedure consisted of placing 1-2 grams of washed and segmented roots in 125 ml Erlenmeyer flasks containing 36 ml of streptomycin sulfate-mercuric chloride solution. The flasks were placed on a rotary shaker at 100 r.p.m. After four days the nematodes were concentrated and counted. The roots were dried at 90 C for 72 hours. Numbers of P. hexincisus were calculated per gram of dry root.

Pratylenchus hexincisus were extracted from 100 cm³ soil from each pot using the centrifuge-flotation method (19). Briefly, this standard technique consists of wet-sieving of the soil to separate most soil particles and debris from the nematodes. Final separation was accomplished by centrifugation.

Measurement of plant parameters

Plant height was measured at each sampling and the foliage and roots of each plant were dried at 90 C for 5 days. Weights of the dried roots used in nematode extractions were added to the total root weights. Discolored root segments were washed with distilled water for 5 min and placed in water agar and potato dextrose agar plates for isolation of suspected pathogenic fungal and bacterial contaminations.

Nematode staining

Microscopic examinations were made of root tissue of plants that were inoculated with P. hexincisus. Free-hand sections were cut with a razor blade, stained with acid-fuchsin by the method described by McBeth, Taylor, and Smith (24), and destained with lacto-phenol. Fine rootlets were stained whole.

Host Range of Pratylenchus hexincisus

Treatments

Forty-four species of small grains, legumes, vegetables, and weeds were tested for susceptibility to P. hexincisus in the greenhouse. The designs were completely randomized with four replications of one to ten plants, depending on the species, growing in a 15 cm diameter clay pot. Two tests consisting of 16 and 26 plant species were conducted from March 2 to June 10 and from April 25 to August 8, 1978, respectively. A third test, from October 27, 1978 to February 4, 1979, consisted of those species that had less than 10 P. hexincisus/g dry root in the previous tests.

Seeds were incubated in petri dishes on wet paper in the laboratory and transferred to 15 cm pots after germination. Steam-sterilized soil (86% sand, 11% silt, 3% clay, 2.5% organic matter, pH 7.0) was used in all tests. Ten cm³ of water containing 2,000 \pm 100 nematodes were pipetted in a 3 cm deep hole near the seedlings three days after planting. Pioneer 216x238 corn was used as a check host in all tests. All plants were fertilized with N-P-K (6-10-4) at 6 g/pot one month after planting.

Sampling and nematode recovery

Three months after inoculation, the plants were cut just above the soil line. The roots and soil were removed from each pot and the soil was gently shaken from each root system and mixed thoroughly and 100 cm³ of soil and the entire root system from each pot were transferred to the laboratory in polyethylene bags. Extraction procedures from roots and soil were the same as in the pathogenicity experiment. The roots were dried at 90 C for 5 days. Numbers of P. hexincisus were calculated per gram of dry root and per root system.

Soil Types and Temperature

Treatments

Population growth of P. hexincisus on corn was measured in four soil types and temperatures during three months in growth chambers. The soils, collected in Iowa, and their descriptions are listed in Table 1. The soils were steam-sterilized at 15 lbs pressure for six hours. Growth chambers were maintained at 15 C, 20 C, 25 C, and 30 C \pm 1 C for three months. Each soil type in each temperature test consisted of twelve 15 cm diameter clay pots, each containing 1500 cm³ of soil. The design was a 4 (soil types) x 4 (temperature) x 3 (sampling dates) factorial experiment with four replications.

One corn seed was planted 2.5 cm deep in the center of each pot and covered with soil. Three days after seed germination, 2,000 \pm 100 nematodes were added around each seedling as in the host range experiments. The pots were watered as needed, and the temperature of the water added was equal to the ambient air temperature of the growth chamber. This

Table 1. Analyses of four soils used in tests with Pratylenchus hexincisus

Soil type	Soil ^a texture	pH	Organic matter	Cation exchange (meq/100 cc soil)	Sand %	Silt %	Clay %	Field capacity %	Saturation percent
Marshall silt loam	Silt loam	5.28	3.80	22.25	18.76	54.81	26.44	20.92	49.04
Clarion silt loam	Silt loam	6.20	5.27	28.75	16.31	57.59	26.11	22.55	48.21
Buckner coarse sand	Sand	6.35	1.85	8.75	85.75	9.07	5.19	5.85	21.67
Haig silty clay loam	Sandy clay loam	5.90	4.17	16.25	59.86	18.69	21.46	19.75	34.86

^aAll soils were collected from depths of 0-50 cm.

was accomplished by placing a bucket of water in the chamber until its temperature reached about that of the growth chamber. Fertilizer (N-P-K 6-10-4) was added at 6 g/pot one month after planting.

Nematode recovery

The same nematode recovery procedures were followed as in the host range experiment for nematode extraction from soil and roots and calculation of nematode numbers/g dry root and /whole root.

Measurement of plant parameters

The same procedures were followed as in the pathogenicity experiment.

Soil analysis

Soil samples from each soil type were analyzed for texture, pH, organic matter, cation exchange capacity, field capacity, and saturation percent. The procedure used for determination of soil organic matter was that outlined by Graham (16). Those for field capacity and texture analysis (sedimentation analysis with pipette sampling) were described by Peters (39) and Day (7), respectively. Cation exchange capacity was determined using modifications of two existing procedures. The soil sample was prepared in the manner described by Chapman (5). Final measurements were made using an ammonium electrode, as described by Banwart et al. (1). Hydrogen-ion concentration was determined using the method described by Peech (38), but with 2.5:1 ratio of water to soil, rather than the published 1:1 ratio. Saturation percent was obtained by measuring the difference in weight between 50 cm³ of soil

when saturated with water and, after drying, saturation percent was then calculated by dividing the weight of water percent at saturation by the weight of the oven-dried soil.

RESULTS

Pathogenicity Test and Symptoms

Corn

Root and top weights of plants inoculated with 5,000 Pratylenchus hexincisus were significantly ($P = 0.05$) less at the third sampling than those plants in the check and wash-water treatments (Table 2). The heights of plants inoculated with 20,000 nematodes were significantly ($P = 0.05$) less than those in other treatments at all samplings (Table 2, Figure 1). Root weights of plants inoculated with 20,000 nematodes were significantly ($P = 0.05$) less at the first two samplings than those in other treatments (Table 2). In the third sampling, however, the root weights of these plants were significantly ($P = 0.05$) less than those plants only in the check and wash-water treatments (Table 2). Table 3 shows the number of P. hexincisus on corn plants during three months in the greenhouse. Figure 6 shows P. hexincisus eggs inside the corn root.

Plants inoculated with P. hexincisus had necrotic dark brown discrete lesions in fibrous and coarse roots by 60 days after inoculation (Figure 2). Lesions nearly covered the entire root system by 90 days, especially in plants inoculated with 20,000 nematodes (Figures 3, 4). Pruning of the fibrous roots was observed at the end of the third month and was severe in plants inoculated with larger numbers of nematodes. P. hexincisus invaded the fibrous roots abundantly and, for the most part, were oriented parallel to the longitudinal axis of the roots (Figure 5). Difficulty was experienced in locating nematodes in the coarse roots, but

Table 2. Heights and dry top and root weights of corn plants during three months after inoculation with Pratylenchus hexincisus in the greenhouse

Treatments	First test ¹								
	Height (cm) ²			Top weight (grams) ²			Root weight (grams) ²		
	Feb 23	Mar 23	Apr 24	Feb 23	Mar 23	Apr 24	Feb 23	Mar 23	Apr 24
5,000 ± 200 <u>P. hexincisus</u> + corn	33.1a	48.6a	90.0a	0.6a	3.0a	20.6a	0.34a	1.2a	5.6a
Wash-water + corn	33.4a	54.1a	110.5a	0.7a	4.0a	27.1a	0.39a	1.7a	7.5b
Corn only (check)	34.2a	52.5a	100.8a	0.7a	3.7a	25.1b	0.45b	1.7a	7.7b
	Second test ³								
	Dec 4	Jan 4	Feb 4	Dec 4	Jan 4	Feb 4	Dec 4	Jan 4	Feb 4
	20,000 ± 400 <u>P. hexincisus</u> + corn	27.6a	42.6a	51.5a	0.6a	3.0a	11.8a	0.2a	1.5a
5,000 ± 200 <u>P. hexincisus</u> + corn	31.0b	58.7b	133b	0.6a	8.6b	33.2b	0.3b	2.6b	4.2a
Wash-water + corn	31.0b	65.1b	156b	0.6a	9.8b	45.8c	0.30b	3.0b	7.8b
Corn only (check)	31.0b	64.3b	152b	0.5a	9.8b	50.0c	0.37b	3.6b	8.2b

¹Inoculation occurred at planting time.

²Means with common letters within a column are not significantly different at P = 0.05 (Duncan's Multiple Range Test).

³Inoculation occurred three days after seed germination.



Figure 1. Corn plants after three months growth in 15 cm diameter pots in the greenhouse. Left, plant inoculated with 20,000 Pratylenchus hexincisus; right, plant inoculated with 5,000 P. hexincisus

Table 3. Numbers of Pratylenchus hexincisus/g of dry root, whole root, and 100 cm³ soil in the greenhouse

Treatments	First test ¹								
	Gram dry root ²			Whole root ²			100 cm ³ soil ²		
	Feb 23	Mar 23	Apr 24	Feb 23	Mar 23	Apr 24	Feb 23	Mar 23	Apr 24
5,000 ± 200 <u>P. hexincisus</u> + corn	794	1407	3757	274	1717	19048	65	51	592
Wash-water + corn	0	0	0	0	0	0	0	0	0
Corn only (check)	0	0	0	0	0	0	0	0	0
5,000 ± 200 <u>P. hexincisus</u> only	--	--	--	--	--	--	39	18	5
	Second test ³								
	Dec 4	Jan 4	Feb 4	Dec 4	Jan 4	Feb 4	Dec 4	Jan 4	Feb 4
20,000 ± 400 <u>P. hexincisus</u> + corn	972a	3322a	25956a	223a	5016a	68783a	84a	783a	8025a
5,000 ± 200 <u>P. hexincisus</u> + corn	655a	1520b	4036b	149b	4013b	17032b	34b	26b	829b
Wash-water + corn	0	0	0	0	0	0	0	0	0
Corn only (check)	0	0	0	0	0	0	0	0	0

¹Inoculation occurred at planting time.

²Means with common letters within a column are not significantly different at P = 0.05 (Duncan's Multiple Range Test).

³Inoculation occurred three days after seed germination.



Figure 2. Brown lesions and pruned fibrous roots caused by Pratylenchus hexincisus on coarse roots of corn. Sixty days after inoculation with P. hexincisus

Figure 3. Corn roots after three months growth in 15 cm diameter pots in the greenhouse. Left, check, no nematodes, no wash-water; center, plant inoculated with wash-water; right, plant inoculated with 5,000 Pratylenchus hexincisus

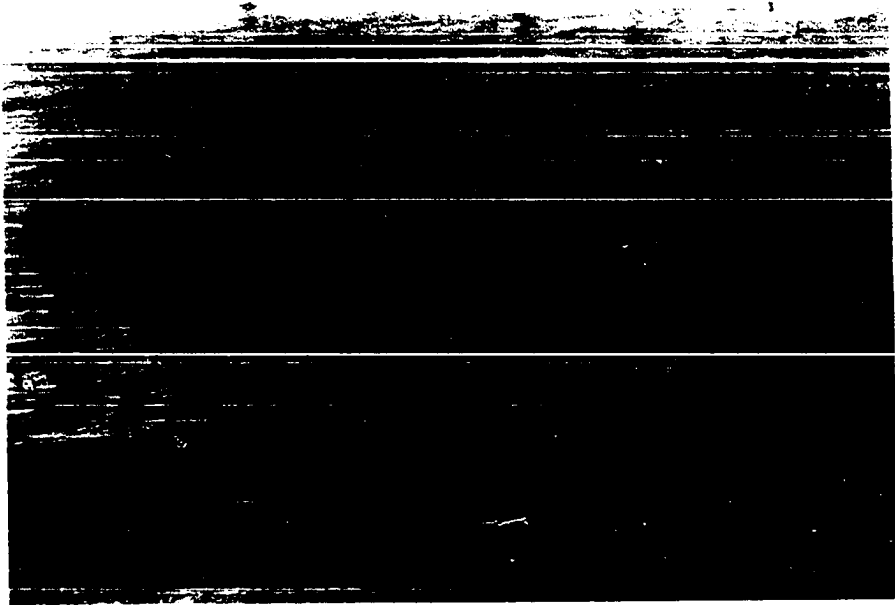


Figure 4. Corn roots after three months growth in 15 cm diameter pots in the greenhouse. Left, check plant, corn only; center, plant inoculated with 5,000 Pratylenchus hexincisus; right, plant inoculated with 20,000 P. hexincisus



Figure 5. Corn root infected with Pratylenchus hexincisus, showing one adult, one juvenile, and three eggs.
100X

Figure 6. Pratylenchus hexincisus eggs inside a corn root.
100X



they were found with ease in fibrous roots. Nematodes were observed only in the cortical parenchyma. Considerable sloughing of the cortical tissue was observed in fibrous roots.

Root cultures were examined for fungal and bacterial growth. Only a few colonies of Murogenella, Rhizopus, Penicillium, and Fusarium were observed, except Fusarium species of these genera are not known to be pathogenic on corn.

Soybean

Soybean plants inoculated with P. hexincisus had significantly ($P = 0.05$) less root weight than those in the check and wash-water treatments at the third sampling (Table 4, Figure 7). Reductions in height and top weight of inoculated plants occurred at the third sampling but were not significant (Table 4, Figure 8). Table 5 shows the numbers of P. hexincisus on soybean plants during three months of growth.

Tomato

The heights and top and root weights of plants inoculated with P. hexincisus were significantly ($P = 0.05$) less than plants in the check and wash-water treatments. The significant decrease in heights occurred at the second sampling, in top weight at second and third samplings, and in root weight at the third sampling (Table 6; Figures 9, 10). Table 7 shows the number of P. hexincisus on tomato plants over a three month growth period. No lesions were observed on the roots (Figure 10). Discolored cells occurred around the stained nematodes inside the root tissue (Figures 11, 12).

Table 4. Heights and dry top and root weights of soybean plants during three months after inoculation with Pratylenchus hexincisus in the greenhouse

Treatments	Height (cm) ¹			Top weight (gram) ¹			Root weight (grams) ¹		
	Dec 4	Jan 4	Feb 4	Dec 4	Jan 4	Feb 4	Dec 4	Jan 4	Feb 4
5,000 ± 200 <u>P. hexincisus</u> + soybean	37a	75a	82a	1.26a	4.5a	5.5a	0.3a	0.6a	0.8a
Wash-water + soybean	35a	66a	79a	1.10a	7.1a	7.4a	0.3a	0.7a	1.1b
Soybean only (check)	36a	84a	71b	1.02a	7.8a	7.9a	0.3a	0.8a	1.1b

¹Means with common letters within a column are not significantly different at P = 0.05 (Duncan's Multiple Range Test).

Table 5. Average number of Pratylenchus hexincisus/g of soybean dry root, whole root, and 100 cm³ soil during three months in the greenhouse

Treatments	Gram dry root			Whole root			100 cm ³ soil		
	Dec 4	Jan 4	Feb 4	Dec 4	Jan 4	Feb 4	Dec 4	Jan 4	Feb 4
5,000 ± 200 <u>P. hexincisus</u> + soybean	582	2639	8046	186	1531	6597	36	16	47
Wash-water + soybean	0	0	0	0	0	0	0	0	0
Soybean only (check)	0	0	0	0	0	0	0	0	0

Figure 7. Root systems of soybean var. Corsoy after three months growth in 15 cm diameter pots in the greenhouse. Left, check, no nematode and no wash-water; center, plant inoculated with wash-water; right plant inoculated with 5,000 Pratylenchus hexincisus

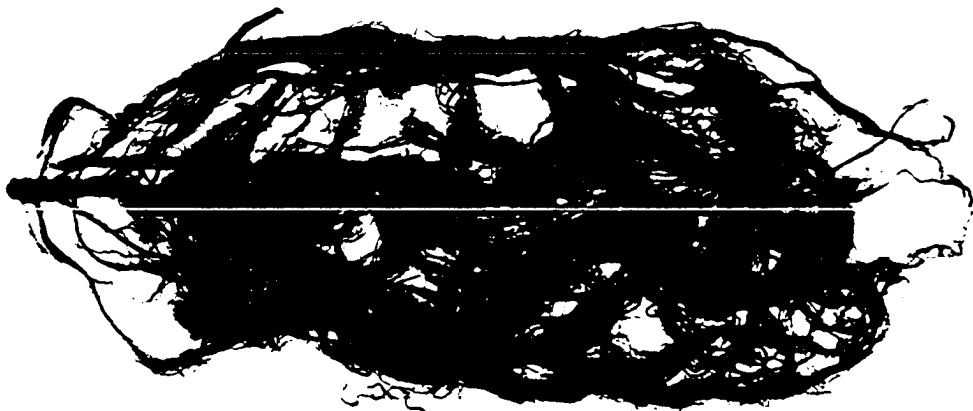




Figure 8. Soybean plants after three months growth in 15 cm diameter pots in the greenhouse. Left, plant inoculated with 5,000 Pratylenchus hexincisus; center, plant inoculated with wash-water; right, check plant, no nematodes and no wash-water

Table 6. Height and dry top and root weights of tomato plants inoculated with Pratylenchus hexincisus during three months in the greenhouse

Treatments	Height (cm) ¹			Top weight (gram) ¹			Root weight (gram) ¹		
	Dec 4	Jan 4	Feb 4	Dec 4	Jan 4	Feb 4	Dec 4	Jan 4	Feb 4
5,000 ± 200 <u>P. hexincisus</u> + tomato	22a	36.85a	66.5a	0.14a	4.12a	12.05a	0.021a	0.72a	1.6a
Wash-water + tomato	24a	43b	69.85a	0.13a	6.63b	22.5b	0.034a	1.2a	3.1b
Tomato only (check)	21a	47.5b	65a	0.19a	6.39b	20.30b	0.055a	1.2a	2.63b

¹Means with common letters within a column are not significantly different at P = 0.05 (Duncan's Multiple Range Test).

Table 7. Average number of Pratylenchus hexincisus/g of tomato dry root, whole root, and 100 cm³ soil during three months in the greenhouse

Treatment	Gram dry root			Whole root			100 cm ³ soil		
	Dec 4	Jan 4	Feb 4	Dec 4	Jan 4	Feb 4	Dec 4	Jan 4	Feb 4
5,000 ± 200 <u>P. hexincisus</u> + tomato	3635	4614	11447	76	3322	18315	34	24	125
Wash-water + tomato	0	0	0	0	0	0	0	0	0
Tomato only (check)	0	0	0	0	0	0	0	0	0



Figure 9. Tomato plants after three months growth in 15 cm diameter pots in the greenhouse. Left, plant inoculated with 5,000 Pratylenchus hexincisus; center, plant inoculated with wash-water; right, check plant, no nematodes and no wash-water

Figure 10. Root systems of tomato plants after three months growth in 15 cm diameter pots in the greenhouse. Left, check, no nematodes and no wash-water; center, plant inoculated with wash-water; right, plant inoculated with 5,000 Pratylenchus hexincisus

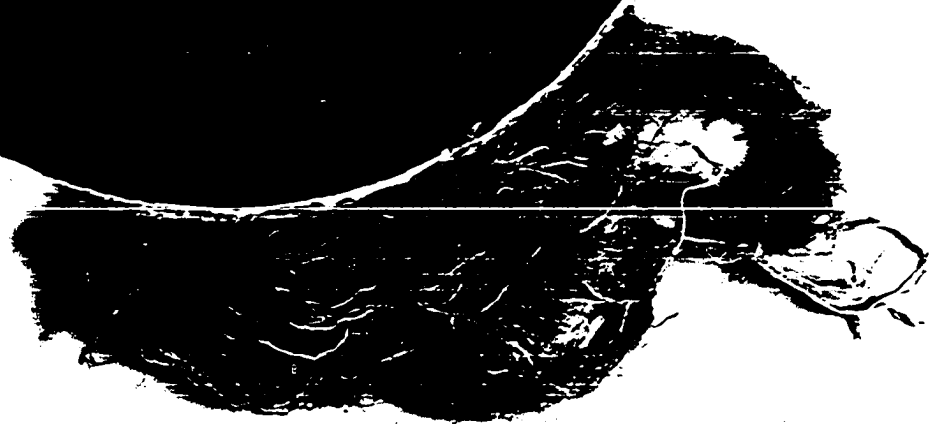
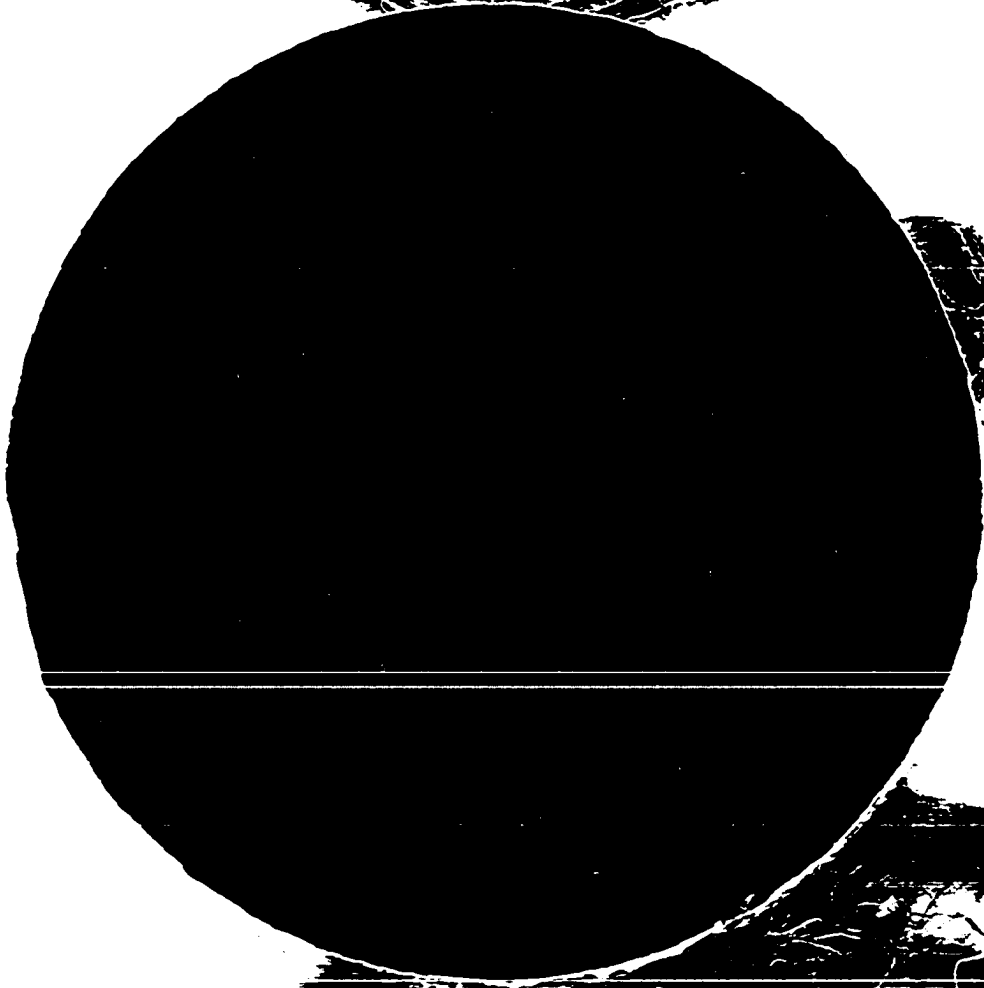
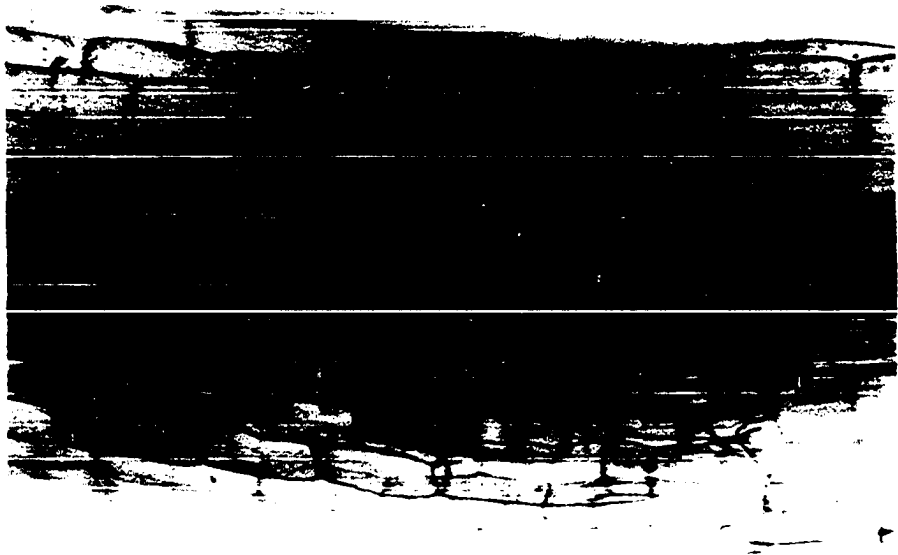
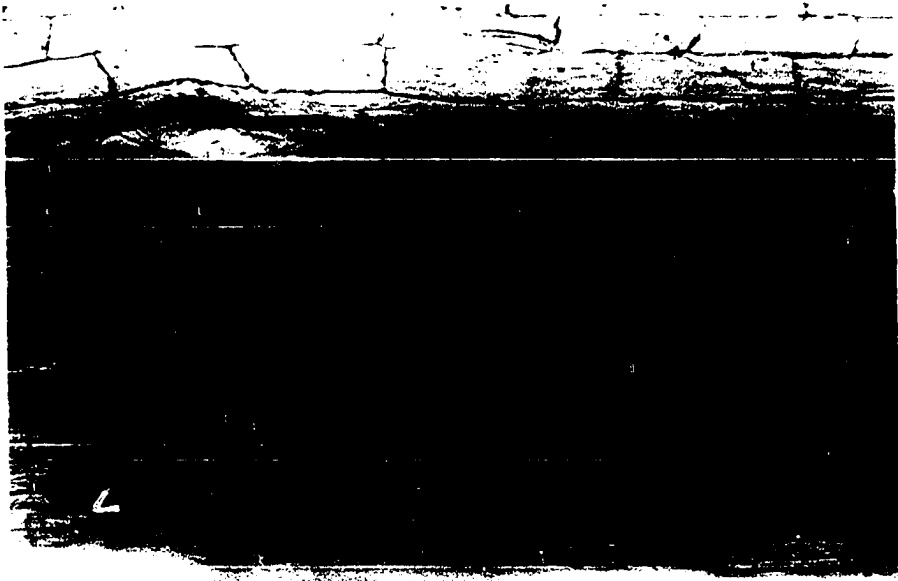




Figure 11. Discoloration of tomato root cells infected with Pratylenchus hexincisus. 100X

Figure 12. Tomato root infected with Pratylenchus hexincisus, showing one adult, one juvenile, and one egg. 100X



Host Range

Pratylenchus hexincisus was recovered after three months from the roots of all 44 plant species or cultivars tested except smooth brome and orchard grass. Final populations of P. hexincisus/pot were larger than the respective initial population in 12 species. Of these, tomato, garden pea, white Dutch clover, velvetleaf, and soybean supported more P. hexincisus/g dry root than the check host (Table 8). Final populations of P. hexincisus/pot in tomato and garden pea were larger than the final population in the corn check.

Soil Types and Temperatures

A square root transformation $\sqrt{x + 1}$ was performed on all data, unless stated otherwise. In all analyses, the assumption was made that there were no growth chamber to growth chamber differences. Numbers of P. hexincisus/g dry root, /whole root system, and /100 cm³ of soil differed significantly ($P = 0.01$, $P = 0.05$) among the four temperature treatments and among soil types in overall analysis (Tables 9, 10, 11). Within each temperature at each sampling, however, numbers of P. hexincisus in soil differed significantly among the four soil types tested at 15 C, 20 C, and 25 C (Figures 13, 14, 15).

Numbers of P. hexincisus/g dry root and per whole root differed significantly ($P = 0.01$) among four soil types in all sampling times at 30 C (Figure 16, 17). Maximum numbers occurred in Buckner coarse sand and minimum numbers in Marshall silt loam (Figures 16, 17). At 25 C, significant ($P = 0.01$) differences in numbers of P. hexincisus in roots and soil among four soil types occurred only in the second sampling. The

Table 8. Numbers of Pratylenchus hexincisus after three months in plants inoculated with 2000 + 100 nematodes per pot

Plant	Numbers of <u>Pratylenchus hexincisus</u>		
	Gram dry root	100 cm ³ soil	Pot (1500 cm ³ soil + roots)
GRAMINEAE			
<u>Agrostis alba</u> L. Redtop	18	2	148
<u>Agrostis tenuis</u> Sibth. Bent-grass	28	2	344
<u>Avena sativa</u> L. cv 'E-76' Oats	193	6	227
<u>Bromus inermis</u> Leyss. Smooth brome	0	2	30
<u>Dactylis glomerata</u> L. cv Pot. Orchard-grass	0	1	15
<u>Echinochloa crusgalli</u> (L.) Barnyard-grass	143	1	76
<u>Festuca arundinacea</u> Schreb. Alta Fescue or Kent. 3l	183	2	113
<u>Festuca rubra</u> L. Red Fescue	13	5	100
<u>Hordeum vulgare</u> L. Barley	28	2	70
<u>Lolium perenne</u> L. Rye-grass	44	3	323
<u>Panicum dichotomiflorum</u> Michx. Fall Panicum	207	19	637
<u>Phalaris arundinacea</u> L. Reed Canary Grass	90	7	729
<u>Phleum pratense</u> L. Timothy	309	76	2944
<u>Poa pratensis</u> L. Kentucky Blue Grass	56	1	104
<u>Secale cereale</u> L. cv 'Balboa' Rye	468	4	795
<u>Setaria lutescens</u> (Weigel) Hubb. Yellow Foxtail	28	1	142

Table 8. (Continued)

Plant	Numbers of <u>Pratylenchus hexincisus</u>		
	Gram dry root	100 cm ³ soil	Pot (1500 cm ³ soil + roots)
<u>Setaria magna</u> Griseb. Giant Foxtail	45	6	256
<u>Setaria viridis</u> (L.) Beauv. Green Foxtail	93	5	387
<u>Sorghum sudanense</u> (Piper) Stapf. cv Piper Sudan Grass	194	21	1268
<u>Sorghum vulgare</u> Pers. cv H-25 Sorghum	94	14	1077
<u>Triticum aestivum</u> L. cv 'Arthur 71' Wheat	310	5	808
<u>Zea mays</u> L. cv Pioneer 216x236 Corn	3192	152	20893
LEGUMINOSAE			
<u>Coronilla varia</u> L. cv Emerald Crown-velch	64	3	202
<u>Glycine max</u> (L.) Merr. cv Corsoy Soybean	4176	61	7881
<u>Lespedeza stipulacea</u> Maxim cv <u>Sericea</u> Korean Clover	30	0	14
<u>Lotus corniculatus</u> L. cv Empire Birdsfoot Trefoil	22	2	43
<u>Medicago sativa</u> (Sown) L. cv Ranger Alfalfa	18	4	100
<u>Melilotus officinalis</u> (L.) Desr. Yellow Sweet Clover	24	12	271
<u>Phaseolus limensis</u> Macf. cv Fordhook 242 Lima Bean	2527	13	2276
<u>Pisum sativum</u> L. Garden Pea	102433	53	57215

Table 8. (Continued)

Plant	Numbers of <u>Pratylenchus hexincisus</u>		
	Gram dry root	100 cm ³ soil	Pot (1500 cm ³ soil + roots)
<u>Trifolium hybridum</u> L. Alsike Clover	322	5	385
<u>Trifolium pratense</u> L. cv Medium Red Clover	62	3	193
<u>Trifolium repens</u> L. cv White Dutch Clover	5422	44	5382
CRUCIFERAE			
<u>Brassica oleracea</u> L. cv Copenhagen Market Cabbage	1958	139	6998
<u>Brassica oleracea</u> cv <u>botrytia</u> L. Cauliflower	605	27	1870
CHENOPODIACEAE			
<u>Beta vulgaris</u> L. cv Detroit Dark Red Beet	369	24	2224
<u>Chenopodium album</u> L. Lambsquarters	343	90	2617
<u>Chenopodium lanceolatum</u> Muhl. Pigweed lambsquarter	25	16	284
MALVACEAE			
<u>Abutilon Theophrasti</u> Medic. Velvetleaf	4205	177	12606
SOLANACEAE			
<u>Capsicum annuum</u> L. cv Bell Pepper	28	2	46
<u>Lycopersicum esculentum</u> P. Mill. cv Bonny Best Tomato	205675	1338	598205

Table 8. (Continued)

Plant	Numbers of <u>Pratylenchus hexincisus</u>		
	Gram dry root	100 cm ³ soil	Pot (1500 cm ³ soil + roots)
UMBELLIFERAE			
<u>Daucus carota</u> L. cv Imperater #408 Carrot	34	2	39
COMPOSITAE			
<u>Helianthus annuus</u> L. Sunflower	159	5	429
POLYGONACEAE			
<u>Polygonum Hydropiper</u> L. Smartweed	350	60	2507

Table 9. Analysis of variance for numbers of Pratylenchus hexincisus/g dry root in four soil types at four temperatures in growth chambers for three months, April 20, 1978 to January 16, 1979

Source of variation	df	Mean square	F value	Prob > F
Temp	3	8326.582	173.26	0.0001
Soil	3	1009.876	21.01	0.0001
Sampling	2	4251.445	88.46	0.0001
Temp * soil	9	768.728	15.99	0.0001
Temp * sampling	6	3516.472	73.17	0.0001
Soil * sampling	6	214.892	4.47	0.0006
Temp * soil * sampling	18	263.837	5.48	0.0001
Residual	<u>144</u>	48.057		
Corrected total	191	405.698		

Table 10. Analysis of variance for numbers of Pratylenchus hexincisus/ whole root system in four soil types at four temperatures in growth chambers for three months, April 20, 1978 to January 16, 1979

Source of variation	df	Mean square	F value	Prob > F
Temp	3	118919.037	169.30	0.0001
Soil	3	12822.701	18.25	0.0001
Sampling	2	78344.076	111.53	0.0001
Temp * soil	9	11495.441	16.36	0.0001
Temp * sampling	6	54085.780	77.00	0.0001
Soil * sampling	6	4018.965	5.72	0.0001
Temp * soil * sampling	18	4970.187	7.07	0.0001
Residual	<u>144</u>	702.399		
Corrected total	191	6254.501		

Table 11. Analysis of variance for Pratylenchus hexincisus/100 cm³ of soil in four soil types at four temperatures in growth chambers for three months, April 20, 1978 to January 16, 1979

Source of variation	df	Mean square	F value	Prob > F
Temp	3	1020.612	167.28	0.0001
Soil	3	16.619	2.72	0.0400
Sampling	2	969.67	158.93	0.0001
Temp * soil	9	12.581	2.06	0.0300
Temp * sampling	6	832.564	136.45	0.0001
Soil * sampling	6	9.724	1.59	0.1500
Temp * soil * sampling	18	9.850	1.61	0.0600
Residual	<u>144</u>	6.101		
Corrected total	191	59.025		

Figure 13. Numbers of Pratylenchus hexincisus in soil and corn roots maintained at 15 C in four soils: Marshall silt loam (MSL), Clarion silt loam (CSL), Buckner coarse sand (BCS), and Haig sandy clay loam (HSCL). M1, M2, and M3 represent first, second, and third month of sampling, respectively. An inoculum consisting of 2,000 P. hexincisus was used. LSD represents least significant difference

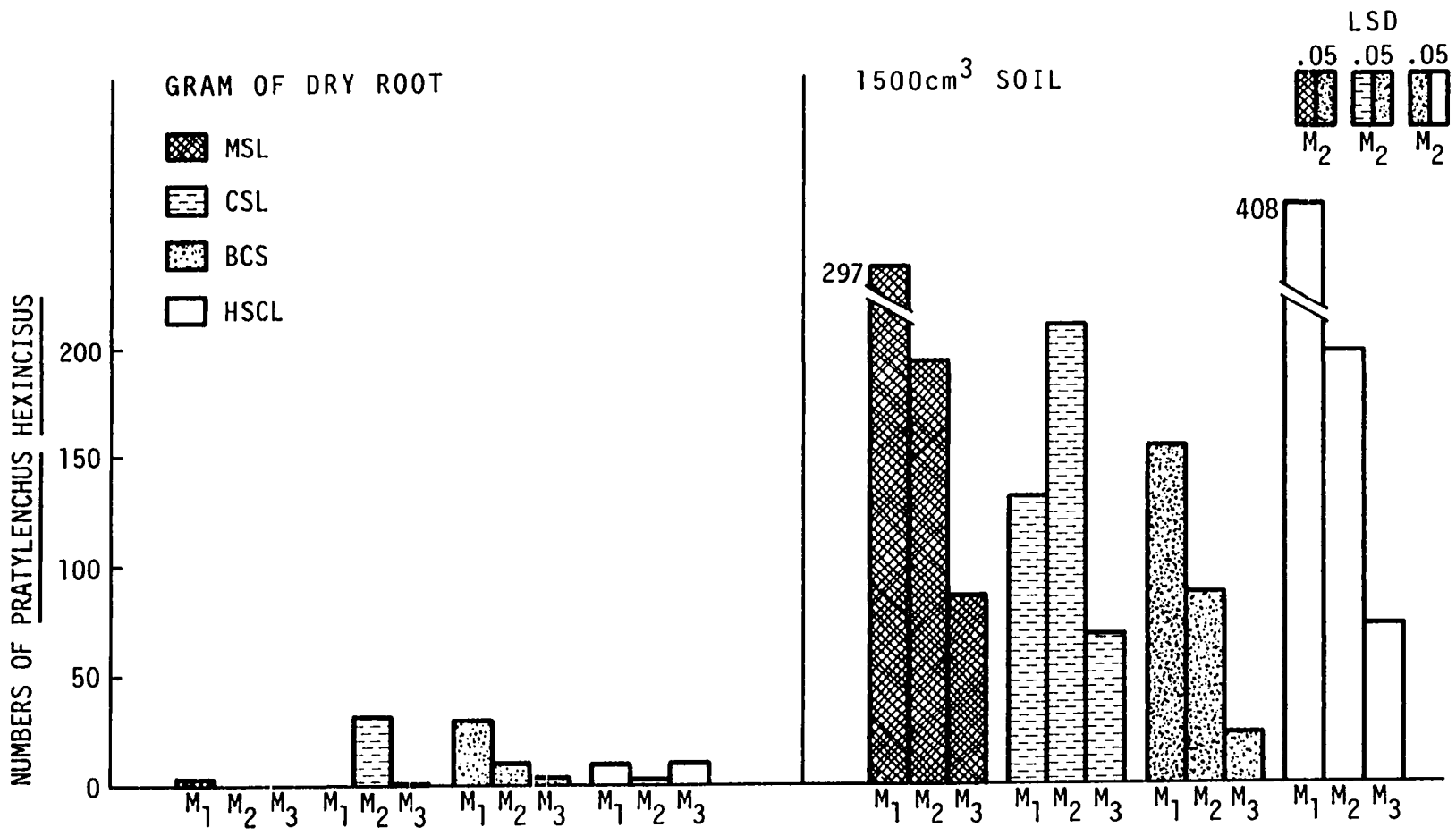


Figure 14. Numbers of Pratylenchus hexincisus in soil and corn roots maintained at 20 C in four soils: Marshall silt loam (MSL), Clarion silt loam (CSL), Buckner coarse sand (BCS), and Haig sandy clay loam (HSCL). M1, M2, and M3 represent first, second, and third month of sampling, respectively. An inoculum consisting of 2,000 P. hexincisus was used. LSD represents least significant difference

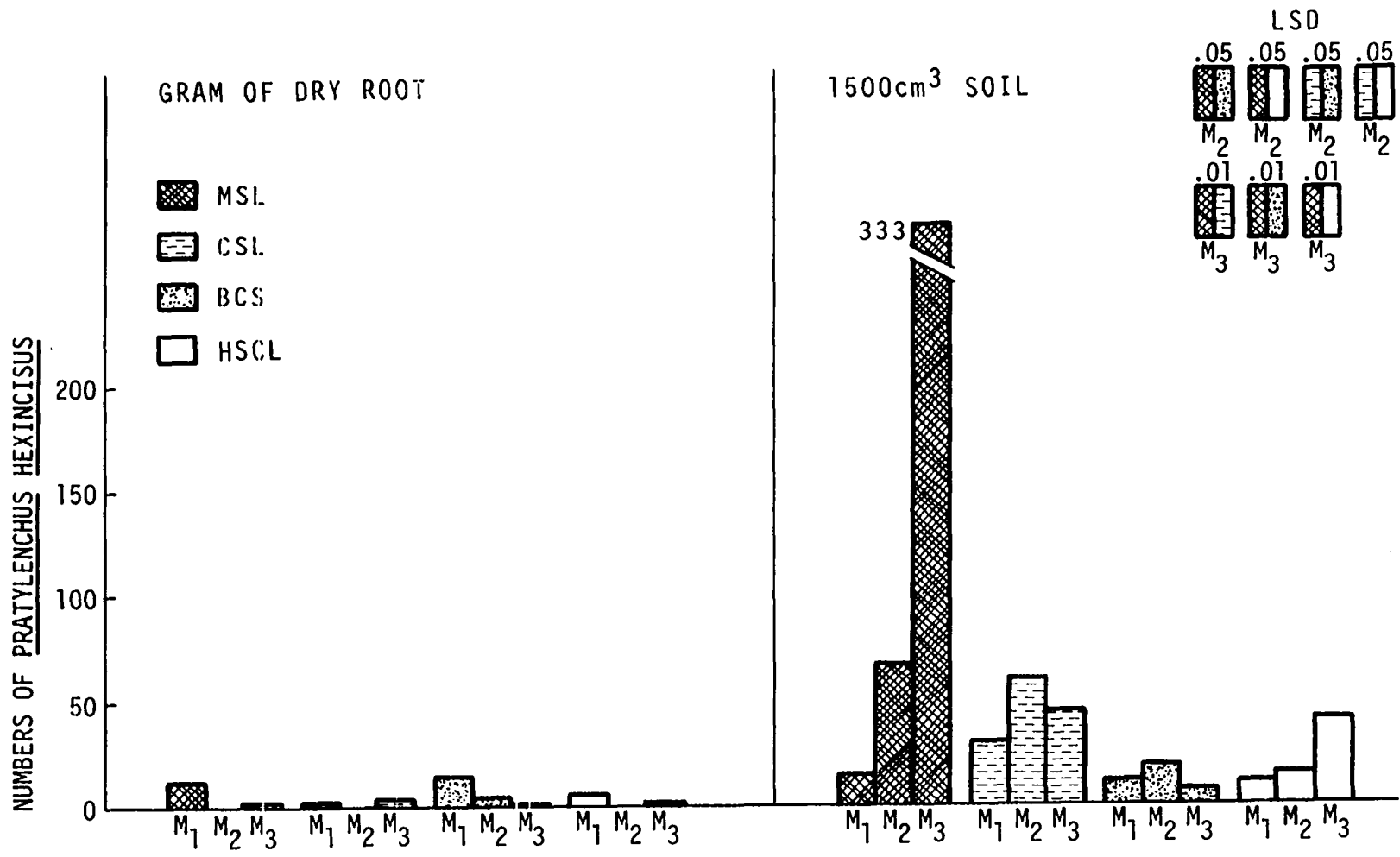


Figure 15. Numbers of Pratylenchus hexincisus in soil and corn roots maintained at 25 C in four soils: Marshall silt loam (MSL), Clarion silt loam (CSL), Buckner coarse sand (BCS), and Haig sandy clay loam (HSCL). M1, M2, and M3 represent first, second, and third month of sampling, respectively. An inoculum consisting of 2,000 P. hexincisus was used. LSD represents least significant difference

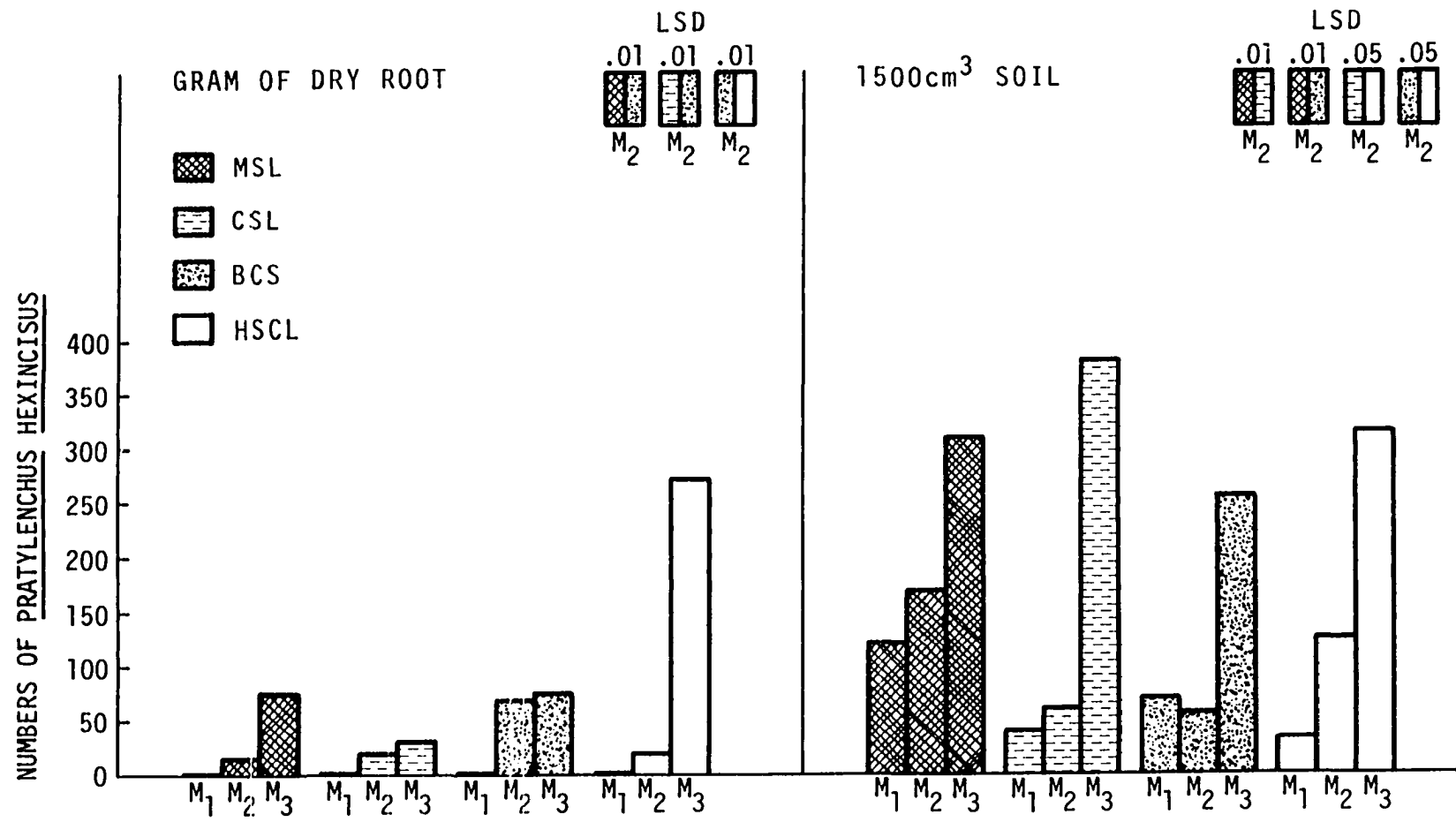


Table 12. Transformed numbers of Pratylenchus hexincisus among four temperatures within each of four soil types over three sampling times. An inoculum consisting of 2,000 P. hexincisus was used

Soil types	Sampling time (month)	Temp (C)	<u>P. hexincisus</u> (mean) ¹ gram dry root	Temp (C)	<u>P. hexincisus</u> (mean) ¹ 100 cm ³ soil
Marshall silt loam	1	30	3.65a	15	4.45a
		20	3.08ac	25	2.20bd
		15	1.58ac	20	1.35cd
		25	1.31bc	30	1.10c
	2	30	11.40a	30	7.10a
		25	3.70b	15	3.67b
		20	1.00c	25	3.50b
		15	1.00c	20	2.30b
	3	30	31.68a	30	24.93a
		25	7.88b	25	5.09b
		20	1.50c	20	4.76c
		15	1.00c	15	2.56c
Clarion silt loam	1	30	6.6a	15	2.98a
		15	4.96a	25	1.90b
		20	1.46b	20	1.72b
		25	1.00b	30	1.00b
	2	30	9.77a	30	7.10a
		25	4.34b	15	3.81b
		15	1.46b	20	2.22c
		20	1.25b	25	2.22c

	3	30	60.29a	30	36.12a
		25	5.63b	25	4.98b
		20	1.65b	15	2.34b
		15	1.00b	20	1.92b
Buckner coarse sand	1	30	10.61a	15	3.33a
		15	5.44b	25	2.32b
		20	3.62bd	20	1.29c
		25	1.00cd	30	1.1c
	2	30	39.54a	30	4.87a
		25	8.69b	15	2.63b
		15	3.08b	25	2.16bd
		20	2.14b	20	1.47cd
	3	30	109.69a	30	25.65a
		25	8.29b	25	4.16b
		15	1.84b	15	1.49b
		20	1.31b	20	1.21b
Haig sandy clay loam	1	30	2.81a	15	4.98a
		15	2.66a	25	1.72b
		20	1.77a	30	1.35b
		25	1.5a	20	1.29b
	2	30	13.62a	30	4.89a
		25	3.72b	15	3.75ab
		15	1.54b	25	2.98bc
		20	1.50b	20	1.37c
	3	30	51.58a	30	27.43a
		25	13.27b	25	4.54b
		15	3.00b	15	2.22b
		20	1.62b	20	1.84b

¹Means with common letters within a column are not significantly different at P = 0.05 (Duncan's Multiple Range Test).

two largest numbers of P. hexincisus in roots were obtained in Haig sandy clay loam and in Clarion silt loam (Figures 15, 17). At 20 C there were significant differences ($P = 0.05$ or $P = 0.01$) in numbers of P. hexincisus/whole root at the first sampling and P. hexincisus/1500 cm³ soil at second and third sampling among four soil types (Figures 14, 17). At 15 C no significant difference was obtained on P. hexincisus in roots, but numbers differed significantly ($P = 0.05$) in the soil among four soil types. At this temperature large numbers of P. hexincisus/g dry root and /1500 cm³ soil occurred at the first sampling in all soil types except Clarion silt loam and decreased through the second and third samplings (Figures 13-16).

The optimum temperature, at least with temperatures tested, for P. hexincisus reproduction in all the soils was 30 C, the final population being significantly ($P = 0.01$) greater than the initial population (Table 12). At 25 C, P. hexincisus invaded the roots and reproduced slightly and only in Haig sandy clay loam did the final population exceed the initial level after three months (Table 12). Few P. hexincisus were recovered from roots growing at 20 C and 15 C in all soil types. Because of a dilution factor due to the increasing root biomass, numbers of P. hexincisus/g dry root decreased through three sampling times at these two temperatures (Table 12). Numbers of P. hexincisus/1500 cm³ of soil was maximum at 30 C and minimum at 20 C.

Eight out of 18 comparisons among various physical and chemical characteristics of the soil with numbers of P. hexincisus in the roots or soil were significantly correlated (Table 13). P. hexincisus numbers in roots were positively correlated with percent sand and pH, and

Figure 16. Numbers of Pratylenchus hexincisus in soil and corn roots maintained at 30 C in four soils: Marshall silt loam (MSL), Clarion silt loam (CSL), Buckner coarse sand (BCS), and Haig sandy clay loam (HSCL). M1, M2, and M3 represent first, second, and third month of sampling, respectively. An inoculum consisting of 2,000 P. hexincisus was used. LSD represents least significant difference

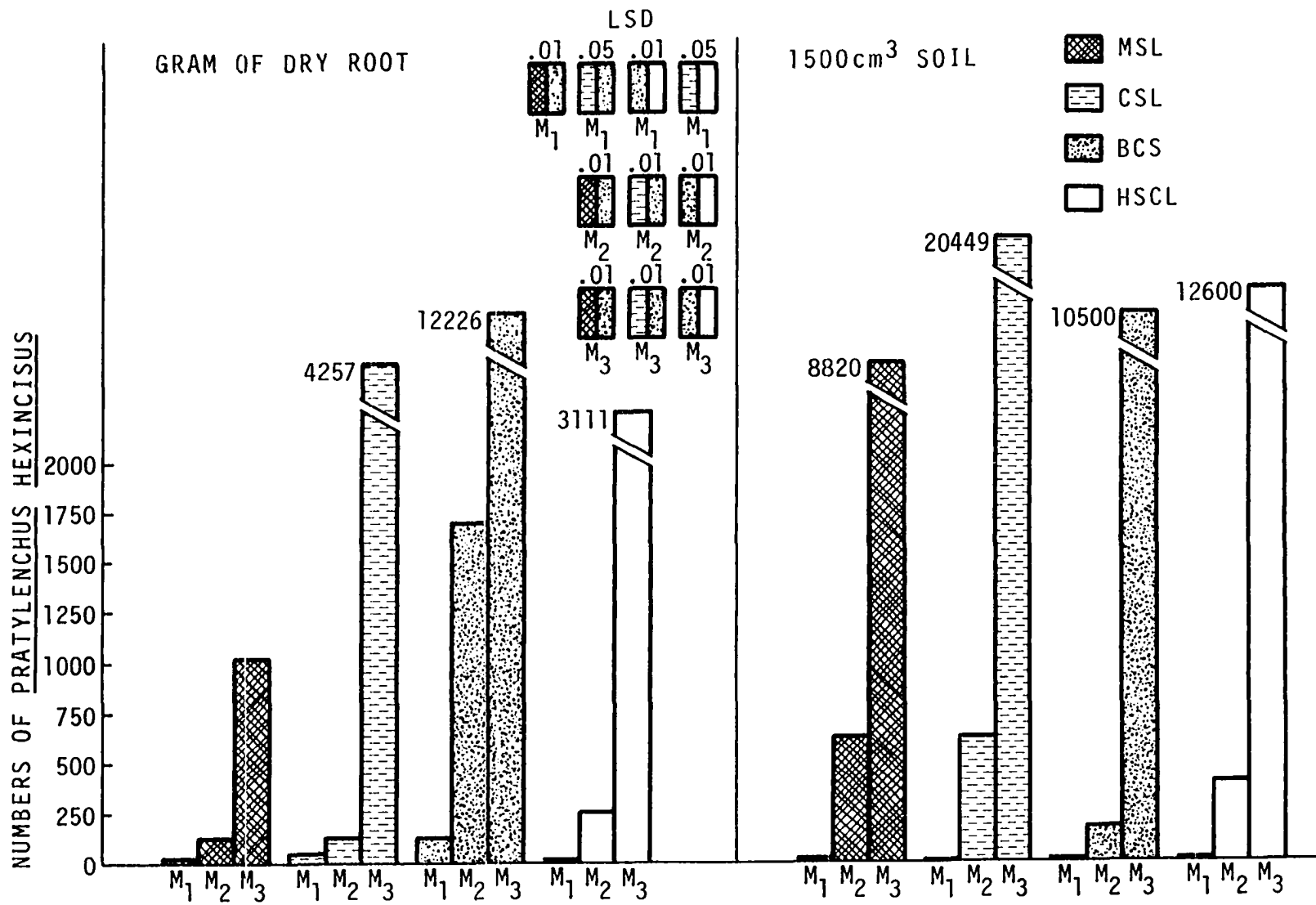


Figure 17. Numbers of Pratylenchus hexincisus per whole root of corn maintained at four temperatures, 15 C, 20 C, 25 C, and 30 C in four soils types, Marshall silt loam (MSL), Clarion silt loam (CSL), Buckner coarse sand (BCS), and Haig sandy clay loam (HSCL). M1, M2, M3 represent first, second, and third month of sampling, respectively. An inoculum consisting of 2,000 P. hexincisus was used. LSD represents least significant difference

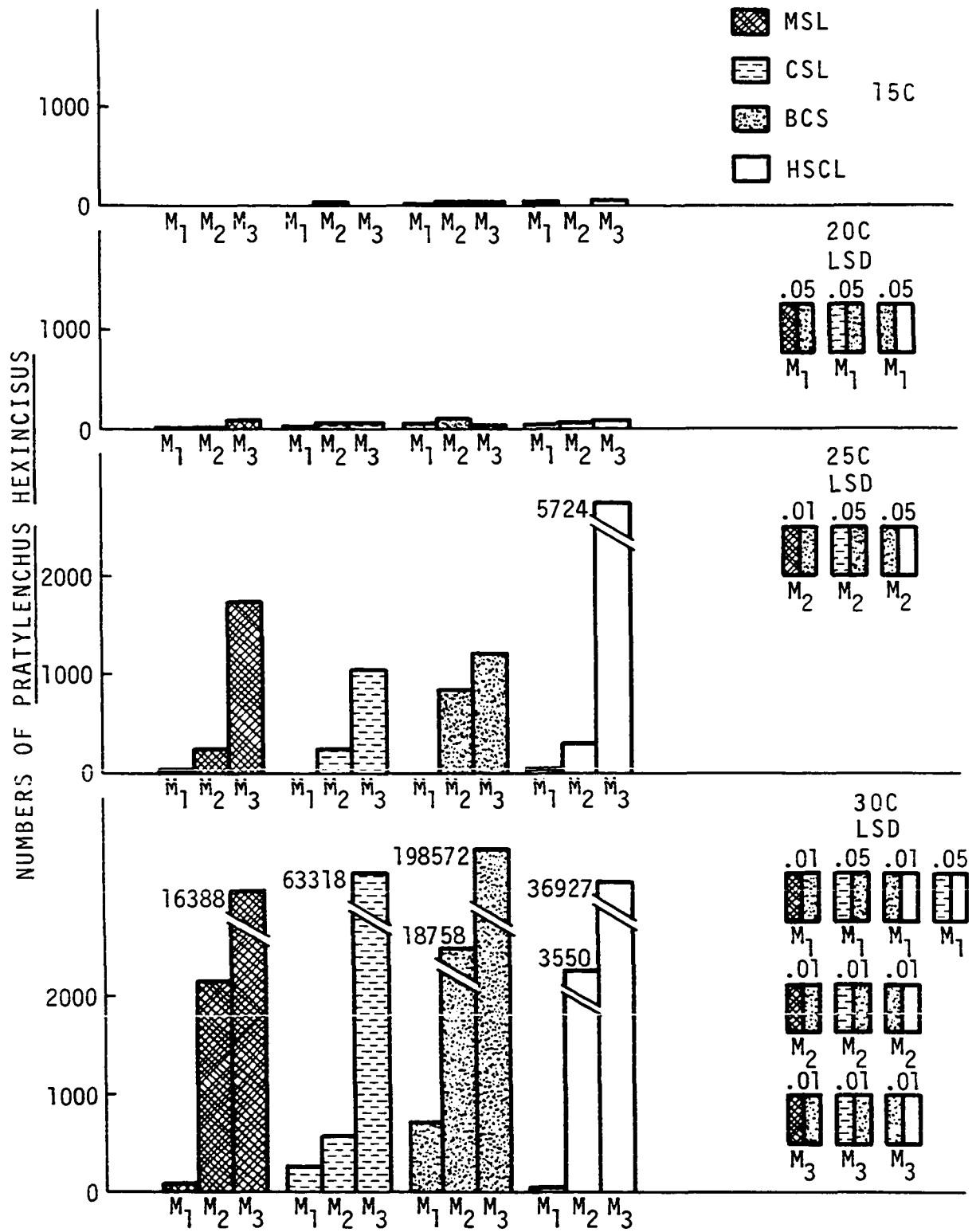


Table 13. Pratylenchus hexincisus correlations with physical and chemical characteristics of four soil types collected from different parts of Iowa in 1976^a

Soil parameter	<u>P. hexincisus</u> numbers	r	p
Percent sand	Soil	-0.08	
	Roots	0.25*	.04
Percent silt	Soil	0.08	
	Roots	-0.22	.07
Percent clay	Soil	0.06	
	Roots	-0.30**	.01
Field capacity	Soil	0.09	
	Roots	-0.28*	.02
Saturation percent	Soil	-0.02	
	Roots	-0.27*	.03
Temperature	Soil	0.65**	.0001
	Roots	0.52**	.0001
pH	Soil	0.07	
	Roots	0.25*	.04
Percent organic matter	Soil	0.12	
	Roots	-0.24*	.05
Cation exchange capacity	Soil	0.11	
	Roots	-0.23	.06

^aProbability of a lesser r value for correlation coefficients without asterisks is less than 10%.

*Probability of a lesser r value is less than 5%.

**Probability of a lesser r value is less than 1%.

negatively with percent silt, percent clay, field capacity, saturation percent, percent organic matter and cation exchange capacity. Numbers of P. hexincisus in soil were positively correlated with percent organic matter and cation exchange capacity.

The time for development of P. hexincisus can be expressed in heat units. Each centigrade degree above 10 C acting for one hour is counted as one heat unit. Population increase of P. hexincisus correlated with heat units at one temperature over time. Numbers of nematodes greatly differed, however, with identical heat units obtained at different temperatures over time (Table 14). P. hexincisus numbers differed with the same heat units in different soil types (Table 14), but in a given soil type, the numbers of P. hexincisus increased with increasing heat units (Table 14).

Table 14. Numbers of Pratylenchus hexincisus relative to heat units and soil types

Temp (C)	Days	Heat units	Soil types	<u>P. hexincisus</u>	
				Whole root	1500 cm ³ soil
15	30	3600	Marshall silt loam	2	296
15	30	3600	Clarion silt loam	15	131
15	30	3600	Buckner coarse sand	20	154
15	30	3600	Haig silty clay loam	25	409
15	60	7200	Marshall silt loam	0	191
15	60	7200	Clarion silt loam	3	210
15	60	7200	Buckner coarse sand	23	90
15	60	7200	Haig silty clay loam	3	199
20	30	7200	Marshall silt loam	10	15
20	30	7200	Clarion silt loam	6	30
20	30	7200	Buckner coarse sand	40	11
20	30	7200	Haig silty clay loam	5	11
15	90	10800	Marshall silt loam	0	86
15	90	10800	Clarion silt loam	0	68
15	90	10800	Buckner coarse sand	18	23
15	90	10800	Haig silty clay loam	45	71
25	30	10800	Marshall silt loam	10	120
25	30	10800	Clarion silt loam	0	41
25	30	10800	Buckner coarse sand	0	71
25	30	10800	Haig silty clay loam	12	34
20	60	14400	Marshall silt loam	11	68
20	60	14400	Clarion silt loam	14	60
20	60	14400	Buckner coarse sand	55	19
20	60	14400	Haig silty clay loam	34	15
30	30	14400	Marshall silt loam	86	3
30	30	14400	Clarion silt loam	236	0
30	30	14400	Buckner coarse sand	723	3
30	30	14400	Haig silty clay loam	46	15

Table 14. (Continued)

Temp (C)	Days	Heat units	Soil types	<u>P. hexincisus</u>	
				Whole root	1500 cm ³ soil
20	90	21600	Marshall silt loam	60	334
20	90	21600	Clarion silt loam	13	45
20	90	21600	Buckner coarse sand	15	8
20	90	21600	Haig silty clay loam	45	41
25	60	21600	Marshall silt loam	240	173
25	60	21600	Clarion silt loam	262	64
25	60	21600	Buckner coarse sand	861	56
25	60	21600	Haig silty clay loam	315	120
30	60	28800	Marshall silt loam	2149	776
30	60	28800	Clarion silt loam	585	746
30	60	28800	Buckner coarse sand	18758	356
30	60	28800	Haig silty clay loam	3544	394
25	90	32400	Marshall silt loam	1771	311
25	90	32400	Clarion silt loam	797	383
25	90	32400	Buckner coarse sand	1236	259
25	90	32400	Haig silty clay loam	5297	315
30	90	43200	Marshall silt loam	16388	8820
30	90	43200	Clarion silt loam	63318	20449
30	90	43200	Bickner coarse sand	198572	10500
30	90	43200	Haig silty clay loam	36927	12600

DISCUSSION

There are many reports on the association of Pratylenchus hexincisus with corn and soybeans in the North Central Region. Norton and Hinz (35) implicated P. hexincisus as a contributing factor to corn decline in sandy soils in Iowa. In my tests, the height and top weights of corn, soybean, and tomato plants were differentially affected by given population levels of P. hexincisus (Tables 2-7). Similar results have been reported with different species of Pratylenchus by Dickerson (8) and Miller (26). These results indicate that different plant species or varieties have varying reactions and tolerances to a given species of Pratylenchus. Significant reduction in stalk height and root and top biomass of corn and soybeans in my pathogenicity experiment support the concept that P. hexincisus is probably an important nematode attacking corn and soybeans in Iowa.

Associations of this nematode with important field crops such as wheat, barley, oats, rye, soybeans, peas, flax, and alfalfa and with different weed species under field conditions have been reported by Taylor, Anderson, and Haglung (48), Taylor and Schleder (47) from Minnesota, and by Norton (33) from Texas. The results of my host range study support the concept that P. hexincisus probably is a widespread nematode associated with different field crops and vegetables such as soybeans, garden pea, white Dutch clover, tomato, beets, and cabbage under field conditions. In addition, different species of weeds are favorable hosts for P. hexincisus. From the evidence presented in my pathogenicity and host range experiments, at least with the cultivars used, crops such as

soybeans, garden pea, and white Dutch clover should not be used as rotation crops with corn to control this nematode. Rotation with resistant crops will not result in the desired control unless susceptible weed plants such as velvetleaf, smartweed, and lambsquarters are controlled in the fields.

Soil types and texture (11, 23, 30, 34, 53) and temperature (2, 8, 34) are important factors for nematode reproduction and survival. Final populations of P. hexincisus in all soil types at 30 C were much larger than those that caused significant reductions in heights and top and root weights of corn, soybean, and tomato plants in the pathogenicity experiment. Although P. hexincisus reproduced in large numbers in all soil types tested at 30 C, numbers/g of dry root and /whole root were significantly higher in Buckner coarse sand than in the other soil types. My results support those of Norton (33), Thorne and Malek (50), Norton and Hinz (35), and Zirakparvar (56) who found P. hexincisus in abundance in heavier and sandy soils. Optimum temperature of 30 C for P. hexincisus reproduction also has been reported by Smolik (42). P. hexincisus required different numbers of heat units under different soil types to reach a given population size. Heat unit requirements for P. hexincisus reproduction changed depending upon the continuous temperature at which it is obtained. In Buckner coarse sand, P. hexincisus required 28,800 heat units, obtained at 30 C for 60 days, to build up to a level (Table 14) that caused significant biomass and height reduction of corn in the pathogenicity test. However, 32,400 heat units obtained at 25 C for 90 days did not result in a large build-up of this nematode in the same soil type. This indicates that probably temperatures above 25 C have a

stimulatory effect on P. hexincisus reproduction, however, this should be further investigated. About 43,000 heat units obtained at 30 C for 90 days caused a build-up of P. hexincisus in the four soil types to levels that were larger than those obtained in the pathogenicity test. The above evidence indicates that P. hexincisus is an important nematode attacking many crops in various soil types in areas where the accumulated heat units in a growing season reach 30,000 to 40,000.

SUMMARY

An inoculum of 5,000 Pratylenchus hexincisus significantly decreased the root and top biomass of corn, soybean, and tomato after three months in the greenhouse. An inoculum of 20,000 P. hexincisus significantly decreased the height and top and root biomass of corn plants in all three monthly samplings. Discoloration of the roots was observed only in corn plants infested with P. hexincisus.

After three months, P. hexincisus was recovered from roots of 42 plant species inoculated with 2,000 nematodes in the greenhouse. In 12 plant species, the final population of P. hexincisus/pot was larger than the initial population. After three months, tomato, garden pea, white Dutch clover, velvetleaf, and soybean roots contained more P. hexincisus/g dry root than the check host, corn.

The optimum temperature for reproduction of P. hexincisus was 30 C in Marshall silt loam, Clarion silt loam, Buckner coarse sand, and Haig silty clay loam soils. The P. hexincisus population was significantly larger in Buckner coarse sand than the other soil types at 30 C. The final population of P. hexincisus equaled or exceeded inoculum levels at temperatures of 25 C or above in Marshall silt loam and Haig silty clay loam, and at 30 C in Clarion silt loam and Buckner coarse sand.

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