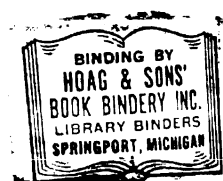


A STUDY OF A MICHIGAN ISOLATE
OF MELOIDOGYNE HAPLA

Thesis for the Degree of M. S.
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ABSTRACT

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By

James K. Brody, Jr.

Various cropping sequences influenced the populations of Meloidogyne hapla Chitwood 1949 in field microplot studies. Populations of M. hapla on celery increased during the growing season. Low populations were noted on carrots, and populations declined in microplots maintained in onions, sweetcorn, or fallow. Greenhouse studies supported the field study. Onions appeared more effective in decreasing M. hapla associated macrodamage to a subsequent crop of carrots than were other cropping sequences studied in the greenhouse. Successive crops of radishes had a trap cropping effect, reducing M. hapla populations.

Host crops affected the generation time of M. hapla. Generation times for M. hapla of 45, 56, and 72 days were found on carrots, celery, and onions, respectively. Sweetcorn appeared to be a nonhost. One generation failed to be completed on radishes.

Meloidogyne hapla penetrated carrot seedlings within 24 hours. Penetration occurred adjacent to the root cap, and the second stage larvae were oriented with their anterior ends towards the distal terminis of the root.

Survival of M. hapla was studied at temperatures of 7 and -14 C, and at ambient room temperature. Greater survival occurred at 7 C than at ambient room temperature or -14 C. Eggs of M. hapla appeared to survive better than the larvae at -14 C. After three days at that temperature, however, neither stage appeared to survive. Bioassays were found to be an important tool since indicator plants often showed galling in soil where no M. hapla larvae were found.

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INTRODUCTION

Northern root-knot nematodes (Meloidogyne hapla Chitwood 1949) are commonly found in Michigan, as well as other states, where numerous agricultural crops serve as host plants. Northern root-knot nematodes are the only nematodes known to cause economic losses to growers of carrots in Michigan, which ranks third in the nation in carrot production (19). Nationally, losses to carrots attributed to nematodes are estimated to be 20% annually (27).

The objectives of this study were: (1) to study the effect of cropping sequences upon population development of a Michigan isolate of M. hapla; (2) to determine the generation time of this isolate of M. hapla on various host crops; (3) to study the rate of larval penetration by M. hapla; (4) to investigate the effects of soil temperature upon the survival of M. hapla. Hopefully, such knowledge will contribute to a better understanding of the northern root-knot nematode in Michigan and assist in the development of improved control methods.

LITERATURE REVIEW

The genus Meloidogyne Goeldi 1887, commonly called the root-knot nematode, was first described as a plant parasite in 1855 when Berkeley (4) described root "excrecences" on the roots of cucumbers in England. In the United States, May (17) described the symptoms caused by root-knot nematodes on violets in 1888. The first studies of root-knot nematodes in the United States were carried out by Neal (21), Atkinson (1), and Stone and Smith (29).

Root-knot nematodes were considered to be of one species until 1949 when Chitwood (9) reclassified Heterodera marioni (Cornu 1879) Goodey 1932 into five species of Meloidogyne, including M. hapla Chitwood 1949, the northern root-knot nematode. Due to numerous name changes, it is difficult to ascertain which species of root-knot nematodes investigators were discussing prior to 1949.

The symptoms identifiable on above ground plant parts of plants infected with root-knot nematodes are quite general and are attributed to a disruption of the vascular system in the roots. Dwarfing, low yield, wilting,

premature death, and poor bloom are common expressions. Below ground symptoms of root-knot nematode infected plants are generally more pronounced than are the above ground symptoms. Gallings, stunting, necrosis and proliferation are common external symptoms on roots.

Profuse lateral root formation is associated with galls caused by the northern root-knot nematode. Smith and Mai (26) describe lateral root formation in onion (Allium cepa L.) roots. The same phenomenon is described by Sasser (24) on peanuts (Arachis hypogaea L.), soybeans (Glycines max (L.) Merr.), and tomatoes (Lycopersicon esculentum Mill.).

Whereas not all plants exhibit external symptoms, the formation of giant cells within the roots is a universal internal symptom (14). Christie (10) states that giant cell formation and gall formation may be a means by which a plant attempts to isolate the nematode. Dropkin and Nelson (12) found, however, that good nematode growth and fecundity were directly associated with giant cells of good quality. Galls with no giant cells, small giant cells, and highly vacuolated giant cells produced slower growing nematodes with a reduced fecundity.

The second stage larva of M. hapla, the stage which emerges from the egg, is the only infective stage. Once established in the root near the root tip with the head in the stele, the second stage larva becomes

sedentary. The larva molts three times within the root, and after each molt the nematode becomes more saccate. After the last molt, the nematode is considered an adult (14).

Meloidogyne hapla has a wide host range as do most root-knot nematodes. Host plants include numerous agricultural and horticultural plants as well as many weeds (24, 31). Crops grown on organic soils and which are known to be susceptible to M. hapla include carrots (Daucus carota L.), onions, and celery (Apium graveolens L.).

Wilson (36) found that after four or five years of continuous carrots in muck soil, 95-98% of the carrots were deformed by M. hapla. Onions have been described as a good host crop for M. hapla by Lewis et al. (16). Olthof et al. (22) estimates a 10% loss in muck grown onions in Ontario due to the northern root-knot nematode. Wilson (35, 36) reported that onions were not good hosts for M. hapla and that M. hapla populations decreased on onions.

The generation time of M. hapla is variable.

Smith and Mai (26) found that egg masses were produced on onions in about 36 days after infection. Using tomatoes as host plants, Bird (5) found that egg laying occurred 29 days after infection. Tyler (32), working with an unidentified species of root-knot nematode inoculated on tomatoes, found that egg laying occurred in 19-27 days and second stage larvae were present in 29-39 days. In

addition, Tyler showed that root-knot nematode generation time is temperature dependent.

Penetration of roots by M. hapla second stage larvae has been found to be quite variable. Bird and Wallace (6) found that 2.9% of a high population (60,000) of M. hapla second stage larvae entered the roots of a tomato plant within 48 hours. Kinlock and Allen (15), also using tomato plants as hosts, found that after ten days the amount of starting inoculum which had penetrated the host roots ranged from 65.3% of an initial larval count of 125 to 47.3% of an initial larval count of 1,000.

Working with M. hapla on onions, Smith and Mai (26) found penetration to occur within 24 hours. They found the second stage larvae within the roots initially oriented with their anterior end towards the distal end of the root. Later, the larvae reoriented so their anterior ends were oriented towards the proximal end of the root. In addition, these investigators found that more than one second stage larva may enter the root through a single site.

M. hapla appears to be able to survive adverse cold conditions since it is a recurring problem in Michigan. Daulton and Nusbaum (11) found that eggs of M. hapla survived under field conditions with temperatures ranging down to 0 C. Bergeson (3) reported that eggs of the northern root-knot nematode survived better at 0 C

than did the second stage larvae. Sayre (25b) found that M. hapla populations under winter field conditions in Ontario declined to 1/4 of the original population, but then remained constant.

MATERIALS AND METHODS

Inoculum for this study came from a Michigan isolate of Meloidogyne hapla which had been maintained on greenhouse grown carrots. A portion of the isolate was mixed with steam sterilized potting soil (5 parts loam, 1 part sand, and 1 part peat) and planted to 'Fireball' tomatoes or pascal celery. The above procedure was repeated as necessary to maintain and increase inoculum.

To facilitate locating nematodes and early gall formation, roots were stained with acid fuchsin in a 1:1 solution of glacial acetic acid and 95% ethanol, a modification of the McBryde method (18). The roots were stained for 24 hours and then cleared for 24 hours in chloral hydrate. After the above procedure had been performed, nematodes within the cleared roots appeared red or pink.

Larvae of M. hapla were recovered from soil by either a modified Baermann funnel technique (2) or the centrifugation-flotation method (20). Soil samples of 100 cc were processed in both cases. Samples from the field were obtained to a depth of 20 cm using a 3 cm

diameter soil sampler. Sufficient cores were obtained from each sampling site to constitute 500 cc of soil. Soil was placed in plastic freezer bags and stored at 7 C until processed.

Nematodes in processed samples were counted in a syracuse watch glass under the dissecting microscope at 40 X.

To determine soil infectivity, bioassays were conducted using one week old tomato seedlings which were transplanted into 7.6 cm plastic pots containing the soil under consideration. After four weeks of growth, the tomato roots were stained as previously described, and the galls 0.5 gm of roots were counted.

Cropping Study

To study the effects of cropping sequences on M. hapla populations, field microplots were established in Houghton muck at the Michigan State University Organic Soils Farm. The microplots consisted of 25 bottomless, galvanized steel garbage cans of 75 liter capacity. The cans were placed in the soil, five cans across and five cans deep on 3 m centers, to within 5 cm of the upper lip.

During the spring and summer of 1971, the following crops were grown in the microplots, five microplots per crop: 'Golden Beauty' sweetcorn (Zea mays L.), 'Yellow Globe' onions, 'Nantes' carrots, and pascal celery. In addition, five microplots were maintained clean fallow.

Prior to planting, each of the microplots was treated with captan fungicide at the rate of 6.7 kg active per hectare and then inoculated with the below ground contents of three 7.6 cm pots of M. hapla inoculum, about 30,000 larvae. The inoculum was incorporated into the top 0.3 m of soil within the microplots. Soil populations were determined three times during the growing season using the modified Baermann funnel technique.

During the spring and summer of 1972, the same crops were grown, but were rotated as shown in Fig. 1. In this manner all possible two year cropping sequences were obtained. Bioassays were conducted with the microplot soil three times during the growing season as were larval counts by the modified Baermann funnel technique.

In conjunction with the field microplot study, a greenhouse study was undertaken to determine the effects of various cropping sequences on M. hapla populations. Three parts potting soil was mixed with one part M. hapla infested soil and placed in 90 clay pots of 20 cm diameter. The following cropping sequences were investigated: carrots followed by cabbage (Brassica oleracea var. capitata L.); carrot followed by rye (Secale cereale L.); carrot followed by onion; carrot followed by one, two, and three crops of radishes (Raphanus sativus L.); carrot followed by one and two crops of carrots; carrot followed by fallow. Larval numbers were determined by the

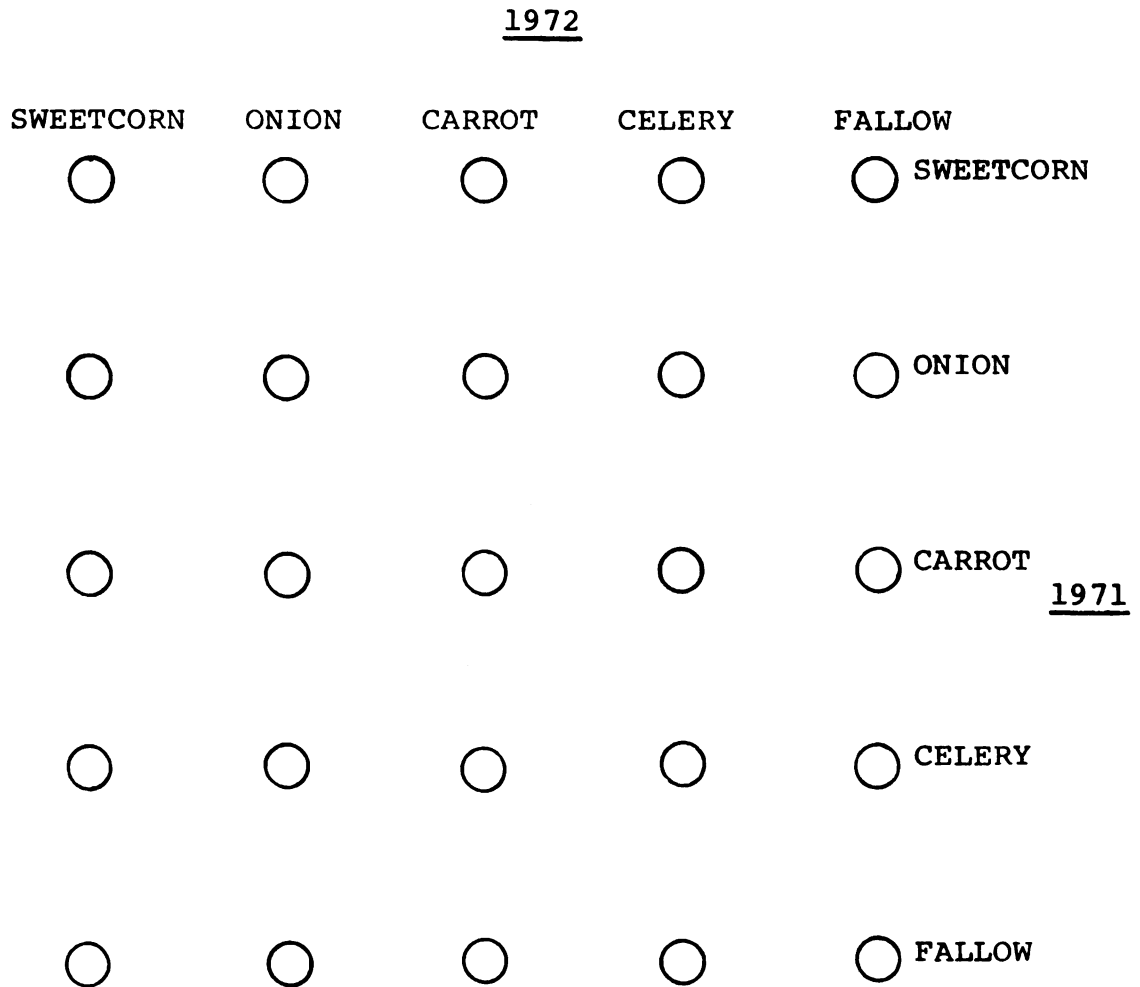


FIG. 1. Microplot cropping diagram showing crops grown in individual microplots in the 1971 and 1972 growing seasons.

centrifugal-flotation method before planting and after harvesting. Crops were allowed to grow for the approximate number of days which are needed in commercial production (after 78 days in the case of rye).

Carrots were planted in the pots of soil which had contained rye, onion, cabbage, and the third crop of radishes and allowed to grow for about 70 days. The number of carrots with galls, split tap roots, and/or a hairy root condition served to indicate the influence of the preceding cropping sequences on M. hapla populations. In addition, the carrot roots of the second and third carrot crops in the carrot, carrot, carrot rotation were similarly evaluated.

Generation Time Study

To determine the generation time of M. hapla on various crops, 'Golden Beauty' sweetcorn, 'Nantes' carrots, 'Yellow Globe' onions, pascal celery, and 'Champion' radishes were grown in the greenhouse in 20 cm clay pots containing sand which had been fumigated with methyl bromide. The fumigated soil was aired for four days prior to planting. Immediately before planting, the pots were inoculated with infected roots from the stock culture by incorporating the infected roots into the sand in the pots. After the pots were inoculated, seeds of the previously mentioned crops were planted.

At approximately 14 day intervals, two pots of each crop were examined. The soil was processed by the centrifugal-flotation method. Roots were stained and the amount of galling, the development of the galls, and the development of the nematodes noted.

Penetration Study

A 24 hour penetration study of M. hapla on 'Nantes' carrot seedlings was conducted using the method described by Chapman and Eason (8). Seeds were treated in 1% Chlorox^{®1} for 15 minutes, rinsed in distilled water, and allowed to germinate on dampened Miracloth^{®2} in a covered petri dish at room temperature. Two day old seedlings were transferred to syracuse watch glasses, two seedlings per glass, which had discs of Miracloth covering the bottoms. The seedling roots were covered with a second disc of Miracloth in such a manner that the seedling roots were sandwiched between discs of Miracloth and the cotyledons and stems were above the sandwich. Each watch glass was then inoculated with 50 M. hapla larvae, which were no more than 24 hours old, in 2 ml of water, covered, and incubated at 21 C in the dark for various lengths of time. Every two hours a dish

^{®1} Registered trademark, The Chlorox Company, P. O. Box 24305, Oakland, California 94623.

^{®2} Registered trademark, Chicopee Mills, Inc., 1450 Broadway, New York, N. Y. 10018.

was removed from the incubator and the roots stained. The occurrence of penetration and the orientation of the nematodes were noted.

Cold Survival Study

Cold survival studies were conducted to determine whether eggs or larvae of M. hapla have the greatest overwintering potential in Michigan. 500 cc quantities of M. hapla infested soil were sealed in plastic freezer bags and placed at either ambient room temperature, 7 C, or -14 C. At the end of two, six, and eight weeks, four bags of soil from each temperature treatment were sampled. Larvae were extracted by the centrifugal-flotation method and the remaining soil in each sample was planted with a tomato indicator plant. In addition to the above procedure, four bags of soil were removed daily for ten days from the -14 C treatment and the soil was processed as described above.

RESULTS AND DISCUSSION

Cropping Study

The cropping study conducted in the field microplots showed that cropping sequences can distinctly affect the northern root-knot nematode populations in the soil (Table 1; Fig. 2). Both in 1971 and in 1972, celery caused M. hapla populations to increase greatly during the growing season. In 1971 the M. hapla population in the celery microplots went from an average of 40 larvae in 100 cc of soil on May 18 to an average of 576 larvae in 100 cc of soil on September 14. With one exception, the M. hapla population in the celery microplots also increased in 1972, though not as dramatically as in 1971. It is interesting to note that on the May 18 and July 20 sampling dates in 1972, only the microplots which had been in celery during the 1971 season had a detectable M. hapla larval population in the soil.

Onions, fallow, and sweetcorn suppressed M. hapla populations, particularly when rotated with each other (Table 2). Rotations of these crops resulted in low mean populations of larvae and low mean number of

TABLE 1. Influence of cropping sequences on M. hapla populations in field microplots.

1971 Season																									
Crop	Corn					Onion					Carrot					Celery					Fallow				
Microplot	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
<u>Date</u>																									
18 May	32 ¹	8	0	0	8	48	64	32	32	16	24	0	16	24	40	32	64	24	64	56	24	0	16	8	0
13 July	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
14 Sept.	0	0	0	0	0	0	0	0	0	0	8	8	24	8	24	968	672	416	608	216	0	0	8	0	0
1972 Season																									
Crop	Corn					Onion					Carrot					Celery					Fallow				
Microplot ²	5	10	15	20	25	4	9	14	19	24	3	8	13	18	23	2	7	12	17	22	1	6	11	16	21
<u>Date</u>																									
18 May	0	0	0	16	0	0	0	0	48	0	0	0	0	32	0	0	0	0	48	0	0	0	0	0	0
20 July	0	0	0	8	0	0	0	0	16	0	0	0	0	8	0	0	0	0	8	0	0	0	0	0	32
29 Aug.	8	0	0	0	0	0	0	0	0	0	16	8	0	8	0	24	24	24	104	0	0	0	0	0	0

1 Numbers are for second stage larvae in 100 cc of soil.

2 Microplot numbers in 1972 season correspond to microplot numbers in 1971 season.

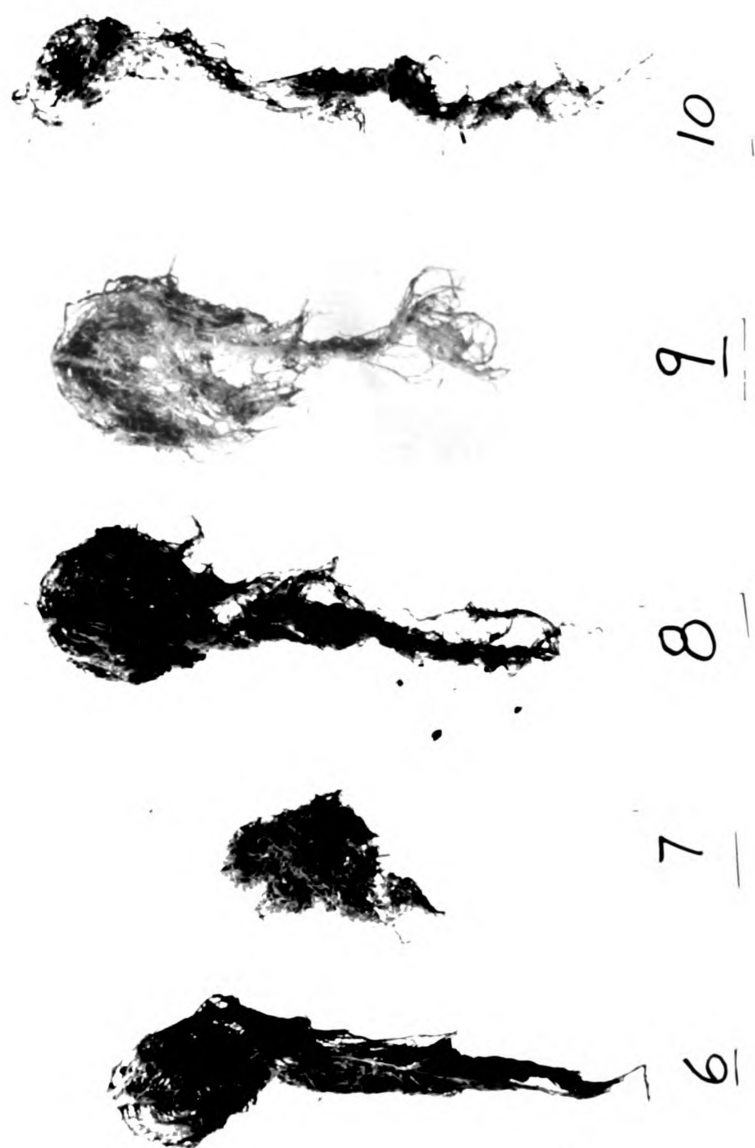


FIG. 2. Effect of various carrot rotations on M. hapla populations as shown by roots of four week old tomato indicator plants.

1 Crops following carrots: (6), clean fallow; (7), celery; (8), carrot; (9), onion; (10), sweetcorn.

TABLE 2. Effect of cropping sequences on the number of galls caused by M. hapla on 0.5 gm of roots of tomato indicator plants in the 1972 season.

1971 Crop	1972 Crop	18 May	20 July	29 Aug.
Onion	Celery	0	2	133
Celery	Celery	86	25	108
Carrot	Celery	57	28	92
Corn	Celery	0	17	71
Fallow	Celery	4	0	38
Onion	Carrot	2	0	0
Celery	Carrot	79	5	32
Carrot	Carrot	14	2	17
Corn	Carrot	0	3	0
Fallow	Carrot	1	0	0
Onion	Onion	6	0	0
Celery	Onion	62	13	17
Carrot	Onion	20	0	6
Corn	Onion	2	0	0
Fallow	Onion	2	0	0
Onion	Corn	0	0	0
Celery	Corn	46	4	0
Carrot	Corn	10	0	0
Corn	Corn	0	0	0
Fallow	Corn	1	0	0
Onion	Fallow	0	0	0
Celery	Fallow	40	31	23
Carrot	Fallow	9	5	0
Corn	Fallow	0	0	0
Fallow	Fallow	1	0	0

galls on tomato indicator plants. M. hapla populations in the onion microplots decreased to the same extent as populations in the sweetcorn and fallow microplots.

Of the crops studied, carrots were intermediate in their ability to support the northern root-knot nematode. M. hapla populations in the carrot microplots were much less than in the celery microplots, but greater than in the sweetcorn, onion, or fallow microplots. Meloidogyne hapla populations in the carrot microplots were less at the end of the 1972 season than at the end of the 1971 season.

The greenhouse cropping study tended to support the findings of the field microplot study. Cabbage greatly increased the number of M. hapla larvae in the soil (Table 3). In the case of radishes, each successive crop decreased the M. hapla population. Successive crops of carrots increased populations of M. hapla while rye and fallow tended to suppress M. hapla populations. Onions maintained a small population of M. hapla.

When considering damage done to carrots planted after various cropping sequences, onions preceeding carrots gave apparent suppression of root-knot nematode symptoms on the carrots (Fig. 3). Continuous carrots resulted in very few carrots showing no symptoms of root-knot nematode damage.

TABLE 3. Effect of cropping sequences on the number of M. hapla second stage larvae in 100 cc of soil under greenhouse conditions.

Crop Rotation ¹	<u>M. hapla</u> Population ²
Cabbage	93.6 a
Onion	13.6 bc
Rye	7.2 bc
Fallow	5.6 c
Radish	8.0 bc
Radish after radish	7.2 bc
Radish after radish after radish	5.6 bc
Carrot	14.4 bc
Carrot after carrot	27.2 b

1 One crop of carrots preceeded all rotations shown.

2 Numbers are means of ten replications. The small letters indicate Duncans new multiple range groupings which do not differ significantly at the 5% level.

FIG. 3. Effect of cropping sequences on producing carrots free of damage caused by M. hapla.

- 1 Cropping sequences, most recent crop listed first: (1) carrot, carrot; (2) carrot, carrot, carrot; (3) carrot, three successive radish crops, carrot; (4) carrot, rye, carrot; (5) carrot, onion, carrot; (6) carrot, cabbage, carrot.

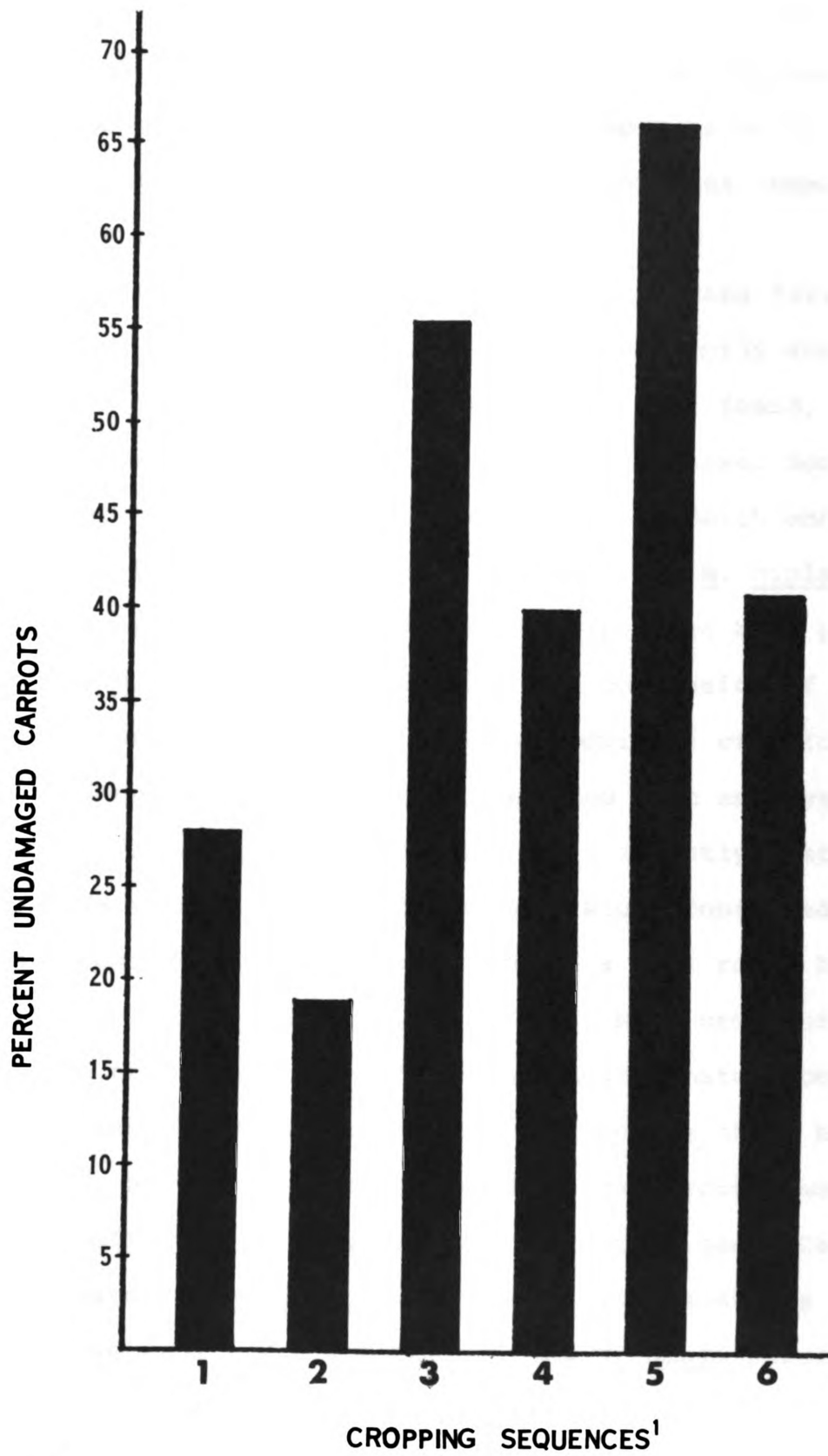


FIG. 3

The symptoms of M. hapla on carrots did not occur with equal frequency (Table 4; Fig. 4). Gallings was always more common than either split taproots or hairy roots. The hairy root condition was the least common in all cases except in the onion rotation.

Brzeski (7) found that small grains and fallow suppressed M. hapla populations and consequently would be appropriate to have in carrot rotations. He found, however, that onions preceeding carrots greatly increased northern root-knot nematode damage to the carrots. Smith and Mai (26) also found onions to be good hosts for M. hapla. As reported by Sasser (24), onions were found to be a poor host for M. hapla, which was also the conclusion of this study. Onions appeared to be an appropriate crop to precede carrots. Sasser (24) reported corn and rye as nonhosts for M. hapla and cabbage as a slightly better host than were carrots. The current study confirmed on a population basis Sasser's findings on a host range basis.

Celery appeared to be the best host crop for M. hapla in this study. Northern root-knot nematode populations increased more on celery than on any other host studied. Whether or not the northern root-knot nematode greatly affects celery yield was not determined. Carrots, while hosts for M. hapla, were not as good hosts as one might have expected. Apparently, low M. hapla populations can cause severe economic problems on carrots.

TABLE 4. Effects of cropping sequences on macrosymptoms associated with M. hapla damage on carrots.

Crop Sequence ¹	% Galled	% Split	% Hairy
1	58.5	43.9	13.4
2	64.9	51.3	13.5
3	34.7	18.4	8.2
4	51.1	29.9	0
5	25.4	8.5	15.3
6	54.5	18.2	11.4

¹ Cropping sequences, most recent crop listed first: (1) carrot, carrot; (2) carrot, carrot, carrot; (3) carrot, three successive radish crops, carrot; (4) carrot, rye; (5) carrot, onion, carrot; (6) carrot, cabbage, carrot.



FIG. 4. Carrots showing galling, forking of the taproot, and a hairy root condition caused by M. hapla.

Generation Time Study

The results of the generation time study indicated that host plants influenced the generation time (second stage larvae to second stage larvae) of the northern root-knot nematode (Table 5). The generation time on carrots was found to be about 45 days. Therefore, two and possibly three generations of M. hapla could develop on carrots in a growing season of 90-120 days.

Limited reproduction occurred on onions, but galling was light. The second generation of second stage larvae was observed in egg masses after 72 days, but these second stage larvae were never observed in the root proper. Lewis et al. (16) found that M. hapla larvae which came from females on onions could infect new onion seedlings. Whether or not the larvae would reinfect the original host was not determined by them. Smith and Mai (26) found numerous egg masses on M. hapla infested onions after 35 days of infection. In the current study, egg masses were observed in 45 days. Temperature differences may account for this difference. Onions could experience only one complete generation in the growing season with a 72 day generation time.

Numerous egg masses were observed on radish, but no second stage M. hapla larvae were observed as a consequence of reproduction on the radish roots. The taproot of the radish never appeared galled or split as

TABLE 5. Development of M. hapla and galling on four different crops.

		Days Since Germination				
Crop	Observations	7	14	21	28	35
Carrot	Nematode Dev. ¹		L ₂			female
	Galling ²		0			2
	Egg Masses ³		0			2
	L ₂ From Eggs ⁴		-			-
	Days ⁵		14			30
Onion	Nematode Dev.		L ₂		L ₄	
	Galling		0		1	
	Egg Masses		0		0	
	L ₂ From Eggs		-		-	
	Days		14		30	
Celery	Nematode Dev.		L ₂		L ₄	
	Galling		0		1	
	Egg Masses		0		0	
	L ₂ From Eggs		-		-	
	Days		14		28	
Radish	Nematode Dev.	L ₂	L ₂	female		
	Galling	0	1	3		
	Egg Masses	0	0	0		
	L ₂ From Eggs	-	-	-		
	Days	5	12	25		

1 (L), larvae; (2, 3, 4), stages of larvae; (female, male), adults.

2 (0), none; (1), 0-10%; (2), 11-30%; (3), 30+% of root galled.

3 (0), none; (1), 0-10%; (2), 11-30%; (3), 30+% of galls with egg masses.

4 Second stage larvae present (+) or not present (-) in galls or egg masses.

5 The number of days since germination.

TABLE 5. (Con't.).

Crop	Time Since Germination							
	42	49	56	63	70	77	84	91
	female		female		female, male		female, L ₃	
	2		2		2		3	
Carrot	2		2		2		2	
	+		+		-		-	
	45		58		72		86	
	female		female		female		female	
	1		1		1		1	
Onion	1		1		2		1	
	-		-		+		-	
	45		58		72		86	
	female, L ₂		female		female		none	
	1		2		1		0	
Celery	0		3		2		0	
	-		+		-		-	
	42		56		70		87	
	female							
	3							
Radish	3							
	-							
	42							

TABLE 5. (Con't.).

Days Since Germination				
Crop	98	105	112	119
	female			female
	3			3
Carrot	2			2
	-			+
	103			117
	none			none
	0			0
Onion	0			0
	-			-
	103			117
	female			
	2			
Celery	1			
	+			
	101			

often occurs with northern root-knot infected carrots. Radishes were grown for 42 days in this study, while radishes are grown commercially for only about 22 days. Hence, radishes could serve as a trap crop for M. hapla.

Hatch in older radish root systems may be hampered by root exudates which are detrimental to hatching. Wuest and Bloom (37) have demonstrated that root emanations are not required for hatch to occur, while Viglierchio and Lownsbery (33) have shown that seedlings do produce exudates which enhance hatching. Perhaps emanations from some older roots retard hatching.

The northern root-knot nematode took 56 days to produce second stage larvae on celery. Slow germination of the celery seed in the inoculated soil may have had an adverse effect on the results of this trial. Celery, then, could experience two generations of M. hapla in one season.

No galling occurred on the roots of sweetcorn and M. hapla larvae were not observed within the roots. The lack of development on corn was in accordance with the findings of Oostenbrink (23) and Sasser (24).

Penetration Study

The results of the 24 hour penetration study showed that M. hapla larvae were able to enter the roots of 'Nantes' carrot seedlings within 24 hours (Table 6). Infectivity

TABLE 6. Penetration time on 'Nantes' carrot seedlings following inoculation with 50 M. hapla larvae.

Elapsed Time (Hrs.)	Penetration ¹	No. of Larvae
2	-	0
4	-	0
6	-	0
8	-	0
10	-	0
12	-	0
14	-	0
16	+	1
18	+	1
20	-	0
22	+	2
24	-	0

1 (-), no penetration; (+), penetration.

was variable, however, and penetration did not always occur after a threshold time had been reached.

Penetration was noted between 14 and 16 hours after inoculation. Larvae were oriented within the roots with their anterior ends toward the distal terminis of the root. The larvae observed within the roots were within 1 cm of the root tip, perhaps indicating a preferred entry site. One larva was observed entering the root adjacent to, but not in, the root cap, and was perpendicular to the root. No intermediate stages of ingression were observed which would indicate how the larvae achieved their orientation once penetration had occurred. The degree of penetration was 0-4%. No good explanation can be given for the lack of penetration at 20 and 24 hours after inoculation.

No galls or cell distortions were observed.

Smith and Mai (26), working with M. hapla on onions, found that penetration occurred within 24 hours. Larvae, at 24 hours, were usually oriented towards the distal end of the root. They found ingression occurring through the root cap, where the root cap is only a few cells thick. At 24 hours they also found no galling and no obvious cell abnormalities.

Wieser (34) found that the most attractive part of excised tomato roots to M. hapla was the region of elongation and not the apical meristem. Wieser, however,

studied attraction and not actual penetration, a subtle but perhaps important difference.

The results of the penetration study indicated that the northern root-knot nematode penetrated carrot roots in a manner similar to the penetration of onion roots. No tap roots appeared to be split, a common symptom of older root-knot infested carrots, or in the process of being split within the 24 hours of this study. Splitting of the taproot may occur at some later time.

Cold Survival Study

Survival of the northern root-knot nematode was affected by temperature. The number of larvae in the room temperature soil treatment was significantly greater than the quantity of larvae in the 7 or -14 C treatments for both two and six weeks (Table 7). At eight weeks, no differences were found in populations recovered from soil maintained at above freezing temperatures. No differences were noted in the galling of indicator plants grown in soil incubated at above freezing temperatures for two and eight weeks. But at six weeks, the 7 C treatment resulted in significantly more galls than the other above freezing treatment (Table 8).

After one day of being frozen at -14 C, the larvae appeared to be dead and failed to respond to a tactile stimulus. With one exception, no galls formed on indicator plants if the soil had been frozen three days or longer.

TABLE 7. Influence of temperature on survival of M. hapla larvae maintained at ambient room temperature, 7, and -14 C for two, six, and eight weeks.

Storage Time (Wk.)			
Storage Temperature (C)	2	6	8
Ambient Room Temp.	766 a ¹	534 a	310 a
7	166 b	254 b	318 a
-14	0 c	0 c	0 b

¹ Numbers are means of four replications. Small letters indicate Duncans new multiple range groupings which are not significant at the 5% level. Comparisons only valid for within a single time.

TABLE 8. Influence of temperature on survival of M. hapla eggs and larvae as indicated by galling on 0.5 gm of roots of tomato plants grown in soil previously maintained at ambient room temperature, 7, and -14 C for two, six, and eight weeks.

Storage Time (Wk.)			
Storage Temperature (C)	2	6	8
Ambient Room Temp.	41.5 a ¹	25.3 b	30.7 a
7	52.0 a	45.0 a	49.7 a
-14	0.25 b	0 c	0 b

¹ Numbers are means of four replications. Small letters indicate Duncans new multiple range groupings which are not significant at the 5%. Comparisons only valid for within a single time.

The numbers of galls formed on indicator plants grown in soil previously frozen at -14 C were significantly different from each other for zero, one, and two days of treatment (Table 9).

Several investigators (11, 25a, 25b) have shown that the second stage larvae and the eggs of M. hapla can survive subfreezing temperatures. Ferris (13) demonstrated that M. hapla larvae can survive better in soil in a refrigerator than in soil kept at room temperature. Ferris's results were supported by the galling of indicator plants in this study. The 7 C soil treatment always resulted in more galls than did the room temperature treatment.

Stein (28) found very few M. hapla larvae in the field in the winter, but galling occurred on indicator plants grown in the "larvaeless" soil, indicating that eggs and not larvae survived the winters. Szczygiel (30) writing of the disappearance of M. hapla larvae at certain times of the year deduced that eggs must be the survival stage of M. hapla. Sayre (25a) could find no precise lower thermal death point for either second stage larvae or eggs of M. hapla but noted that precooling egg masses prior to freezing failed to improve the survival of the eggs.

While conducting microplot field studies, some light was shed on the overwintering of the northern root-knot nematode. Sampling in the spring of 1972

TABLE 9. Influence of exposure time at -14 C on the survival of M. hapla eggs and larvae in soil as indicated by galling on 0.5 gm of roots of tomato plants grown in soil previously maintained at -14 C for 0-3 days.

	Days at -14 C			
	0	1	2	3
No. of Galls ¹	96.7 a ²	47.0 b	0.5 c	0 c

1 Means of four replications.

2 Small letters indicate Duncans new multiple range groupings which do not differ significantly at the 5% level.

(May 18), few larvae were found, but tomato indicator plants subsequently planted in the same soil showed much galling. Brzeski (7) found a similar situation occurring in Poland and concluded that eggs, in egg masses, are the overwintering stage for M. hapla.

The results of the current study indicate that the larvae of the Michigan isolate of M. hapla fail to survive subfreezing temperatures and that the eggs fare only slightly better than the larvae. Eggs in the inoculum were probably freed from the gelatinous matrix which surrounds them, and this may have adversely affected the survival of the eggs.

SUMMARY AND CONCLUSIONS

Field microplot cropping studies indicated that celery allowed M. hapla populations to increase during the growing season. Regardless of the crop planted subsequent to celery, populations remained higher than in plots which did not have celery in the rotation. The economic importance of the northern root-knot nematode on carrots appeared to be disproportionate to the M. hapla populations maintained. Carrots maintained only a small population of M. hapla when compared to celery. Onions, sweetcorn, and clean fallow suppressed M. hapla populations equally well. At the end of the growing season, it was rare to find larvae in the soil or galls on indicator plants grown in soil previously maintained in onions, sweetcorn, or clean fallow.

Greenhouse cropping studies indicated M. hapla populations increased under the influence of cabbage and successive crops of carrots. Successive crops of radishes apparently acted as trap crops and reduced M. hapla populations. Based upon larval populations, onions were moderate hosts. Damage to a subsequent crop of carrots,

however, indicated that onions may be effective in suppressing northern root-knot nematode damage to the carrots, more effective than the other cropping sequences studied in the greenhouse.

Generation time (second stage larvae to second stage larvae) studies demonstrated an apparent dependence of generation time of M. hapla on the particular host crop. Sweetcorn appeared to be a nonhost for M. hapla, with no penetration or reproduction taking place. M. hapla produced numerous egg masses on radishes but no second stage larvae were ever observed as a result of reproduction on the radish roots. Meloidogyne hapla had a generation time of 72 days on onions. The generation time of M. hapla on celery was 56 days. Forty-five days were required for M. hapla to complete one generation on carrots. Consequently, one generation of northern root-knot nematode may occur on onions in a growing season, and two generations may occur on celery and carrots.

Penetration of two day old carrot seedlings by M. hapla occurred within 24 hours. The shortest time for penetration to occur was 16 hours. Penetration occurred in the vicinity of the root cap, but apparently not through the root cap. The second stage larvae within the root tip were oriented with their anterior ends towards the distal terminis of the root.

Cold survival studies indicated that neither the eggs nor the larvae of M. hapla survived well in soil at a temperature of -14 C. At -14 C, all the second stage larvae appeared to be dead within one day. Gallings, however, occurred on indicator plants grown in soil previously frozen at -14 C for up to two days. Apparently, eggs survived -14 C for this duration, then hatched after the soil was thawed, producing infectious larvae. Spring sampling of field microplots showed few M. hapla larvae, but indicator plants subsequently planted in the same soil frequently became galled. Eggs apparently are the overwintering stage of M. hapla in Michigan soils.

Bioassays were an important diagnostic tool in determining the presence of M. hapla in the soil. Meloidogyne hapla often appeared on indicator plants when the centrifugation-flotation or Baermann funnel methods of nematode extraction failed to indicate its presence.

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