Relationship between *Meloidogyne hapla* Density and Damage to Carrots in Organic Soils

T. C. VRAIN[1]

Abstract: Field and growth chamber experiments were conducted to determine the effect of five initial densities (Pi – 20–240/100 cm³ soil) of *Meloidogyne hapla* on carrot development and yield of storage roots at maturity. Carrots growing in infested and noninfested organic soil were harvested after 15, 29, 44, 59, and 106 days of growth in controlled environment chambers and after 110 days in field plots. Nematodes affected weight of roots and foliage, weight and length of the storage portion of tap roots, and induced malformations (forking), galling, and hairiness of tap roots. In most cases the data could not be represented satisfactorily by the exponential model of Seinhorst: $y = m + (1\text{-}m)\, Z^{P-t}$. In growth chambers the weight of mature storage roots was not correlated to initial nematode density, but there was a significant negative correlation between weight of storage roots and initial nematode density in field plots. Tolerance levels were calculated as points where the regression lines reached the growth level on noninoculated plants. The tolerance levels of foliage were higher than those of roots, and increased with age of plants. The tolerance level of marketable weight in field plots, average crop value, and a hypothetical control cost function are used to discuss the possibility of optimizing chemical control of root-knot nematode in organic soils. *Key words:* root-knot nematode, population dynamics, tolerance levels, damage function.

Journal of Nematology 14(1):50-57. 1982.

Carrot (*Daucus carota sativa* L.) is a very susceptible host of *Meloidogyne hapla* Chitwood in temperate climates (2,3,14,16, 21). High densities of the nematode at planting (several hundreds/100 cm³ soil) induced loss of weight of foliage and of roots, severe galling of roots, extreme malformations (forking) of storage roots, and a total loss of the crop (3,18,21). Quality requirements for carrot production are long and smooth tap (storage) roots, but even with low densities of root-knot nematodes (less than 40/100 cm³ soil), storage roots can be malformed and unmarketable, although their weight is not affected. Because of this low tolerance level, i.e., nematode density beyond which the crop in infested soil becomes quickly unprofitable, carrot growers rely heavily on chemical control measures when root-knot nematode is detected. Soils rich in organic matter bind organic pesticides, and high dosages of fumigant are required to reduce the initial nematode density before planting. The recommended treatment of infested carrot fields in Quebec organic soils is to apply 292 liter/ha broadcast of 1,3-dichloropropene nematicide. This standard practice is costly and lacks flexibility, but no information is available from

Received for publication 26 May 1981.

[1]Agriculture Canada Research Station, 6660 N. W. Marine Drive, Vancouver, B.C. V6T 1X2.

I thank Y. Fournier, G. Belair, and M. Dmytriw for their technical assistance and J. Hall for his advice on statistical analysis.

which to predict crop losses and optimize control measures. Slinger (13) has shown how the ontogeny of the carrot plant is affected by a low density of *M. hapla* and developed a preliminary model, based on greenhouse experiments, to predict the growth of infected carrots using leaf surface area and fresh weight of tap roots. Damage functions describing the loss of yield as a function of nematode density can vary with geographic, edaphic, and climatic conditions, with cultivars, and with the economics of the crop in a particular year (4,5), but estimates of tolerance levels and control costs (even for only one set of conditions) may prove useful towards maximizing crop return after nematode control.

The object of this study was to determine the effect of initial densities of *M. hapla* on carrot development and yield in a controlled environment and in field conditions, and to estimate the parameters needed to develop tolerance levels and damage functions necessary to optimize control costs.

MATERIALS AND METHODS

Field experiment: Forty-eight bottomless galvanized steel frames (1 × 2 × 0.65 m) were buried 0.5 m deep in organic soil (over 80% organic matter) at the Agriculture Canada Experimental Farm, Ste. Clotilde, Quebec. These microplots were fumigated with methyl bromide (0.22 kg/m^2) and allowed to air for 13 days before infestation. The nematode inoculum, propagated on tomato, *Lycopersicon esculentum,* cv. Rutgers in the greenhouse, consisted of infested organic soil and infected tomato roots bearing numerous egg masses. The roots were cut into pieces 1 cm long. Soil and chopped roots were mixed thoroughly and five 100 cm^3 samples were taken to determine nematode density. Roots from these samples were separated from soil. Second-stage juveniles in the soil were extracted for 7 days using modified Baermann pans, and eggs from egg masses were extracted by maceration of roots (7). Quantities of the soil and root mix were incorporated into the plots to produce infestation levels of 20, 40, 80, 160, and 240 nematodes/100 cm^3 of soil. Control plots received a similar volume of soil-root mix from noninfested tomato cultures. Plots were arranged in eight randomized complete blocks, and 400 carrot seeds cv. Gold Pak were sown in two rows 0.5 m apart in each plot. The seedlings were thinned to 200 per plot after 7 days. Fungicides and insecticides were applied uniformly as needed during the growth period. Soil temperature at 20-cm depth was recorded continuously. After 110 days carrots were harvested. Tap roots were graded according to nematode damage and marketability, counted, and weighed. Nematode densities were determined when carrots were harvested by taking 10 cores of soil 20 cm deep from each plot using a 2.5-cm-d soil probe. Nematodes were extracted for 7 days from 100-cm^3 subsamples on modified Baerman pans. Nematode densities were transformed to $\log_{10}$ (P + 1), and regression analyses were used to relate yield and nematode densities at harvest (Pf) to initial nematode densities (Pi) (9). Quadratic models were utilized only when they made a statistically significant contribution. A tolerance level of nematode density T, below which no yield loss could be measured, was estimated as the intersection of the horizontal line Y = yield of carrots in noninoculated soil, and the damage function y = f (Pi). An iterative nonlinear regression program (1) was used to estimate the coefficients of Seinhorst exponential model: $y = m + (1 - m)\, Z^{P-t}$, when $P \geq t$ (11). This program needs starting values of m, Z, and t, and produces a list of estimates of the coefficients and the residual sums of squares determined for each iteration performed.

Growth chamber experiment: The organic soil taken from the field for this experiment had a pH of 5.6–5.8, 43.4% carbon, 2.5% nitrogen, and 18% mineral matter. It was steam sterilized, then aerated for 4 wk before use. Fine sand was added (1:2 v/v) to improve drainage, and a commercial fertilizer (6–12–14) was added at 0.2 g/liter. The nematode inoculum was propagated on tomato cv. Rutgers in the greenhouse. Eggs and second-stage larvae were obtained by macerating roots with egg masses (7) and hatching the eggs (15) on a 500-mesh sieve (26 μm pores). The nematode suspension (70% eggs and 30% larvae) was pipetted

onto the organic soil at the rate of 100 ml suspension in 20 liter of soil and mixed in a cement mixer for 2 min. Initial nematode densities (Pi) were the same as in the field experiment. Carrots were grown in two types of containers, depending upon whether they were to be harvested during their growth or at maturity. Early carrot growth was studied using 6-cm-d polyvinyl chloride pipes 0.5 m long cut longitudinally into halves and rejoined with tape. These tubes were filled with 1.4 liter of inoculated soil and packed to provide a medium compaction (20,21). Three carrot seeds were sown in each tube. Carrots to be harvested at maturity were grown in plastic containers (0.20 × 0.27 × 0.27 m). Ten liters of inoculated soil were placed in each container, slightly compacted, and 30 seeds were sown in each container. The tubes and containers were placed in growth chambers in seven and five randomized complete blocks, respectively. Air relative humidity was maintained around 60% throughout the experiment. The day/night temperature regime was 22/18 C, and the photoperiod was 16 h/d. Light intensity at the level of the foliage was kept between 20 and 30 hlux by moving vertically the set of fluorescent and incandescent lights. After germination the seedlings were thinned to one per tube and 10 per large container. Carrots growing in tubes were harvested and examined after 15, 29, 44, and 59 days. The roots were washed of soil in situ with a gentle stream of water. The fresh weight of foliage, fresh weight and length of roots, fresh weight and length of storage portion of tap root, and number of nematode-induced galls on roots were recorded. Carrots growing in large containers were harvested at maturity (106 days). Storage roots and foliage were weighed separately. Length of undamaged storage root was recorded, as well as length from the crown to the nearest bifurcation or gall when the root was forked or galled. The storage roots were indexed on a scale of 1–4 for abundance of galls, and 1–4 for abundance of high order roots (ramification). Nonforked roots were given a value of 1 and forked roots a value of 4. A growth index (17) was calculated as

$$\frac{\log_{10}(\text{length in cm} \times \text{weight in g})}{\log_{10}[(\text{forking} \times \text{galling} \times \text{ramification}) + 1]}.$$

Nematode densities were determined when carrots were harvested at maturity, by taking five cores of soil, 20 cm deep, in each container. Data transformations, regression analyses, and estimation of tolerance levels of nematode densities were done in the same manner as for the field experiment.

RESULTS

Field experiment: The effect of initial densities of *Meloidogyne hapla* on fresh weight of roots are shown in Table 1. The regression analysis of these effects are shown in Table 2. There was a significant negative correlation between weight of storage roots and initial nematode density. A number of roots were rendered unmarketable (truncated) by a cause other than nematodes in several noninoculated plots. An estimate of the tolerance level, density below which no damage could be measured, was obtained assuming 85% of storage root weight would have been marketable in the noninoculated soil. This percentage represents a conservative estimate of an average marketable yield from a noninfested field, for carrots can be damaged by a number of causes (17) and growers normally experiment 15 to 20% losses of roots in fields where no root-knot nematodes can be detected. The yields from these microplots could not be fitted satisfactorily to Seinhorst's exponential model, presumably because of an insufficient number of levels of nematode infestation. The effect of initial nematode densities (Pi) on density at harvest (Pf) are shown in Fig. 1.

Growth chambers: Emergence of seedlings was not affected by nematodes. The effect of nematode densities on growth and yield of carrots is shown in Table 1. A summary of the regression analysis with the calculated tolerance levels is shown in Table 2. The growth of seedlings was delayed by nematodes (Fig. 2), but there were no significant differences in weight at 59 days at densities of 80–240/100 cm^3 soil. The relative shoot-to-root ratio was affected by nematode densities and increased considerably during the development of the plants (Fig. 3). Root weight of infected plants was increased at 15 days but decreased afterwards relative to shoot weight; the effect was more pronounced at the higher nematode densities. However, the lower leaves of nonin-

Table 1. Effects of *Meloidogyne hapla* on carrot development and yield.

Growth parameter	Time of development (days)	Initial nematode density/100 cm³ soil 0	20	40	80	160	240	LSD ($P = 0.05$)
Microplot experiment†								
Weight of storage roots (kg/microplot)	110	21.1	16.8	15.8	14.2	13.0	12.9	1.3
Weight of marketable storage roots (kg/microplot)	110	—	10.8	6.9	4.3	1.3	1.8	2.0
Growth chamber experiment‡								
Weight of roots (g/plant)	15	0.04	0.03	0.03	0.05	0.06	0.05	0.01
	29	3.2	2.9	3.2	1.0	1.7	1.5	NS
	44	39.1	27.8	34.7	11.8	16.8	18.9	10.0
	59	61.9	57.4	40.9	31.5	27.9	32.5	14.1
Weight of leaves (g/plant)	15	0.06	0.04	0.05	0.04	0.05	0.04	0.02
	29	1.9	1.9	1.9	0.5	0.7	0.7	1.0
	44	19.4	19.3	21.9	8.5	11.9	12.0	5.0
	59	38.8	42.7	36.8	34.0	32.3	34.5	NS
Length of storage root (cm/plant)	44	14.4	10.6	13.5	7.7	8.5	8.6	3.7
	59	23.3	19.8	17.6	14.8	12.8	13.8	4.5
	106	16.6	14.4	13.4	13.6	10.3	7.8	2.8
Weight of storage root (g/plant)	59	31.1	30.8	21.5	15.9	15.1	15.7	9.6
	106	62.6	45.5	42.8	45.9	49.8	40.6	15.8
Percent forked storage roots	106	0.0	0.0	2.2	8.0	59.0	57.0	20.0
Galling index (1–4)	106	1.0	1.7	2.1	2.3	3.3	3.7	0.5
Ramification index (1–4)	106	1.0	1.2	1.7	1.5	2.9	3.3	0.1
Growth index§	106	10.0	5.9	4.2	4.3	1.9	1.7	1.3

†Values of growth parameters are the average of eight replicated microplots.

‡Values of growth parameters are the average of seven replicated tubes for havest at 15, 29, 44, and 59 days, and of five replicated pots for harvest at 106 days.

§Growth index calculated as $\frac{\log (\text{length cm} \times \text{weight g})}{\log [(\text{forking} \times \text{galling} \times \text{ramification}) + 1]}$.

oculated plants started to senesce after 80 days while the leaves of carrots growing in inoculated soil did not. This delay in maturity of the foliage accounts for some of the large increases in relative shoot-to-root ratios at 106 days. Tuberization of the tap root was slight but noticeable at 44 days. The tuberization of tap roots in infested soil was delayed at 59 days (Fig. 4). The weight of storage roots at maturity was affected by nematodes (Table 1) but not correlated to their initial densities (Table 2). Length of the tuberized portion of the tap roots was correlated negatively with Pi at 44, 59, and 106 days (Table 2). Damage to the tap roots was evident, as forked roots with nematode-induced swellings (galls) and secondary ramifications were numerous in the presence of nematodes. Percentages of forked carrots and galling index, ramification index, and the growth index are shown in Table 1. The weight of tuberized roots at 106 days was poorly correlated with nematode density, indicating that, given time, heavily infected plants are able to develop their tuberized root. Nematode

Table 2. Regression analysis of effects of *Meloidogyne hapla* on carrot development and yield.

Growth parameter	Time of development (days)	Regression of growth vs nematode density (log Pi/100 cm³ soil)			Tolerance level†
		Intercept	Slope	r	
Microplot experiment					
Weight of storage root (kg/microplot)	110	22.1	−4.01	−0.78*	1.8
Weight of marketable storage roots (kg/microplot)	110	21.7	−8.87	−0.86*	2.7‡
Growth chamber experiment					
Weight of roots (g/plant)	15	0.0	0.02	0.52*	...
	29	5.3	−1.73	−0.35*	15.6
	44	47.9	−13.72	−0.45*	4.3
	59	228.7	$-187.75X + 44.10X^2$	−0.66*	18.4
Weight of leaves (g/plant)	29	3.7	−1.36	−0.50*	22.2
	44	32.4	−9.41	−0.55*	24.3
	59	51.2	−8.05	−0.33§	35.0
Length of storage root (cm/plant)	44	16.3	−3.45	−0.35*	3.5
	59	27.7	−6.33	−0.53*	5.1
	106	22.8	−5.76	−0.71*	11.6
Weight of storage root (g/plant)	59	45.9	−13.85	−0.53*	11.7
	106	46.0	−0.48	0.01NS	...
Growth index	106	11.2	−4.01	−0.85*	1.9

*Indicate significance at $P = 0.05$.

†Tolerance level of Pi (initial nematode density per 100 cm³ of soil) below which no effect could be measured, calculated as the intersect of a horizontal line (y = mean value of growth parameter for uninoculated plants), and the linear regression line of growth parameter vs initial nematode density.

‡Tolerance level of Pi assuming 85% of storage root weight in uninoculated plots should have been marketable.

§Significant at $P = 0.058$.

densities (Fig. 1) decreased during the growth of the carrot, and the regression line of log Pf vs log Pi is below the maintenance line.

DISCUSSION

Only five levels of initial nematode density were used, and this proved insufficient to fit the exponential model of Seinhorst (11) during most of the carrot development. Perhaps the computer programs used (1) were not adequately suited to determine these parameters in all cases. Ferris et al. (6) have since described an algorithm that allows the determination of m, Z, and t of a Seinhorst curve for any data set. Only at 59 days in controlled environment did length and weight of storage roots fit the model with values of the parameters, respectively, m = 0.57 and 0.49, Z = 0.976 and 0.954, and t = 3.21 and 19.62 nematodes/100 cm³ soil.

The linear regression models allowed the estimation of tolerance levels (T) and followed the tolerance of roots and foliage during their development. In the first 59 days of growth the effect of nematodes on foliage was less pronounced than on roots. The tolerance level for foliage weight was fairly high and increased from 29 to 59 days. A similar effect was recorded by Seinhorst (12) in a study of the damage to carrots by *Rotylenchus uniformis;* he noted that the increase of tolerance of plants with

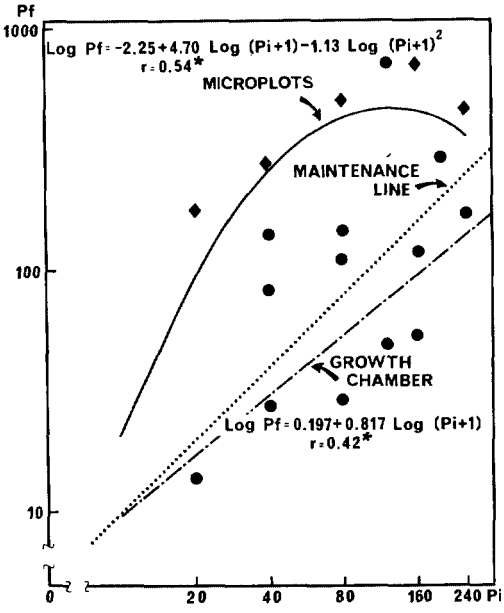

Fig. 1. Relationship of *Meloidogyne hapla* density at planting (Pi) to density at harvest (Pf) in microplots and in growth chambers.

age could be the result of a decrease of nematode density (per unit volume of root) due to fast growth of the root system. In this study with *Meloidogyne hapla* the tolerance level for weight of roots increased somewhat from 29 to 59 days. There was also an increase in tolerance for length of

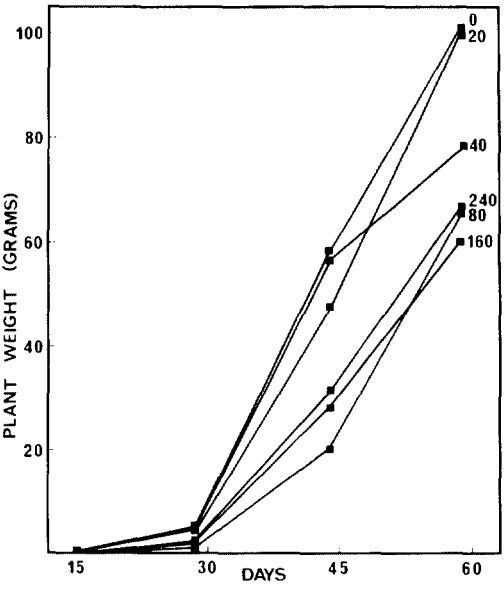

Fig. 2. Effect of five initial densities of *Meloidogyne hapla* (Pi = 20–240/100 cm³ soil) on early growth of carrots.

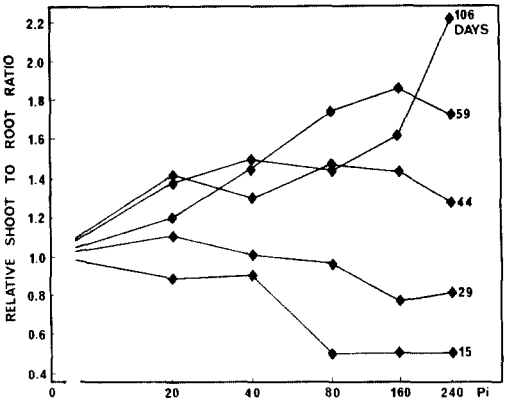

Fig. 3. Influence of *Meloidogyne* hapla (Pi, initial densities/100 cm³ soil, log scale) on the shoot to root ratio of carrots.

storage root from 44 to 106 days, but during the latter part of the development of the plant the increase of the root system is mostly in the storage root and not in the functional roots where the infection sites are.

The lack of correlation between weight of storage roots at maturity and nematode densities (Pi) between 20 and 240/100 cm³ soil was also observed in two preliminary experiments where carrots were harvested after 95 and 115 days of development. This lack of correlation in growth chambers is in contrast with the field study where there was a significant negative correlation between weight of storage roots and initial nematode densities. Possibly the lack of additional stresses other than nematodes enabled plants approaching maturity in growth chambers to compensate somewhat for nematode effects. Regardless of nematode infestation, weight and length of carrot roots were always less in growth chambers than in field conditions. The growth

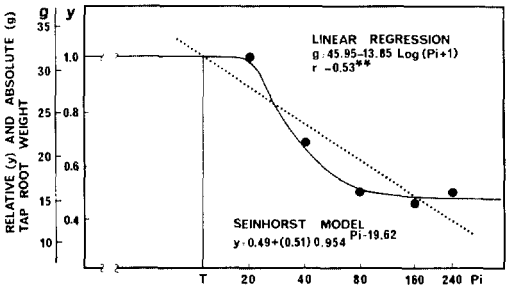

Fig. 4. Relationship of initial *Meloidogyne hapla* densities (Pi) and the development of tap roots of carrots after 59 days.

index gives a good estimate of quality of roots (17). It proved to be a very sensitive index to measure nematode damage, much more than any one of its components singly. According to this growth index the tolerance level of carrots grown in growth chambers was 1.9 nematodes/100 cm^3 soil, while the tolerance level for weight of marketable roots in field plots was 2.7 nematodes/100 cm^3

A linear damage function can be developed from the results of the field experiment. Average production in noninfested organic soil in southeastern Quebec is 50 t/ha at a cost of production of $1,250/ha (Canadian currency) with a value of $60/t (1980 prices). The tolerance level from Table 2 and relative yields at five initial nematode densities in field plots allow to calculate a damage function: y (return per ha in dollars) = 1,750 for Pi $<$ 2.7 nematodes/100 cm^3 and y = 1,824.90–27.74 Pi for Pi $\geq$ 2.7. The economic threshold (8, 10), density of nematodes at which the potential loss in crop value is equal to the cost of control, can be calculated with the assumption that carrot growth in 2-m^2 microplots approached field conditions. The standard practice in the organic soils of Quebec is to fumigate with 292 liter/ha broadcast of 1,3-dichloropropene nematicide, at a cost of $2.05/liter (Canadian currency, 1980 prices) and an estimated application cost of $50/ha for a total cost of $648.60/ha. Substitutiing the damage function, the economic threshold is Pi = 9.37 nematodes/100 cm^3 soil, and the profit per acre after treatment is $1,750 – 648.60 = $1,101.04. This economic threshold, while relatively high, would still be difficult to detect in organic soils and may explain the common practice of recommending fumigation of land planned for carrot cropping where *Meloidogyne hapla* is detected, however low the initial nematode density. Root-knot nematode infestation is far from uniform (21), and the standard practice of applying, uniformly, a high dosage of fumigant (292) liter/ha) is probably excessive. More thorough assessment of density in fields where the nematode is detected could result in considerable savings by adapting the dosage of chemical used to the nematode density, thus maximizing the efficiency of chemical control. Ferris (4) has developed a theoretical model of control and shown that the optimizing nematode threshold is the population level at which the derivatives of the damage function (linear in our study) and the control cost function intersect. For example, if a portion of a field had a population of 9.37 nematodes/100 cm^3 soil, and assuming that 292 liters/ha of 1,3-dichloropropene reduced the population in organic soil by 90%, the optimizing threshold using Ferris's model would become 2.84 nematodes/100 cm^3 soil; the cost of reducing the nematode density from 9.37 to 2.84/100 cm^3 soil would be $247.45/ha, the loss of yield (since the optimizing threshold would be higher than the tolerance level of 2.7 used in the damage function) would be $10.15/ha. The profit per hectare using this method of maximizing the efficiency of control would be $1,492.40/ha, as compared to the $1,101.40/ha using the standard economic threshold and fumigation method. These theoretical considerations must be based on a detailed knowledge of initial nematode density in soil, and would be subject to environmental and economical factors as noted by Ferris (4,5). The need for reliable tolerance levels and damage functions developed from field tests becomes apparent, if optimum return from infested carrot fields is to be achieved.

LITERATURE CITED

1. Brown, M. B., ed. 1977. Biomedical computer programs, P-series. Berkeley: University of California Press.

2. Brzeski, M. W., and Z. Bojda. 1974. The northern root-knot nematode (Meloidogyne hapla Chitw.) on carrot—pathogenicity and control. Zeszyty Problemowe Postepow Nauk Rolniczych NR 154:159-172.

3. Crete, R. 1977. Diseases of carrots in Canada. Publication 1615, Canada Department of Agriculture, Information Division, Ottawa.

4. Ferris, H. 1978. Nematode economic thresholds: derivation, requirements and theoretical considerations. J. Nematol. 10:341-350.

5. Ferris, H. 1980. Nematology—status and prospects: Practical implementation of quantitative approaches to nematology. J. Nematol. 12:164-170.

6. Ferris, H., W. D. Turner, and L. W. Duncan. 1981. An algorithm for fitting Seinhorst curves to the relationship between plant growth and preplant nematode densities. J. Nematol. 13:300-304.

7. Hussey, R. S., and K. R. Barker. 1973. A comparison of methods of collecting inocula of Meloidogyne sp., including a new technique. Plant Dis. Rep. 57:1025-1028.

8. Olthof, T. H., and J. W. Potter. 1972. Relationship between population densities of Meloidogyne hapla and crop losses in summer maturing vegetables in Ontario. Phytopathology 62:981-986.

9. Oostenbrink, M. 1966. Major characteristics of the relation between nematodes and plants. Meded. Landbou. Wageningen 66:1-46.

10. Potter, J. W., and T. H. Olthof. 1974. Yield losses in fall maturing vegetables relative to population densities of Pratylenchus penetrans and Meloidogyne hapla. Phytopathology 64:1072-1075.

11. Seinhorst, J. W. 1965. The relation between nematode density and damage to plants. Nematologica 11:137-154.

12. Seinhorst, J. W., and J. Kozlowska. 1977. Damage to carrots by Rotylenchus uniformis with a discussion on the cause of increase of tolerance during the development of the plant. Nematologica 23:1-23.

13. Slinger, L. A. 1976. Ontogeny of Daucus carota in relation to Meloidogyne hapla with a preliminary endomycorrhizal study. Ms. thesis, Michigan State University.

14. Stein, W. 1963. Investigations on the infestation of carrots by Meloidogyne hapla Chitwood in two successive years of cultivation. Z. Pflanzenkr. Pflanzenpathol. Pflanzenschutz. 70:217-224.

15. Vrain, T. C. 1977. A technique for the collection of larvae of Meloidogyne spp. and a comparison of eggs and larvae as inocula. J. Nematol. 9:249-251.

16. Vrain, T. C. 1978. Dissémination et importance des nématodes phytoparasites dans les sols organiques du Québec. Phytoprotection 59:186 (Abstr.)

17. Vrain, T. C., and L. R. Baker. 1980. Reaction of hybrid carrot cultivars to Meloidogyne hapla. Can. J. Plant Path. 2:163-168.

18. Vrain, T. C., Y. Fournier, and R. Crete. 1981. Carrot yield increases after chemical control of root-knot nematode in organic soil. Can. J. Plant Sci. 61, in press.

19. White, J. M. 1978. Soil preparation effects on compaction, carrot yield, and root characteristics in organic soil. J. Amer. Soc. Hort. Sci. 103:433-435.

20. White, J. M., and J. O. Strandberg. 1978. Early root growth of carrots in organic soil. J. Amer. Soc. Hort. Sci. 103:344-347.

21. Wilson, J. D. 1957. A distribution pattern of root-knot nematode infestation on muck grown carrots. Down to Earth, Summer:4-7.