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Dynamics of damage to tomato by *Meloidogyne incognita*

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Summary - Damage due to *Meloidogyne incognita* on tomato (cv. Moneymaker) growing in large pots (3.65 kg dry soil) was related to initial population density (*Pi*). Damage was assessed after 42 and 135 days and only the highest *Pi* used (20 viable eggs/g soil) caused a statistically significant decrease in growth at both harvests. After 135 days, growth was also decreased significantly by *Pi* = 0.8 and 4.0 eggs/g soil. The decrease in growth was proportionally much greater after 135 days than after 42 days, as was reflected by the estimated values of *m* (the minimum yield), *T* (the tolerance limit) or *z* (a constant close to 1.0 which reflects the damage caused by a single nematode or where several generations are involved, all its progeny). The increased damage after 135 days was associated with a greatly increased final population density (*Pf*) of *M. incognita*. The greatest increase in population density (*Pf*/*Pi* = 273) occurred at the lowest effective initial population density used (*Pi* = 0.03 eggs/g soil), but the greatest final population (*Pf* = 90 000 nematodes/pot) occurred with *Pi* = 0.16 viable eggs/g soil. The effects on tomato growth of drought and of *M. incognita* were additive, and both decreased stomatal conductance. However, neither affected the ^{13}C discrimination in either upper or lower leaves, but the percentage of N in the leaf dry matter was greater in infested than uninfested plants. © Orstom/Elsevier, Paris

Résumé - ***Dynamique des dommages causés à la tomate par* Meloidogyne incognita** - Les dommages causés par *Meloidogyne incognita* à des tomates cv. Moneymaker poussant dans des pots de grande taille (3,65 kg de sol sec) ont été mis en relation avec la densité initiale de la population du nématode (*Pi*). Les dommages sont estimés après 42 et 135 jours de culture : seule la *Pi* la plus élevée (20 œufs viables/g de sol) cause une diminution statistiquement significative de la croissance aux deux dates de récolte. Après 135 jours, la croissance est également significativement diminuée par des *Pi* de 0,8 et 4,0 œufs/g de sol. La diminution de croissance après 135 jours était proportionnellement beaucoup plus forte qu'après 42 jours, ainsi qu'il l'était indiqué par les valeurs estimées de *m* (récolte minimum), *T* (limite de tolérance) et *z* (une constante proche de 1,0 reflétant les dommages causés par un seul nématode ou, dans le cas de plusieurs générations, par la totalité de sa descendance). Cette augmentation des dégâts après 135 jours est associée au très fort accroissement de la population finale (*Pf*) de *M. incognita*. La plus forte augmentation de la densité de population (*Pf*/*Pi* = 273) se rencontre aux densités efficaces les plus faibles de la population initiale (*Pi* = 0,03 œuf/g de sol), mais la population finale la plus élevée (*Pf* = 90 000 nématodes par pot) résulte d'une *Pi* = 0,16 œufs viables/g de sol. Les effets de la sécheresse et de *M. incognita* sur la tomate s'additionnent et l'une et l'autre diminuent la conductivité des stomates. Cependant, ni l'une ni l'autre n'affectent la discrimination du ^{13}C entre feuilles inférieures et supérieures ; toutefois, le pourcentage de N dans la matière sèche des feuilles est plus élevé chez les plantes infestées que chez les témoins. © Orstom/Elsevier, Paris

Keywords: Drought, host status, *Meloidogyne incognita,* modelling, stable isotopes, stomatal conductance, tolerance, tomato, water relations.

Several equations have been used to describe the relationship between crop yields and the initial population density of nematodes (*Pi*) with a single generation per year, such as potato cyst nematode (PCN or *Globodera* spp.). These include a log linear model suggested by Oostenbrink (1966):

$$Y = a + b\ (\log Pi) \qquad (1)$$

where Y is the expected yield for a given value of *Pi*, and the exponential model of Seinhorst (1965):

$$\{Y = Ymax\ [m + (1 - m)z^{Pi - T}] \text{ if } Pi > T \text{ and } Y = Ymax \text{ if } Pi > T\} \qquad (2)$$

with four parameters: *Ymax* - the expected yield in the absence of nematodes; T - the 'tolerance' limit, the threshold for *Pi* below which no damage occurs; z - the rate at which the increasing *Pi* decreases expected yield; and m - the ratio of minimum yield at high values of *Pi* and the maximum value when $Pi = 0$. Later Seinhorst (1986) used a modified version of equation (2) based on a number of experiments where he had observed that $z^T = 0.95$, thus giving

$$\{Y = Ymax\ [m + (1 - m)0.95^{(Pi/T - 1)}] \text{ if } Pi > T \text{ and } Y = Ymax \text{ if } Pi = T\} \qquad (3)$$

Elston *et al.* (1991) proposed an inverse linear model which has two parameters. Whilst the log-linear and inverse linear models are descriptive, that of Seinhorst (1965) includes the concept of a tolerance limit (T), and of a minimum yield (m). The value of m is expressed as a proportion of the nematode-free growth and depends on several factors, including the amount of growth achieved during the initial delay before nematode attack started. The longer the delay (or the larger the planting material), the greater the growth before attack and the greater the value of m (Seinhorst & Kozlowska, 1977; Seinhorst, 1995).

The Seinhorst equation has also been used to model damage by nematodes with several generations per growing season. Duncan and Ferris (1983) modelled the effects of the root-knot nematodes *Meloidogyne javanica* and *M. incognita* on the growth of cowpea cv. Californian Blackeye No 5 and showed that damage was greater with the *M. javanica* than with *M. incognita* for which the cowpea was a poorer host. McSorley *et al.* (1992) found that the effect of *M. arenaria* on the yield of peanut could be effectively modelled using the Seinhorst equation but observed values of T as low as 0.01 eggs/cm^3 soil, and recently Di Vito *et al.* (1997) obtained a low estimate of T (0.13 eggs/cm^3 soil) for kenaf growing in microplots and attacked by *M. incognita*. In contrast, Vrain (1982) found that damage to carrots by *M. hapla* could not be satisfactorily modelled by the Seinhorst equation.

Seinhorst (1995) simulated up to three generations of *Heterodera avenae* by applying the inoculum at sowing and/or once or twice at later dates. Except at the highest inoculum densities (more than 14 eggs/g soil), the relative weights of oat plants after 97 days growth were found to be the products of the relative weights that would have been obtained after single inoculations on the same dates. The greater sensitivity to additional damage observed with the most heavily infested plants was attributed to their development being retarded and to the effects of the 'second mechanism of damage'.

The severity of crop loss resulting from root-knot nematode attack is influenced by environmental factors. Various authors (Niblack *et al.*, 1986; Windham & Barker, 1986; Barker & Noe, 1987; Barker & Weeks, 1991; McSorley *et al.*, 1992) showed that damage by root knot nematodes is influenced by host crop and cultivar, by soil type and by the year in which the experiment was done. Understanding how the effects of the nematode and of the environment might interact depends on understanding how the crop is damaged by the nematode. Drought coupled with infection of roots by potato cyst nematodes decrease leaf water potentials (Haverkort *et al.*, 1991) and the two can interact to further decrease yield. Similarly, O'Bannon and Reynolds (1965) reported that mild water stress decreased water consumption of cotton plants infected with *M. incognita* much more than that of uninfected plants and Meon *et al.* (1978) reported that *M. javanica* infection increased the suction pressure in tomato roots, especially when soil water was restricted, without greatly affecting transpiration. Rahi *et al.* (1988) observed that both *M. incognita* and *M. javanica* increased water stress within the leaves of tobacco and greatly decreased the efficiency of water use per gram of dry matter produced and Wheeler *et al.* (1991) found that the effects on the yield of tobacco of drought and of *M. incognita* were additive. Meon *et al.* (1978) proposed that damage by *M. javanica* to tomato involved disruption of the xylem within the galls and, consequently, increased water stress in infected plants. It seems likely, therefore, that the availability of soil water may be one of the environmental factors that could influence the relationship between yield and nematode density.

A recent survey involving countries in east and west Africa, in the Caribbean and South America suggested that damage by root knot nematode was widespread and severe in crops of tomato grown by local farmers (average gall indices of 5 to 6, on a 1-10 scale; Triviño, unpubl.). It appears that, in general, it is the good hosts for root knot nematodes which are most severely damaged. The main objective of the experiment reported here was to test the hypothesis that on a good host (tomato) initially non damaging populations of *M. incognita* can multiply so greatly that they become damaging later in the growing season. The experiment also examined the effects of *M. incognita* on the water relations of tomato and the interaction with drought.

Materials and methods

A single experiment was done in large pots (23 cm diam, 5.4 dm^3) in a glasshouse with heating and vents set at 26°C during the day and 22°C at night. The effects on the growth of well-watered susceptible tomato cv. Moneymaker of six population densities of *M. incognita* were determined and compared at three of those population densities with the effects on plants which were partially subjected to drought. Plants (five replicates) were harvested 42 days and 135 days after inoculation so as to be able to compare the effects on growth early in the growing season with those that occurred later.

To produce the inoculum, 4-week-old tomato plants cv. Moneymaker were transplanted into 15 cm diam. pots filled with a peat/sand mixture and infected with chopped roots containing egg-masses of *M. incognita*. The pots were maintained at 25-26°C in a glasshouse for 2 months, after which the infected roots with egg-masses were cleaned of debris, cut in 2 to 5 cm long pieces, and then stirred for 4 min in 1% NaOCl

solution at 23°C to release the eggs (Hussey & Barker, 1973). The suspension of eggs was collected on a 38 µm mesh sieve and quickly washed several times with tap water. As some eggs are killed during extraction with NaOCl (Ehwaeti *et al.*, 1998) a hatching test was performed to determine the proportion of hatchable eggs.

The pots were inoculated with 360 ml of egg suspension during filling with 3.8 kg (3.65 kg dry weight) of sterilised, moist, mechanically mixed soil (40% peat and 60% sand). Six densities of *M. incognita* (0, 160, 800, 4 000, 20 000, 100 000 eggs/pot; equivalent to 0.0, 0.044, 0.22, 1.1, 5.5, and 27.4 eggs/g dry soil) were applied and mixed in by hand during filling to try to ensure that the eggs were distributed throughout the soil. Each pot was placed in a saucer and a 2-cm layer of polythene granules added to the surface to prevent water loss. The pots were watered to 33% moisture and 3 days later planted with a tomato seedling with two true leaves. There were ten replicates of each treatment.

A further ten replicates inoculated with 0, 4 000, or 100 000 eggs/pot received the reduced watering treatment (drought). These pots were initially watered to 22% moisture. Two pots without tomato plants were used to estimate water losses from the soil surface.

The pots were watered to a standard weight (progressively adjusted to allow for the estimated increase in plant weight) equivalent to 30 and 20% moisture for the well-watered and drought-subjected, respectively. The amounts of water added to each pot were recorded. The well-watered plants were watered when they had used more than 200 g of water, but the drought-subjected plants were not watered until they showed signs of wilting. The frequency of watering depended on water use, so that smaller plants with lower transpiration rates were watered less frequently than larger plants

The pots were randomised within ten blocks that were rotated and plants re-randomised each week to try to ensure each pot experienced a similar environment. Fertiliser was added weekly as a liquid feed (Vitafeed III; N 19%, P_2O_5 19%, K_2O 19%), a balanced feed, suitable for a wide range of plants and conditions. The plants were sprayed twice with Benlate and Calixin to control powdery mildew.

Five blocks were harvested 42 days after planting (first harvest) and the remaining five blocks after 135 days (second and final harvest). At each harvest the plant height and fresh and dry weight of the tops were determined. The roots were washed free of soil and the numbers of galls counted (first harvest), or scored on a 1-5 scale (second harvest). The nematodes per root system were determined by staining the whole root system (first harvest), or 10% of the chopped and mixed root system (second harvest), with acid fuschin in lactic acid. The roots were examined in glycerol (first harvest) under a stereomicroscope, or after blending in 150 ml water for 30 s at low and then high speed (second harvest), and the numbers of nematodes present and stages of development determined.

The effects of *M. incognita* and of drought on water relations were determined once a week between 13-16 h over 15 weeks by measuring the stomatal conductance (gs) on the abaxial and adaxial side of the upper leaflet of the youngest full-grown leaf of every plant using a diffusion porometer (MK3, Delta-T Devices Ltd, Cambridge, UK). In addition, immediately before the final harvest, two leaves were taken from each plant. One came from the lower part of the plant (the ninth leaf) and one from the upper part (the fourth leaf) counting from the top of the plant. The leaves were dried at 80°C for 48 h, ground to a fine powder and sub-samples of 1 mg were combusted, and the resulting CO_2 was analysed for the relative abundance of ^{13}C and ^{12}C, the ratio of which is thought to reflect stomatal conductance (Farquhar *et al.*, 1982). The ratios of $^{13}C/^{12}C$ of the samples were expressed as $^{13}\delta C$ (per thousand); the carbon isotopic composition was determined in 1 mg samples relative to Pee Dee Belemnite standard ($^{13}\delta C$) using a ratio mass spectrometer (Europa Scientific, UK). During the carbon analysis, the percentage of N and its isotope composition were also determined.

Results

In the hatching test, 73% of the eggs hatched and this is assumed to reflect the true level of viability. The effective inoculum densities were, therefore, 0.0, 0.03, 0.16, 0.80, 4.0, and 20.0 viable eggs/g dry soil. These are the values used hereafter.

At the first harvest, the numbers of galls and estimated numbers of nematodes per root system and per g root progressively increased with inoculum densities up to 4.0 viable eggs/g soil, but slightly decreased with 20 eggs (Table 1). Percentage invasion was low throughout; ranging from 4.9% with the lowest inoculum density to 0.7% of viable eggs with the highest inoculum density. The numbers of galls and the total numbers of nematodes were slightly lower in the drought treatments compared with the corresponding well-watered plants but the differences were not significant.

After 135 days, the greatest number of nematodes was produced by plants inoculated with 0.16 eggs/g soil and the greatest number per g root by those inoculated with 0.8 eggs (Table 1). The highest inoculum density (20 eggs/g soil) produced plants with the smallest root systems (Table 2) and the fewest nematodes at the second harvest. The reproduction rate (final population/initial population [*Pf/Pi*]) progres-

Table 1. *Number of galls or gall index and numbers of nematodes per plant or per pot 42 and 135 days after planting.*

Eggs, *Pi* Per pot	Viable/g	First harvest (42 days)			Second harvest (135 days)		
		Number of galls	Number of nematodes/plant		Gall index *	Total nematodes per pot (*Pf*)	Pf/Pi
			Females	J2			
			Well watered				
0	0.00	0	0	0	1.0	0	0
160	0.03	5	4	1	2.2	43 642	273
800	0.16	16	13	3	3.6	90 052	113
4 000	0.80	89	81	11	3.2	78 892	19.7
20 000	4.00	531	512	54	2.6	16 221	0.81
100 000	20.00	486	487	45	2.7	11 015	0.11
			Drought-subjected				
0	0.00	0	0	0	1.0	0	0
4 000	0.80	63	58	7	3.2	74 204	18.6
100 000	20.00	427	416	35	2.8	19 680	0.2
LSD 5%		167	188	36	1.0	47 939	-

Results are means of five replicates.
*On a 1-5 scale.

Table 2. *Growth of tomato plants 42 days after planting.*

Pi Viable eggs per g soil	Number of leaves	Plant height (cm)	Fresh weight				% dry matter (shoot only)	Shoot to root ratio
			Shoot (g)	Root (g)	Total (g)	Shoot (dry weight) (g)		
				Well watered				
0.00	16.2	55	90	4.4	94.4	9.4	10.4	21
0.03	15.4	55	85	4.3	89.3	9.8	11.6	20
0.16	15.8	56	89	5.0	94.0	9.7	10.9	18
0.80	16.6	57	79	3.4	82.4	9.2	11.5	23
4.00	16.0	55	77	3.8	80.3	8.8	11.5	20
20.00	14.0	42	57	4.1	63.1	7.8	13.6	14
				Drought-subjected				
0.00	14.8	48	62	2.6	64.6	7.0	11.3	24
0.80	14.6	48	58	2.4	50.4	6.4	11.1	24
20.00	14.0	34	37	2.7	39.7	4.1	11.8	13
LSD 5%	4.8	12	27	1.5	28.3	2.4	1.3	6.2

Results are means of five plants.

sively decreased from × 273 at $Pi = 0.03$ eggs/g, to × 0.11 at $Pi = 20$ eggs/g soil for well-watered plants and × 0.20 for the equivalent drought-subjected plants (Table 1). When the mean numbers of nematodes per plant at first harvest were used as the Pi, then the reproductive rate increased to × 8728 for the plants inoculated with 0.03 eggs and to × 21 for those with 20 eggs/g soil.

At the first harvest, compared with the uninfected control plants, only the highest inoculum density (20 viable eggs/g dry soil) affected plant growth; mean plant weight, shoot fresh weight, total fresh weight and the root/shoot ratio were all significantly ($P \leq 0.05$) decreased (Table 3). Leaf number was not significantly affected, but height was decreased ($P \leq 0.05$), indicating that internode length had been decreased. It was calculated that the highest inoculum density also decreased leaf size; a mean of 4.1 g of top weight per leaf for the most heavily infested plants compared with 5.6 g per leaf for the uninfected control plants. The highest inoculum density significantly increased percentage dry matter in the shoot (Table 3).

At the second harvest (Table 2), shoot fresh weight and numbers of fruit and total fresh weight were significantly ($P \leq 0.01$) decreased by the three highest inoculum densities (0.8, 4.0 and 20.0 eggs/g soil). The shoot/root ratio was also significantly ($P \leq 0.05$) decreased with 0.16, 0.80, and 4.0 eggs/g soil. However, compared with the uninoculated plants, the lowest inoculum density (0.032 eggs/g soil) significantly ($P \leq 0.05$) increased shoot and root fresh and total dry weight.

Using equation (1), the relationship between total plant weight as a percentage of the uninoculated value at the first harvest against inoculum density (log viable eggs/pot) was examined and showed (Fig. 1) a significant negative, log-linear relationship ($P \leq 0.05$) accounting for 68% of the variance.

$$Y = - 10.55(\log Pi) + 123$$

At the second harvest there was a much greater negative, linear relationship ($P \leq 0.0007$, variance accounted for 98%) between log Pi and total fresh weight as a percentage of the uninoculated (Fig. 1).

$$Y = - 38.07(\log Pi) + 199$$

It could be argued that six data points are insufficient to fit the Seinhorst equations, but nonetheless an attempt was made to estimate the parameters in equations (2) and (3) (Table 4). Parameters were estimated using maximum likelihood in the statistical package GENSTAT (Payne *et al.*, 1987). Simultaneously estimating all four parameters in equation (2) proved difficult without fixing the estimates of at least two of them. Equation (3) presented no difficulty in estimating all parameters simultaneously and described the data more effectively than equation (2) (Fig. 2). For the first harvest the model accounted for 86.2% of the variation and for the second harvest

Table 3. *Growth of tomato plants 135 days after planting.*

Pi Viable eggs per g soil	Plant height (cm)	Fresh weight				Dry weigth shoot + fruit (g)	Shoot to root ratio
		Shoot (g)	Root (g)	Fruit (g)	Total (g)		
			Well watered				
0.00	124	232	53	107	391	43	4.4
0.03	131	302	90	69	461	51	3.4
0.16	119	183	91	84	388	39	2.0
0.80	115	157	68	30	255	40	2.3
4.00	103	71	32	30	132	28	2.3
20.00	91	55	14	0	69	19	3.9
			Drought-subjected				
0.00	109	199	45	36	280	35	4.4
0.80	99	139	58	22	219	28	2.5
20.00	69	28	9	0	37	10	3.2
LSD 5%	12	27	22	45	82	6.3	2.0

Results are means of five plants.

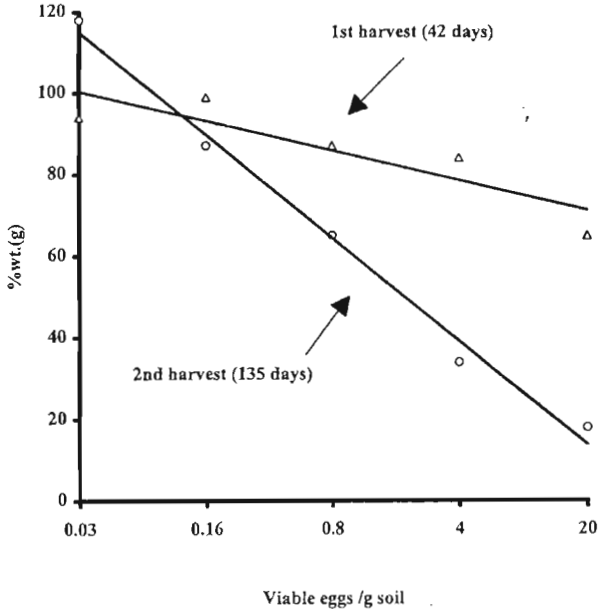

Fig. 1. Total fresh weight at the first (42 days) and second harvests (135 days) as a percentage of the uninoculated treatment regressed against viable eggs per g soil on a log. scale. Results are means of five replicates.

94.6% of the variation. Using equation (2), with the estimated values of T and of $Ymax$ fixed, the value of z was estimated to have decreased from 0.88 at the first to 0.42 at the second harvest (Table 4). With equation (3), the estimates of m and of T had decreased from 0.66 (of $Ymax$) and 0.34 eggs per g soil respectively at the first harvest to 0.22 (of $Ymax$) and 0.06 eggs per g soil at the second harvest.

Plant height, shoot fresh and dry weight and root fresh weight were less, at both harvests, for drought-subjected compared with the equivalent well watered plants (Tables 2, 3). At the first harvest, the percentage differences (-32%, -27%, and -35%) in total fresh weight were similar for the three inoculum densities (0.0, 0.8, and 20 eggs/g soil, respectively). At the second harvest, the relative decrease associated

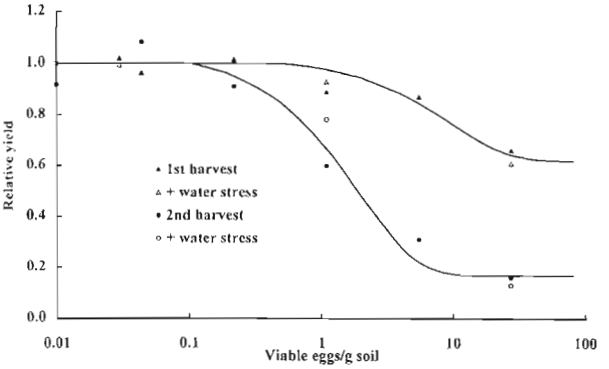

Fig. 2. Relationship between total fresh weight at the first (42 days) and second harvests (135 days) and viable eggs per g soil as described by fitting parameters to the Seinhorst yield loss model (equation 3).

with the drought treatment was less than at the first harvest for plants with 0.0 and 0.8 eggs/g soil (-29% and -14%, respectively) but greater for the plants inoculated with 20 eggs/g (-47%). However, there was no significant interaction between the nematodes and the drought treatment (Fig. 2).

Water used per plant was decreased overall *ca* 35% by drought, and increasingly by the three highest inoculum densities with the most heavily infested, well watered and drought-subjected plants having 72 and 76% decreases, respectively, compared with the corresponding uninfected plants. After 42 days, water use per g dry matter produced had been largely unaffected by drought or by the *M. incognita*, but after 135 days drought and the highest inoculum density had significantly ($P \leq 0.05$) increased water use efficiency (data not shown).

The porometer measurements before the first harvest showed that, compared with the well watered, uninoculated plants, stomatal conductance (Table 5) tended to be decreased by drought but was unaffected by *M. incognita*, except perhaps for the highest inoculum density. Between the first and second harvests, stomatal conductance was slightly decreased by the drought treatment and by 0.16, 0.8 and 4.0 eggs/g soil

Table 4. *Estimates parameters for equations (2) and (3) based on total fresh weights (* indicates parameters fixed).*

Harvest	Equation (2)					Equation (3)		
	T^*	Y_{max}^*	m	Z	Z^T	T	Y_{max}	m
First	0.3	91	0.61	0.88	0.90	0.342	96.28	0.664
Second	0.032	415.0	0.23	0.42	0.92	0.057	422.6	0.221

Table 5. *Mean stomatal conductance measured weekly.*

Pi viables eggs per g soil	Stomatal conductance (cm/s)			
	To 42 days		To 135 days	
	Watered	Drought-subjected	Watered	Drought-subjected
0.00	1.53	1.34	0.29	0.25
0.03	1.72	-	0.35	-
0.16	1.84	-	0.26	-
0.80	1.75	0.92	0.27	0.22
4.00	1.90	-	0.24	-
20.0	0.96	1.24	0.19	0.19
LSD (5%)	0.92		0.09	

Results 42 and 135 days are means of ten plants measured on three occasions, and of five plants measured on nine occasions, respectively.

of *M. incognita*. The highest inoculum density (20 eggs/g soil) significantly ($P \leq 0.05$) decreased the stomatal conductance of the well-watered plants, which was not further decreased by drought.

Neither nematodes nor drought had any effect on the ^{13}C composition of either the lower leaves or upper leaves (Table 6). All the values were close to -28 and, with one exception, none were significantly different from the well-watered, uninoculated control plants. The exception was the drought-subjected plants with 0.8 viable eggs/g soil where the value discrimination in favour of ^{12}C increased ($P \leq 0.05$) rather than decreased as might have been expected if the plants were water stressed.

The percentage of N was significantly ($P \leq 0.05$) increased in the lower leaf dry matter by both drought and by the highest level of nematode infection and in the upper leaves by the highest level of nematodes only (Table 6). The intermediate level of infection had no consistent effect on the lower leaves but consistently increased the percentage of N in the upper leaves of the drought-subjected plants.

Table 6. *13δC values for upper and lower leaves and percentage of nitrogen (%N) in leaf dry matter at 135 days.*

Pi viable eggs per g soil	$^{13}\delta C$		%N	
	Lower leaves	Upper leaves	Lower leaves	Upper leaves
		Well watered		
0.00	-28.3	-28.3	4.75	4.95
0.03	-28.1	-27.7	4.90	5.03
0.16	-28.3	-27.7	4.70	5.27
0.80	-27.9	-27.6	4.58	4.77
4.00	-28.0	-27.3	4.53	5.28
20.00	-27.9	-27.7	5.94	6.31
		Drought-subjected		
0.00	-28.1	-28.2	5.66	4.63
0.80	-29.9	-27.7	4.91	5.72
20.00	-27.9	-27.8	6.22	6.72
LSD (5%)	-0.60	-0.70	0.86	0.99

Discussion

We hypothesised that on good hosts, initially small populations of *M. incognita* which cause little or no damage at the start of the growing season, can increase so greatly that they cause substantial damage later in the growing season. Tomato cv. Moneymaker fulfilled the requirement of being a good host, as shown by the rates of the population increase of the *M. incognita* at the lower population densities, and the results obtained with it broadly supported the original hypothesis.

The general principle (Seinhorst, 1967) that the multiplication rate of sedentary nematodes is density dependent was again confirmed with the greatest rate of increase observed at low values of *Pi*. With an inoculum of 0.03 eggs/g soil there was a × 273 increase in the population density over the whole of the experiment compared with × 0.11 increase with a *Pi* of 20.0 eggs/g soil. However, as found with *M. arenaria* by McSorley *et al.* (1992), the highest *Pf* was produced by intermediate values of *Pi* and it was again demonstrated that on a good host, and in favourable conditions, small populations can become very large during the lifetime of one crop.

A much greater proportional effect on plant growth (total plant weight) of the intermediate inoculum densities at the second as compared with the first harvest was demonstrated, supporting the hypothesis that initially non-damaging population densities can increase so greatly that they become damaging during the lifetime of an annual crop. Also, the slope of the linear regression of total plant weight as a proportion of the uninoculated yield against increasing log inoculum density was much greater at the second than at the first harvest. Using the Seinhorst equation (3) to fit lines to the data showed that, for the first and second harvests, the respective estimates of T (the tolerance limit) and of m (the minimum yield) were always higher for the first harvest data then for the second harvest data. As at planting, the plants were small (< 2.0 g), their contribution to m at either harvest would have been small. Estimating z in equation (2) showed that it was also much higher for the first harvest (where it measures the damage caused by a single nematode) compared with the second harvest (where it measures the damage caused by a single nematode and all its progeny).

Equation (3) has the assumption that $z^T = 0.95$, which is largely derived for observations with *Globodera* spp. (Seinhorst, 1986) with one generation in a growing season. Applying this assumption to the data for the second harvest, where there will have been up to three generations of the *M. incognita*, still resulted in equation (3) providing a better fit to the data than equation (2). This approach has also been successfully used by Di Vito *et al.* (1985, 1997) to describe yield losses in relation to *M. incognita* on *Capsicum* and *Hibiscus*, respectively.

An important conclusion from these results is that the estimated threshold for damage (T) at the second harvest of 0.057 viable eggs per g soil is so low that it is on the border line of detection, *i.e.*, 11.4 viable eggs of J2 in 200 g soil. In a series of studies of *M. arenaria* damage to peanut in microplots McSorley *et al.* (1992) reported closer fits with the Seinhorst equation (r^2 ranged from 0.79 to 0.98) and similar low T values (as low as 1.0 egg/100 g soil), except in one trial where the value of T was higher (23 eggs/100 g soil). Their estimated value of z varied from 0.90 to 0.99, indicating that *M. arenaria* was much less damaging to peanut than was *M. incognita* to tomato.

The lowest inoculum density (0.03 eggs/g soil) had apparently increased tomato growth at the end of the experiment. A similar increase was observed with a *Pi* of 0.31 eggs per g soil by Niblack *et al.* (1986) with *M. incognita* on partially resistant soybean cultivars Bragg and Braxton in two microplot experiments. In contrast the yield of the susceptible cultivar GaSoy 17 was greatly reduced at *Pi* of 0.31 eggs.

Under ideal growing conditions, nematodes may cause only moderate damage, whereas under periods of drought, or other stress factors, they may cause considerably more damage (Wallace, 1973). Moreover, the relative host sensitivity of a plant measured under glasshouse conditions, where there is minimal stress, frequently differs from that of plants grown under field conditions. The drought stress applied in this experiment decreased the growth of the uninfested and of the *M. incognita* infested plants equally and generally reduced their stomatal conductance compared with the comparatively well watered plants. However, there was no evidence of an interaction between the stress induced by the drought and that of the *M. incognita*. Wheeler *et al.* (1991) also found that the effects of damage to tobacco by *M. incognita* and the effects of drought were additive and not interactive.

Although both the *M. incognita* and drought decreased stomatal conductance, indicating that both increased water stress, this was not reflected in the ratio of the stable isotopes 12/13 of carbon. Drought generally decreases the degree of discrimination so that a value of -21 is typical of drought stressed plants (Haverkort & Valkenburg, 1992). However all our plants had similar values close to -28. That the drought-subjected plants grew less well than the well watered plants suggests that they were water stressed for at least some of the time but this was not reflected in the carbon isotope ratio. Consequently, the stomatal conductance results are the more meaningful and they support the hypothesis that a damaging infection with *M. incognita* does increase water stress, leading to

reduced growth. The increased percentage nitrogen in the leaf dry matter of the most heavily infested plants indicates that they were not N deficient, and that some other factor, such as water stress, must have been responsible for their poor growth. However, the possibility has not been excluded of other physiological effects being involved in reducing the growth of *M. incognita* infested plants.

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References

BARKER, K.R. & NOE, J.P. (1987). Establishing and using threshold population levels. *In*: Veech, J.A. & Dickson, D.W. (Eds). *Vistas on nematology: a commemoration of the twenty-fifth anniversary of the Society of Nematologists*: 75-81.

BARKER, K.R. & WEEKS, W.W. (1991). Relationships between soil and levels of *Meloidogyne incognita* and tobacco yield and quality. *J. Nematol.*, 23: 82-90.

DI VITO, M., GRECO, N. & CARELLA, A. (1985). Populations densities of *Meloidogyne incognita* and yield of *Capsicum annuum*. *J. Nematol.*, 17: 45-49.

DI VITO, M., PISCIONERI, S., PACE, G. & CATALANO, F. (1997). Pathogenicity of *Meloidogyne incognita* on kenaf in microplots. *Nematol. medit.*, 25: 165-168.

DUNCAN, L.W. & FERRIS, H. (1983). Validation of a model for prediction of host damage by two nematode species. *J. Nematol.*, 15: 227-234.

EHWAETI, M.E., PHILLIPS, M.S. & TRUDGILL, D.L. (1998). The viability of *Meloidogyne incognita* eggs released from egg masses of different ages using different concentrations of sodium hypochlorite. *Nematologica*, 44: 207-217.

ELSTON, D.A., PHILLIPS, M.S. & TRUDGILL, D.L. (1991). The relationship between initial population density of potato cyst nematode *Globodera pallida* and the yield of partially resistant potatoes. *Revue Nématol.*, 14: 213-219.

FARQHAR, G.D., O'LEARY, M.H. & BERRY, J.A. (1982). On the relationship between carbon isotope discrimination and the intercellular carbon dioxide concentration in leaves. *Aust. J. Pl. Physiol.*, 9: 121-137.

HAVERKORT, A.J., FASAN, T. & VAN DE WAART, M. (1991). The influence of cyst nematodes and drought on potato growth. 2. Effects on plant water relations under semi-controlled conditions. *Neth. J. Pl. Pathol.*, 97: 162-170.

HAVERKORT, A.J. & VALKENBURG, G.W. (1992). The influence of cyst nematodes and drought on potato growth. 3. Effects on carbon isotope fractionation. *Neth. J. Pl. Pathol.*, 98: 12-20.

HUSSEY, R.S. & BARKER, K.R. (1973). A comparison of methods of collecting inocula of *Meloidogyne* spp., including a new technique. *Pl. Dis. Reptr*, 57: 1025-1028.

MCSORLEY, R., DICKSON, D.W., CANDANEDO-LAY, E.M., HEWLETT, T.E. & FREDERICK, J.J. (1992). Damage functions for *Meloidogyne arenaria* on peanut. *J. Nematol.*, 24: 193-198.

MEON, S., WALLACE, H.R. & FISHER, J.M. (1978). Water relation of tomato (*Lycopersicon esculentum* Mill. cv. Early Dwarf Red) infected with *Meloidogyne javanica* (Treub) Chitwood. *Phys. Pl. Path.*, 13: 275-281.

NIBLACK, T.L., HUSSEY, R.S. & BOERMA, H.R. (1986). Effects of environments, *Meloidogyne incognita* inoculum levels, and *Glycine max* genotype on root-knot nematode-soybean interactions in field microplots. *J. Nematol.*, 18: 338-346.

O'BANNON, J.H. & REYNOLDS, H.W. (1965). Water consumption and growth of root-knot nematode infected and uninfected cotton plants. *Soil Sci.*, 99: 251-255.

OOSTENBRINK, M. (1966). Major characteristics of the relation between nematode and plants. *Meded. Landb-Hogesch. Wageningen*, 66: 3-46.

RAHI, G.S., RICH, J.R. & HODGE, C. (1988). Effect of *Meloidogyne incognita* and *M. javanica* on leaf water potential and water use of tobacco. *J. Nematol.*, 20: 516-522.

PAYNE, R.W., LANE, P.W., AINSLEY, E.E., BICKNELL, K.E., DIGBY, P.G.N., HARDING, S.A., LEECH, P.K., SIMPSON, H.R., TODD, A.D., VERRIER, P.J. & WHITE, R.P. (1987). *Genstat 5 reference manual*. Oxford, UK, Clarendon Press, 749 p.

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

SEINHORST, J.W. (1967). The relationship between population increase and population density in plant parasitic nematodes. II. Sedentary nematodes. *Nematologica*, 13: 157-171.

SEINHORST, J.W. (1986). Effects of nematode attack on growth and yield of crop plants. *In*: Lamberti, F. & Taylor, C.E. (Eds). *Cyst nematodes*. New York & London, Plenum Press: 211-224.

SEINHORST, J.W. (1995). The effect of delayed attack of oats by *Heterodera avenae* on the relation between initial nematode density and plant growth, plant weight, water consumption and dry matter. *Nematologica*, 41: 487-504.

SEINHORST, J.W. & KOZLOWSKA, J. (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.

VRAIN, T.C. (1982). Relationship between *Meloidogyne hapla* density and damage to carrots in organic soils. *J. Nematol.*, 14: 50-57.

WALLACE, H.R. (1973). *Nematode ecology and plant disease*. London, UK, Arnold, 228 p.

WHEELER, T.A., BARKER, K.R. & SCHNEIDER, S.M. (1991). Yield-loss model for tobacco infected with *Meloidogyne incognita* as affected by soil moisture. *J. Nematol.*, 23: 365-371.

WINDHAM, G.L. & BARKER, K.R. (1986). Effects of soil type on the damage potential of *Meloidogyne incognita* on soybean. *J. Nematol.*, 18: 331-338.