Interaction of Meloidogyne hapla and M. javanica Infecting Tomato[1]

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Abstract: A soil temperature of 20 C was equally suitable for the invasion and development of *M. hapla* and *M. javanica*. However, *M. javanica* predominated in a mixed species infection at this temperature. Predominance increased with increasing mixed-species inoculum levels. Invasion by *M. hapla* was more density-dependent than *M. javanica*. *M. hapla* produced a greater incidence of terminal galls and lateral roots.
Key Words: Temperature, Inoculum level, Invasion, Development, Sex ratio, Terminal galls, Lateral roots.

In California many crops are susceptible to the root-knot nematodes *Meloidogyne hapla*, *M. javanica*, and *M. incognita*, all of which are widespread in the state. Although plants may be infected by more than one species of *Meloidogyne* (5), mixed species infections are not common in the field.

We conducted experiments to determine: whether dominance of one species results from coincident infections by *M. hapla* and *M. javanica* on tomato; whether certain temperature and inoculum levels might favor one species; and whether host response might be more favorable to one species.

MATERIALS AND METHODS

Tomato plants (*Lycopersicon esculentum* Mill.) 'Rutgers', a host of both *M. hapla* Chitwood, and *M. javanica* (Treub) Chitwood, were used throughout this study.

Stock cultures of *M. hapla* and *M. javanica*, reared initially from single egg masses, were maintained separately on tomato plants. Larvae for inocula were hatched from egg masses supported on funnels in a mist chamber.

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Plants in all experiments were grown in a 1:1 *v/v* mixture of ovendried coarse river sand and garden loam soil. Constant (±1 C) temperatures were maintained in water bath temperature tanks.

In studies following the experiment involving root-halves, roots were washed free of soil in a fine spray of water and stained in cold acid fuchsin in lactophenol for 24 hr, then destained and stored in clear lactophenol prior to dissection.

MIXED AND SINGLE SPECIES INFECTIONS OF ROOT-HALVES: The following experiment was conducted to determine whether one species would dominate in a coincident infection. A soil temperature of 20 C was chosen to favor infection by *M. hapla*, generally regarded as the least virulent of the two species.

Tomato seedlings approximately 10 cm tall, grown in individual containers, were carefully removed from the soil and their tap roots and stems split longitudinally to a point midway between the crown and the cotyledons. Each half of a split root system was transplanted into separate adjacent 825-ml containers of soil mixture. The plants were watered with half-strength Hoagland's solution and allowed to become re-established for one week at ambient glasshouse temperatures prior to inoculation.

Each whole plant comprised one replicate. Approximately 4,000 *M. hapla* larvae were added to the soil surrounding each half-root

system of 5 plants, and 4,000 larvae of *M. javanica* were added to each of the other half-root systems of the same plants. In the mixed species inoculations, 2,000 larvae of each species were transferred to each half-root system of another 5 plants.

Plants were maintained at 20 C and harvested after 40 days. The mature females were removed from the root tissue by a modification of the technique described by Dropkin *et al.* (4). Roots were stained 4 min in boiling acid fuchsin in lactophenol prior to a 12 hr agitation in 33% Pectinol 59L® (Rohm and Haas Company, Philadelphia, PA. 19105), then washed through nested screens with tap water. The relative numbers of *M. hapla* and *M. javanica* females in the mixed species replicates were determined by pouring all females from one replicate into a petri dish and selecting 100 at random for specific perineal pattern identification (7).

INFLUENCE OF TEMPERATURE ON INVASION: The invasion of tomato roots by *M. hapla* or *M. javanica* was studied under constant temperatures of 15, 20, 25, and 30 C. A sealed pot system was employed to aid stabilization of soil moisture levels during the experiment. Single tomato seeds were planted in 35 ml of soil mixture maintained in 100-ml translucent polyethylene beakers (Fig. 1). The soil was watered with 8 ml of diluted plant nutrient (1 part 12% N, 6% P, 6% K: 760 parts water). The pots were sealed with polypropylene tissue secured with a rubber band. The seeds were allowed to germinate at ambient glasshouse temperatures. When the seedlings had reached the first true-leaf stage (Fig. 1), 180 larvae of either *M. hapla* or *M. javanica* were added to the soil in 1 ml of suspension by puncturing the seal with a hypodermic syringe (No. 18 needle aperture). The puncture in the cover then was sealed. The amount of soil in the pots was adequate for root growth during the experiment. The total amount of water added to each pot gave a maximum soil moisture content of 13.2% by weight. Pots were maintained at the desired soil temperatures. Every 24 hr after inoculation, 4 replicates from each species and temperature treatment were removed and the number of invading larvae counted.

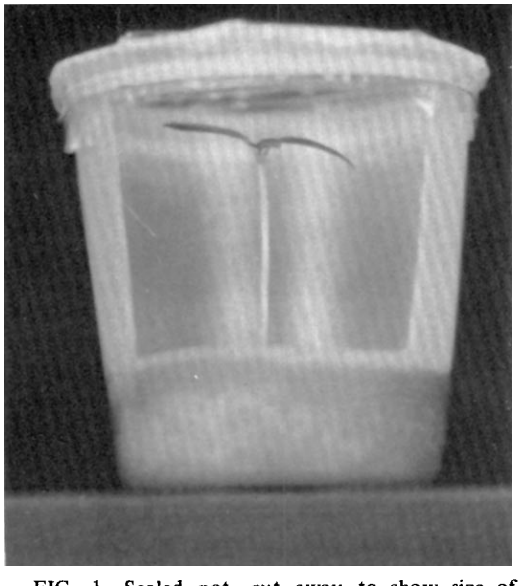

FIG. 1. Sealed pot, cut away to show size of tomato seedling at time of inoculation.

INFLUENCE OF TEMPERATURE ON DEVELOPMENT: The rate of development within single species populations of *M. hapla* and *M. javanica* was studied at 15, 20, 25, 30, and 35 C. The sealed pot system was used to ensure relatively constant moisture levels during the period of invasion. One hundred twenty-five larvae were introduced into each of a series of pots. The seals were removed after one week. Due to the small volume of soil, the pots were checked several times daily and irrigated, when needed, with water adjusted to the test temperature. Nematode development was followed by examining specimens from one plant from each species and temperature treatment at various time intervals.

INFLUENCE OF TEMPERATURE ON SEX RATIO: The influence of temperature on the adult sex ratio of single species populations of *M. hapla* and *M. javanica* was studied using an open-pot system. Single tomato seeds were germinated in 50 ml of soil mixture in 100-ml polyethylene beakers. Three hundred larvae of either species were added to the pots when the seedlings were 2 weeks old. Pots were maintained at 15, 20, 25, 30, and 35 C. After an adequate time to allow for development of adults at each temperature, 4 replicates from each species treatment were removed and the numbers of adult males and females determined.

INFLUENCE OF INOCULUM LEVEL ON INVASION AND DEVELOPMENT: Invasion by *M. hapla* and *M. javanica* larvae was studied at different inoculum levels (Inoculum Test-1). A series of seedlings in sealed pots was exposed to 100, 200, and 400 larvae of either

species/seedling. Five replicates from each species and population level were removed and examined at 48 hr intervals for 8 days.

The influence of larval numbers on the invasion and development of single and mixed species populations was also studied (Inoculum Test-2). Tomato seedlings in open pots were exposed to 125, 250, 500, 750, and 1,000 larvae of either species/seedling. Additional seedlings were exposed to a mixture of equal numbers of larvae of both species to give the same population levels.

Ten days after exposure 5 replicates from each species and inoculum treatment were harvested and the number of invading larvae counted. This period was considered long enough to allow the majority of larvae to invade the roots. Five replicates were harvested 35 days after addition of the larvae to allow time for maturation of adults at the experimental temperature. The number of nematodes and the relative percentage of the various life stages were determined. In the mixed species infections all mature females were identified by the perineal patterns.

A soil temperature of 20 C was chosen for both experiments since this temperature was close to the optimum for invasion and development of both species.

INFLUENCE OF INOCULUM LEVEL ON GALLING CHARACTERISTICS: A comparison of the influence of inoculum level on the host-parasite relationships as exhibited by number of nematodes per gall, lateral root production, and incidence of terminal galls was included in Inoculum Test-2. Nematodes per gall were recorded for all replicates. The position of each gall was recorded as either terminal or non-terminal; a terminal gall being one that incorporated the root tip. Lateral roots emanating from each gall were also counted.

RESULTS

MIXED AND SINGLE SPECIES INFECTIONS OF ROOT-HALVES: The total number of females recovered from single species infection was not significantly different from the number recovered in the mixed species infection (t-test, $P<0.05$). In single species infections there were 2 X as many females of *M. javanica* present as of *M. hapla* (Table 1). However, in the mixed species infection there were almost 16 X as many females of *M. javanica* as of *M. hapla*. It is apparent that *M. javanica* predominated at the expense of *M. hapla* in the mixed species infection.

TABLE 1. Number of females of *Meloidogyne hapla* and *M. javanica* resulting from single and mixed species infections of split-root tomatoes, 40 days after inoculation with approximately 4,000 second-stage larvae per half-root system. Each figure is the mean of five replicates ± SE.

	M. hapla/ half-root	*M. javanica*/ half-root	Combined
Single species infection	661 ± 88	1305 ± 152	1967 ± 167
Mixed species infection	6 ± 1%†	94 ± 1%†	2311 ± 54

† Based on 100 specimens

INFLUENCE OF TEMPERATURE ON INVASION: Of the temperatures tested, 20 C was closest to the optimum for invasion by either species (Fig. 2). Maximum invasion by *M. javanica* was achieved approximately 2 days before maximum invasion by *M. hapla*. *M. hapla* did not invade roots at 35 C.

INFLUENCE OF TEMPERATURE ON DEVELOPMENT: Populations of both species matured slowly at 15 C, with *M. hapla* maturing slightly earlier than *M. javanica* (Fig. 3). Both species required similar times to reach maturity at 20 and 25 C, but *M. javanica* matured earlier than *M. hapla* at 30 C. Development of *M. hapla* was most rapid at 25 C, but there was little difference in the developmental time of *M. javanica* reared at 25, 30, and 35 C.

All males of *M. javanica* recovered from the 35 C treatment showed varying degrees of abnormality (Fig. 4). They were incompletely elongated and had conspicuous swellings near the base of the testes.

INFLUENCE OF TEMPERATURE ON SEX RATIO: The percentage of males of *M. hapla* recovered was constant, irrespective of temperature treatment. There was a significant increase in the percentage of *M. javanica* males at temperatures above 20 C (Table 2). The greatest number of nematodes was recovered at 20 C for both species. Compared with the experiment on the influence of temperature on invasion, considerably more *M. javanica* were recovered than *M. hapla*. Since different batches of larvae were used in each experiment, variation in the infectivity of the batches could account for these differences.

INFLUENCE OF INOCULUM LEVEL ON INVASION AND DEVELOPMENT

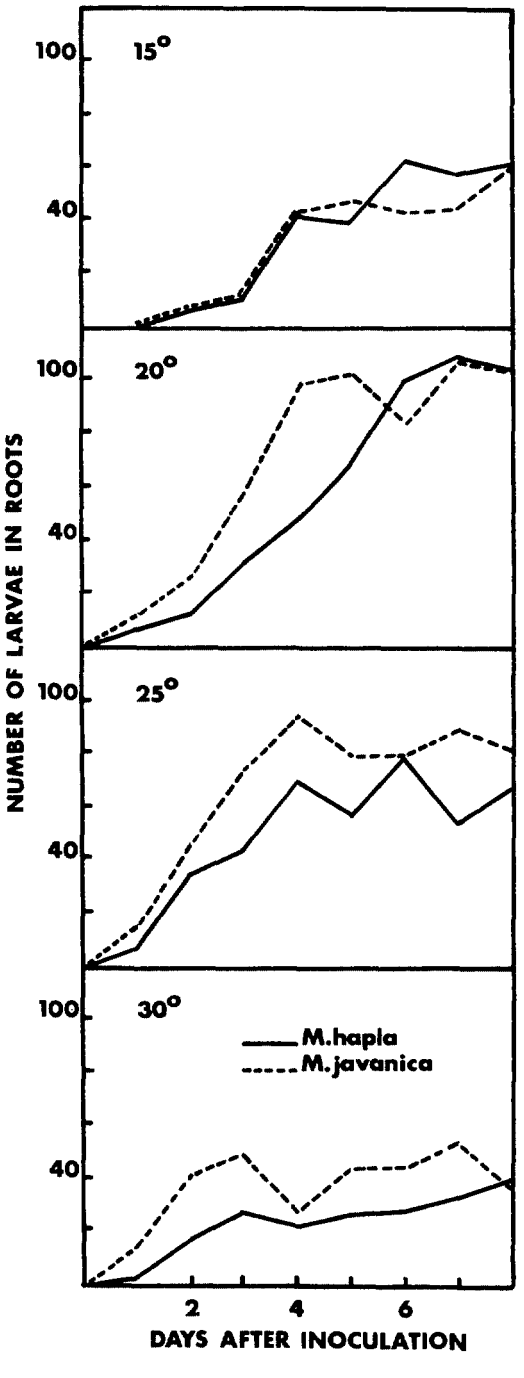

FIG. 2. Influence of temperature on the invasion of tomato roots by *M. hapla* and *M. javanica*. Each point represents the mean of four replicates. Inoculum - 180 larvae per seedling.

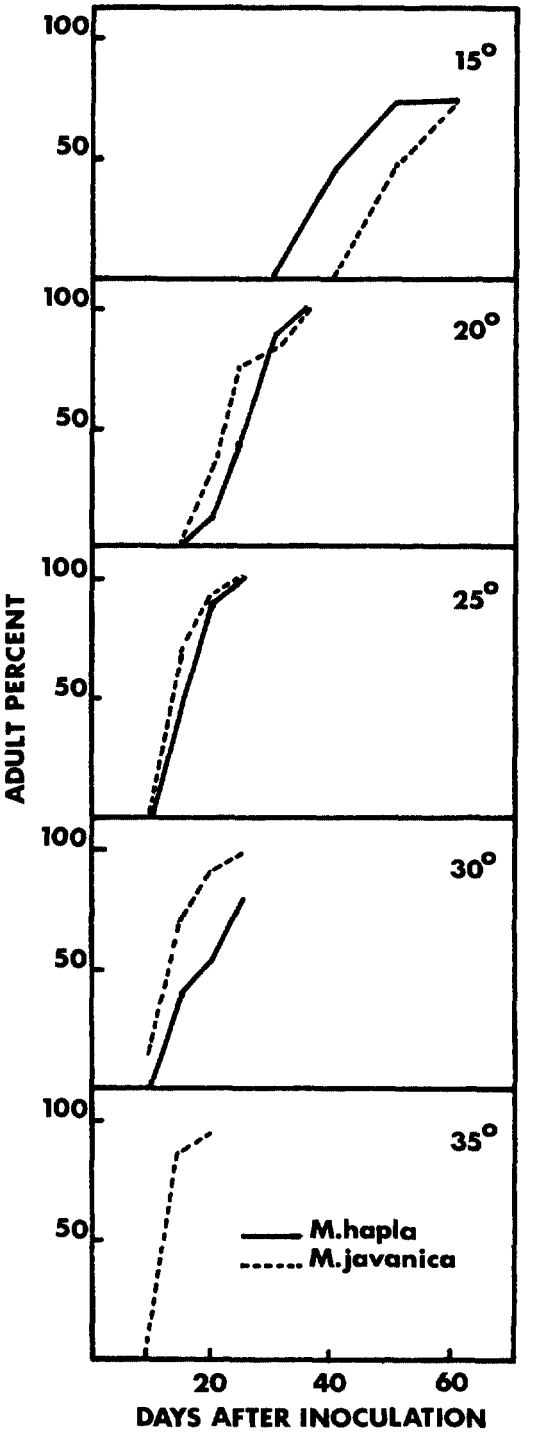

FIG. 3. Influence of temperature on the development of *M. hapla* and *M. javanica*. The appearance of adults expressed as a percentage of total infection.

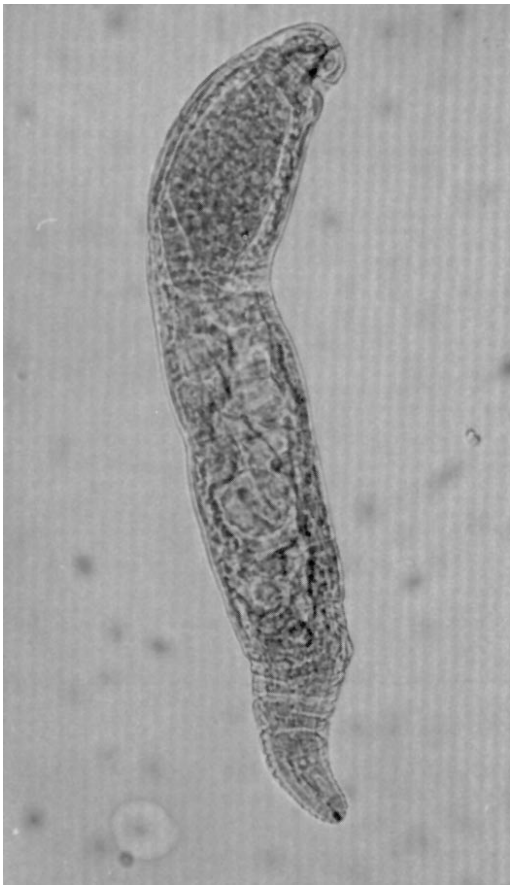

FIG. 4. Abnormal male of *M. javanica* recovered from tomato maintained at 35 C.

(INOCULUM TEST-1): The total number of larvae of *M. javanica* which penetrated the roots was constant in relation to the inoculum levels used (Fig. 5). The mean percentage penetrations at the 8th day after inoculation were 75.0, 71.2, and 71.5% for inoculum levels of 100, 200, and 400 larvae/plant, respectively. The mean percentage penetrations of *M. hapla* were 52.0, 50.5, and 34.8%. The reduction in percentage penetration at the 400 larval inoculum level was statistically significant (t-test, $P<0.01$).

In a second experiment there were differences in the percentage penetrations between the single species infections of *M. hapla* and *M. javanica* recovered 10 days after inoculation (Table 3). There was a marked reduction in the percentage penetration of *M. hapla* at the higher inoculum levels. There was no significant change in the percentage penetration due to different inoculum levels in either the *M. javanica* or mixed species infections. At the 1,000 level, there were significantly greater numbers of *M. javanica* recovered than in the mixed species (t-test, $P<0.05$) or *M. hapla* infections (t-test, $P<0.01$).

Differences occurred in the level of infections at 10 days and at 35 days after exposure in both single species infections. At the low inoculum levels, more nematodes were found in the roots after 35 days in both species treatments. At higher inoculum levels, there was a reduction in the number of nematodes present after 35 days in the single species infection by *M. javanica*. This probably was due to a loss of roots. Many of the roots and galls in the 750 and 1,000 larvae treatments with this species were necrotic. There was an increase in the infection by *M. hapla* at all inoculum levels. Many of the late penetrating larvae of this species had invaded the lateral roots produced in response to the earlier invasions. Root necrosis was not so evident in the *M. hapla*

TABLE 2. Influence of temperature on the adult sex ratio of *Meloidogyne hapla* and *M. javanica*. Figures are the mean of five replicates. (Inocula were 300 larvae per plant.)

Species	Temp. (C)	Time (days)	Nematodes per plant	Percentage		
				Larvae	Females	Males
M. hapla	15	80	123	2.2	82.4	15.4a†
	20	33	166	1.8	78.4	19.8a
	25	26	146	2.6	80.0	17.4a
	30	27	136	1.0	78.4	20.6a
	35	27	0	0	0	0
M. javanica	15	85	163	1.3	94.9	3.8a
	20	33	276	2.5	92.6	4.9a
	25	23	255	2.3	82.9	14.8b
	30	23	180	0.2	76.6	23.2c
	35	23	141	0.4	56.9	42.7d

† Values in each column not followed by the same letter differ significantly ($P<0.01$).

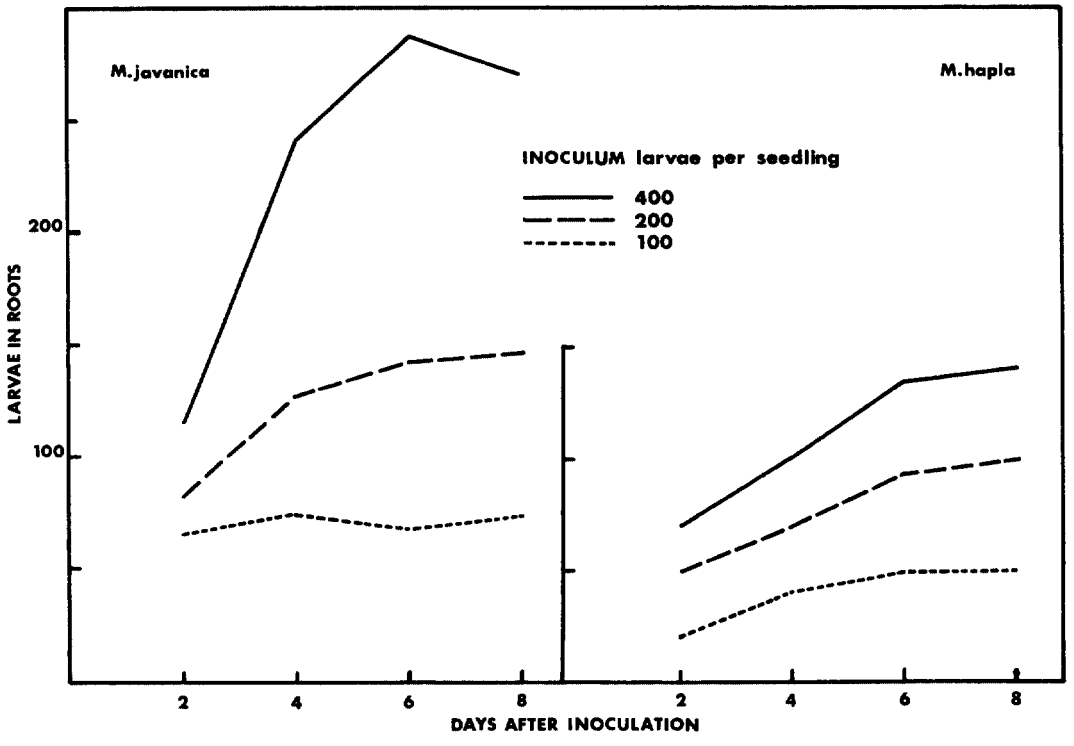

FIG. 5. Influence of inoculum level on the invasion of tomato roots by *M. hapla* and *M. javanica*. Each point represents the mean of five replicates.

TABLE 3. Number of *Meloidogyne hapla, M. javanica* and a mixture of both infecting tomato at 20 C after 10 days and 35 days. Figures are the mean of five replicates.

Species	Inoculum (No. of Larvae)	After 10 days		After 35 days	
		Nematodes in roots	% of inoculum	Nematodes in roots	% of inoculum
M. hapla	125	82	65.3ab†	120	96.3a†
	250	190	76.2a	239	95.6ab
	500	323	64.7ab	370	74.1c
	750	441	58.7bc	489	65.3cd
	1000	473	47.3c	618	61.8d
M. javanica	125	75	59.8a	114	91.5a
	250	168	67.1a	175	70.3b
	500	335	67.0a	315	63.1bc
	750	561	74.7a	417	55.6cd
	1000	731	73.1a	500	50.1d
Mixed	125	83	66.2a	113	90.1a
	250	133	53.1a	176	70.3b
	500	317	63.4a	368	73.6b
	750	396	52.7a	426	56.8c
	1000	566	56.6a	558	55.8c

† Values in each column not followed by the same letter differ significantly ($P<0.05$).

TABLE 4. Percentage of life stages of single and mixed species infections of *Meloidogyne hapla* and *M. javanica* after 35 days at 20 C following inoculation with various inoculum levels. Each figure is the mean of five replicates.

Species	Inoculum Level	Total Nematodes	2nd Stage	3-4th Stage	Adult		
					Males	Imm.♀♀	Mature♀♀
M. hapla	125	120	10.3	3.2	4.1	16.7	65.7
	250	239	16.5	3.1	5.3	18.5	56.6
	500	370	21.3	2.8	15.6	12.8	47.5
	750	490	29.2	3.7	15.5	15.8	35.8
	1000	618	23.9	4.6	23.9	14.6	33.0
M. javanica	125	114	9.3	1.5	0.2	12.0	77.0
	250	176	11.8	1.3	0.8	14.1	72.0
	500	315	15.7	1.3	5.0	20.9	57.1
	750	417	26.9	2.1	6.5	21.7	42.8
	1000	501	17.4	3.1	10.6	25.0	43.9
Mixed	125	113	13.5	0.9	3.3	14.6	67.7
	250	176	12.3	1.4	4.9	11.7	69.7
	500	368	18.7	2.0	9.8	10.0	59.5
	750	426	11.6	2.9	11.0	16.9	57.6
	1000	558	15.9	2.6	15.2	19.3	47.0

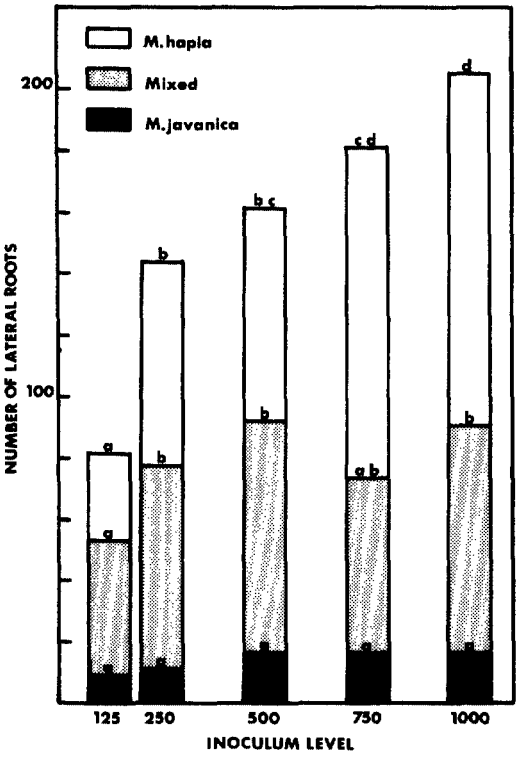

FIG. 6. Total number of lateral roots from galls in infections by *M. hapla, M. javanica*, and a mixture of both, 35 days after inoculation with various inoculum levels. Mean of five replicates. Columns under the same letter in individual species treatments are not significantly different ($P<0.05$).

TABLE 5. Ratio of mature females of *Meloidogyne hapla* and *M. javanica* from mixed species infections at various inoculum levels. Figures are the mean of five replicates.

Inoculum level	Mature Females	Ratio *hapla:javanica*
125	76	1:1.2a†
250	123	1:1.4a
500	216	1:1.7a
750	244	1:2.9b
1000	261	1:3.6c

† Values not followed by the same letter differ significantly ($P<0.01$).

infections. Total numbers in the mixed species infections were about the same at the 10th and 35th day after inoculation.

The percentage of mature females declined with increasing inoculum levels in all species treatments (Table 4). At the low levels of mixed species infections, there were approximately 50% more *M. javanica* than *M. hapla* (Table 5). With increasing inoculum levels, the disparity between the numbers of mature females of each species increased. There were three to four times as many mature females of *M. javanica* as *M. hapla* at the highest infection level.

INFLUENCE OF INOCULUM LEVEL ON GALLING CHARACTERISTICS: Very few lateral roots were associated with galls in the single species infections of *M. javanica* (Fig. 6). The number of lateral roots induced by *M. hapla* increased with an increase of infecting nematodes. All females of *M. hapla* were found at the base of lateral roots.

A comparison of the infection sites revealed differences in the location of galls between the infections of either species. The great majority of *M. hapla* from tests of 500 or more larvae were in terminal galls whereas the majority of *M. javanica* were in non-terminal galls (Table 6). In the higher infections of the mixed species treatments, an intermediate condition prevailed with the nematodes equally distributed between terminal and non-terminal galls.

The mean number of nematodes in a terminal or non-terminal gall increased with increasing infection levels of *M. javanica*. In the case of *M. hapla*, the mean number infecting a non-terminal gall remained constant at all infection levels. There was no significant increase in the mean number infecting a terminal gall in infections resulting from inoculum containing > 500 larvae.

DISCUSSION

The species ratio among the immature females, males, and larval stages in the mixed species infections in Inoculum Test-2 could not be determined. However, if the mature female ratios are applied to the total nematodes recovered from the higher inoculum infections, the estimated numbers of *M. hapla* (*i.e.* means of 136, 109, and 121 from mixed species inoculum levels of 500, 750, and 1,000 larvae, respectively) are equivalent to the mean number recovered from the 125-larva infection

TABLE 6. Mean number of larvae in terminal and non-terminal galls in single and mixed species infections of *Meloidogyne hapla* and *M. javanica* at various inoculum levels. Each figure is the mean of five replicates.

Species	Inoculum (no. larvae)	Non-Terminal Galls		Terminal Galls	
		Total Nematodes	Mean per gall	Total Nematodes	Mean per gall
M. hapla	125	65a†	1.3a†	55a†	3.5a†
	250	121ab	1.6a	118a	3.5a
	500	122ab	1.9a	248b	4.8b
	750	142b	2.0a	348c	4.6b
	1000	163b	1.9a	455d	5.4b
M. javanica	125	100a	1.6a	14a	1.7a
	250	137a	1.7a	39a	3.0ab
	500	230bc	2.2ab	85b	3.8ab
	750	294cd	2.5b	123b	4.2b
	1000	325d	3.6c	176c	7.3c
Mixed	125	96a	1.4a	17a	3.1a
	250	115a	1.6ab	61a	3.6ab
	500	181b	2.0bc	187bc	5.0ab
	750	219b	2.3c	207c	5.3ab
	1000	273c	2.3c	285d	5.6b

† Values in each column not followed by the same letter differ significantly ($P<0.01$).

of *M. hapla* (*i.e.* 120). Lateral roots are initiated by second-stage larvae of *M. hapla*. In the mixed species infections, there were no significant differences in lateral root production in infections from inoculum levels of 250 or more larvae. These levels of mixed species infection produced lateral roots equivalent in number to the production from 125 larvae of *M. hapla*.

The ratio of mature females of *M. hapla* and *M. javanica* in the mixed species infections confirms the dominance of the latter species as found in the experiment involving tomato root-halves. It has been suggested that the prevalence of either *M. hapla* or *M. javanica* would depend on the prevailing soil temperature (2). The mixed species experiments in this study were conducted at temperatures equally suitable for the invasion and development of both species. Hence factors other than temperature must have been responsible for the predominance of *M. javanica*.

The percentage of *M. javanica* penetrating the roots was not reduced by increased inoculum levels. Since the higher inoculum levels in Inoculum Test-2 gave sufficient levels of infection to produce root loss, destruction of roots probably occurred before a maximum level of penetration was reached. It follows that invasion of tomato roots by *M. javanica* is less density-dependent than invasion by *M. hapla* which exhibited a significant reduction in percentage penetration from the higher inoculum levels in both experiments.

Terminal galls likely result from a cessation of the mitotic activity of the root meristem (3). Since these galls were more prevalent in infections by *M. hapla* than in infections by *M. javanica*, the former species apparently is more capable of suppressing mitotic activity.

M. hapla is a species which apparently can suppress tomato root elongation during invasion and promote lateral root initiation after settling at the feeding site. *M. javanica* suppresses root tip elongation to a lesser degree and is relatively incapable of promoting lateral roots. There is evidence to suggest that these differences are associated with changes in the hormonal balance within the host.

Street *et al.* (6) found both indoleacetic acid (IAA) and indoleacetonitrile (IAN) capable of inhibiting tomato root growth but that IAN enhances the frequency of lateral roots, whereas IAA either does not affect or decreases lateral root development. Yu and Viglierchio (8) determined the presence of IAA and IAN and indoleacetic acid ethylester (IAE) in the egg masses, larvae, and galls of *M. hapla* infecting tomato. With *M. javanica*, IAE was not detected but IAA and IAN occurred in the relative amounts of 10:1, respectively. With *M. hapla* this ratio was reversed and greater, *i.e.* 1:100. The different complement of plant growth regulators associated with *M. hapla* may also be responsible for the marked suppression of root tip meristems by this species.

Bird (1) reported that gall tissue is as attractive as root tips to *M. javanica* and becomes more attractive with an increase in larval density. In this study, increasing inoculum produced significant increases in the mean number of *M. javanica* in both terminal and non-terminal galls. However, the mean number of *M. hapla* in non-terminal galls remained constant at all inoculum levels, and the mean number in terminal galls did not significantly increase beyond those in plants inoculated with 500 larvae. It is possible that the larvae of *M. hapla*, requiring an active meristem as an invasion site, are not attracted to, or incapable of penetrating galled tissue. This limitation of invasion sites and the subsequent suppression of root tip meristems limits the number of *M. hapla* per gall and would explain both the small gall size associated with this species and the reduction in the percentage penetration by this species with increasing inoculum.

In a coincident infestation by *M. hapla* and *M. javanica* at medial soil temperatures, the host response favors a more rapid invasion by *M. javanica* and this species would be relatively more capable of invading the roots with increasing nematode density levels.

There was no evidence in this study to suggest that the predominance of *M. javanica* was due to any greater competitive ability of this species once penetration had taken place.

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