

## PATHOGENICITY OF *MELOIDOGYNE HAPLA* ON ONION

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### ABSTRACT

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*Meloidogyne hapla* and *M. incognita* can both cause severe damage in onion production. Thus the objective of this study was to examine the relationship between different initial densities of *M. hapla* and the subsequent yield loss of onion. 'Tioga' onion seedlings were inoculated with *M. hapla* at 0, 3,000, 6,000, 9,000, or 12,000 J2/1500 cm<sup>3</sup> pot in the greenhouse and 0, 40,000, 80,000, 160,000 *M. hapla* or 80,000 *M. incognita* J2/20,000 cm<sup>3</sup> plot in the field microplot experiment. Data was collected on fresh and dry weights of the shoot, root and whole plant, bulb diameter and weight, and nematode populations in the roots and soil were collected at harvest. Onion weight was reduced with the increasing inoculum levels of *M. hapla*, and significant reduction began at 3,000 *M. hapla* J2/pot or 40,000 *M. hapla* J2/microplot. Root dry weight reduction ranged from 30.2% to 48.2%, with a total plant dry weight reduction of 40.6% to 59.6% in greenhouse tests. In field microplots, the maximum reduction in bulb fresh weight was 41.3% at 160,000 *M. hapla* J2/microplot and significantly more damage was caused by *M. hapla* than *M. incognita* at the same rate of 80,000 J2/microplot. Linear regressions indicated that for an increase of 40,000 in the initial inoculum density of *M. hapla* J2, there was a reduction of 0.4 cm in bulb diameter and 12 gram in bulb weight. The damage caused by *M. incognita* at 80,000 J2/microplot was not different from that caused by *M. hapla* at 40,000 J2/plot.

*Key words:* *Allium cepa*, *Meloidogyne hapla*, *Meloidogyne incognita*, onion, pathogenicity.

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### RESUMEN

Pang, W., S. L. Hafez, and P. Sundararaj. 2009. Patogenicidad de *Meloidogyne hapla* en cebolla. *Nematropica* 39:225-233.

Tanto *Meloidogyne hapla* como *M. incognita* pueden causar daño severo a la producción de cebolla. El objetivo de este estudio fue examinar la relación entre diferentes densidades iniciales de *M. hapla* y el daño causado en la producción de cebolla. Se inocularon plántulas de cebolla 'Tioga' con *M. hapla* a razón de 0, 3,000, 6,000, 9,000, ó 12,000 J2/maceta de 1500 cm<sup>3</sup> en el invernadero y a razón de 0, 40,000, 80,000, 160,000 *M. hapla* ó 80,000 *M. incognita* J2/microparcela de 20,000 cm<sup>3</sup> en experimentos de campo. Se midió el peso fresco y seco de la parte aérea, las raíces, y la planta completa, el diámetro y peso de los bulbos, y la población de nematodos en las raíces y el suelo al momento de la cosecha. El peso de las cebollas se redujo con el incremento de los niveles de inóculo de *M. hapla*, y la reducción significativa se inició con 3,000 *M. hapla* J2/maceta ó 40,000 *M. hapla* J2/microparcela. La reducción del peso seco de raíces fluctuó entre 30.2% y 48.2%, con una reducción del peso seco de la planta completa de 40.6% a 59.6% en los ensayos de invernadero. En las microparcels en el campo, la máxima reducción del peso fresco de los bulbos fue del 41.3% con 160,000 *M. hapla* J2/microparcela. Se observó significativamente mayor daño con *M. hapla* que con *M. incognita* al mismo nivel de inóculo de 80,000 J2/microparcela. Las regresiones lineales indicaron que para un incremento de 40,000 en la densidad de inóculo inicial de *M. hapla* J2, se observa una reducción de 0.4 cm en el diámetro del bulbo y 12 g en el peso del bulbo. El daño causado por *M. incognita* a razón de 80,000 J2/microparcela no fue diferente al daño causado por *M. hapla* con 40,000 J2/microparcela.

*Palabras clave:* *Allium cepa*, cebolla, *Meloidogyne hapla*, *Meloidogyne incognita*, patogenicidad.

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## INTRODUCTION

Onion (*Allium cepa*) is a common vegetable that is cultivated in more than 175 countries of the world. The U.S.A. is a leader in onion production and produces over 7% of the world onion crop (National Onion Association, 2007). Idaho and Oregon are the primary onion producing states in the U.S.A., collectively producing 35% of the bulb onion crops for the nation (Martin *et al.*, 2004). However, root-knot nematode (*Meloidogyne* spp.), one of the most common nematodes in Idaho fields, can severely reduce plant vigor and onion yield. It was reported that several species of *Meloidogyne* damage onions (Johnson and Roberts, 1996; Gergon *et al.*, 2002) and the threshold of *M. hapla* on onion was 2 J<sub>2</sub>/cm<sup>3</sup> soil (Barker and Olthof, 1976; Potter and Olthof, 1993; Merrifield, 1999). Marketable yields of onion were reduced by 31%, 72%, and 64%, respectively at 2, 6, and 18 *M. hapla* J<sub>2</sub>/cm<sup>3</sup> soil (Olthof and Potter, 1972a; Merrifield, 1999). Reduction in the bulb yield up to 70% was observed in heavily infested commercial fields in New York (Widmer *et al.*, 1999). *Meloidogyne incognita* population densities greater than 1 egg and juvenile/cm<sup>3</sup> soil can cause significant yield loss of onion in sandy loam soil (Corgan *et al.*, 1985; Babu and Sivagami, 1989).

There is no clear agreement on the damage threshold of *M. hapla* or *M. incognita* on onion production in Idaho. It is also unknown which *Meloidogyne* species is more damaging to onion. Little information is available on the relationship between population densities of *M. hapla* and onion growth and yield. Thus, the objectives of this study were to quantify the relationship between population densities of *M. hapla* and the yield loss of onion, and to compare the damage potential of *M. hapla* and *M. incognita* on onion.

## MATERIALS AND METHODS

### *Inoculum preparation*

*Meloidogyne hapla* and *M. incognita* were maintained on roots of tomato 'Payette' grown in pots in the greenhouse. The nematode cultures were extracted by uprooting the plants and rising the soil off the roots with tap water. Roots were then dipped in 0.5% NaOCl for 1 minute, cut into 1-cm-long pieces, and placed in Baermann funnels under a mist for 2 weeks. Second stage juveniles were collected every other day during this 2-week period. The nematode suspensions were concentrated using a 25- $\mu$ m pore sieve. The nematodes were maintained at 4°C for 24 hours with aeration until used.

### *Greenhouse experiments*

This experiment was conducted from June to September 2006 and repeated in 2007 to test the pathogenicity of *M. hapla* on onion. The experiment was arranged in a randomized complete block design with five replications. 'Tioga' onion seeds were sowed 1.5-cm deep in plastic pots filled with 1500 cm<sup>3</sup> of steam-sterilized soil mix (1 sand:1 soil, 1.7% organic matter, 39.0 mg/kg total nitrogen, 45 mg/kg phosphorus, 4066 mg/kg calcium, and pH 7.9). After germination, seedlings were thinned to one per pot. When the onion seedlings were four-week-old, soil in each treatment was infested with *M. hapla*. Before inoculation, suspensions of *M. hapla* were removed from the refrigerator, concentrated to 1500 J<sub>2</sub>/ml, and aerated at room temperature for 3 hours. Four 1.5-cm deep holes were made 1 cm from the base of the seedlings, and 0, 3,000, 6,000, 9,000, or 12,000 J<sub>2</sub> were divided into the holes in each 1500 cm<sup>3</sup> pot. Holes were covered with a layer of soil, and moistened by a mist of water. During the experiment, temperature ranged

from 25°C to 34°C under natural daylight. Onions were watered daily and fertilized weekly with 1 g of 20-20-20 N-P-K in 50 ml water per pot. Six weeks after planting, plants were sprayed weekly with the insecticide mixture of imidacloprid and cyfluthrin at 197 ml/ha, and with carbaryl at 2.3 L/ha.

Onions were harvested 15 wk after planting. Plants were separated from the soil by hand and kept moist by rolling them in a wet paper towel. Plant height was measured from the base of the stem to the tip of the longest leaf and the whole plant fresh weight, root fresh weight, and top fresh weight were recorded. After drying the plant shoots at 60°C for 96 hr, the dry shoot weight was recorded. Roots were washed and chopped into 1-cm long pieces and placed in the mist chamber for 10 days to extract *M. hapla*. Roots were then dried at 60°C for 96 hours and weighed. Soil from each pot was mixed by hand and nematodes extracted from a 500-cm<sup>3</sup> sample using decanting and sieving followed by centrifugal sugar flotation. Nematode counts from soil, roots, final population (Pf, population from both the roots and soil), nematode population per gram of dry root, and the reproductive factor (Rf = Pf/Pi, Pi was the initial nematode population) were recorded.

This experiment was repeated in 2007 from March to June. In addition to N-P-K fertilization, sulfur was applied at 0.4 ml in 50 ml of water to each pot once every 2 weeks. Data was collected as in 2006.

#### *Field microplot experiments*

Damage of *M. hapla* and *M. incognita* to onion was determined in field microplots between May to October 2006 and again from March to August 2007. Buckets with the volume of 20,000 cm<sup>3</sup> (30-cm diameter × 28-cm deep) were filled with natural field

slit loam soil (1.7% organic matter, 39.0 mg/kg total nitrogen, 45 mg/kg phosphorus, 4,066 mg/kg calcium, and pH 7.9) and fumigated with metam sodium at 468 L/ha. Two weeks later, buckets were set 25 cm below the soil surface spaced 1.2 m apart. Ten onion seeds were planted 1.5-cm deep in each microplot on 20 March 2007, and after germination seedlings were thinned to three per plot. Microplots were set in a randomized complete block design with five replications and five nematode population levels of 0, 40,000, 80,000, 160,000 *M. hapla* or 80,000 *M. incognita* J2/20,000 cm<sup>3</sup> microplot. A drip irrigation system was installed to irrigate the microplots and soil was watered to field capacity twice a week. Onions were fertilized weekly with 3 g 20-20-20 N-P-K in 150 ml water per plot and sprayed weekly with the insecticide mixture of imidacloprid and cyfluthrin at 197 ml/ha and carbaryl at 2.3 L/ha. Onions were harvested 180 days after planting and microplots were removed from the ground and shaken to separate the soil from the plants. Plants were separated from the soil and kept moist by rolling them in a wet paper towel. Soil in each microplot was mixed and a 500-cm<sup>3</sup> subsample was collected. Data parameters collected from this experiment were identical to those for the greenhouse experiment except that plant height was not recorded. In addition, onion bulb diameters were measured by a pair of vernier calipers and bulb fresh weight was recorded.

#### *Statistical Analysis*

All data were subjected to analysis of variance (ANOVA), and the differences among means were compared by the Fisher's protected least significant difference test at ( $P \leq 0.05$ ). No significant interaction was observed between the data of 2006 and 2007, so the two sets of data were combined

for analysis. Data were also subjected to regression analysis. Plant growth and the final nematode population were regressed as the dependent variable with the initial inoculum density as the independent variable. Statistical analysis was conducted by using SAS program (SAS Institute Inc., 2004).

## RESULTS AND DISCUSSION

### Greenhouse studies

*Meloidogyne hapla* and *M. incognita* had an overall significant effect on the plant growth parameters and nematode population at harvest ( $P \leq 0.05$ ). All plant growth parameters of inoculated onions were less than those of the noninoculated control (Table 1). Maximum reduction in root dry weight was 48.2% at 12,000 *M. hapla* J2/1500 cm<sup>3</sup> pot. Regression showed that there was a reduction of 2.1 g in root fresh weight with every increase in the inoculum level of 3000 *M. hapla*/1500 cm<sup>3</sup> pot (Fig. 1A). MacGuidwin *et al.* (1987) and Gergon *et al.* (2002) also found that onion root weight was negatively correlated with the initial population densities of *M. hapla* and *M. graminicola*, respectively. Galls caused by *M. hapla* were observed on onion roots. Reduction in plant total dry weight ranged from 40.6% to 59.6% with the increasing inoculum levels. MacGuidwin (1987) and Babu and Sivagami (1989) also found that total plant weight was reduced up to 60% with 40,000 eggs/plant under greenhouse conditions. It was found that with the increase of 3000 *M. hapla* in each pot, onion total fresh weight was reduced by 18 grams (Fig. 1B). However, we found no difference between 3,000 and 6,000 or 9,000 and 12,000 *M. hapla*/1500 cm<sup>3</sup> pot for root dry weight, and shoot and total fresh and dry weights. Onion plant height was progressively reduced as the inoculum levels

Table 1. Effects of *Meloidogyne hapla* inoculum levels on onion top height, fresh and dry weights for root, shoot, and whole plant under greenhouse conditions.<sup>a</sup>

Inoculum level ( <i>M. hapla</i> per 1500 cm <sup>3</sup> pot)	Root fresh weight <sup>b</sup> (g)	Root dry weight (g)	Shoot fresh weight (g)	Shoot dry weight (g)	Top height (cm)	Total fresh weight (g)	Total dry weight (g)
0	15.44 a	2.72 a	114.68 a	16.62 a	64.08 a	130.12 a	19.34 a
3,000	10.46 b	1.90 b	71.37 b	9.59 b	54.94 b	81.83 b	11.49 b
6,000	9.87 b	1.76 b	67.68 b	9.06 b	52.97 c	77.54 b	10.82 b
9,000	7.68 c	1.55 c	49.67 c	6.56 c	47.58 d	57.34 c	8.10 c
12,000	6.52 d	1.41 c	45.99 c	6.40 c	46.10 d	52.51 c	7.81 c
LSD ( $P \leq 0.05$ )	0.99	0.16	4.76	0.82	1.95	5.02	0.94

<sup>a</sup>Each value represents five replications from two tests.

<sup>b</sup>The means within each column followed by the same letters were not significantly different when means were compared using Fishers' LSD ( $P \leq 0.05$ ).

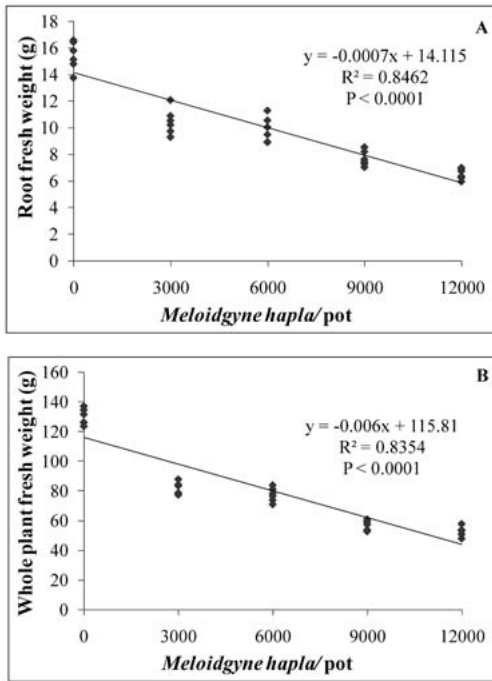


Fig. 1. Effect of initial inoculums density of *Meloidogyne hapla* on A, onion root fresh weight, and B, onion total fresh weight under greenhouse conditions.

increased. Reductions in height have been reported by Corgan *et al.* (1985) with *M. incognita* in onion. In another study, Khan (2003) found that the height of onion was reduced by *M. incognita* at less than 1 J2/cm<sup>3</sup> soil.

Final nematode population at harvest decreased with the increasing inoculum levels (Table 2). The Rf was greater than 1 in all treatments, with the maximum up to 10.05, which indicated that ‘Tioga’ onion supported the multiplication of *M. hapla*. That fact that onion was a good host in this study confirmed the earlier study that onion supported a high degree of *M. hapla* reproduction (Olthof and Potter, 1972a, 1972b). The negative relationship between the initial inoculum level of *M. hapla* and Rf was also reported by Khan and Yetunde (1993), which was

Table 2. Effects of *Meloidogyne hapla* inoculum levels on nematode population at harvest under greenhouse conditions.\*

Inoculum level ( <i>M. hapla</i> per 1500 cm <sup>3</sup> pot)	Nematodes in 1500 cm <sup>3</sup> soil <sup>b</sup>	Nematodes per gram dry root	Total population <sup>c</sup> per pot	Reproductive factor (Rf) <sup>d</sup>
3,000	8250 a	11524 a	30148 a	10.05 a
6,000	6600 ab	9671 b	23698 b	3.95 b
9,000	6300 ab	8163 c	18935 c	2.10 c
12,000	6150 b	7309 d	16440 d	1.37 d
LSD ( <i>P</i> ≤ 0.05)	2070	804	1265	0.27

\*Each value represents five replications from two tests.

<sup>b</sup>The means within each column followed by the same letters were not significantly different when means were compared using Fishers’ LSD (*P* ≤ 0.05).

<sup>c</sup>Total population = Nematode population from both the roots and soil in each pot.

<sup>d</sup>Rf = Final total population/ initial population.

due to the competition for invasion and feeding sites at high inoculum densities.

#### Field microplot studies

Inoculation of *M. hapla* and *M. incognita* negatively affected plant growth in the microplots. *M. hapla* and *M. incognita* also caused a reduction on onion plant growth parameters as observed under greenhouse conditions (Table 3). Root fresh and dry weight decreased compared to the noninoculated control ( $P \leq 0.05$ ), and the differences in root weight among vinculum levels were also significant (Table 3). Leaf fresh weight at 40,000 *M. hapla*/plot was not different from the noninoculated control. The difference between 40,000 and 80,000 *M. hapla*/plot was not significant (Table 3), which agrees with the results of the greenhouse study (Table 1). However, Babu and Sivagami (1989) showed that at 10 J2/pot, *M. incognita* reduced onion shoot weight under greenhouse conditions. They also found that the reductions in shoot weight among 100, 1000, 10000 J2/pot were not different from each other. Nematodes at either 40,000 *M. hapla* or 80,000 *M. incognita*/plot did not reduce the leaf dry weight compared to the noninoculated control, but did reduce leaf dry weight at 80,000 or 160,000 *M. hapla*/plot, and the reduction was different between the latter two levels ( $P \leq 0.05$ ). The range of reduction in leaf dry weight was from 0.3% to 18.9%. Total plant fresh weight was reduced by 14.0% at 40,000 *M. hapla*/plot and up to 35.9% at 120,000 *M. hapla*/plot (Table 3).

Onion bulbs were formed in all treatments of this study. However, MacGuidwin *et al.* (1987) found that there was no bulb formation when onions were inoculated with 15,000 to 40,000 *M. hapla* eggs/plant. The reduction in bulb diameter ranged from 12.5% to 30.7%, with the bulb weight

Table 3. Effects of *Meloidogyne hapla* and *M. incognita* inoculum levels on onion root, leaf, bulb, and total weights and bulb diameter in field microplots.<sup>a</sup>

Inoculum level (nematodes per 20,000 cm <sup>2</sup> plot)	Root fresh weight <sup>b</sup> (g)	Root dry weight (g)	Leaf fresh weight (g)	Leaf dry weight (g)	Bulb diameter (cm)	Bulb fresh weight (g)	Total fresh weight (g)
0	11.02 a	1.77 a	40.59 a	3.69 a	5.79 a	94.35 a	145.96 a
40,000 <i>M. hapla</i>	8.65 b	1.40 b	30.95 bc	3.00 b	4.95 c	65.42 c	105.01 c
80,000 <i>M. hapla</i>	7.47 c	1.03 c	28.49 c	2.77 b	4.31 d	54.41 d	90.37 d
160,000 <i>M. hapla</i>	6.73 c	0.72 d	25.30 d	2.35 c	3.96 e	47.18 e	79.21 e
80,000 <i>M. incognita</i>	8.85 b	1.40 b	32.30 b	2.95 b	5.17 b	72.98 b	114.13 b
LSD ( $P \leq 0.05$ )	0.738	0.218	3.121	0.362	0.131	3.261	5.685

<sup>a</sup>Each value represents five replications from two tests.

<sup>b</sup>The means within each column followed by the same letters were not significantly different when means were compared using Fishers' LSD ( $P \leq 0.05$ ).

reduced from 17.5% to 41.3% (Table 3). The maximum reduction occurred at 160,000 *M. hapla*/plot. Higher order regressions were tested but we found that the linear regression was the best fit. Linear regressions indicated that for an increase of 40,000 in the initial inoculum density, there was a reduction of 0.4 cm in bulb diameter and 12 gram in bulb weight (Figs. 2A, B). Widmer *et al.* (1999) reported that bulb weights of onion 'Norstar' and 'Paragon' were reduced by 50% at 20 eggs of *M. hapla*/cm<sup>3</sup> soil in a microplot experiment, whereas the reduction reached up to 70% in nematode infested commercial fields. As shown in all plant parameters, the reduction in onion growth caused by *M. incognita* was significantly less than that caused by *M. hapla* at the same inoculum level of 80,000 J2/cm<sup>3</sup> soil, but was not different from the damage at 40,000 *M. hapla*/cm<sup>3</sup> soil, which indicated that *M. hapla* was more damaging than *M. incognita* on 'Tioga' onion. Furthermore, both greenhouse and microplot experiments demonstrated that *M. hapla* caused significant reductions in root and bulb growth at the lowest inoculum level except for the leaf fresh and dry weights.

Nematode population densities at harvest increased significantly as the inoculum levels increased in the microplot experiments (Table 4). Soil nematode populations in all treatments were higher than the initial populations. However, for the greenhouse study, final nematode population densities in soil at 9,000 and 12,000 *M. hapla*/pot were lower than the initial population, which could be due to the greater competition for limited volume of soil and pot size as the nematode population increased. There was a significant increase in *M. hapla* population/g dry root at each increasing inoculum level (Table 4). The maximum population was 41934g dry root at 160,000 *M. hapla*/20,000 cm<sup>3</sup> plot. The population of *M. incognita* in roots was not

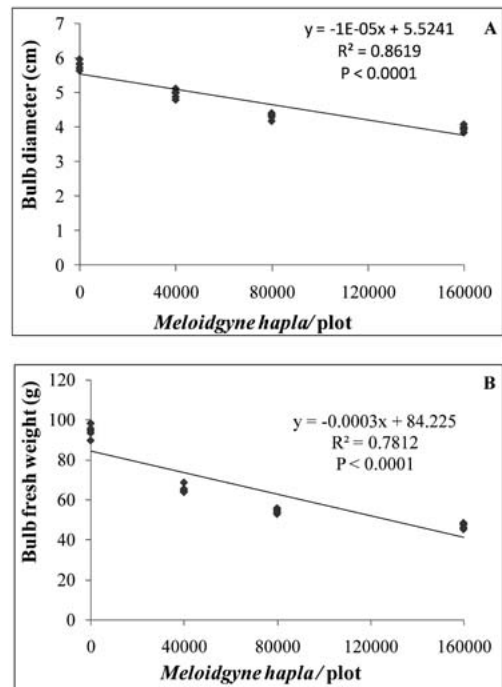


Fig. 2. Effect of initial inoculum density of *Meloidogyne hapla* on A, onion bulb diameter, and B, onion bulb fresh weight in field microplot.

different from that of *M. hapla* at 40,000 J2/plot. However, *M. hapla* population/g dry root was much lower under greenhouse conditions compared to the microplot experiment, ranging from 7309 to 11524 nematodes/g dry root (Table 2). *Meloidogyne hapla* population in roots was increased as the inoculum levels increased, which conflicted with the results of greenhouse study. The reason could be that *M. hapla* multiplied on onion ( $R_f > 1$  in all treatments), but the roots available for the nematode feeding were limited under greenhouse conditions, and more root weight was reduced with the increasing inoculum levels, which constrained the feeding of *M. hapla*. Furthermore, the symptoms of stunting and low vigor were observed in plants at high inoculum levels

Table 4. Effects of *Meloidogyne hapla* and *M. incognita* inoculum levels on nematode population at harvest in field microplots.<sup>z</sup>

Inoculum level (nematodes per 20,000 cm <sup>3</sup> plot)	Nematodes in 20,000 cm <sup>3</sup> soil <sup>y</sup>	Nematodes per gram dry root	Reproductive factor (Rf) <sup>x</sup>
40,000 <i>M. hapla</i>	128000 c	18842 c	4.28 a
80,000 <i>M. hapla</i>	228000 b	30311 b	3.52 b
160,000 <i>M. hapla</i>	354000 a	41934 a	2.55 c
80,000 <i>M. incognita</i>	268000 b	17036 c	3.90 ab
LSD ( $P \leq 0.05$ )	41800	4779	0.7568

<sup>z</sup>Each value represents five replications from two tests.

<sup>y</sup>The means within each column followed by the same letters were not significantly different when means were compared using Fishers' LSD ( $P \leq 0.05$ ).

<sup>x</sup>Rf = Final total population/ initial population.

of 9,000 and 12,000 J<sub>2</sub>/cm<sup>3</sup> soil, which could reduce the availability of healthy roots and further restrain feeding of the nematodes. In contrast, the bigger and more vigorous plants in microplot provided more feeding sites, so more nematodes were able to penetrate and reproduce. The Rfs were greater than 1 in both greenhouse and microplot experiments (Tables 2 and 4), which indicated that 'Tioga' supported the multiplication of *M. hapla*. The Rfs decreased as the nematode inoculum levels increased (Table 4) as the greenhouse study (Table 2). The same decreasing trend in reproductive rate was also found in *M. incognita* on onion by Babu and Sivagami (1989), which could be due to a competition for the feeding sites in roots at higher inoculum levels. The Rf value of *M. incognita* was not significantly different from those of *M. hapla* at 40,000 or 80,000 J<sub>2</sub>/plot.

In summary, *M. hapla* reduced onion yield at the lowest initial population densities of 3,000 J<sub>2</sub>/pot or 4,000 J<sub>2</sub>/plot, respectively for the greenhouse and microplot experiments. *Meloidogyne incognita* caused yield reduction in this study; however, Corgan *et al.* (1985) and Babu and Sivagami (1989) reported that *M.*

*incognita* density less than 1 egg and J<sub>2</sub>/cm<sup>3</sup> soil caused significant yield loss of onion in sandy loam soil. Khan (2003) reported that *M. incognita* was damaging to onion at population densities below 1 J<sub>2</sub>/cm<sup>3</sup> soil.

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