CONCOMITANT INTERACTION OF *PRATYLENCHUS PENETRANS* AND *MELOIDOGYNE HAPLA* ON ONION

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ABSTRACT

Pang, W., S. L. Hafez, and P. Sundararaj. 2009. Concomitant Interaction of *Pratylenchus penetrans* and *Meloidogyne hapla* on Onion. Nematropica 39:297-303.

Pratylenchus penetrans and *Meloidogyne hapla* often exist together in Idaho onion fields; however, there is no information available about the yield loss of onion when they exist simultaneously in soil. The objectives of this research were to determine onion yield loss when the two species were inoculated simultaneously; and to determine the effects of each nematode on the multiplication of the other. Seedlings of 'Tioga' onion were inoculated with 6000 *P. penetrans*, 6000 *M. hapla*, or 6000 *P. penetrans* + 6000 *M. hapla*/ pot in a greenhouse and compared to a non-inoculated control. Onion bulb diameter, shoot height, whole plant fresh and dry weights, and nematode population in the root and soil were recorded at harvest. *Meloidogyne hapla* and *P. penetrans* alone reduced onion growth compared with the non-inoculated control ($P \leq 0.05$). More damage was induced by *P. penetrans* compared to *M. hapla*. However, maximum reduction in plant growth occurred when *P. penetrans* and *M. hapla* were simultaneously inoculated. The two nematode species suppressed the multiplication of each other on onion and the suppressive effect of *P. penetrans* on *M. hapla* was more severe than that of *M. hapla* on *P. penetrans*.

Key words: Allium cepa, interaction, *Meloidogyne hapla*, onion, *Pratylenchus penetrans*.

RESUMEN

Pang, W., S. L. Hafez, and P. Sundararaj. 2009. Interacción concomitante de *Pratylenchus penetrans* y *Meloidogyne hapla* en cebolla. Nematropica 39:297-303.

En los campos de cebolla de Idaho es común encontrar a *Pratylenchus penetrans* y *Meloidogyne hapla* coexistiendo. Sin embargo, no hay información acerca de las pérdidas que ocurren en la producción de cebolla cuando estos dos organismos están presentes simultáneamente en el suelo. Los objetivos de esta investigación fueron determinar las pérdidas causadas por la infección concomitante de estas dos especies y determinar los efectos de cada especie sobre la reproducción de la otra especie. Para esto, se inocularon plántulas de cebolla 'Tioga' con 6000 *P. penetrans*, 6000 *M. hapla*, ó 6000 *P. penetrans* + 6000 *M. hapla*/ maceta en el invernadero y se compararon con controles sin inocular. Se midió el diámetro de los bulbos, la altura de los tallos, el peso fresco y peso seco de las plantas y la población de los nematodos en las raíces y en el suelo en el momento de la cosecha. Se observó reducción en el crecimiento de la cebolla con *Meloidogyne hapla* solo y con *P. penetrans* solo, comparados con los controles sin inocular ($P \leq 0.05$). *Pratylenchus penetrans* causó mayor daño que *M. hapla*, pero el mayor nivel de daño se observó cuando se inocularon ambas especies de manera simultánea. Ambas especies redujeron la reproducción de la otra especie, y el efecto supresivo de *P. penetrans* sobre *M. hapla* fue más severo que el de *M. hapla* sobre *P. penetrans*.

Palabras clave: Allium cepa, cebolla, interacción, *Meloidogyne hapla*, *Pratylenchus penetrans*.

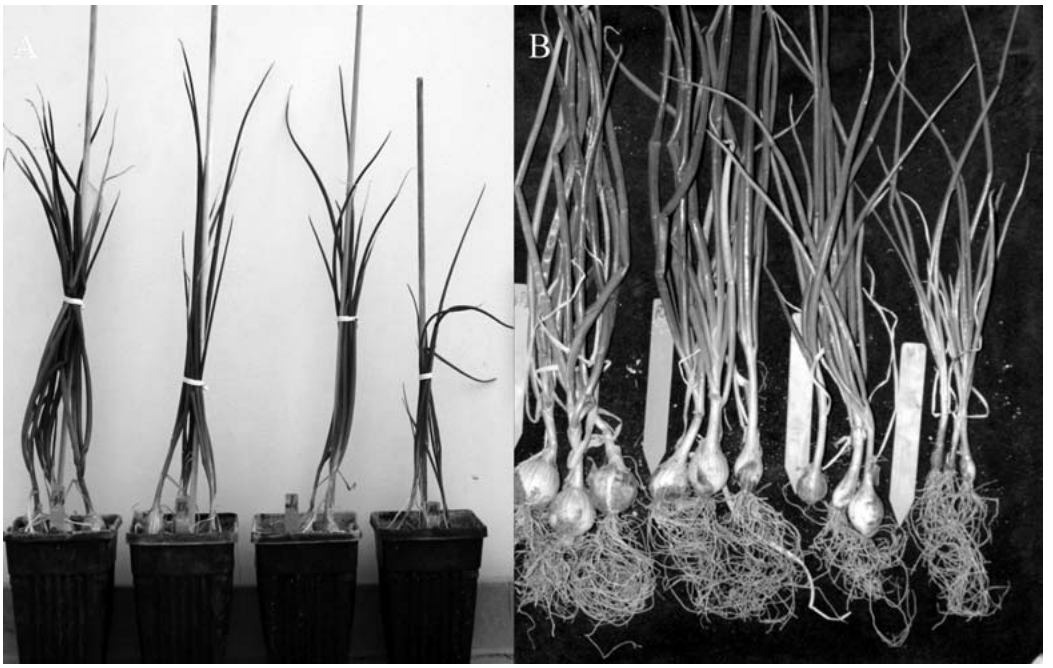

Fig. 1. Onion growth responses to *Pratylenchus penetrans* and *Meloidogyne hapla* alone or in combination under greenhouse conditions. A) Onion growth before harvest. B) Onion after harvest. The treatments were: No nematode, 6000 *M. hapla* J2, 6000 adults and J of *P. penetrans*, and 6000 *M. hapla* + 6000 *P. penetrans*/ pot (left to right, respectively).

In the U.S., onions are the third most frequently consumed fresh vegetable (National Onion Association, 2007). Idaho and Oregon collectively produce 35% of the bulb onion crop for the U.S. (Martin *et al.*, 2004). *Pratylenchus penetrans* or *Meloidogyne hapla* can reduce onion yield when each occurs singularly in the field (Olthof and Potter, 1973; Viaene and Abawi, 1998), but according to the diagnosis of the nematode assay lab at University of Idaho, the two nematode species often exist together in Idaho onion fields.

Combinations of *Pratylenchus spp.* and *Meloidogyne spp.* have been studied on numerous crops. Haseeb and Shukla (2000) found that in concomitant and sequential inoculations of *P. thornei* and *M. incognita* on *Mentha arvensis*, the reproduction of both nematodes was suppressed compared to when the nematodes were inoculated individually. In contrast, Amosu and Taylor (1975) found that *M. hapla* alone or combined with *P. penetrans* decreased the shoot and root weights of red clover with no difference between the damage caused by *M. hapla* alone or combined with *P. penetrans*. However, Sujatha and Mehta (1997) reported a negative interaction between *P. zeae* and *M. javanica* on sugarcane. Both qualitative and quantitative characters of sugarcane were reduced when the two nematodes coexisted, and the nematode populations in soil and roots were higher when the nematodes occurred singly compared to concomitant infections. Al-Rehiayani *et al.* (1999) showed that potato tuber yield was lower compared to a non-inoculated control when *M. chitwoodi* was alone or in combina-

tion with *P. neglectus*. The presence of *P. neglectus* reduced the population of *M. chitwoodi* and tuber infection by *M. chitwoodi*. The population of both nematodes was suppressed when they were inoculated simultaneously compared to individually.

Effects of *M. hapla* and *P. penetrans* on onion are unknown. This information is essential to give onion growers guidance for those situations when both nematodes exist concurrently in soil. The objectives of this project were to study the yield loss of onion when *P. penetrans* and *M. hapla* were inoculated simultaneously, and to determine the effects of each nematode on the multiplication of the other.

Pratylenchus penetrans was maintained on excised corn roots growing on Gamborg's B5 medium (Gamborg *et al.*, 1976) in tissue culture (Huettel, 1990). The 'AP162' corn seeds were incubated on 1% water agar filled petri dishes at 24°C for 7 days. After germination, when the root tips were 3 cm long, roots were cut from the shoot and transferred to Gamborg's B5 medium and incubated at 24°C without light for 7 days before inoculation with nematodes. Nematodes previously established on excised corn roots were transferred to jars with newly excised roots. Six months later, corn roots were chopped into 1-cm-long pieces and placed in Baermann funnels in a mist chamber to extract *P. penetrans*. All vermiform stages were collected every other day for 10 days. The nematodes were concentrated using a 25-µm pore sieve and stored at 4°C with aeration until used.

Meloidogyne hapla was maintained on tomato 'Payette' grown in pots in a greenhouse. Four-month-old tomato plants were uprooted and soil was rinsed off from roots with tap water. Roots were then dipped in 0.5% NaOCl solution for 1 minute, cut into 1-cm-long pieces and J2s of *M. hapla* were extracted for 2 weeks by using the mist chamber technique.

The interaction of *P. penetrans and M. hapla* was established as a randomized complete block design with five replications and the trial was conducted sequentially in a greenhouse in 2007. Treatments consisted of non-inoculated control, onions inoculated with *P. penetrans* and *M. hapla*, respectively, and onions inoculated with both nematode species. 'Tioga' onion seeds were sown 2-cm deep in plastic pots filled with 1500 cm^3 steam-pasteurized sterilized soil mixture (1 sand: 1 soil, 1.7% OM, 39.0 mg/ kg N, 45 mg/ kg P, 4066 mg/ kg Ca, and pH 7.9). One week after germination, seedlings were thinned to three per pot. Another week later, soil was infested with nematodes at 0, 6000 J2 of *M. hapla*, 6000 vermiform *P. penetrans*, or a combination of 6000 vermiform *P. penetrans* and 6000 J2 of *M. hapla*/1500 cm^3 soil. The inoculum levels were comparable to the nematode population levels in Idaho onion fields. Before inoculation, suspensions containing the nematodes were removed from the refrigerator, concentrated to 1500 nematodes/ ml, and maintained at room temperature with aeration for 3 hours. Four 1.5-cm-deep holes were made 1 cm from the base of the seedlings near the root zone in each pot, and 4 ml of the suspension containing either *P. penetrans* or *M. hapla*, or both species was inoculated into the holes with a syringe in the above treatments except the non-inoculated control. The holes were then covered with a light layer of soil followed with a light water mist. Pots were arranged on a bench in a greenhouse maintained at 25°C to 34°C under natural day light. Onions were watered daily and fertilized weekly with 1g 20-20-20 N-P-K in 50 ml of water per pot. Sulfur was applied at the rate of 0.4 ml in 50 ml of water per pot every 2 weeks. Plants were sprayed weekly with a mixture of imidacloprid and cyfluthrin at the rate of 197 ml/ ha, and 6 weeks after planting

with carbaryl at 2.3 L/ ha to treat onion thrips and other insects.

Onions were harvested three months after inoculation. Plants were separated from the soil by hand and rolled in wet paper towels. Plant height was measured from the base of the stem to the tip of the longest leaf, and the whole plant fresh weight, and bulb diameter were recorded. The plant shoot was dried at 60°C for 96 hours and the dry weight was recorded. A 500 cm^3 soil sample was taken after evenly mixing the soil in each pot from which nematodes were extracted by decanting and sieving followed by centrifugal sugar flotation at 1.2 g. Onion roots from each pot were washed with tap water, chopped into 1-cm long pieces and nematodes extracted by using the mist chamber for 10 days. Roots were then dried at 60°C for 96 hours and root dry weight was recorded. Nematode populations from the roots, soil, final total population (Pf), nematode number/ g dry root, and the reproductive factor (Rf) were recorded.

No significant interaction over time was observed between the two trials ($P \leq 0.05$), so the data were combined for analysis. Data were subjected to analysis of variance (ANOVA), and the differences among means were compared by using Fisher's protected least significant difference test at $P \leq 0.05$. Statistical analysis was conducted by using the SAS program (SAS Institute Inc., 2004).

Nematode species had an impact on onion growth. Bulb diameter, plant shoot height, and the whole plant fresh and dry weights were reduced by *M. hapla* or *P. penetrans*, and significantly more reduction was caused by *P. penetrans* than *M. hapla* (Table 1). However, maximum reductions in plant parameters occurred when the two nematode species were inoculated simultaneously rather than with either species alone. For the bulb diameter, *M. hapla* alone caused 31.5% reduction compared with the non-inoculated control, while the reduction caused by *P. penetrans* was 49.0%. When both nematode species were inoculated, bulb diameter was reduced by 65.8%. *M. hapla* alone reduced the onion total fresh weight by 35.4% compared with the non-inoculated control; a 60.0% reduction was induced by *P. penetrans*. A maximum reduction of 79.7% in total fresh

Table 1. Effects of *Pratylenchus penetrans* and *Meloidogyne hapla* alone or in combination on 'Tioga' onion bulb diameter, top height, and total fresh and dry weights three months after inoculation under greenhouse conditions.[y]

Treatments	Bulb diameter (cm)	Top height (cm)	Total fresh weight (g)	Total dry weight (g)
No nematode	2.92 a	50.37 a	95.04 a	26.16 a
M. hapla 6000/ pot[z]	2.00 b	44.24 b	61.35 b	10.50 b
P. penetrans 6000/ pot[z]	1.49 c	40.23 c	38.03 c	6.81 c
P. penetrans + *M. hapla* (6000 + 6000)/ pot[z]	1.00 d	31.87 d	19.25 d	3.37 d
LSD ($P \leq 0.05$)	0.110	0.870	3.768	1.164

[y]Each value was the means of five replications from two runs of the test. The means within each column followed by the same letters were not significantly different when means were separated by using LSD ($P \leq 0.05$).
[z]Each pot contains 1500 cm^3 soil.

weight occurred when both nematode species were inoculated. The results agreed with the results of Rakesh (2001), who reported that the maximum reduction in the growth and oil yield of menthol mint occurred when *M. incognita* was inoculated in combination with *P. thornei* rather than with each nematode alone. The results also agreed with Sujatha and Mehta (1997) in that both *P. zeae* and *M. javanica* significantly reduced the cane weight of sugarcane at inoculum levels of 100 or 1000/ pot, and more damage was observed when both species were inoculated simultaneously at a 1:1 ratio at the above two densities.

The addition of *M. hapla* to pots did not change *P. penetrans* populations in soil at harvest (Table 2). However, inoculation of *M. hapla* with *P. penetrans* significantly reduced the population of *M. hapla* in soil at harvest. In contrast, Haseeb and Shukla (2000) reported that soil populations of both *M. incognita* and *P. thornei* were reduced when the two species were inoculated together compared with alone.

Inoculation of both nematode species caused a significant reduction in nematode population/ g dry root (Table 2). The population of *P. penetrans* was reduced by 37.4% from 1009 to 722 (vermiform) when *M. hapla* was present, whereas the population of *M. hapla* was reduced by 48.2%, from 5286 to 2739 (J2s) with the inoculation of *P. penetrans*. This reduction of population could be due to the competition between the two species for feeding sites and penetration in roots. It is also possible that each nematode could have altered the root structure or physiology so that the feeding sites for each other were reduced (Kodira and Ferris, 1994). Similar results were observed by Dickson and McSorley (1990) in that populations of *P. brachyurus*

Table 2. Effects of concomitant inoculation of both nematodes on final population of *Pratylenchus penetrans* on onion 'Tioga' three months after inoculation.[w]

Treatments	*P. penetrans* population/ soil pot	*P. penetrans* population/g dry root	Total population per pot[y]	Reproductive factor (Rf)[z]
P. penetrans 6000/pot[x]	1830 a	1009 a	2492 a	0.42 a
P. penetrans + *M. hapla* (6000 + 6000)/pot[x]	1845 a	772 b	2186 b	0.36 b
LSD ($P \leq 0.05$)	183	29	146	0.0242
	M. hapla population/ soil pot	*M. hapla* population/g dry root	Total population per pot	Reproductive factor (Rf)
M. hapla 6000/pot[x]	2214 a	5286 a	7222 a	1.20 a
P. penetrans + *M. hapla* (6000 + 6000)/pot[x]	592 b	2739 b	1790 b	0.30 b
LSD ($P \leq 0.05$)	326	364	784	0.1307

[w]Each value was the means of five replications from two runs of the test. The means within each column followed by the same letters were not significantly different when means were separated by using LSD ($P \leq 0.05$).
[x]Each pot contains 1500 cm^3 soil.
[y]Total population per pot = population from soil + population from onion roots.
[z]Rf = Final total population/initial population.

and *M. incognita*/ g dry root of corn were reduced with the two species existing together compared with when they were inoculated singly.

Simultaneous inoculation of both species significantly reduced the final total population of both species compared to *P. penetrans* or *M. hapla* alone, respectively (Table 2). The Rf value of *P. penetrans* was 0.42 when it occurred alone but when *M. hapla* was added, the value was reduced to 0.36. The Rf value was 1.20 when *M. hapla* was alone and also decreased to 0.30 with the additional inoculation of *P. penetrans*. Similar results were reported by Herman et al. (1988) in that the reproduction of *M. incognita* and *P. brachyurus* was mutually suppressed on a soybean cultivar that was susceptible to both nematodes. It was also concluded from this study that the reproductive rate of *M. hapla* on onion was higher than that of *P. penetrans* at the same initial population density. This agrees with Haseeb and Shukla (2000) in that the reproduction rate of *M. incognita* was higher than *P. thornei* at equal initial population density, and in concomitant inoculations of the two nematodes, nematode reproduction was suppressed compared to when the nematodes were inoculated individually. Furthermore, based on the reduction in Rf when the two species existed together, it can be inferred that *P. penetrans* suppressed the multiplication of *M. hapla* much more than *M. hapla* did *P. penetrans*. This research will not only help predict the onion yield reduction when both *M. hapla* and *P. penetrans* exist in the field, but also expect the soil nematode population after growing of onion. All information would help onion growers make decisions about whether or not to grow onion when both nematode species are in soil.

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LITERATURE CITED

Al-Rehiayani, S., S. L. Hafez, M. Thornton, and P. Sundararaj. 1999. Effects of *Pratylenchus neglectus*, *Bacillus megaterium*, and oil radish or rapeseed green manure on productive potential of *Meloidogyne chitwoodi* on potato. Nematropica 29:37-49.

Amosu, J. O., and D. P. Taylor. 1975. Interaction of *Meloidogyne hapla*, *Pratylenchus penetrans*, and *Tylenchorhynchus agri* on Kenland red clover, *Trifolium pratense*. Indian Journal of Nematology 4:124-131.

Dickson, D. W., and R. McSorley. 1990. Interaction of three plant-parasitic nematodes on corn and soybean. Journal of Nematology 22:785-789.

Gamborg, O. L., T. Murashige, T. A. Thorpe, and I. K. Vasil. 1976. Plant tissue culture media. In Vitro 12:473-478.

Haseeb, A., and P. K. Shukla. 2000. Effect of *Pratylenchus thornei* and *Meloidogyne incognita*, alone and in combinations, on the growth and oil yield of *Mentha arvensi*. Nematologia Mediterranea 28:151-155.

Herman, M., R. S. Hussey, and H. R. Boerma. 1988. Interactions between *Meloidogyne incognita* and *Pratylenchus brachyurus* on soybean. Journal of Nematology 20:79-85.

Huttel, R. N. 1990. Monoxenic culturing of plant parasitic nematodes using carrot discs, callus tissue, and root-explants. Pp 163-172 *in* B. M., Zuckerman, W. F. Mai, and L. R. Krusberg, Eds. Plant Nematology Laboratory Manual. The University of Massachusetts Agricultural Experiment Station. Amberst, Massachusetts.

Kodira, C. U., and H. Ferris. 1994. Influence of temperature and host plant on the interaction between *Pratylenchus neglectus* and *Meloidogyne chitwoodi*. Journal of Nematology 26:65-71.

Martin, M., C. V. Ransom, and W. M. Thompson. 2004. Effect of volunteer potato density on bulb onion yield and quality. Weed Science 52:754-758.

National Onion Association. 2007. About onions: bulb onion production. http://www.onions-usa.org/about/production.asp Accessed by 15 August 2007.

Olthof, T. H. A., and J. W. Potter. 1973. The relationship between population densities of *Pratylenchus penetrans* and crop losses in summer-maturing

vegetables in Ontario. Phytopathology 63:577-582.

Rakesh, P. 2001. Influence of *Meloidogyne incognita*, *Pratylenchus thornei* and *Tylenchorhynchus vulgaris* on growth and oil growth of menthol mint. Indian Journal of Nematology 31:111-114.

SAS Institute Inc., 2004. SAS Online Doc 9.13, Cary, NC, USA.

Sujatha, K., and U. K. Mehta. 1997. Relative pathogenicity of *Pratylenchus zeae* and *Meloidogyne javanica* to sugarcane crop. International Journal of Nematology 7:221-224.

Viaene, N. M., and G. S. Abawi. 1998. Management of *Meloidogyne hapla* on lettuce in organic soil with sudangrass as a cover crop. Plant Disease 82:945-952.

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