PATHOGENICITY OF *PRATYLENCHUS PENETRANS* ON ONION

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ABSTRACT

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Pathogenicity of *Pratylenchus penetrans* on onion was tested both under greenhouse and microplot conditions. For the greenhouse study, seedlings of onion (*Allium cepa*) cultivar Tioga were inoculated with *P. penetrans* at 0, 3000, 6000, 9000, or 1200 females + juveniles/ pot. In field microplots, onions were inoculated 0, 26500, 53000, 106000, or 212000 females + juveniles of *P. penetrans*/ microplot. Cultural practices recommended by onion production in Idaho were carried out and data on fresh and dry weight of the plant top and roots, bulb diameter and weight, and nematode population in roots and soil were collected at harvest. Increasing inoculum levels led to significant reductions in onion growth and increased nematode population at harvest. Significant reduction in plant growth began at 3000 *P. penetrans*/ pot or 26500/ microplot. Bulb weight reduction ranged from 31.5% to 64.2% at the inoculum level of 26500 and 212000 *P. penetrans* / microplot, respectively, in field microplots. Maximum reduction in plant total dry weight was 84.2% at 12000 *P. penetrans*/ pot under greenhouse conditions. This study will help onion growers in Idaho make decisions on whether or not to grow onions in a field with a specific population density of *P. penetrans* in the soil.

Key words: Allium cepa, onion, pathogenicity, *Pratylenchus penetrans*.

RESUMEN

Pang, W., S. L. Hafez, P. Sundararaj, B. Shafii, and E. Fallah. 2009. Patogenicidad de *Pratylenchus penetrans* en cebolla. Nematropica 39:35-46.

Se evaluó la patogenicidad de *Pratylenchus penetrans* en cebolla en invernadero y en microparcelas. En el ensayo de invernadero, se inocularon plántulas de cebolla (*Allium cepa*) cultivar Tioga con 0, 3000, 6000, 9000, ó 1200 hembras + juveniles de *P. penetrans* /maceta. En las microparcelas, se inocularon las cebollas con 0, 26500, 53000, 106000, ó 212000 hembras + juveniles de *P. penetrans*/microparcela. Se utilizaron las prácticas culturales recomendadas para la producción de cebolla en Idaho y se colectaron datos del peso fresco y seco de la parte aérea de las plantas y de las raíces, diámetro y peso de los bulbos, y densidad de población de los nematodos en suelo y raíces al momento de la cosecha. A mayor nivel de inóculo, mayor reducción en el crecimiento de las plantas y mayor población de nematodos al momento de la cosecha. Se observó reducción significativa en el crecimiento de las plantas con 3000 *P. penetrans*/maceta y con 26500 nematodos/microparcela. La reducción en el peso de los bulbos osciló entre 31.5% y 64.2% con inóculo de 26500 y 212000 *P. penetrans* /microparcela, respectivamente, en el ensayo de microparcelas. La reducción máxima del peso total seco de plantas fue del 84.2% con 12000 *P. penetrans*/maceta en el ensayo de invernadero. Este estudio ayuda a los cultivadores de Idaho a tomar la decisión de cultivar o no cebolla con base en la densidad de población de *P. penetrans* presente en el suelo.

Palabras clave: Allium cepa, cebolla, patogenicidad, *Pratylenchus penetrans*.

INTRODUCTION

Onion (*Allium cepa*) is an important vegetable for consumption in the U.S., and Idaho is one of the leading states in onion production (Greene, 1991). The area for onion production in Idaho is 3200 ha with cash receipts from onion totaling $58.5 million, which ranks Idaho third among states in onion production (IASS, 2005). However, plant parasitic nematodes are common in Idaho onion fields, which can seriously limit bulb yield and quality. The severity of damage depends on the species and population densities of nematodes present in soil at the time of planting. Olthof and Potter (1973) reported that the marketable yield of onion was inversely correlated with pre-plant numbers of *Pratylenchus penetrans*. Losses in yield of onions were 14% and 71% at infestation levels of 0.6 and 18 *P. penetrans*/cm^3 soil, respectively. However, other authors reported that a root-lesion nematode population of more than 0.01 nematode/cm^3 soil resulted in injury to onions (Ferris, 1962; Olthof and Potter, 1973). Moreover, several authors concluded that the threshold level of *P. penetrans* on onion was one nematode/cm^3 soil (Barker and Olthof, 1976; Potter and Olthof, 1993; Merrifield, 1999).

The available information about the pathogenicity of *Pratylenchus spp.* on onion is controversial. These conflicting results may be due to each researcher using different onion cultivars, and the variability in nematode species or populations. In Idaho, although yield loss by *P. penetrans* is potentially an important constraint in onion production, no information is available on the relationship between nematode densities and onion growth and yield. It is essential for growers to know the effects of *P. penetrans* on growth and yield of onion in order to decide whether to grow onions in a field. The objective of this research was to study the relationship between different initial population densities of *P. penetrans* and onion growth and yield.

MATERIALS AND METHODS

Culturing of nematodes

Pratylenchus penetrans was maintained on excised corn roots growing on Gamborg's B5 medium (Gamborg *et al.,* 1976). A modification of Huettel's (1990) method was used for establishing the excised roots. The petri dishes with corn seeds ('AP162') placed on 1% agar were incubated at 24°C for 7 days. After germination, when the root tips were 3-cm long, they were cut and transferred to B5 medium in 112-ml jars and incubated at 24°C in the dark for 7 days before inoculation with nematodes. Nematodes were originally obtained from an Idaho field and established on corn roots in an *in vitro* culture. They were then transferred to jars with newly excised roots and fresh medium every 6 months under aseptic conditions.

Inoculum preparation

Pratylenchus penetrans was extracted from corn roots maintained on the 6-month-old cultures. Corn roots with the media were chopped into 1-cm-long pieces and placed in Baermann funnels under a mist chamber sprayed with a fine mist of water for 1 minute every 10 minutes for a period of 10 days. Nematodes were collected every other day for 10 days, and the nematode suspensions were concentrated by using a 25-µm (500-mesh) sieve. Suspensions were kept in a refrigerator and exposed to air bubbles until used.

Greenhouse studies

The experiment was arranged in a randomized complete block design with five

treatments and five replications. On 30 May 2006, seeds of onion cultivar Tioga were sowed 1.5-cm deep in plastic pots containing 1500 cm^3 of a mixture (1:1) of steam-sterilized sand and soil (1.7% organic matter, 39.0 mg/ kg total nitrogen, 45 mg/ kg phosphorus, 4, 066 mg/ kg calcium, and pH 7.9). One week after germination, seedlings were thinned to one per pot and one week later, each pot was inoculated with initial populations (Pi) of 0, 3000, 6000, 9000, or 12000 females and juveniles of *P. penetrans*. Before inoculation, suspensions of *P. penetrans* were taken out of the refrigerator, concentrated to 1500 nematodes/ml, and exposed to air bubbles at room temperature 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 the appropriate volume of suspension was inoculated into the holes. The holes were then covered with a light layer of soil, and moistened with a light water mist. Onions were grown at 25°C to 34°C under natural daylight, watered daily to field capacity and fertilized weekly beginning 1 week after inoculation with 1 g 20-20-20 N-K-P in 50 ml water per pot. Six weeks after planting, onions were sprayed weekly with carbaryl at 2.3 L/ha and an insecticide mixture of imidacloprid and cyfluthrin at 197 ml/ ha.

Onions were harvested 14 weeks after planting. Plants were separated from soil by hand and the whole plant including the roots, stem, and leaves were carefully separated (no bulb was formed). Plants were kept fresh by rolling them in a wet paper towel. Plant top 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. The plant top was dried at 60°C for 96 hours and the dry top weight was recorded. Onion roots from each pot were washed free of soil and chopped, and *P. penetrans* were extracted in the mist chamber for 10 days. Roots were then dried at 60°C for 96 hours to determine the dry root weight along with the whole plant dry weight. Soil in each pot was mixed evenly by hand and a 500 cm^3 sample was taken. Nematodes were extracted from each sample using Cobb's decanting and sieving technique (Flegg, 1967) followed by the modified centrifugal sugar floatation technique. Nematode population from the 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) were recorded.

This experiment was repeated in 2007 from March to June. Three plants were kept in each pot, and besides NPK, 0.4 ml sulfur in 50 ml water was applied to each pot in every other week. Parameters measured in 2007 were similar to those in 2006.

Field microplot studies

This experiment was carried out in field microplot at Parma Research and Extension Center, University of Idaho, in 2007. Three-and-a-half-gallon buckets (30-cm diameter × 28-cm deep) were filled with natural field 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. Each bucket represented a microplot, and they were set in a randomized complete block design with five replications and five treatments of 0, 26500, 53000, 106000, or 212000 females and juveniles of *P. penetrans*/plot, respectively. Ten seeds of 'Tioga' onion were sowed in each microplot on 21 March, and after germination, seedlings were thinned to three per

plot. On 12 May onions were inoculated with nematodes as described above for the greenhouse experiment. A drip irrigation system with a single emitter at each plot was used to irrigate the microplots and soil was watered to field capacity twice a week. The microplots were weeded when necessary and fertilized weekly with 3 g of 20-20-20 N-K-P in 150 ml of water per microplot. Sulfur was applied at the rate of 1.5 ml in 150 ml water per microplot once every 2 weeks. Plants were sprayed weekly with the insecticide mixture of imidacloprid and cyfluthrin at 197 ml·ha-1 and carbaryl at 2.3 L·ha-1. On 20 August, onions were harvested and microplots were dug out of the ground and shaken to separate the soil from the plants. Plants were separated and picked out of the soil by hand. Soil in each plot was mixed evenly and a 500 cm^3 sample was collected. Data were collected from this experiment as described for the greenhouse experiment except that plant height was not recorded. In addition, onion bulb diameter was measured utilizing 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 Fishers protected least significant difference test at ($P \leq 0.05$). Data were also subjected to regression analysis. Plant growth as well as the final nematode population was regressed ad the dependent variable with the initial inoculum density and the independent variable. Homogeneity of variance was tested, and 'nematode population per gram dry root' from greenhouse study was transformed by natural log before analysis. Statistical analysis was conducted by using SAS program (SAS Institute Inc., 2004).

RESULTS AND DISCUSSION

Greenhouse studies

Onion growth parameters were reduced with the increasing *P. penetrans* inoculum levels as observed visually in Fig. 1A and 1B. Linear regression indicated that there was a negative relationship between the increasing inoculum levels and onion root and top fresh weight at harvest (Figs. 2A and B). Root fresh and dry weights at all inoculum levels were significantly reduced ($P \leq 0.05$) compared to the uninoculated control, and the reduction was also significant between inoculum levels (Table 1). Olthof and Potter (1973) also found that onion fresh root weight decreased with increasing inoculum levels of *P. penetrans* with the maximum reduction at the highest inoculum level. Fresh and dry weights of the top and whole plant were significantly reduced at all inoculum levels, and significant reduction began at the lowest inoculum level of 3000 *P. penetrans*/ pot. Reduction in whole plant dry weight ranged from 47.7% to 84.2%, with the maximum reduction at 12000 *P. penetrans*/ pot (Table 1). Similar results was reported by Olthof and Potter (1973) that the marketable yield of onion was inversely correlated with pre-plant number of nematodes, and yield losses ranged from 14% to 71% at inoculum levels of 0.6 to 18 *P. penetrans*/ cm^3 soil. The same trend of reduction was found in plant height, and stunting in onions was observed at the seedling age (Figs. 1A and B), which could contribute to the reduction in plant weight. It was concluded from this study that onion was very sensitive to *P. penetrans*, since plant growth was significantly reduced even at 3000 nematodes/ pot.

The nematode population at harvest increased with the increasing inoculum levels (Table 2). Maximum soil population

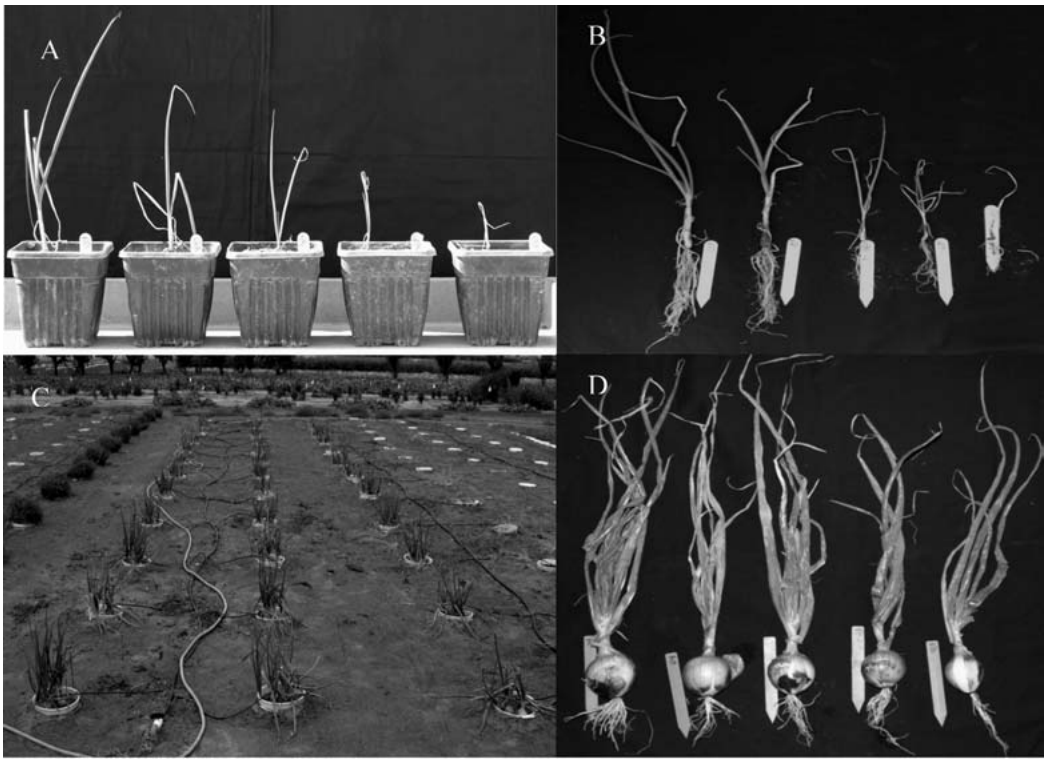

Fig. 1. From left to right, onion growth response to *Pratylenchus penetrans* inoculum levels of 0, 3000, 6000, 9000, or 12000 adults and juveniles per pot in greenhouse study 1: A) plants before harvest; B) plants after harvest; C) overview of the microplot study; D) from left to right, onions harvested from microplots inoculated with 0, 26500, 53000, 106000, or 212000 per microplot.

was at the highest inoculum level, and soil population at all inoculum densities was lower than the initial population, which was contradictory to the results of Olthof and Potter (1973) except at their highest inoculum level. A positive relationship was found between the final nematode population per gram dry root and the increasing initial inoculum density (Fig. 2C). The number ranged from 315 at 3000 *P. penetrans*/pot to 7298 at 12000 *P. penetrans*/pot. However, the nematode reproductive factors were decreased from 0.379 to 0.358 with the increasing inoculum levels (Fig. 2D). Reproductive factors at 3000 and 12,000 *P. penetrans*/pot were significantly different ($P \leq 0.05$), but there was no differences among other inoculum levels (Table 2). Such a decrease in nematode multiplication could be due to the more competition in soil and roots at higher nematode population densities. The low multiplication rate of *Pratylenchus spp.* on onion was reported by Machado and Inomoto (2001) as only 0.73, 0.13, and 0.17 for *P. brachyurus* in three experiments, while Charchar and Huang (1981), and Khan (1992) showed the Rf of *P. brachyurus* on onion ranged from 0.28 and to 0.6.

Field microplot studies

Results of the filed study supported those of the greenhouse study. In general,

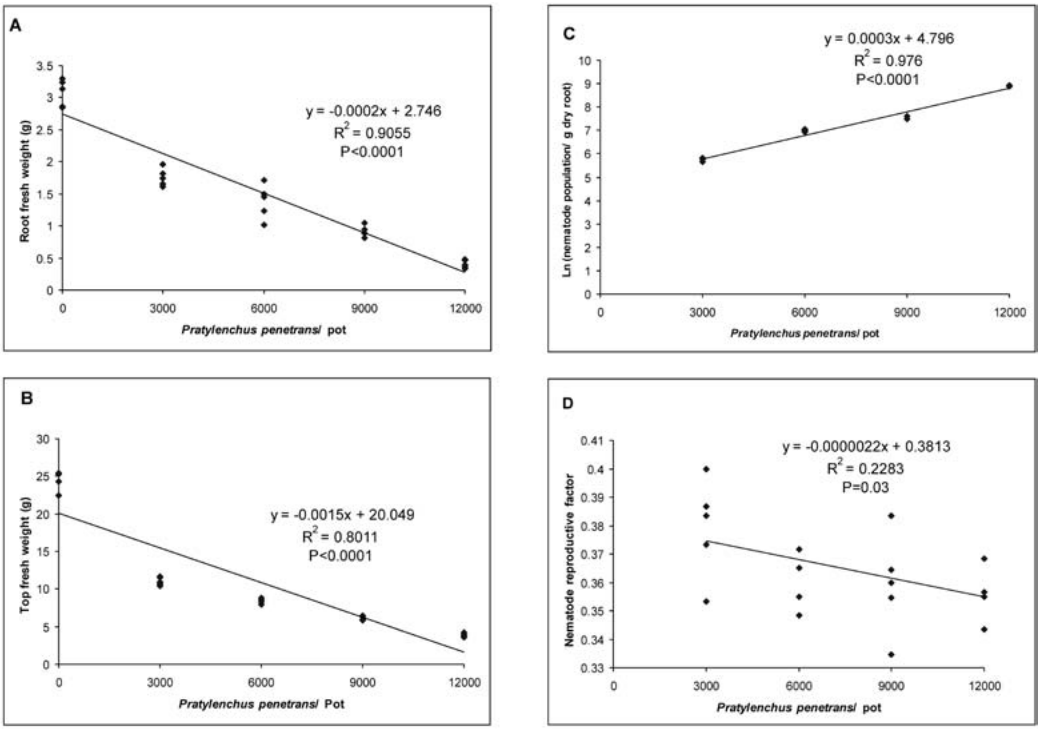

Fig. 2. Effect of initial inoculum density of *Pratylenchus penetrans* on A, onion root fresh weight, B, onion shoot fresh weight, C, Ln nematode population per gram dry root, and D, nematode reproductive factor under greenhouse conditions.

increasing initial population density negatively affected the onion growth (Figs. 1D, 3A, 3B, 3C, and 3D). Onion growth was significantly reduced ($P \leq 0.05$) at all inoculum levels compared with the uninoculated control (Tables 3). Root, bulb, and whole plant fresh weights were reduced by the inoculation of *P. penetrans*, and the reductions between inoculum levels were also significant. The reductions ranged from 26.5% to 48.5%, 31.5% to 64.1%, and 26.9% to 53.3%, respectively for onion root, bulb, and whole plant weight compared with the uninoculated control. Regression showed that onion bulb and total plant fresh weights were reduced 13 and 15 gram, respectively by increasing the inoculum level at 50,000 *P. penetrans*/plot. Compared with the results of the greenhouse study (Tables 1 and 3), more damage was observed in onion under greenhouse conditions than in microplots. Onion total plant fresh weight was reduced from 26.9% to 53.3% in field, while the reduction was high as 53.8% to 84.3% under greenhouse conditions. This probably was due to the more favorable growing conditions in the field microplots compared to the greenhouse, where temperature and soil type were more favorable for nematode feeding and multiplication.

Pratylenchus penetrans also affected fresh and dry weights of onion leaves in microplots (Table 3). All inoculated onions showed a significant reduction in leaf fresh and dry weight compared with the uninoc-

Table 1. Effects of *Pratylenchus penetrans* inoculum levels on onion root, top, and total fresh and dry weights and top height under greenhouse conditions.[z]

Inoculum level (*P. penetrans* per pot)	Root fresh weight (g)	Root dry weight (g)	Top fresh weight (g)	Top dry weight (g)	Top height (cm)	Total fresh weight (g)	Total dry weight (g)
Uninoculated control	3.08 a	0.62 a	24.57 a	2.48 a	39.29 a	27.64 a	3.10 a
3000	1.76 b	0.47 b	11.01 b	1.15 b	32.76 b	12.76 b	1.62 b
6000	1.39 c	0.34 c	8.46 c	0.85 c	30.22 c	9.85 c	1.19 c
9000	0.93 d	0.15 d	6.19 d	0.70 d	23.48 d	7.11 d	0.85 d
12000	0.41 e	0.04 e	3.93 e	0.45 e	20.07 e	4.34 e	0.49 e
LSD ($P \leq 0.05$)	0.23	0.04	0.89	0.10	0.98	0.95	0.12

[z]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 ($P \leq 0.05$).

Table 2. Effects of *Pratylenchus penetrans* inoculum levels on nematode population at harvest under greenhouse conditions.[x]

Inoculum level (*P. penetrans* per pot)	Nematodes per cm^3 soil	Nematodes per gram dry root	Total Population[y] per pot	Reproductive factor (Rf)[z]
3000	0.7 d	315 d	1138 d	0.379 a
6000	1.2 c	1085 c	2174 c	0.362 ab
9000	2.0 b	1899 b	3234 b	0.359 ab
12000	2.7 a	7298 a	4300 a	0.358 b
LSD ($P \leq 0.05$)	0.11	128.19	159.01	0.02

[x]Each value was the means of five replications. The means within each column followed by the same letters were not significantly different when means were separated by using LSD ($P \leq 0.05$).
[y]Total population = Nematode population from both the roots and soil in each pot.
[z]Rf = Final total population/ initial population.

ulated control. Leaf fresh weight at 212000 *P. penetrans*/plot was reduced by (16.4%) more than the other inoculum levels, but there were no significant differences among the inoculated treatments. More effects on top weight were shown in the greenhouse than in microplots, which could be due to the fact that *P. penetrans* only feeds on the roots of onion, and the damage on roots could affect the top growth especially at the seedling age, which is the case in greenhouse. In contrast, in field microplots, onions were harvested when mature bulbs formed, and leaf growth had ceased (Chris, 2006). Thus, the damage was reflected more in bulb weight rather than leaf weight. Bulb diameter and fresh weight (Table 3) were significantly reduced ($P \leq 0.05$) with the increasing inoculum levels as well. Linear regressions indicated that for one increase in the initial inoculum density, there was a reduction of 0.00001 cm in bulb diameter and 0.0003 gram in bulb weight (Figs. 3C and D). Maximum reductions in bulb diameter and fresh weight were 44.2% and 64.2%, respectively at 212000 *P. penetrans*/ plot. Moreover, the microplot study showed that significant reductions in most plant parameters began at the lowest inoculum level, which corroborated with the greenhouse study.

Nematode population at harvest showed the same trend as the greenhouse study. Final soil nematode populations were not different from each other at the initial inoculum levels of 26500/ plot and 53000/ plot (Table 4). Similar to the greenhouse study, there was also a positive relationship between the nematode population per gram dry root and the increasing inoculum levels. It was showed that for one increase in the initial inoculum density, there was an increase of 0.0062 nematodes in one gram of dry root at harvest (Fig. 3E). However, higher nematode populations per gram of dry root occurred under greenhouse conditions (Tables 2 and 4), which explained the increase in damage observed in the greenhouse. In the microplot study, as in the greenhouse study, nematode Rf's decreased from 0.13 to 0.05 with the increasing initial inoculum levels (Fig. 3F, Table 4). This study found that nematode RF were lower than 0.4 in both greenhouse and microplot studies, which indicated that 'Tioga' is a poor host for *P. penetrans*. Based on the reduction in the total plant dry weight (84.2%) and the fresh bulb

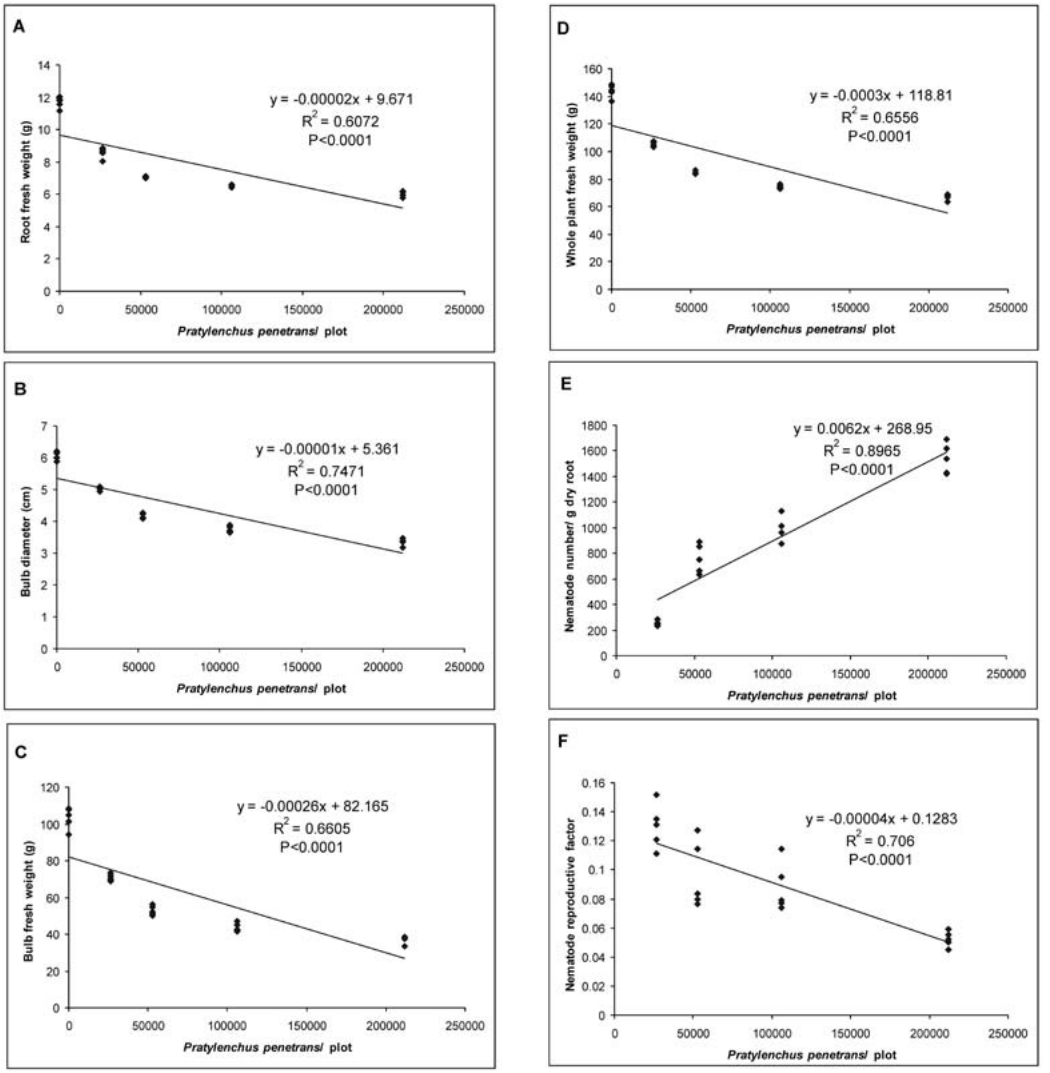

Fig. 3. Effect of initial inoculum density of *Pratylenchus penetrans* on A, onion root fresh weight, B, onion bulb diameter, C, onion bulb fresh weight, D whole plant fresh weight, E, nematode population per gram dry root, and F, nematode reproductive factor in field microplot.

weight (64.2%), it can be concluded that onion is very sensitive to *P. penetrans*.

Results from this study indicate that *P. penetrans* can cause severe damage on onions in Idaho. Based on the results of the initial soil nematode population at planting, this study can guide onion growers in Idaho in deciding whether or not grow onions and what plant growth reductions they may encounter. Resistant onion cultivars could be selected in the future to reduce the damage of *P. penetrans* to plant growth and bulb development.

Table 3. Effects of *Pratylenchus penetrans* inoculum levels on onion root, leaf, bulb, and total weights and bulb diameter in field microplots.[z]

Inoculum level (*P. penetrans* per pot)	Root fresh weight (g)	Root dry weight (g)	Leaf fresh weight (g)	Leaf dry weight (g)	Bulb diameter (cm)	Bulb fresh weight (g)	Total fresh weight (g)
Uninoculated control	11.69 a	2.02 a	28.83 a	2.95 a	6.04 a	103.31 a	143.83 a
26500	8.59 b	1.21 b	25.79 b	2.57 b	5.04 b	70.79 b	105.17 b
53000	7.06 c	0.98 c	25.02 b	2.29 c	4.19 c	52.79 c	84.87 c
106000	6.48 d	0.79 d	24.29 b	2.27 c	3.75 d	43.58 d	74.36 d
212000	6.02 e	0.56 e	24.11 b	2.26 c	3.37 e	37.04 e	67.17 e
LSD ($P \leq 0.05$)	0.31	0.15	1.69	0.14	0.15	4.40	3.82

[z]Each value was the means of five replications. The means within each column followed by the same letters were not significantly different when means were separated by using LSD ($P \leq 0.05$).

Table 4. Effects of *Pratylenchus penetrans* inoculum levels on nematode population at harvest in field microplots.[y]

Inoculum level (*P. penetrans* per microplot)	Nematodes in soil per plot	Nematodes per gram dry root	Reproductive factor (Rf)[z]
26500	3127 c	261 d	0.13 a
53000	4346 c	759 c	0.10 b
106000	8533 b	989 b	0.09 b
212000	10229 a	1537 a	0.05 c
LSD ($P \leq 0.05$)	1402.50	122.01	0.02

[y]Each value was the means of five replications. 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]Rf = Final total population/ initial population.

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