

SCREENING OF ONION CULTIVARS FOR RESISTANCE AND TOLERANCE TO *PRATYLENCHUS PENETRANS* AND *MELOIDOGYNE HAPLA*

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ABSTRACT

Pang, W., S. L. Hafez, and P. Sundararaj. 2009. Screening of onion cultivars for resistance and tolerance to *Pratylenchus penetrans* and *Meloidogyne hapla*. *Nematropica* 39:47-55.

Greenhouse experiments were conducted to evaluate commercially grown onion cultivars for resistance and tolerance to *Pratylenchus penetrans* and *Meloidogyne hapla*. Onion seedlings of cv. Tioga, Orizaba, Caveat, Citation, Vision, R10 Tinto, Sanfan, Sweet Sunrise, Nirvana, and Mercury were inoculated with female adults and juveniles of *P. penetrans* or second stage juveniles of *M. hapla* at four nematodes/cm³ soil, respectively. Eight weeks after inoculation plants were harvested and resistance and tolerance were evaluated based on nematode reproductive factor and the reduction in onion total plant dry weight. Cultivars Mercury, R10 Tinto, and Caveat were both tolerant and resistant to *P. penetrans* infection. Caveat has the best tolerance to *P. penetrans*. Mercury and R10 Tinto were both tolerant and resistant to *M. hapla*, and they were tolerant and resistant to both nematode species. Sanfan and Sweet Sunrise were susceptible to *P. penetrans*, whereas Tioga, Citation, and Vision were susceptible to *M. hapla*. No cultivar tested was susceptible to both nematode species. Cultivars with both tolerance and resistance can be used as a standard for future screening and are potential cultivars for nematode management in field production.

Key words: *Meloidogyne hapla*, onion cultivars, *Pratylenchus penetrans*, resistance, tolerance.

RESUMEN

Pang, W., S. L. Hafez, and P. Sundararaj. 2009. Evaluación de la resistencia y tolerancia de variedades de cebolla a *Pratylenchus penetrans* y *Meloidogyne hapla*. *Nematropica* 39:47-55.

En experimentos de invernadero, se evaluó la resistencia y tolerancia a *Pratylenchus penetrans* y *Meloidogyne hapla* de variedades de cebolla cultivadas comercialmente. Se inocularon plántulas de cebolla de la variedades Tioga, Orizaba, Caveat, Citation, Vision, R10 Tinto, Sanfan, Sweet Sunrise, Nirvana y Mercury con hembras adultas y juveniles de *P. penetrans* o con juveniles de segundo estadio de *M. Hapla*, a razón de cuatro nematodos/cm³ de suelo, respectivamente. Ocho semanas después de la inoculación, se cosecharon las plantas y se evaluó la resistencia y la tolerancia de las variedades con base en el factor reproductivo del nematodo y la reducción del peso total seco de las plantas de cebolla. Las variedades Mercury, R10 Tinto y Caveat fueron tolerantes y resistentes a *P. penetrans*. Caveat tuvo la mejor tolerancia a *P. penetrans*. Mercury y R10 Tinto fueron resistentes y tolerantes a *M. hapla*, y fueron tolerantes y resistentes a ambas especies de nematodos. Sanfan y Sweet Sunrise fueron susceptibles a *P. penetrans*, mientras que Tioga, Citation y Vision fueron susceptibles a *M. hapla*. Ninguna de las variedades evaluadas fue susceptible a ambas especies de nematodos. Las variedades con tolerancia y resistencia pueden usarse como referencia en futuros estudios de evaluación de variedades y tienen el potencial de utilizarse para manejar los problemas de nematodos en el campo.

Palabras clave: *Meloidogyne hapla*, variedades de cebolla, *Pratylenchus penetrans*, resistencia, tolerancia.

INTRODUCTION

Resistance to nematodes is usually defined as a plant's ability to inhibit nematode reproduction (Roberts, 2002). Resistant cultivars should suppress while susceptible cultivars support the multiplication of nematodes (Zhou and Starr, 2003). Nematode resistance is usually determined by the nematode reproductive factor (Rf) which is the final nematode population at harvest (Pf) divided by the initial nematode population at inoculation (Pi). Tolerance is the ability of a plant to withstand the damage caused by nematodes. Tolerant plants suffer little yield damage while intolerant plants suffer great damage under nematode infection (Roberts, 2002). Plant growth or yield reduction caused by nematodes is usually used to determine tolerance. *Pratylenchus penetrans* and *Meloidogyne hapla* are common nematode species in Idaho onion fields, which can reduce onion bulb yield up to 70% (Olthof and Potter, 1973). The resistance of onion cultivars to *Pratylenchus spp.* has been tested before. Machado and Inomoto (2001) indicated that onion cv. Baia Periforme was resistant to *P. brachyurus* infection, and same result had been reported by Khan (1992). Bergeson (1962) tested 32 onion cultivars for resistance to *P. penetrans*, and he found that none of the cultivars was immune to *P. penetrans*, but two cultivars were more resistant than several commonly grown cultivars. Moreover, Wong and Ferris (1968) and Olthof and Potter (1973) reported that onion was a very good host for *P. penetrans*. Different results were also reported on the resistance of onions to *M. hapla*. Kotcon *et al.* (1985) and Belair (1992) reported that onion cv. Krummery Special, MSU 8155 × 826, Downing Yellow Globe, Spartan Banner, Spartan Sleeper, and Pronto were moderate hosts for *M. hapla*. However, Viaene and Abawi (1998)

reported that onion was a good host for *M. hapla*.

Although some onion cultivars have been tested for nematode resistance by different authors, there is no information available about the resistance or tolerance of onion cultivars commercially grown in Idaho and Oregon in the United States of America. Utilization of resistant and tolerant cultivars is one of the best economic options for sustainable nematode management in onion. It is essential to provide growers information on which onion cultivars are tolerant withstanding the least yield reduction in a field for each nematode species and populations densities at planting. It is also essential to determine the relative increase or decrease in nematode populations after a season of onion production, which will impact the subsequent crop (Park *et al.*, 2007). The objectives of this study were to evaluate the resistance and tolerance level of onion cultivars commercially grown in Idaho and Oregon to *P. penetrans* and *M. hapla*, and to identify cultivars that can be used in a crop rotation for nematode management.

MATERIALS AND METHODS

Plant materials

Onion cv. Tioga, Caveat, Citation, Vision, Safan, Sweet Sunrise, Mercury, R10 Tinto, Nirvana, and Orizaba were tested. Orizaba and Mercury were white and red cultivars, respectively, and the remaining eight were yellow. Seeds were obtained from onion growers in Idaho.

Inoculum preparation

Lesion nematodes were maintained on excised corn roots growing on Gamborg's B5 medium (Gamborg *et al.*, 1976) in tissue culture. Based on Huettel's (1990) method on establishing the excised roots, there

were some modifications as follows. The petri dishes with corn seeds (AP162) placed on 1% agar were incubated at 24°C for 7 days. Three-cm long root tips 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 either *P. penetrans* or *M. hapla*. The nematodes were obtained from cultures that had been established on corn roots, and were transferred to jars with newly excised roots and fresh medium every 6 months under aseptic conditions. *Pratylenchus penetrans* were extracted from corn roots maintained on the 6-month-old cultures. Corn roots were cut into 1-cm-long pieces and placed in Baermann funnels under a mist chamber sprayed with a fine mist of water for 60 seconds every 10 minutes for a period of 10 days. Nematodes were collected and concentrated by sieving (25- μ m) every other day for 10 days. The number of juveniles and adult females were quantified from 1-ml aliquots. Suspensions were then kept at 4°C and aerated with bubblers for 12 hours until used.

Meloidogyne hapla were maintained on roots of tomato cv. Payette grown in pots in greenhouse. Four-month-old plants were uprooted and soil was rinsed from the roots with tap water. Roots were then dipped in 0.5% NaOCl solution for 1 minute, cut into 1-cm-long pieces and the second stage juveniles (J2) were extracted from the roots for 2 weeks by using the mist chamber technique. *Meloidogyne hapla* were collected, quantified and stored using the same techniques as for *P. penetrans*.

Screening onion cultivars for resistance to P. penetrans and M. hapla

Onion seeds were sown in jiffy pots filled with 100 cm³ of a steam-sterilized mixture of 3 sand:1 peatmoss:1 soil (1.7% organic matter, 39.0 mg·kg⁻¹ total nitrogen, 45 mg·kg⁻¹

phosphorus, 4, 066 mg·kg⁻¹ calcium, and pH 7.9) in September of 2006. After germination, 2-week-old seedlings were transplanted to plastic pots filled with 250 cm³ steam-sterilized 1 sand:1 soil mixture and seedlings were thinned to three per pot 1 week later. One month later, oil in each pot was inoculated with either 4 females and juveniles of *P. penetrans*/cm³ or 4 J2/ of *M. hapla* per cm³ soil. The suspensions were inoculated into four 1.5-cm deep holes made 1 cm from the base of the seedlings near the root zone and the holes were covered with a light layer of soil, and moistened with water. Both *P. penetrans* and *M. hapla* inoculated and non inoculated control onion cultivars were arranged in a randomized complete block design and replicated six times. Plants were grown at the temperature of 25°C to 34°C under natural day light, watered daily to field capacity and fertilized weekly with 0.5 g 20-20-20 N-P-K solved in 15 ml water per pot. Plants were sprayed weekly with the insecticide mixture of imidacloprid and cyfluthrin at 197 ml·ha⁻¹ and 8 weeks after planting with carbaryl at 2.3 L·ha⁻¹.

Onions were harvested 8 weeks after inoculation. Root fresh weight and whole plant fresh weight were recorded. Plant top dry weight was recorded after drying the top at 60°C for 96 hours. Nematodes were extracted from the entire soil mass of each pot by Cobb's decanting and sieving (Flegg, 1967) followed by the modified centrifugal sugar floatation. Onion roots were washed with tap water, chopped into 1-cm-long pieces and nematodes were extracted in an intermittent mist chamber for 10 days. Roots were then dried at 60°C for 96 hours and dry root weight was recorded. Total plant dry weight was calculated as the sum of top and root dry weight. Nematode populations in the roots and soil, final population (Pf), nematode population per gram of dry root, and the reproductive factor (Rf) were recorded for each cultivar.

This experiment was repeated twice in 2007. Onion seeds were sowed 1.5-cm deep in plastic pots filled with 250 cm³ steam-sterilized 1 sand:1 soil mixture on 8 March 2007. *Pratylenchulus penetrans* was inoculated at the same density as the previous study when the seedlings were 4-week-old. Seven replications were kept for each cultivar and in addition to 20-20-20 N-P-K, sulfur was applied at the rate of 0.2 ml in 15 ml water per pot in every 2 weeks. Onions were harvested 8 weeks after inoculation and data parameters measured were the same as the previous study.

Data analysis

Resistance was determined by the nematode reproductive factor (Rf). If the Rf was greater than 1, the cultivar was considered susceptible. However, if the Rf was less than 1, then the cultivar was considered resistant to the nematode damage. Tolerance was determined by the reduction in plant total dry weight using the following standards. The plant was considered tolerant if the reduction was less than 32%; moderately tolerant if the reduction ranged from 32% to 50%; and intolerant if the reduction was more than 50%. All data were subjected to analysis of variance (ANOVA), and the differences among means were compared using Fishers protected least significance difference test ($P \leq 0.05$). Statistical analysis was conducted by using SAS program (SAS Institute Inc., 2004). No significant interaction was observed between the two tests of 2007, so the two sets of data were combined for analysis.

RESULTS

Screening onion cultivars for resistance and tolerance to *P. penetrans*

Inoculation with *P. penetrans* reduced plant growth in all onion cultivars. Signifi-

cant reductions ($P \leq 0.05$) of both root and whole plant fresh and dry weight were observed among onion cultivars following nematode inoculation compared with the uninoculated control (Table 1). Tioga, Orizaba, and Sweet Sunrise, which were considered as intolerant cultivars with at least a 50% reduction in total plant dry weight. Nirvana, Citation, Sanfan, and Vision were moderate tolerant cultivars. Mercury, R10 Tinto, and Caveat were identified as tolerant to *P. penetrans* with less than 31% reduction in total plant dry weight (Table 1). Nematode reproduction rate also showed differences among cultivars at harvest (Table 1). The two cultivars, Sanfan and Sweet Sunrise, were susceptible to *P. penetrans* supporting Rf of greater than 1.0. The remaining eight cultivars were all resistant with Rf of less than 1.0 (Table 1). Mercury, R10 Tinto, and Caveat were both tolerant and resistant to *P. penetrans*. Mercury, R10 Tinto, and Caveat plant weight reductions were 30.3%, 30.2%, and 20.7% with corresponding Rf values of 0.79, 0.62, and 0.82 respectively. On the other hand, Sweet Sunrise was intolerant (51.1% growth reduction) and susceptible (Rf = 1.06) to *P. penetrans*. Cultivars Vision, Citation, and Nirvana did not maintain the population of *P. penetrans* with Rf values of less than 1.0; however, they were only moderately tolerant to *P. penetrans* with 32.4%, 49.0% and 49.5% reductions in plant growth. Although Tioga and Orizaba were resistant to *P. penetrans*, they were intolerant cultivars suffering greater than 50% reductions in total plant mass. Among all cultivars, Caveat suffered significantly less ($P \leq 0.05$) reductions in plant growth than all other cultivars tests. Nematode reproductive factor was the lowest on cv. Tioga.

Screening onion cultivars for resistance and tolerance to *M. hapla*

Inoculation of onion with *M. hapla* also significantly affected plant growth param-

Table 1. Effects of *Pratylenchus penetrans* inoculated at 1000 (females + juveniles)/pot on root and total fresh and dry weight of different onion cultivars and nematode population at harvest in a greenhouse.^x

Cultivar	Root fresh weight reduction (%) ^y	Root dry weight reduction (%) ^y	Total fresh weight reduction (%) ^y	Total dry weight reduction (%) ^y	Nematodes per gram dry root	Total population per pot	Reproductive Factor (RF) ^z
Tioga	57.0 a	54.0 a	58.3 ab	55.3 a	1341 fg	556 g	0.56 g
Orizaba	50.0 ab	59.0 a	60.4 a	54.5 a	1512 ef	594 g	0.59 g
Sweet Sunrise	44.5 ab	44.2 ab	57.0 ab	54.1 a	4866 a	1064 a	1.06 a
Nirvana	36.6 bcd	47.0 ab	43.2 c	49.5 ab	3827 c	677 ef	0.68 ef
Citation	42.8 abc	58.1 a	52.0 abc	49.0 ab	1593 e	899 b	0.90 b
Sanfan	26.4 de	10.7 c	49.9 bc	44.3 b	3664 c	1040 a	1.04 a
Vision	27.2 cde	9.6 c	45.4 c	32.4 c	672 h	733 de	0.73 de
Mercury	7.4 g	33.1 b	27.0 d	30.3 c	2244 d	794 cd	0.79 cd
R10 Tinto	14.2 ef	34.5 b	22.2 d	30.2 c	4084 b	617 fg	0.62 fg
Caveat	24.6 de	9.9 c	25.3 d	20.7 d	1244 g	819 c	0.82 c
LSD ($P \leq 0.05$)	16.20	19.28	9.84	9.10	230.1	74.2	0.074

^xEach value was the mean of two trails with seven replications each. The means of each column followed by the same letters were not significantly different by Fishers LSD at ($P \leq 0.05$).

^yReduction (%) = percent change relative to the uninoculated control.

^zRf = final total population/initial population.

ters and nematode population at harvest (Table 2). Orizaba, Sanfan, Vision, and Citation were considered as intolerant cultivars with at least a 43% reduction in total plant dry weight. Caveat and Sweet Sunrise were moderate tolerant cultivars. R10 Tinto, Tioga, Mercury, and Nirvana were identified as tolerant to *M. hapla* with less than 32% reduction in total plant dry weight (Table 2). Nematode reproduction rate also showed differences among cultivars (Table 2). The three cultivars, Vision, Tioga, and Citation, were susceptible to *M. hapla* supporting Rf of greater than 1.0. The remaining seven cultivars were all resistant with Rf of less than 1.0 (Table 2). Mercury, R10 Tinto, and Nirvana were both tolerant and resistant to *M. hapla*. The plant total dry weight reductions for Mercury, R10 Tinto, and Nirvana were 31.5%, 30.0%, and 31.6%, with corresponding Rf values of 0.71, 0.76, and 0.62 respectively. On the other hand, Vision and Citation were intolerant (more than 43.0% in growth reduction) and susceptible (Rf \geq 1.51) to *M. hapla*. Cultivars Caveat and Sweet Sunrise did not maintain the population of *M. hapla* with Rf values of less than 1.0; however, they were only moderately tolerant to *M. hapla* with 40.1% and 33.8% reductions in plant growth. Although Sanfan and Orizaba were resistant to *M. hapla*, they were intolerant cultivars suffering greater than 50% reductions in total plant mass. Among all cultivars, nematode reproductive factor was the lowest on Caveat (0.35).

DISCUSSION

This study showed differences in nematode reproduction rate among cultivars, which supported the conclusion that the reproduction rate of *M. hapla* was affected by onion cultivars (Kotcon *et al.*, 1985; Belair 1992). Onion resistance to root-knot

nematodes was also found in *Meloidogyne javanica* (Khan and Yetunde, 1993). Future research is needed to test onion resistance to other root-knot nematode species.

Overall, none of the cultivars tested were susceptible to both nematode species. Mercury and R10 Tinto were resistant and tolerant to both nematode species, and could be used as a standard for the future cultivar evaluation. It is also possible that these two cultivars should be incorporated into a rotation scheme in Idaho and Oregon due to its nematode tolerance and resistance. Other cultivars could also be used depending on the purpose. If to *P. penetrans* is the pathogen problem, Mercury, R10 Tinto, or Caveat can be used as a standard for future cultivar screening. Cultivars that are resistant but not tolerant can be used in a crop rotation to suppress the nematode populations in the soil, which could be an economic nematode management option if yields remain economical. On the other hand, susceptible but tolerant cultivars could be planted in the next rotation for higher onion yields.

Previous research has reported that nematode tolerance was positively correlated with plant yield (Trudgill, 1991). It was also reported that plant height, number of leaves, root numbers and length all had positive, linear, and highly significant relationship ($P \leq 0.05$) with onion bulb yield (Islam *et al.*, 2007). Total plant dry weight instead of only root dry weight was used in this study to determine tolerance. Evaluating total plant dry weight is a convenient way for greenhouse screening, because bulb production may not occur in the greenhouse setting. However, bulb quality such as weight and diameter could be used for tolerance evaluation in a field study.

Differences in plant growth were shown between uninoculated control and inoculated treatment by the end of this experi-

Table 2. Effects of *Meloidogyne hapla* inoculated at 1000 (females + juveniles)/pot on root and total fresh and dry weight of different onion cultivars and nematode population at harvest in a greenhouse.^x

Cultivar	Root fresh weight reduction (%) ^y	Root dry weight reduction (%) ^y	Total fresh weight reduction (%) ^y	Total dry weight reduction (%) ^y	Nematode sper gram dry root	Total population per pot	Reproductive Factor (Rf) ^z
Tioga	19.5 de	52.6 cd	14.9 c	30.9 d	5552 b	1591 ab	1.59 ab
Orizaba	33.8 abcd	71.2 a	44.8 a	53.0 a	3389 d	579 c	0.58 c
Sweet Sunrise	32.7 bcd	61.1 abc	25.4 bc	33.8 cd	4524 c	771 c	0.77 c
Nirvana	22.1 cd	64.3 abc	25.7 bc	31.6 d	2306 e	620 c	0.62 c
Citation	19.6 cde	65.8 abc	29.4 b	43.0 abc	8473 a	1510 b	1.51 b
Sanfan	16.9 de	56.3 abcd	44.3 a	50.4 ab	2550 e	634 c	0.63 c
Vision	50.0 a	54.7 bcd	45.8 a	48.4 ab	5868 b	1723 a	1.72 a
Mercury	40.0 ab	26.0 e	32.5 b	31.5 d	4388 c	709 c	0.71 c
R10 Tinto	4.5 e	43.1 d	18.0 c	30.0 d	4275 c	756 c	0.76 c
Caveat	36.7 abc	68.1 ab	23.1 bc	40.1 bcd	1035 f	351 d	0.35 d
LSD ($P \leq 0.05$)	17.11	15.48	10.84	10.43	389.4	197.2	0.197

^xEach value was the mean of two trails with seven replications each. The means of each column followed by the same letters were not significantly different by Fishers LSD at ($P \leq 0.05$). ^yReduction (%) = percent change relative to the uninoculated control.

^zRf = final total population/initial population.

ment, which indicated that 8 weeks of the duration (from inoculation to harvest), was sufficient for onion cultivar screening under greenhouse conditions. However, if nematode eggs were used as the inoculum, longer screening time would be needed. Furthermore, 10 cultivars were tested in pots under greenhouse conditions in this study. If a large number of cultivars were tested, containers instead of pots would be preferred due to the limitation of greenhouse space and labor.

In conclusion, onion cultivars showed differences in resistance and tolerance to *P. penetrans* and *M. hapla*. Cultivar Caveat was both tolerant and resistant to *P. penetrans* infection, while Mercury and R10 Tinto were resistant and tolerant to both nematode species. These cultivars could be used as a standard for future onion cultivar screening. It was also found that no cultivar was susceptible to both nematode species. Tolerant and resistant cultivars can be used in crop rotations depending on the purpose. Tolerant cultivars could be planted to achieve the yield requirement, and resistant cultivars can be planted in the following rotation to suppress the nematode multiplication and achieve the goal of nematode management.

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