DIFFERENTIATION OF TWO GEOGRAPHICALLY ISOLATED POPULATIONS OF *PRATYLENCHUS NEGLECTUS* BASED ON THEIR PARASITISM OF POTATO AND INTERACTION WITH *VERTICILLIUM DAHLIAE*

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ABSTRACT

Hafez, S. L., S. Al-Rehiayani, M. Thornton, and P. Sundararaj, 1999. Differentiation of two geographically isolated populations of *Pratylenchus neglectus* based on their parasitism of potato and interaction with *Verticillium dahliae.* Nematropica 29:25-36.

Relationships of two populations of *Pratylenchus neglectus,* one from Parma, Idaho (IPN) and the other from Ontario, Canada (CPN) to Russet Burbank potato and *Verticillium dahliae* (Vd) were evaluated. Early egression of IPN and CPN was measured on a resistant potato cultivar (Butte). Egression of CPN from Butte roots was lower (79.4%) than that of IPN (93.7%). CPN reproduced faster and averaged twice the population size on cultivars Russet Burbank and Butte ($P < 0.05$) compared to IPN. IPN with Vd had an effect on tuber yield that was similar to the effect of Vd alone, while the interaction of *P. penetrans* (PP) or CPN with Vd was greater than that of Vd alone. Area under the disease progress curve (AUDPC) for plants grown in soil infested with *P. penetrans* plus V*d* was higher ($P <$ 0.05) than those for Vd alone or IPN plus Vd. AUDPC values produced by CPN plus Vd were not different from those produced by *P. penetrans* plus Vd ($P > 0.05$). IPN, CPN and *P. penetrans* did not affect root and shoot growth of Russet Burbank ($P > 0.05$) but tuber yield was reduced at inoculum levels of 10,000, or 20,000 nematodes of CPN per plant, and at 5 000, 10 000, or 20 000 nematodes of*P.* penetrans per plant ($P < 0.05$). At inoculum levels of 5 000 and 10 000, CPN reproduced more on potato than did IPN. At all inoculum levels, *P. penetrans* reproduced in significantly greater numbers than IPN or CPN. Based on these findings IPN and CPN appear to be distinct and differ in their behavior. PCR amplification of the ITS1 region of rDNA from individual IPN and CPN nematodes yielded fragments of different size suggesting a possible need for taxonomic reevaluation of this species.

Key words: intraspecific variation, potato, *Pratylenchus neglectus, Verticillium dahliae.*

RESUMEN

Hafez, S. L., S. Al-Rehiayani, M. Thornton y P. Sundararaj. 1999. Diferenciación de dos poblaciones separadas geograficamente de *Pratylenchus neglectus* en base a sus relaciones con la papa y el *Verticillium dahliae.* Nematrópica 29:25-36.

La relación de dos poblaciones de *Pratylenchus neglectus,* una de Parma, 'Idaho' (IPN) y otra de Ontario, Canadá (CPN) a la papa 'Russet Burbank' y a el *Verticillium dahliae* (Vd) fueron evaluadas. La salida temprana de IPN y CPN se midió en una variedad de papa ('Butte') resistente. La salida de CPN de las raíces de 'Butter' fue menor (79.4%) que la de IPN (93.7%). La reproducción de las dos poblaciones en las dos variedades de papa 'Russet Burbank' y 'Butte', mostró que la primera variedad tenía mayores números de nematodos ($P < 0.05$) en las raíces, que la ultima. CPN se reprodujo más rápido y promedio dos veces la población en ambas variedades comparado a IPN. La interacción de IPN y CPN con Vd se midió en papa 'Russet Burbank', en macetas con suelo infestado con Vd solo, con cada especie de nematodo por separado (IPN, CPN, o *P. penetrans* incluido como control) o en combinación con Vd o el control no infestado. *P. penetrans* más Vd-suelo infestado siempre produjo más síntomas, los que además ocurrieron una semana antes, en comparación a los otros tratamientos. IPN con Vd tuvo un efecto en el rendimiento del tubérculo que fue similar al efecto del Vd solo, mientras la interacción de *P. penetrans* (PP) o de CPN con Vd fue mayor que la de Vd solo. El área bajo la curva de progreso de la enfermedad (AUDPC), para plantas crecidas en suelo infestado con *P. penetrans,* fue significativamente mayor que aquella para Vd solo o IPN más Vd. Los valores de AUDPC producidos por CPN más VD no se diferenciaron de aquellos producidos por *P.penetrants* más Vd ($P < 0.05$). El efecto de varias densidades poblacionales iniciales de IPN, CPN y *P.penetrans* en el rendimiento de 'Russet Burbank' también se investigó en el invernadero. IPN, CPN y *P.penetrans* no afectaron el crecimiento de la raíz o la hoja de 'Russet Burbank' a ningún nivel de inoculo ($P < 0.05$). Sin embargo, el rendimiento del tubérculo se redujo a niveles de inóculo de 10 000 ó 20 000 nematodos de CPN por planta, y a 5 000, 10 000 ó 20 000 nematodos o *P. penetrans* por planta ($P > 0.05$). La población de IPN no afectó el rendimiento del tubérculo a ningún nivel de inóculo. A niveles de inóculo de 5 000 y 10 000, CPN se reprodujo más en la papa que IPN. A cualquier nivel de inóculo, *P.penetrans* se reprodujo en números significativamente mayores que IPN o CPN. De acuerdo a estas observaciones IPN y CPN parecen ser distintos y diferir en su comportamiento biológico. Estas observaciones están respaldadas por el análisis de ADN, en el que la amplificación por PCR de la región ITS1 del ADN de IPN y CPN rindió fragmentos diferentes.

Palabras claves: papas, *Pratylenchus neglectus,* variaciones entre-specificas, *Verticillium dahliae.*

INTRODUCTION

The root lesion nematode, *Pratylenchus neglectus* (Rensch) Filipjev & Schuurmans Stekhoven is a migratory endoparasite, endemic in the western United States and Canada (Thorne, 1961). It has been found associated with alfalfa, grain, rangeland grasses, and wheat (Umish and Ferris, 1992). In Idaho it has been reported on a wide range of crop plants including potato (*Solanum tuberosum* L.) (Hafez, 1992).

Little is known about the effect of *P. neglectus* on potatoes and reported results have been conflicting. Brodie (1984) stated that *P. neglectus* causes little damage to potato. Davis *et al.* (1992) found no correlation between populations of *P. neglectus* in soil and yield of potato. In contrast, Olthof (1990) reported a 22% yield reduction in Russet Burbank potato in the presence of *P. neglectus.* Pathogenicity of this nematode reportedly varies with soil type and host plant. In clay soil, *P. neglectus* did not affect the yield of potatoes but in loamy sand it reduced the total number and weight of tubers (Olthof, 1990). In Idaho, after five consecutive years of cropping with potato cv. Butte, few *P. neglectus* were observed in soil or roots of Russet Burbank potatoes grown the following year (Davis *et al.*, 1992).

Conflicting results have also been reported about the relationship of *P. neglectus* to *Verticillium dahliae* Kleb., the fungus that causes Verticillium wilt or the early dying disease in potato. Sholte and s'Jacob (1989) reported that *P. neglectus* may have a synergistic effect with *V. dahliae* in reducing yield, while Davis *et al.* (1992, 1996) found no significant interaction between *V. dahliae* and *P. neglectus.*

Population variability within nematode species has also been suggested to explain differences among *Pratylenchus* species in reproduction on potato (France and Brodie, 1996; Townshend and Anderson, 1976) and possibly in relationships with *V. dahliae.* Intraspecific differences in pathogenicity to alfalfa have been noted for isolates of *P. neglectus* from different geographic origins (Griffin, 1991) and between isolates of *P. penetrans* on tobacco, celery (Olthof, 1968), and potato (France and Brodie, 1996). More recently, genetic diversity in isolates of *P. goodeyi* was identified by RAPD-PCR

(Pinochet *et al.*, 1997). It is not known, however, if isolates or populations of *P. neglectus* differ in pathogenicity or virulence to potatoes or in relation to *V. dahliae.* The possible existence of different physiological populations of *P. neglectus* will complicate management strategies. The objective of this study was to test the hypothesis that the population of *P. neglectus* from Parma, Idaho has less impact on potato yield and behaves differently than a population of *P. neglectus* from Ontario, Canada, which is reported to be highly virulent on Russet Burbank potato (Olthof, 1990).

MATERIALS AND METHODS

Nematode culture and inoculum preparation: Two geographically isolated populations of *Pratylenchus neglectus* were used in these studies and were denoted as Idaho population (IPN) and Canadian population (CPN). An Idaho population (IPN) originated from several females collected from a potato field in Parma, Idaho. This population has been reared on corn in the greenhouse since 1988 and on corn tissue culture in the laboratory since 1997. A Canadian population (CPN) originated from a single female isolated from a potato field in Alliston, Southern Ontario in 1995. The CPN population has been maintained in the laboratory on corn tissue culture (provided by Dr. Qing Yu, Pest Management Research Center, Agriculture Canada, Vineland, Ontario, Canada). One population of *P. penetrans* from Ohio (provided by Dr. Paulette Pierson, Department of Plant Pathology, Ohio State University) was included in some of these studies to compare its behavior with the *P. neglectus* populations.

All nematode species were cultured on excised corn roots growing on Gamborg's B5 medium (Gamborg *et al.*, 1976). Methods for establishing nematodes on excised roots described by Huettel (1990) and France and Brodie (1995) were followed with some modification. The plates were incubated at 21°C for about one week. When root tips were about 0.5 cm in length, they were transferred to B5 medium in 112-ml jars. The excised roots in jars were incubated at 21°C in the dark for at least 2 weeks before nematode transfer. CPN and the Ohio population of *P. penetrans* had already been established in corn tissue cultures in Petri dishes by the providers. Nematodes were transferred to jars with fresh medium and newly excised roots every three months.

Inoculum was obtained from three-month-old cultures and consisted of nematodes of mixed life stages. Nematodes were recovered from corn tissue culture by placing the corn roots with medium on Baermann funnels. Funnels then were placed in an intermittent mist for 10 days. Nematodes were collected daily on a 25-µm-pore sieve and nematode suspension was aerated and refrigerated until use within 10 days.

Verticillium dahliae inoculum preparation: *Verticillium dahliae* isolate V-5 RI was provided by Leland H. Sorensen, Aberdeen Research and Extension Center, University of Idaho, Aberdeen, Idaho. The fungus was maintained on potato dextrose agar (PDA). Conidial inocula were prepared in 1 000-ml Erlenmeyer flasks containing 500 ml of potato dextrose broth (PDB). Flasks were inoculated with mycelial plugs from PDA dishes and incubated on a horizontal shaker for one week in the dark at 21°C. Conidial suspensions were filtered through cheesecloth, and conidia densities were determined with a hemacytometer and adjusted to the desired inoculum level by addition of distilled water.

Egression experiment: Early egression of *Pratylenchus* spp. from roots is an indication of resistance in potato (France and Brodie, 1995; 1996). Single, two-week-old seedlings of potato cv. Butte were transplanted into plastic cones filled with 200 cm^3 of sterile sand and soil mix (3:1). Ten plants (replications) per nematode population were used. Plants were arranged in a complete randomized design on a greenhouse bench and were allowed to grow for a week at 23-24°C with 14 hours of light. Seven days after transplanting, each potato plant was inoculated with 1 000 nematodes. One week after inoculation, plants were removed from their containers and their root systems washed. The root system was placed in a 112-ml jar filled with 100 ml distilled water. Jars were then placed in a growth chamber at 21°C. At 24-hour intervals for three days, the water was removed from each jar and replaced with fresh water, and juveniles and females were counted from 5-ml suspensions. After three days, root systems were washed and stained by boiling (80-85°C) for 3 minutes in a solution of acid fuchsin-lactoglycerol (Byrd *et al.*, 1983). Roots were then rinsed in tap water 3 times and placed in a solution of 50 ml distilled water plus 50 ml of glycerol for 24 hr. The number of nematodes remaining inside root tissue was counted. Percentages of egressed nematodes were calculated from the total number of nematodes (the number of egressed nematodes plus number of nematodes remaining in root tissue). Nematode percentages were subjected to analysis of variance and the means were separated by LSD.

Reproduction experiment: Reproduction rates of IPN and CPN were assessed on two commercial potato cultivars: Russet Burbank, a cultivar reported to be susceptible to *P. neglectus* (Davis *et al.*, 1992, Olthof, 1990), and Butte, a cultivar reported to be highly resistant to *P. neglectus* (Davis *et al.*, 1992). A single three-week-old potato seedling was transplanted into a 6-inch plastic pot filled with a 3:1 mixture of sterile sandy loam soil (64% sand, 32% silt, 4% clay, 8.1 pH, 0.6% organic matter). Two days after transplanting, each seedling was inoculated with a mixture of 700 juveniles and adults of *P. neglectus.* Pots were arranged in a randomized complete block design on greenhouse benches. Pots were watered daily and fertilized weekly with 5 g of NPK (20-20-20) in 100 ml water per pot. Plant growth conditions were as previously described. The experiment was terminated 56 days after inoculation. Soil from each pot was separated from roots and nematodes were extracted from 500-cm^3 soil by sieving followed by sugar flotation method (Jenkins, 1964). Individual root systems were chopped and placed in Baermann funnels in an intermittent mist chamber for 10 days and then oven dried at 65°C. The collected nematode suspensions were concentrated using a 0.025-mm (500-mesh) sieve. The number of eggs and juveniles were counted from 1-ml aliquots and results were extrapolated to the total volume of the suspension. Final nematode population densities (Pf), nematodes per root system and per gram of dry root, and reproductive factors (Rf, where Rf = Pf/Pi) were subjected to analysis of variance. Final nematode population data were log transformed before analysis. Fisher's protected LSD was used for mean separation.

Interaction with Verticillium dahliae: The relationship of IPN and CPN and the Ohio population of *P. penetrans* (PP) (included as a standard pathogen) to Verticillium wilt and *Verticillium dahliae* (Vd) was measured on Russet Burbank potato in a greenhouse experiment. Four-week-old seedlings were transplanted in plastic pots containing 1 500 cm^3 of sterile sandy loam soil (64% sand, 32% silt, 8% clay, 0.8 pH, 0.8% organic matter). Each of 7 replicate pots received 1.94×10^6 colony forming units (cfu) of Vd alone, 1 500 vermiforms from each nematode species alone, or combinations of Vd with each nematode species, or no inoculum (control). Plant growth conditions were as previously described. Each plant was evaluated weekly for wilt symptoms. A subjective 0-5 rating scale was used (Botseas and Rowe, 1994) where 0 = no wilt symptoms, 1 = chlorosis of leaves in the lower half of the plant, 2 = chlorosis and necrosis of leaves in the lower half of the plant, 3 = chlorosis of leaves on the entire plant, 4 = chlorosis and necrosis of leaves on the entire plant, and 5 = dead plant. Areas under the disease progress curves (AUDPC) were calculated for each plant by using equation 8.29 in Campbell and Madden (1990). Analysis of variance of AUDPC values was used to evaluate significant differences in symptom development among treatments. LSD test and single degree of freedom contrasts were used to test for significant differences among AUDPC values or total tuber yield per pot. At harvest, tubers per plant were counted and weighed. The root system and a soil sample (500 cm^3) were collected from each plant for determination of final nematode population as previously described. Nematode count data were transformed to $\log_{10}(x + 1)$ values before analysis, although untransformed arithmetic means are presented in the tables. The means were compared using LSD.

Pathogenicity experiment: The relationships between initial population densities of IPN and CPN and Russet Burbank potato yield, and the reproduction and final density levels of these nematodes on Russet Burbank were evaluated in the greenhouse. IPN and CPN were compared with *P. penetrans* (PP), a reported pathogen of potato (Wheeler and Riedel, 1994). Certified Russet Burbank potato seed pieces (45-55 g) were planted in pots (single seed piece/pot) filled with 5 000 cm^3 sterile sandy loam soil (74% sand, 21% silt, 5% clay, 1.5% organic matter, and 8.0 pH). Pots were arranged in a randomized complete block design on greenhouse benches and grown under conditions as described previously. Eight plants were each inoculated after 15 days with mixed stages of *P. neglectus* or *P. penetrans.* Control plants were not inoculated. The inoculum levels were 0, 5 000, 10 000, or 20 000 nematodes per 5 000 cm^3 soil. The duration of the experiment was 130 days. Plants were fertilized with 15 g of NPK (20-20-20) in 300 ml water per pot every two weeks for the first three months and no fertilizer was added in the last month of the growing period. At harvest, the foliage was cut and weighed. The root systems were separated from soil and weighed. Tubers were counted and weighed. Nematodes were extracted from roots and 500-cm^3 soil samples as described previously. Data were subjected to analysis of variance and LSD was used for mean separation.

PCR test: Restriction analysis of the ITS1 region of rDNA from IPN and CPN, using the enzyme Tag I, was conducted by Tom Powers University of Nebraska (Powers *et al.*, 1997).

RESULTS

Egression experiment: The two populations of *P. neglectus* differed in the number of nematodes that egressed from the roots of Butte potato (Fig. 1). Egression of IPN (93.7%) was greater ($P < 0.05$) than that of CPN (79.4%).

Nematode reproduction experiment: Two months after inoculation, Russet Burbank had higher numbers of nematodes ($P < 0.05$) in roots than did Butte, and final populations of CPN were higher on both of the cultivars compared to IPN (Table 1). Roots from both cultivars supported an average of two times more CPN than IPN.

Interaction with Verticillium dahliae: Populations of CPN, IPN, and PP were significantly lower in roots infected with Vd (Table 2).

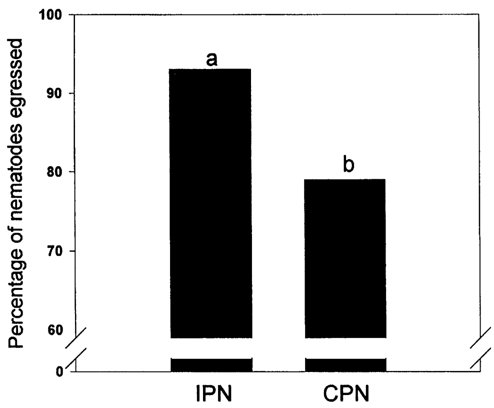

Fig. 1. Percentage of nematodes egressed from Butte potato inoculated with two geographically isolated populations of *Pratylenchus neglectus*. IPN = Parma (Idaho), CPN = Ontario (Canada). Bars with different letters are different ($P < 0.05$) according to the LSD test.

Nematodes reproduced well in potato roots in the absence of the fungus and the population densities of both PP and CPN in roots were higher ($P < 0.05$) than those of IPN. Nematodes in combination with Vd reduced the tuber yield ($P < 0.05$) while nematodes alone did not affect tuber yield (Table 2). IPN plus Vd had an affect on tuber yield that was similar to the effect of Vd alone, whereas the effect of PP or CPN with Vd was greater than that of Vd alone (Table 2).

Symptoms of Verticillium wilt were observed within three weeks after the experiment was established. The disease progress curves showed that *Verticillium dahliae* (Vd) plus *P. penetrans* (PP) produced more disease symptoms than other treatments and induced symptoms on the plants one week earlier than other treatments (Fig. 2). Vd plus CPN treatments also produced greater disease than Vd alone. Vd and IPN treatments produced less disease symptoms than Vd alone in the first six weeks of the experiment, but they induced greater symptoms after the seventh week of the experiment. Area under disease progress curve (AUDPC) was significantly higher for plants grown in soil infested with Vd plus PP than for plants grown in soil infested with Vd alone or infested with Vd plus IPN (Fig. 3). CPN plus Vd produced AUDPC values that were not significantly different from those produced by PP plus Vd.

Table 1. Reproduction of two isolates from geographically separated populations of *Pratylenchus neglectus* on potato cultivars two months after inoculation with 700 nematodes per pot.

Nematode population	Potato cultivars	Nematodes per root system[x]	Nematodes per gram dry root	Nematodes per 500 cm^3 soil	Final nematode population (Pf)/pot	Reproductive factor (Rf)[y]
IPN[z]	Butte	181 b	991 c	336 b	1 190 c	1.7 c
IPN	Russet Burbank	892 a	5 355 ab	257 b	1 665 bc	2.4 bc
CPN	Butte	281 b	2 104 bc	771 a	2 595 ab	3.7 ab
CPN	Russet Burbank	1 272 a	8 729 a	616 a	3 121 a	4.5 a

[x]Values are means of eight replications. Means in each column followed by the same letter are not different ($P = 0.05$) according to LSD.

[y]Rf = (Pf / Pi).

[z]The two nematode populations of *P. neglectus* were obtained from Parma, Idaho (IPN), and from Ontario, Canada (CPN).

Table 2. Reproduction of isolates from two geographically separated populations of *Pratylenchus neglectus* and one isolate of *P. penetrans* on Russet Burbank potato and tuber yield of plants grown in pots containing soil infested with *Verticillium dahliae.*

Nematodes	*Verticillium dahliae* 1.94 × 10^6 cfu/ 1 500 cm^3 soil	Nematodes per root system[x]	Nematodes per gram dry root	Nematodes per 500 cm^3 soil	Root dry weight (g)	Tuber weight (g)
None	–	0	0	0	1.04 a	140.1 a
None	+	0	0	0	0.35 b	57.3 b
IPN[y]	–	1 461 b	1 392 ab	114 bc	1.04 a	132.7a
IPN	+	219 c	690 b	119 bc	0.25 b	41.0 bc
CPN	–	3 454 a	4 211 ab	513 a	0.98 a	126.9 a
CPN	+	1 100 bc	3 650 ab	391 ab	0.22 b	34.3 c
PP	–	2 810 a	3 236 ab	176 bc	1.01 a	118.6 a
PP	+	926 bc	6 126 a	44 c	0.17 b	20.9 c

[x]Values are means of seven replications. Means in each column followed by the same letter are not different (P = 0.05) according to LSD.

[y]The two nematode populations of *P. neglectus* were obtained from Parma. Idaho (IPN), and from Ontario, Canada (CPN). The population of *P. penetrans* was obtained from Ohio (PP).

Pathogenicity Experiment: The two populations of *P. neglectus* (IPN and CPN) and the population of *P. penetrans* (PP) did not affect root and shoot growth of Russet Burbank at any inoculum level (Table 3). However, tuber yield was reduced at inoculum levels of 10 000, or 20 000 of CPN per plant,

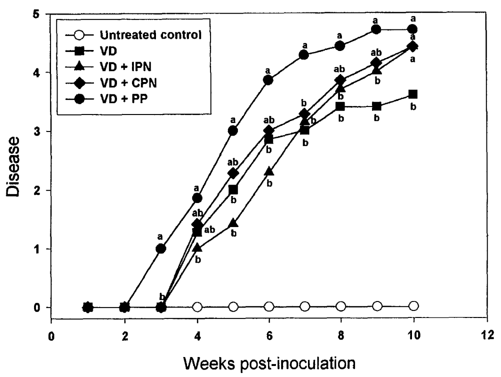

Fig. 2. Disease progress curves for Verticillium wilt on Russet Burbank potato growing in soil infested with *V. dahliae* and *Pratylenchus* spp. Vd = *Verticillium dahliae*, IPN = *P. neglectus* population from Parma (Idaho), CPN = *P. neglectus* from Ontario (Canada), PP = *P. penetrans* population from Ohio. Data at each week with different letters are different (P < 0.05) according to LSD test.

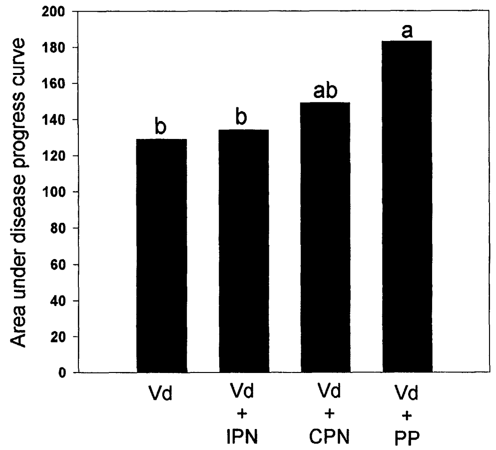

Fig. 3. Area under the disease progress curve (AUDPC) of potato growing in soil infested with *V. dahliae* and *Pratylenchus* spp. Vd = *Verticillium dahliae;* IPN = *P. neglectus* population of Parma, Idaho; CPN = *P. neglectus* population of Ontario, Canada; PP = *P. penetrans* population of Ohio. Bars with different letters are different (P < 0.05) according to the LSD test.

Table 3. Effect of three initial soil population densities of two geographically isolated populations of *Pratylenchus neglectus* and one population of *P. penetrans* on growth and tuber yield of Russet Burbank potato after 4 months of growth in greenhouse pots.

Nematodes[y]	Inoculum level Nem/5 000 cm^3 soil	Root dry wt (g)	Shoot dry wt (g)	Tuber wt (g)
0	0	2.7 a[z]	38.1 a	358.4 a
IPN	5 000	2.7 a	39.0 a	349.6 ab
IPN	10 000	2.6 a	36.8 a	317.9 abc
IPN	20 000	2.7 a	39.2 a	318.9 abc
CPN	5 000	2.6 a	37.7 a	313.3 abc
CPN	10 000	2.4 a	38.5 a	308.5 bc
CPN	20 000	2.5 a	38.2 a	300.9 c
PP	5 000	2.4 a	39.2 a	302.5 c
PP	10 000	2.5 a	36.2 a	298.1 c
PP	20 000	2.5 a	38.3 a	297.8 c

[y]The two nematode populations of *P. neglectus* were obtained from Parma, Idaho (IPN) and from Ontario, Canada (CPN). The population of *P. penetrans* was obtained from Ohio (PP).
[z]Values are means of 8 replicates. Means in each column followed by the same letter do not differ ($P < 0.05$) according to LSD test.

and at 5 000, 10 000, or 20 000 of PP per plant. IPN did not affect tuber yield at any inoculum level.

Reproduction of IPN, CPN and PP varied significantly among inoculum levels ($P <$ 0.05) (Table 4). PP reproduced more than IPN or CPN at all inoculum densities. At inoculum levels of 5 000 and 10 000, CPN reproduced more on potato than did IPN.

PCR test: Separate analyses of rDNA from several individual nematodes from each population showed unique fragments in the internal transcribed spacer (ITS1) region that were characteristic for either IPN or CPN (Fig. 4).

DISCUSSION

Pratylenchus neglectus is endemic in virgin and cultivated soils of the western United States and Ontario, Canada (Thorne, 1960). Despite its wide distribution, little is known about its intraspecific variability or the relationship of this nematode species to potato. The data from these experiments provide evidence that the population of *P. neglectus* from Idaho differs from the population of *P. neglectus* from Canada.

IPN egressed at higher rates from the resistant clone Butte, reproduced on Butte and Russet Burbank at slower rates, and was less virulent to potato than CPN. Moreover, PCR amplification of the ITS1 region from IPN and CPN indicated clear differences in size. This genetic marker may provide a tool to identify these two populations, as was shown recently for *P. coffeae* and *P. loosi* populations in Japan (Uehara, *et al.*, 1998).

Virulence of a nematode may result from the reproduction rate, and the intrinsic ability of a given number of nematodes to induce physiological alterations (Sarah

Table 4. Reproduction of two isolates from geographically separated populations of *Pratylenchus neglectus* and one isolate of *P. penetrans* on Russet Burbank potato 130 days after inoculation with three initial nematode densities under greenhouse conditions.

Nematodes	Inoculum level (Pi) Nem/5 000 cm^3 soil	Nematodes per root system[x]	Nematodes per gram dry root	Nematodes per 500 cm^3 soil	Final nematode population (Pf)/pot	Reproductive factor (Rf)[y]
IPN[z]	5 000	17 124 e	6 706 f	445 e	21 574 e	4.3 e
IPN	10 000	30 965 e	12 232 f	1 275 de	43 715 de	4.4 e
IPN	20 000	63 518 cd	23 722 df	4 374 a	107 255 c	5.4 de
CPN	5 000	39 219 de	15 547 ef	1 871 cd	57 931 d	11.6 bc
CPN	10 000	66 533 c	26 252 d	3 243 b	98 958 c	9.9 c
CPN	20 000	69 968 c	28 695 cd	5 336 a	123 330 bc	6.2 d
PP	5 000	86 283 c	35 272 c	1 515 cd	101 433 c	20.3 a
PP	10 000	117 848 b	47 115 b	2 493 bc	142 773 b	14.3 b
PP	20 000	187 661 a	76 037 a	4 668 a	234 336 a	11.7 bc

[x]Values are means of 8 replications. Means in each column followed by the same letter are not different (P = 0.05) according to LSD.
[y]Rf = (Pf / Pi).
[z]The two nematode populations of *P. neglectus* were obtained from Parma. Idaho (IPN), and from Ontario, Canada (CPN). The population of *P. penetrans* was obtained from Ohio (PP).

et al., 1993). In our studies, CPN always developed higher numbers in potato roots than did IPN, though not as high as *P. penetrans.* Griffin and Gray (1991) also have shown that differences in virulence of *P. neglectus* to alfalfa are related to differences in the rate of nematode population increase within plant tissues.

Differences in the behavior between these two geographical isolates provide further evidence of the existence of intraspecific variability in *P. neglectus* populations (Griffin and Gray, 1991) as well as other *Pratylenchus* species (France and Brodie, 1995; 1996; Pinochet *et al.*, 1993; 1994). Griffin and Gray (1991) reported that a population of *P. neglectus* from Utah behaved more aggressively on alfalfa than three other isolates collected in Utah and Wyoming. Formation of a pathotype population of nematodes may result from monoculture of a given host (Dropkin, 1988). These results clarify earlier conflicting reports concerning the relationship of *P. neglectus* to Russet Burbank potato (Davis *et al.*, 1992 and Olthof, 1990). Our study confirmed that CPN, collected from the same area of Ontario, Canada as that used by Olthof (1990), was pathogenic on Russet Burbank potato while IPN was not pathogenic even at high initial population densities (4 nematodes per 1 cm^3 soil). Our results also demonstrate that the *P. penetrans* population from Ohio was pathogenic to Russet Burbank even at low initial population densities (1 nematode per 1 cm^3), and was more virulent to potato than CPN. Pathogenicity of *P. penetrans* on potato has also been demonstrated in Michigan and Ontario (Bernard and Laughlin, 1976; Olthof, 1983). This finding, while supporting an earlier study in

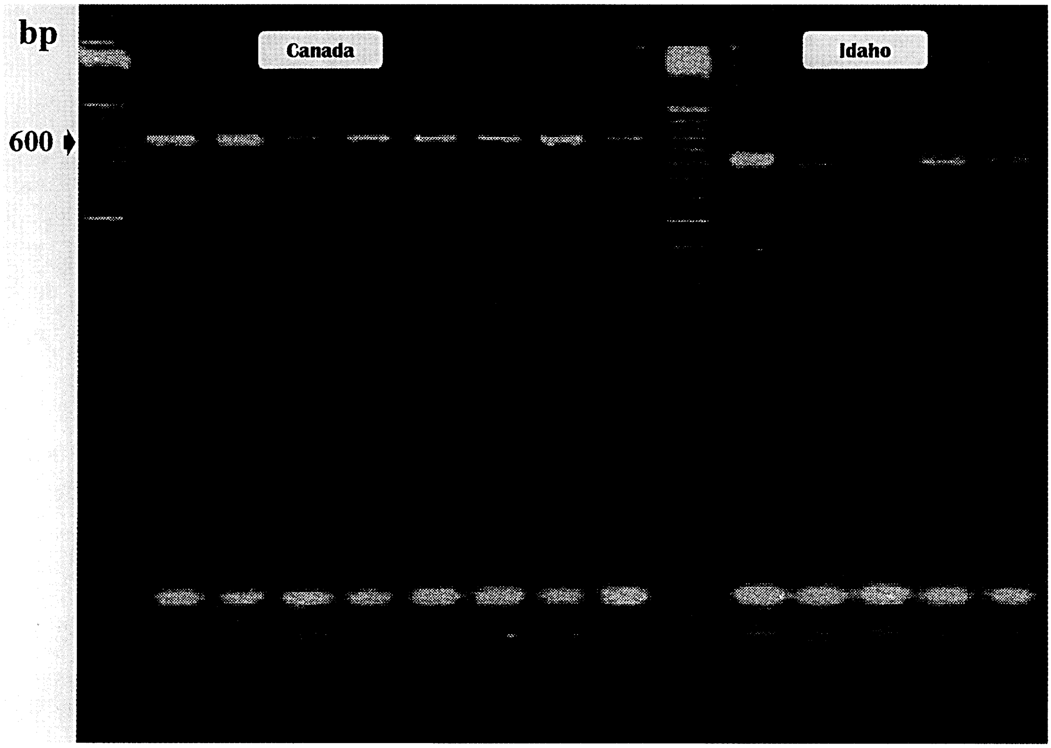

Fig. 4. Hinf I digest of ITS1 amplification from two populations of *Pratylenchus neglectus*. Eight and 5 individual nematodes were used from the Canada and Idaho populations, respectively.

Ohio (Wheeler and Riedel, 1994), does not agree with a report by Olthof (1990) indicating that the damage threshold of Russet Burbank to CPN is lower than it is to *P. penetrans*. Olthof (1990) reported up to 24% loss in potato tuber yield in CPN-infested soil with an initial nematode population density of 1 884/kg soil, but only a 15.7% loss in tuber yield in *P. penetrans*-infested soil with initial population density of 1 850/kg. The Ohio population of *P. penetrans* may be more virulent to Russet Burbank than the Canadian population of *P. penetrans* (France and Brodie, 1996). IPN, CPN, and *P. penetrans* had no negative effect on root or shoot dry weight at any level of initial inoculum, as reported previously (Kotcon and Loria, 1986; Olthof, 1990). Plant growth in the greenhouse may have been limited by cultural and environmental conditions, thus preventing the full expression of nematode-induced damage on roots or shoots. Initial population densities of *P. penetrans* is reported to be related to potato yield loss (Kimpinski and McRae, 1988). In our study, however, there were no differences among the three inoculum levels of *P. penetrans* in terms of their effect on tuber yield. This may be due to the fact that *P. penetrans* was able to reproduce at high rates and rapidly reached population densities capable of causing damage.

Pratylenchus penetrans not only causes economic losses to potato when acting alone (Wheeler and Riedel, 1994), but can cause even more severe losses by interacting with *Verticillium dahliae*, to increase Ver-

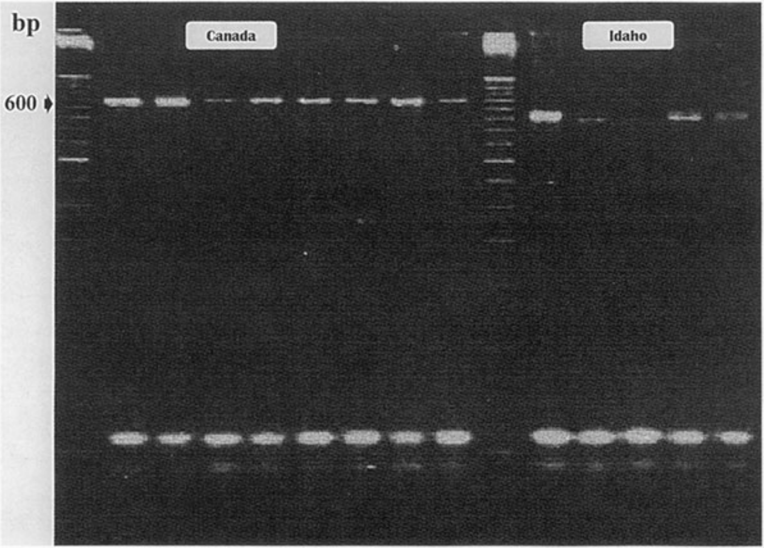

Fig. 4. Hinf I digest of ITS1 amplification from two populations of *Pratylenchus neglectus*. Eight and 5 individual nematodes were used from the Canada and Idaho populations, respectively.

ticillium wilt or potato early dying (Riedel *et al.*, 1985). Our results were consistent with previous reports that the effect of *P. penetrans* and *V. dahliae* on tuber yield is synergistic and that Verticillium wilt symptom development was more pronounced when *P. penetrans* was present (Riedel *et al.*, 1985; Wheeler and Riedel, 1994). Our results also indicated that the interaction of IPN, CPN, or *P. penetrans* with *V. dahliae* is dependent not only on the species involved, but also on their population. CPN interacted with *V. dahliae* to increase severity of wilt symptoms and to reduce tuber yield while IPN did not. Earlier symptom expression by Pp and Vd may be due to faster and more extensive colonization by the more aggressive nematodes.

The fact that the population densities of these nematode species were significantly lower in *Verticillium*-infected plants than those in plants free of the fungus, agree with earlier studies that showed suppression of *P. penetrans* in the presence of *V. dahliae* (Riedel *et al.*, 1995). Nematodes in *Verticillium*-infected plants encounter extensive root destruction.

Further field data is needed to confirm the relationship between CPN, IPN, potato and Vd. However, if consistent differences are found to exist between IPN and CPN with regard to their relationship to potato or their ability to interact with Vd, management decisions for potato or Verticillium wilt will be affected. The geographic distribution of *P. neglectus* populations in commercial potato production areas should be investigated. If CPN or other pathogenic populations are predominant, control measures should be taken, especially when Vd is present. Steps also may be taken to prevent introduction of CPN to other fields. If some potato production areas are presently infested with IPN and not CPN, steps might be taken only to prevent Verticillium wilt. Diagnostic services should consider variability among and within *Pratylenchus* species as well as nematode population densities when recommending control measures to potato growers.

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