

Characterization of *Pratylenchus penetrans* from Ten Geographically Isolated Populations Based on Their Reaction on Potato

R. A. FRANCE AND B. B. BRODIE¹

Abstract: Single female cultures of *Pratylenchus penetrans* were established from soil and root samples collected from 10 geographically isolated locations in North America. The resultant isolates were used to evaluate nematode egression from and multiplication on roots of potato clones to distinguish intraspecific differences among isolates. The 10 nematode isolates were statistically separated into four groups based on percentage of nematodes that egressed from the *P. penetrans*-resistant potato clone L118-2. The Cornell (CR), Wisconsin (WI), Long Island (LI), and Adirondack (AD) isolates, selected as representative isolates of each of the four groups, exhibited 53%, 39%, 25%, and 10% egression from L118-2, respectively. Reproduction of these four isolates was measured on three potato cultivars (Russet Burbank, Butte, and Hudson) and two breeding lines (NY85 and L118-2). The LI and AD isolates reproduced well on all five potato clones. The CR isolate reproduced well on Russet Burbank and NY85 but significantly less on Butte, Hudson, and L118-2. Reproduction of the WI isolate was less than the LI and AD isolates but more than the CR isolate on all potato clones tested except Russet Burbank. Reproduction of the WI isolate on Russet Burbank was less than the other three isolates. Based on these results, four distinct intraspecific variants of *P. penetrans* are proposed: Cornell, Wisconsin, Long Island, and Adirondack.

Key words: biotype, egression, lesion nematode, pathotype, potato, *Pratylenchus penetrans*, reproduction, resistance, *Solanum tuberosum*.

The lesion nematode *Pratylenchus penetrans* (Cobb) Chitwood & Oteifa is a widespread and destructive plant-parasitic nematode, distributed chiefly in the temperate zones around the world (4,16). In North America, this species is a major pest of potato (*Solanum tuberosum* L.) in the states and provinces around the Great Lakes, the St. Lawrence river area, the northeastern states of the United States, and the Maritime provinces of Canada (19,29).

Several reports describe the amount of damage to potato caused by *P. penetrans* and its reproduction on different potato genotypes, but there have been conflicting results (2,3,5,13,21). The cultivar Hudson is reported to be resistant to *P. penetrans* by some authors (3,7,8) because it supports less nematode multiplication than other cultivars. On the other hand, this cultivar is considered to be susceptible by others (14) because it supported the highest number of *P. penetrans* among several cultivars

evaluated. Further, the cultivar Russet Burbank was reported to be tolerant of *P. penetrans* infection because of no measurable loss in yield in the presence of high nematode populations (2,15,17). However, Olthof (21,22) found Russet Burbank to suffer yield reductions ranging from 15% to 61% in the presence of *P. penetrans*.

The existence of biological races has been suggested to explain differences in *P. penetrans* reproduction on and damage to potato (3). Intraspecific differences in the reproduction of *P. penetrans* have been reported on tobacco and celery (20). France and Brodie (9) found that two isolates of *P. penetrans* from New York State differed significantly in their ability to reproduce on selected potato clones. The objective of this study was to determine the extent of variability among 10 populations of *P. penetrans* collected throughout North America.

MATERIALS AND METHODS

Nematode culturing and species determination: Single-female isolates were established from 10 populations of *P. penetrans* collected from various regions of the

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¹ Department of Plant Pathology and USDA ARS, Cornell University, Ithaca, NY 14853. Current address of first author: Estación Experimental Quilamapu, INIA, Casilla 426, Chillán, Chile.

E-mail: bbb2@cornell.edu

± northeastern United States, south-central provinces of Canada, states around the Great Lakes, and the Pacific Northwest. The code, host, and origin of the different *P. penetrans* populations are as follows. The AD population came from potato in Franklin County, New York; CR from sour cherry in Monroe County, New York; CT from strawberry in Hartford, Connecticut; LI from potato in Suffolk County, New York; ME from potato in Presque, Maine; MI from alfalfa in Montcalm, Michigan; ON from potato in Vineland, Ontario, Canada; OR from Easter lily in Brooking, Oregon; WA from apple in Prosser, Washington; and WI from potato in Madison, Wisconsin.

Nematodes were extracted from soil by centrifugal flotation and from roots by the pie-pan method (18). Individual females were collected from each sample, placed in a small BPI dish (22 mm-diam.), and disinfested with contact lens solution as previously described (9). Sterile females were transferred individually to small petri dishes (35 mm-diam.) containing alfalfa callus tissue grown on White's medium and incubated at 25 °C in continuous darkness (24). When mature nematodes were observed on the agar, a small piece of the infected callus was aseptically removed and covered with tap water. From 10 to 20 females were collected from each culture and fixed with hot formalin-glacial acetic acid (4:1) mixture for microscopic observation and species identification (25). Characters used to diagnose *P. penetrans* were the three annules in the lip region, stylet length, round spermatheca, number of lines in lateral field, and tail terminus shape (4,16). All cultures that produced either females without these characteristics or no males were eliminated. Successful cultures were transferred to larger plates (60 mm-diam.) containing alfalfa callus and subcultured approximately every 3 months. Three days before inoculation, nematodes were extracted from the callus tissue using the pie-pan method and used for inoculum.

Egression experiment: Early egression,

particularly of females, is reported to be associated with resistance in potato to *P. penetrans* (1,9). Egression of nematodes from the resistant clone L118-2 (3) was used in this experiment to discriminate among *P. penetrans* isolates. Single 15-day-old explants of the potato clone L118-2, previously determined to be resistant to *P. penetrans* (9), were transplanted into plastic containers (13.5 cm long × 4 cm-diam.) filled with sand. At transplanting, 1,000 nematodes (primarily adults collected on a screen with 53- μ m openings) of the desired *P. penetrans* isolate were added to two holes in the sand on opposite sides of the plant. The plants were placed in a growth chamber for 3 days at 24 °C with 14 hours of light (light intensity = 320 μ E/sec/m²) and watered twice daily. Afterwards, the plants were removed from their containers and their root systems washed. Each plant with root system intact was then placed in a 125-ml flask filled with 100 ml of deionized water that covered the entire root system and placed in a growth chamber at 24 °C. Individual flasks were wrapped in aluminum foil to exclude light. At 24-hour intervals for 72 hours, the water was collected from individual flasks and examined for nematodes. After 72 hours the root systems were washed, stained with acid fuchsin-lactoglycerol, and homogenized in a blender (1). The number of nematodes that remained inside the roots was determined from two 1-ml aliquots of the homogenate, and the results were averaged and extrapolated to the total volume of suspension. Percentages of egressed nematodes were calculated from the total number egressed and those that remained inside the roots.

Due to the physical limitations of handling 10 isolates in a single experiment, the isolates were arbitrarily divided into two groups for study. Group 1 included isolates from the AD, ME, OR, and WA populations, and group 2 included isolates from the CT, MI, ON, and WI populations. In both groups, isolates from the CR and LI populations were included as reference isolates (9). The experiment with

each group consisted of six isolates in a completely randomized design replicated 10 times. Each experiment was repeated once and subjected to analysis of variance. Fisher's protected LSD was used for mean separation. For comparisons among isolates evaluated in different groups, multiple chi-square tests across the two trials on 2×2 contingency tables were performed (10). The hypothesis tested was that the proportion of egressed nematodes is similar between any two isolates, and the two possible outcomes were egressed or non-egressed nematodes of each isolate.

Nematode reproduction experiment: The AD, CR, LI, and WI isolates of *P. penetrans* were selected for this experiment based on their differences in egression from potato clone L118-2. These isolates were tested for their ability to reproduce on five potato clones. These clones included the commercial cultivars Russet Burbank, which is reported both tolerant (2,15,17) and susceptible (21,22) to *P. penetrans*; Butte, which is resistant (5); and Hudson, which is reported to be both resistant (3,7,8) and susceptible (14) to *P. penetrans*. The other two clones were NY85 and L118-2 from the Cornell potato breeding program, which are susceptible and resistant, respectively, to the CR isolate of *P. penetrans* (3). Tubers of each clone were sprouted and the resulting plants used as mother plants for cuttings. Stem pieces (10 cm long) with an axillary bud were rooted to obtain plants for the experiment. The proximal end of the cuttings was dipped in a commercial formulation of auxin (Rootone) to stimulate rooting. The explants were kept for 15 days in moist vermiculite to promote root initiation before use.

Single 15-day-old explants of each clone were transplanted to 7.5-cm-diam. clay pots filled with a 1:1 mixture of sterile loamy sand (87% sand, 7.8% silt, 5.2% clay; pH in water 8.23) and a sterile medium fine white sand (98% of grains between 100–500 μm). At transplanting, a mixture of 2,000 juveniles and adults of *P. penetrans* was added around the root system and covered with the sand-soil mix-

ture. The plants were placed in a growth chamber for 30 days at a constant temperature of 24 °C with 14 hours of light (light intensity = 320 $\mu\text{E}/\text{sec}/\text{m}^2$). Pots were watered daily and fertilized weekly with a commercial solution of NPK (23-19-17).

At the conclusion of the experiment (30 days), the root systems were washed in tap water and then stained by boiling in a solution of acid fuchsin-lactoglycerol, as previously described (9). After cooling at room temperature, the stained roots were washed in acidified water and homogenized in a blender three times for 10 seconds each. This suspension was then sieved through a screen with 250- μm openings to eliminate coarse debris. Eggs, juveniles, and adults in two 1-ml aliquots were counted, and the results were averaged and extrapolated to the total volume of suspension.

The experiment was a 4×5 complete factorial, with four *P. penetrans* isolates (AD, CR, LI, and WI) and five potato clones (Russet Burbank, Butte, Hudson, NY85, and L118-2) arranged in a completely randomized design with five replications. The experiment was repeated once, and the data from each trial were subjected to analysis of variance. Fisher's protected LSD was used for mean separations.

RESULTS

Egression experiment: Based on female egression from clone L1182, four groups of *P. penetrans* were distinguishable. Group 1 included the CR and ME isolates; group 2 the WI isolate; group 3 the LI, ON, CT, MI, WA, and OR isolates; and group 4 the AD isolate. More females of the CR and ME isolates than of the other isolates egressed from the roots of potato clone L118-2, with approximately 53% of the *P. penetrans* females of these two isolates that penetrated the roots egressing within 6 days after inoculation (Fig. 1). Female egression of the WI isolate was less than the CR and ME isolates but greater than the LI, ON, CT, and MI isolates ($P < 0.05$).

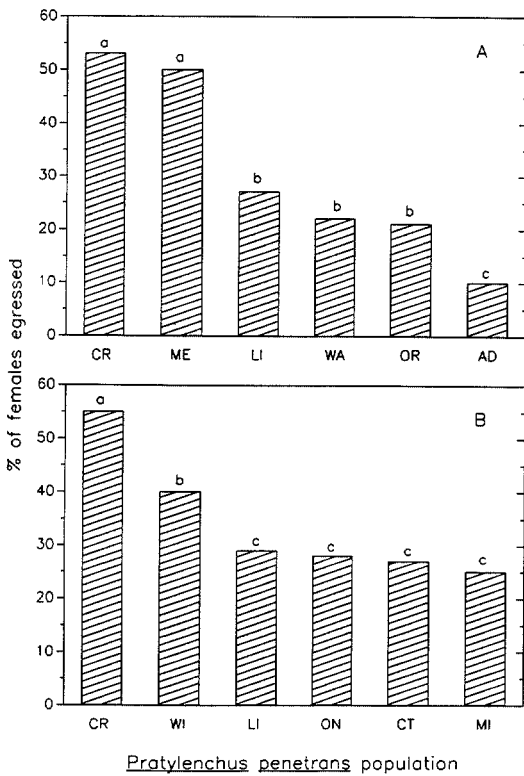


FIG. 1. A, B) Percentage of females that egressed from *Solanum tuberosum* clone L118-2 inoculated with single-female isolates from 10 different geographical populations of *Pratylenchus penetrans*. CR = Cornell, ME = Maine, LI = Long Island, WA = Washington, OR = Oregon, AD = Adirondack, WI = Wisconsin, ON = Ontario (Canada), CT = Connecticut, and MI = Michigan isolates of *P. penetrans*. Bars with different letters are significantly different ($P < 0.05$) according to the LSD test.

(Fig. 1A,B). There were no differences in the number of egressed females of the WA, OR, ON, CT, MI, and LI isolates from clone L118-2, with an average of 25% of the females of these isolates egressing within 6 days after inoculation ($P > 0.05$) (Fig. 1A,B). Fewer females of the AD isolate than of the other isolates egressed from clone L118-2, with only 10% that penetrated the roots egressing within 6 days after inoculation ($P < 0.05$) (Fig 1A).

These data were subjected to the Chi-square tests which confirmed the existence of four distinct groups based on female egression from the resistant clone L118-2 (Table 1). Based on statistical differences in female egression among isolates, these groups consisted of the CR and ME populations; the WA population; the LI, ON, CT, MI, and OR populations; and the AD population.

Reproduction experiment: Generally, the numbers of eggs were about 25% greater than the numbers of nematodes, except for CR on L118-2, Hudson, and Butte, which were similar to the numbers of nematodes on those lines. The rank ordering of the four nematode populations was similar for eggs and nematodes on the different potato clones (Fig. 2). Numbers of nematodes of the four selected isolates of *P. penetrans* were similar on potato clone NY85 but differed on the other four clones (Fig. 2A). The number of nema-

TABLE 1. Comparison of 10 geographical populations of *Pratylenchus penetrans* based on female egression over 6 days from *Solanum tuberosum* clone L118-2.

	<i>P. penetrans</i> populations ^a								
	CR	ME	WI	LI	ON	CT	MI	WA	OR
AD	*** ^b	***	***	***	***	***	***	***	***
OR	***	***	***	NS	NS	NS	NS	NS	***
WA	***	***	***	NS	NS	NS	NS		
MI	***	***	***	NS	NS	NS			
CT	***	***	***	NS	NS				
ON	***	***	**	NS					
LI	***	***	***						
WI	**	**							
ME	NS								

^a CR = Cornell, ME = Maine, WI = Wisconsin, LI = Long Island, ON = Ontario (Canada), CT = Connecticut, MI = Michigan, WA = Washington, OR = Oregon, and AD = Adirondack isolates of *P. penetrans*.

^b The number of asterisks indicates level of differences between two nematode isolates according to the chi-square test, with ** = $P < 0.01$ and *** = $P < 0.001$.

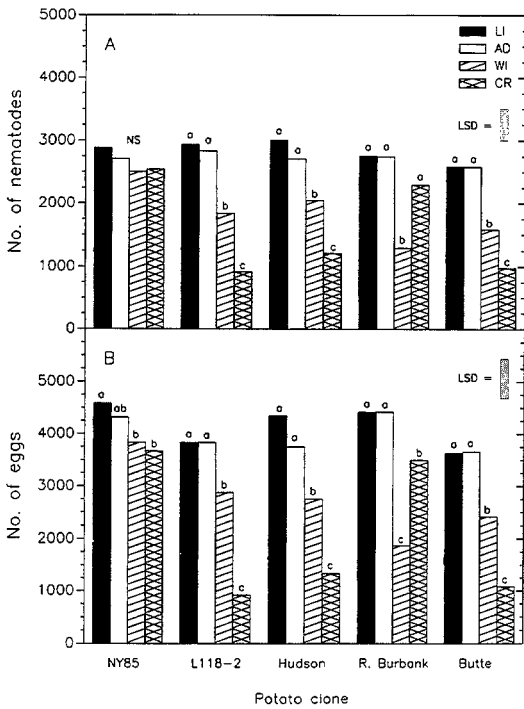


FIG. 2. Number of nematodes A) and eggs B) per root system of five *Solanum tuberosum* clones 30 days after inoculation with single-female isolates from four different geographical populations of *Pratylenchus penetrans*. LI = Long Island, AD = Adirondack, WI = Wisconsin, and CR = Cornell isolates of *P. penetrans*. Bars with different letters indicate significant differences ($P < 0.05$) among the four nematode populations on a single potato clone.

todes (adults and juveniles) of the LI and AD isolates recovered from L118-2, Hudson, and Butte was greater 30 days after inoculation than were the numbers of nematodes from the other recovered isolates ($P < 0.05$) (Fig. 2A). There was no difference in the number of nematodes of the LI, AD, and CR isolates recovered from Russet Burbank 30 days after inoculation. Fewer nematodes of the WI isolate than of other isolates were recovered from Russet Burbank ($P < 0.01$). There were no differences in the numbers of nematodes of the LI and AD isolates recovered from the five potato clones. More nematodes of the WI isolate were recovered from NY85 and Hudson than from Butte and Russet Burbank ($P < 0.05$). Numbers of nematodes of the WI isolate recovered from L118-2 were lower than from NY85 but

similar to the other clones. More nematodes of the CR isolate were recovered from NY85 and Russet Burbank than from L118-2, Hudson, and Butte ($P < 0.01$).

The number of eggs per root system was similar to the number of juveniles and adults, except for NY85 and Russet Burbank (Fig. 2B). The LI isolate produced more eggs on NY85 than did the WI and CR isolates ($P < 0.05$). The LI or AD isolates produced more eggs on Russet Burbank than did the CR and WI isolates ($P < 0.01$). The CR isolate produced more eggs on Russet Burbank than did the WI isolate ($P < 0.01$).

DISCUSSION

Pratylenchus penetrans is a migratory endoparasite that penetrates roots of resistant and susceptible potato plants in equal numbers, but resistant plants induce early egression and delay the development of those nematodes that remain inside the root (1). Consequently, we used egression of nematodes from potato roots as a measure of resistance in potato to *P. penetrans*. Because females penetrate roots earlier and in greater numbers than do males or juveniles (28), we considered female egression to be a more reliable measure of resistance than egression of other nematode stages. Previous studies using the resistant potato clone L118-2 and the CR and LI isolates of *P. penetrans* confirmed this behavior (9).

Our experiments separated 10 geographical populations of *P. penetrans* into four different behavioral groups based on significant differences in female egression of single-female isolates of these populations from potato clone L118-2. The LI, AD, WI, and CR isolates were selected as representative of each of these four groups. However, differences in reproduction of these four isolates on the resistant potato clone L118-2 differentiated only three groups of *P. penetrans* represented by the LI, WI, and CR isolates. For example, the AD isolate egressed less than

other isolates from clone L118-2, but it reproduced more on L118-2 than all other isolates except the LI isolate. These results indicate that early egression and reproduction of *P. penetrans* on this resistant potato clone are not always correlated. Although it is uncertain how representative the single-female isolates are of natural field population, the probability of selecting a frequent genotype when establishing a single female culture is greater than selecting a rare one.

Pratylenchus penetrans is distributed worldwide and is widely disseminated by infested nursery stocks (27). Siddiqui et al. (26) found that *P. penetrans* was the most frequently intercepted species from interstate shipments of plants into California over a 20-year period. The ability of this nematode to spread freely by infected plant tissues could explain the similarity in early egression from clone L118-2 that a majority of our geographically isolated populations exhibited.

The CR population has been completely isolated for the last 30 years by continuous subculturing on sterile alfalfa callus (9). The optimum conditions and the minimal selection pressure experienced by this isolate in tissue culture could have reduced its aggressiveness. The AD isolate originated from soil collected from a farm in Franklin County, New York, that has been used traditionally for seed potato production. Differences associated with this *P. penetrans* isolate could be a result of its long-term association with potato. However, the effects of long-term isolation of *P. penetrans* on development of distinctive variants remains to be investigated.

The WI isolate was from potato and was reared on sterile corn roots for the last 8 years (17). This isolate was intermediate to the LI and CR isolates in egression from L118-2 and reproduction on all clones except Russet Burbank, which was the least suitable clone for this isolate. Studies in Wisconsin (17) indicated that this isolate does not affect the yield of Russet Burbank; a different *P. penetrans* population in Ontario, Canada, reduced the yield of

Russet Burbank (21,22), which supports our findings that some geographical isolates of *P. penetrans* behave differently.

The occurrence of intraspecific variability in other species of *Pratylenchus* indicates that parasitic differences may exist in local populations as well as geographically isolated populations. Griffin (11) reported that a *P. neglectus* isolate from Utah behaved more aggressively on alfalfa than three other isolates collected in Utah and Wyoming. Studies with *P. vulnus* populations collected in different countries demonstrated that isolates differed in parasitic fitness and damage to peach-almond hybrid and apple rootstocks (23). Also, Olthof (20) reported variation in host suitability for *P. penetrans* isolates collected from different hosts in Ontario, Canada. However, Griffin (12) did not find differences in virulence or reproduction on alfalfa among four isolates of *P. penetrans* collected in Utah and Colorado, suggesting that intraspecific variation in *Pratylenchus* spp. is not a universal phenomenon.

In a previous study, we differentiated two populations of *P. penetrans* on the basis of their egression and reproduction and proposed that they be referred to as the Cornell and Long Island pathotypes (9). Dropkin (6) defined pathotype as a nematode population whose members have similar parasitic ability on a host with resistance to other populations. Accordingly, we propose that the WI population is one additional pathotype. Although the isolate of the AD population differed from the isolates of other populations in egression from clone L118-2, it does not fit Dropkin's description of a pathotype. We evaluated the reproduction of these isolates on only five potato clones. The probability of detecting variability in parasitism increases with the number of differential hosts evaluated. If more differential hosts are used, the AD population could possibly be identified as a different pathotype.

The difficulty in detecting intraspecific differences and providing terms to characterize them is complicated, in part, because of the narrow characters used for measur-

ing variability. The use of DNA analysis should provide a more reliable measure of variability than biological behavior. However, our studies of parasitic abilities clearly indicate the existence of intraspecific variants of *P. penetrans*.

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