Journal of Nematology 27(3):339–345. 1995.


# Differentiation of Two New York Isolates of *Pratylenchus penetrans* Based on Their Reaction on Potato

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*Abstract:* The behavior of two isolates of *Pratylenchus penetrans* on six potato clones was assessed to test the hypothesis that these nematode isolates from New York were different. Four potato cultivars (Superior, Russet Burbank, Butte, and Hudson) and two breeding lines (NY85 and L118-2) were inoculated with nematode isolates designated Cornell (CR) and Long Island (LI). Population increase and egression of nematodes from roots were used to distinguish resistance and susceptibility of the potato clones. Based on numbers of eggs, juveniles, and adults in their roots 30 days after inoculation, potato clones Butte, Hudson, and L118-2 were designated resistant to the CR isolate and susceptible to the LI isolate. More eggs were found in the roots of all plants inoculated with the LI isolate than with the CR isolate. The clones NY85 and L118-2 were inoculated with the CR and LI isolates in a 2 × 2 factorial experiment to assess differences in nematode egression. Egression was measured, beginning 3 days after inoculation, for 12 days. The rates of egression were similar for the four treatments and fit linear regression models, but differences were detected in numbers of egressed nematodes. More nematodes of the CR isolate than the LI isolate egressed from L118-2. Differences in egression of females was particularly significant and can be used as an alternative or supplement to reproduction tests to assess resistance in potato to *P. penetrans* and to distinguish variation in virulence.

*Key words:* egression, lesion nematode, nematode, pathotypes, potato, *Pratylenchus penetrans,* resistance, *Solanum tuberosum.*

*Pratylenchus penetrans* (Cobb) Chitwood and Oteifa is one of the most important and frequently encountered plant-pathogenic nematodes in northeastern North America (15,27). It is an important pest of potato (*Solanum tuberosum* L.), producing extensive lesions on roots, rhizomes, and tubers (3). Economic losses between 15 to 73% of marketable tubers have been reported (2,11,17,18). Furthermore, the presence of *P. penetrans* can enhance infection by *Verticillium dahaliae* in the potato early dying disease (13,14,22). This disease is one of the most important limiting factors in potato production (19).

Some potato cultivars are resistant and (or) tolerant to *P. penetrans* (2,4,6,8,9,17, 18). However, there is conflicting evidence concerning the degree of resistance or susceptibility of some cultivars (4,8,12). In two studies, the cv. Hudson supported fewer nematodes than the cv. Katahdin (8,9), suggesting that Kathahin was the more suitable host of the two cultivars for *P. penetrans.* In contrast, experiments on Long Island, New York, showed that as many or more *P. penetrans* developed on Hudson as on Katahdin and the highly susceptible cv. Superior, indicating that Hudson was equally as suitable as Katahdin to *P. penetrans* (12). More recently, a study evaluating resistance in potato to *P. penetrans* showed that fewer *P. penetrans* developed on Hudson than on known susceptible cultivars (4).

The objective of this study was to determine the differences in reproduction of two geographically isolated New York populations of *P. penetrans* on potato. Because early egression was recently identified as one of the principal mechanisms of resistance in potato to *P. penetrans* (1), differences in egression from resistant and susceptible potato clones inoculated with these two isolates were also assessed.

## Materials and Methods

*Nematode origin:* Two isolates of *P. penetrans* were used in these studies and were designated the Cornell (CR) and Long Island (LI) isolates. The CR isolate originated from a single female collected from a sour cherry orchard in Monroe County,

Received for publication 29 August 1994.

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New York. This isolate has been reared on alfalfa callus culture at Cornell University since 1963 (W. F. Mai, pers. comm.). The LI isolate originated from several females collected in 1992 from a potato field on Long Island, New York.

*Sterilizing nematodes:* We developed a simple technique to sterilize *P. penetrans.* Briefly, nematodes extracted from soil were transferred to a small BPI dish (22-mm-d) filled with sterile tap water and left for 15 minutes. The nematodes were then transferred under sterile conditions with a pipet and a minimum amount of water to a different BPI dish. Approximately 1 ml of Bausch and Lomb Disinfecting Solution with thimerosal (for soft contact lenses) was added to the dish and left for 15 minutes. The nematodes were then transferred to another BPI dish with fresh Bausch and Lomb solution and left for another 15 minutes. Then, gravid females were individually transferred, with a nematode pick, to petri dishes containing sterile alfalfa callus growing on White's medium (20). After the nematodes were transferred to the alfalfa callus culture, they recovered their motility completely as evidenced by their ability to find and penetrate roots.

Three days before each experiment, nematodes were extracted from the callus tissue using the pie-pan method (12). The resultant suspensions were counted and used for inoculum.

*Potato clones:* Six potato clones were used. Cultivars Superior and Russet Burbank are susceptible to *P. penetrans* (2), Butte is resistant to *P. penetrans* (and *P. neglectus* (Rensch) Filipjev and Schuurmans Stekhoven) (6), and Hudson has been reported to be both resistant (4,8,9) and susceptible (12) to *P. penetrans.* Two lines from the Cornell potato breeding program, NY 85 and L118-2, are susceptible and resistant, respectively, to the CR isolate of *P. penetrans* (4).

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 experiments. The proximal end of the cuttings was dipped in a commercial formulation of auxins (Rootone) to stimulate rooting. The explants were kept for 15 days in wet vermiculite to promote root initiation.

*Nematode reproduction experiment:* Single potato explants were transplanted into 7.5-cm-d clay pots filled with a 1:1 mixture of sterile loamy sand (87% sand, 7.8% silt, 5.2% clay, pH in water—7.2) 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* were placed around the root system and covered with the sand-soil mixture. The plants were then placed in a growth chamber for 30 days at a constant temperature of 24 C with 14 hours of light (light intensity = 320 $\mu E/sec/m^2$). 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 with tap water and then stained by boiling in a solution of acid fuchsin-lactoglycerol (0.1 g acid fuchsin, 200 ml lactic acid, 200 ml glycerol, and 200 ml distilled water). After cooling at room temperature, the stained roots were washed with acidified water (150 ml distilled water and 0.1 ml hydrochloric acid) and homogenized in a blender at high speed three times for 10 seconds each (1). This suspension was then sieved through a screen with 250-μm openings to eliminate coarse debris. The numbers of eggs, juveniles, and adults were counted from three 1-ml aliquots and the results averaged and extrapolated to the total volume of the suspension.

The experimental treatments, replicated six times, were randomly arranged in a 2 × 6 complete factorial with two *P. penetrans* populations (CR and LI) and six potato clones (Superior, Russet Burbank, Butte, Hudson, NY85, and L118-2). 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.

*Egression experiments:* The methodology described by Arévalo (1) was used to study nematode egression from potato lines NY85 and L118-2. Single 15-day-old explants were transplanted into cone-shaped plastic containers (13.5 cm long × 4 cm-d) filled with sand (98% of grains between 100–500 μm). At transplanting, a suspension containing 1,000 juveniles and adults of *P. penetrans* was added to two depressions in the sand surface on opposite sides of the plant. Inoculated plants were placed in a growth chamber for 3 days. The plants were then removed from their containers and the root systems washed with tap water. Each plant was then placed in a 125-ml flask filled with 100 ml of deionized water that covered the entire root system. The flasks were placed in a growth chamber. At 24-hour intervals for 12 days, the water was collected from each flask and replaced with fresh water. The suspensions collected from individual flasks for three consecutive days were combined and examined for nematodes. Juveniles, males, and females were counted from a 5-ml subsample of each sample. On day 12, the root systems were stained and the number of nematodes that remained inside the roots were counted. Percentages of egressed nematodes were calculated based on the number of egressed plus those that remained inside the roots.

The experiment, performed twice, was a 2 × 2 factorial (two *P. penetrans* isolates [CR and LI] and two potato clones [NY85 and L118-2]) arranged in a completely randomized design with 10 replications. Regression analysis was applied to each clone × nematode isolate combination. Homogeneity of regression coefficients was tested to compare the regression lines among treatments. In addition, mean comparisons were made for the intercept value of each regression using the LSD test (10).

In order to increase accuracy in measuring the number of egressed adults, a second experiment was performed using only potato clone L118-2 and two *P. penetrans* isolates (CR and LI). The proportion of adults in the inoculum was increased by passing the nematode suspension three times through a sieve with 53-μm openings. Rooted cuttings of L118-2 were transplanted, inoculated, and incubated as described for the first test. Egressed nematodes were collected 1, 3, and 5 days after inoculation and then counted and recorded as male or female. The percentage of egressed nematodes was determined as before. The experiment was arranged in a completely randomized design with 10 replications. The experiment was repeated once and an analysis of multiobservation data was performed (10). Mean separation for each day was performed using the LSD test.

## Results

*Nematode reproduction experiment:* Thirty days after inoculation, a statistical interaction was detected between *P. penetrans* isolate and potato clone. When inoculated with the CR isolate, the potato clones Superior, Russet Burbank, and NY85 had higher numbers of nematodes ($P < 0.01$) in their roots than did Hudson, Butte, and L118-2. All the potato clones inoculated with the LI isolate had similar ($P > 0.05$) numbers of nematodes per root system (Fig. 1A). The LI isolate reproduced more than the CR isolate on all clones, but nematode numbers were different ($P < 0.01$) only for Hudson, Butte, and L118-2. These three potato clones supported an average of 2.5 times more nematodes of the LI isolate than of the CR isolate.

The number of eggs per root system followed a trend similar to that of the number of juveniles and adults. The number of eggs per root system of all potato clones was higher ($P < 0.05$) for the LI isolate than for the CR isolate (Fig. 1B). There were no differences ($P > 0.05$) in the number of eggs per root system among clones inoculated with the LI isolate. The potato clones Superior, Russet Burbank, and NY85 supported more ($P < 0.01$) egg production of the CR isolate than did Hudson, Butte, and L118-2.

*Egression experiments:* The two isolates of

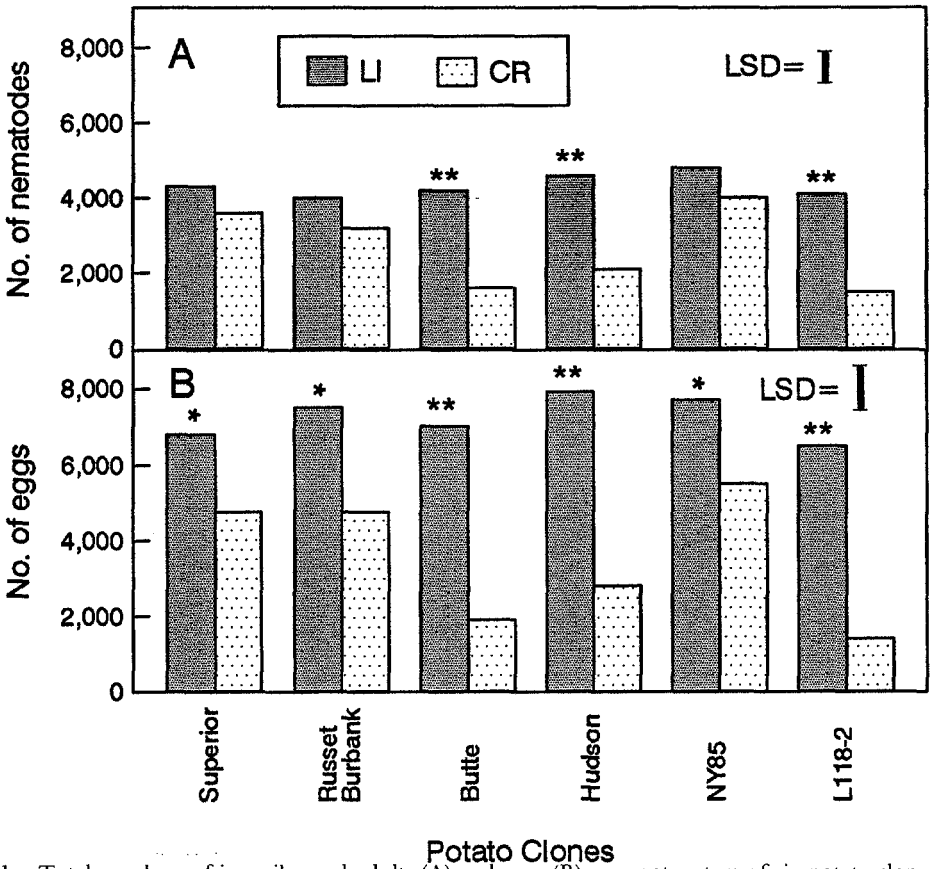


FIG. 1. Total numbers of juveniles and adults (A) and eggs (B) per root system of six potato clones at 30 days after inoculation with two different isolates of *Pratylenchus penetrans* (CR = Cornell isolate and LI = Long Island isolate). LSD ($P < 0.05$) is for comparisons of the same nematode isolate among potato clones. Number of asterisks indicates level of differences between the two nematode isolates on a single clone with * = $P < 0.05$ and ** = $P < 0.01$.

*P. penetrans* differed in the number of nematodes that egressed from the roots of potato clones L118-2 (resistant) and NY85 (susceptible). The regression equations for juvenile and male egression (data not shown) were similar to those of female egression; all regression models were linear and had high $R^2$ values (Fig. 2). Furthermore, the slopes of the regression lines were similar for all treatments, indicating that the rate of egression was similar for both clones. However, intercepts were different, suggesting that differences in egression occurred within the first 24 hours (Table 1). Female egression was affected more by the clones than were males or juveniles. A higher number ($P < 0.001$) of females of the CR isolate egressed from clone L118-2 than from NY95 during the first 24 hours of evaluation. There were no differences ($P > 0.05$) in the number of nematodes of the LI isolate that egressed from the two potato clones (Fig. 2).

In the second egression experiment, only the resistant potato clone L118-2 was used and most of the inoculum consisted of adults (76%) with the remaining inoculum consisting mainly of fourth-stage juveniles. Large differences were detected in the number of adults that egressed. More males (Fig. 3A) and females (Fig. 3B) of the CR isolate than of the LI isolate egressed ($P < 0.01$) at all evaluation days. Twice as many CR females as LI females egressed at all three dates (Fig. 3B).

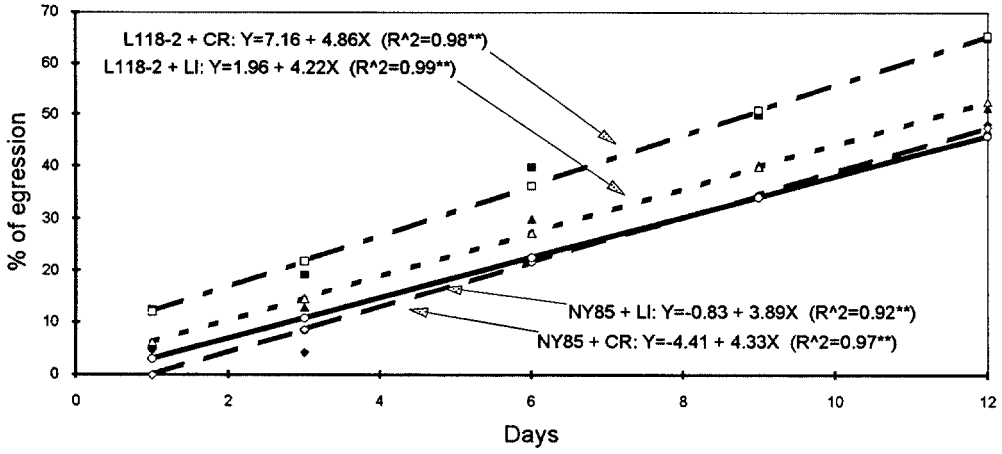


FIG. 2. Percentage of females that egressed from potato clones L118-2 and NY85 inoculated with a Cornell (CR) or Long Island (LI) isolate of *Pratylenchus penetrans*. The regression lines are predicted values of the treatment means; each point represents the average of 10 observations.

## DISCUSSION

*Pratylenchus penetrans* is a highly polyphagous nematode, with approximately 350 hosts in the temperate zone around the world (5). Despite its wide distribution and numerous hosts, little is known about its intraspecific variability. The first suggestion of races of *P. penetrans* was made by Slootweg (23) in 1956. Later, Olthof (16) identified two races based on host suitability and pathogenicity on tobacco and celery. Morphological characteristics, based on the tail terminus of females, were used by Townshend et al. (28) for identification of two pathotypes. Also, environmental factors and host plants influence quantitative and qualitative characteristics in *P. penetrans* (24,26).

Our results, based on reproduction of *P. penetrans* and its egression from roots of different potato clones, indicate the existence of intraspecific differences in two geographical isolates of this nematode species in New York. Furthermore, these results clarify earlier conflicting reports concerning the host status of the cultivar Hudson (4,8,9,12) for *P. penetrans*. Hudson was first reported as resistant to *P. penetrans* in 1973 (8). In our studies, we used the same isolate of *P. penetrans* that was used in the 1973 study and concluded that after 20 years this isolate still reproduces poorly on the cultivar Hudson. We also confirmed that our Long Island isolate, collected from the same area of Long Island as the one used by Kotcon et al. (12), reproduces well on Hudson. The cultivars Hudson

TABLE 1. Slopes (S) and intercept (I) values for predicted regression lines for juveniles, males, and females of *Pratylenchus penetrans* that egressed from roots of potato clones L118-2 and NY85 inoculated with Cornell (CR) or Long Island (LI) isolate.

| | | Juveniles | | Males | | Females | |
|---|---|---|---|---|---|---|---|
| Isolate | Potato clone | S | I | S | I | S | I |
| CR | L118-2 | 5.04 | 10.66 | 5.74 | 13.94 | 4.86 | 7.16 |
| CR | NY85 | 4.14 | 3.97 | 4.90 | 2.74 | 4.33 | −4.41 |
| LI | L118-2 | 4.65 | 9.99 | 5.12 | 11.15 | 4.22 | 1.96 |
| LI | NY85 | 4.26 | 4.69 | 5.49 | 4.06 | 3.89 | −0.83 |
| LSD ($P < 0.05$) | | NS | 5.53 | NS | 8.38 | NS | 4.49 |
| P-value | | 0.31 | 0.03 | 0.41 | 0.02 | 0.15 | <0.01 |

and Superior supported similar numbers of the LI *P. penetrans* isolate, thus also confirming the results of Kotcon et al. (12). However, more nematode isolates or populations need to be tested before it can be definitely concluded that all populations of *P. penetrans* on Long Island are similar in behavior.

The egression experiments showed that the rate of egression of the two *P. penetrans* isolates from clones L118-2 and NY85 was similar and probably reflects the normal mobility of this species (5,15). However, differences in the numbers of nematodes that egressed, particularly differences in the number of females, occurred soon after root penetration by the different nematode stages. Apparently, females egress sooner because they usually penetrate roots earlier and in greater numbers than males and juveniles (25). In addition, females may be more sensitive to host status because, without a suitable food source, they stop laying eggs (15).

The resistance in clone L118-2 results in early egression of nematodes from the roots. Arévalo (1) concluded that egression of nematodes from roots is one of the mechanisms of resistance, probably stimulated by penetration, in clone L118-2 against *P. penetrans*. The factor(s) that induce early egression in clone L118-2 is (are) unknown, but the LI isolate either does not activate it or is able to overcome the process involved.

Egression experiments are simple and would be appropriate for screening either a large number of potato clones or different *P. penetrans* isolates against specific clones. The percentage of egressed females was a reliable measure for identifying the resistance and susceptibility of given clones and for assessing the relative aggressiveness of the two isolates. It is comparable to measurements based on reproduction capability of the nematode, and also requires less space and time.

Some caution should be exercised in screening potato germplasm for resistance to *P. penetrans*. The resistance mechanisms in clone L118-2 are known to some extent (1), but it is possible that other mechanisms may be operating in resistant germplasm. Combinations of different sources of resistance may eventually be used to produce more durable resistance that would enable potato clones to have more defense against different pathotypes of *P. penetrans*.

Several methods for sterilizing plant nematodes for axenic culture have been described (21). However, the commercial disinfecting solution we used proved to be a simple and effective method for sterilizing *P. penetrans*. Nematodes of different stages survived treatment up to 30 minutes or more in the disinfecting solution without apparent damage.

The possibility exists that the CR isolate may have changed after 30 years in axenic culture due to its complete isolation and minimal selection pressures. Intraspecific differences can arise in response to isolation and selection pressures (7). The LI isolate, on the other hand, could have be-

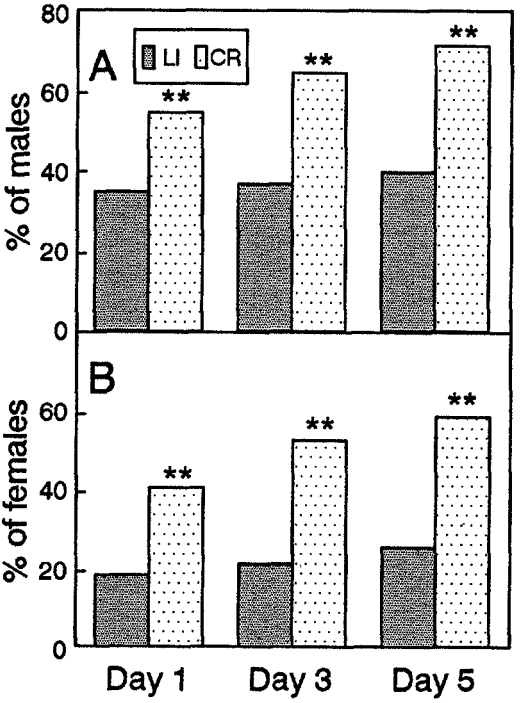


FIG. 3. Percentage of males (A) and females (B) egressing from clone L118-2 inoculated with a Cornell (CR) or Long Island (LI) isolate of *Pratylenchus penetrans*. ** = Significant differences ($P < 0.01$) according to the LSD test.

come more aggressive because it had to adapt to an intensive cropping system. Many experiments have been conducted with the CR isolate in different laboratories since 1963. Our results emphasize the need to evaluate more isolates and to further elucidate the degree of aggressiveness of the CR and LI isolates.

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