JOURNAL OF NEMATOLOGY

VOLUME 20 JULY 1988 NUMBER 3

Journal of Nematology 20(3):335–339. 1988.

Effects of Host Resistance on Second-stage Juveniles and Adult Males of *Globodera rostochiensis*[1]

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Abstract: Potato cultivars Katahdin (susceptible) and Rosa (resistant) were exposed to infective second-stage juveniles (J2) of *Globodera rostochiensis* for varying periods of time, after which root systems were washed and plants were placed in Hoagland's solution to assess J2 egression and male emergence. After transfer to liquid culture, many J2 egressed from both cultivars, but significantly more egressed from the resistant Rosa than from Katahdin. Juveniles that egressed from Rosa invaded a second host, resistant or susceptible, in significantly fewer numbers than did juveniles that egressed from Katahdin. Also, significantly fewer males developed in and emerged from resistant host roots, relative to susceptible ones. These effects of resistance may be an important component of the tolerance to invasion by *G. rostochiensis* exhibited by Rosa.

Key words: Globodera rostochiensis, host resistance, male development, juvenile invasion, potato, *Solanum tuberosum.*

In the United States, only Race R_1A of *Globodera rostochiensis* is known to occur. Control of this pathogen relies heavily on the use of resistance conditioned by a single gene, H_1, derived from *Solanum tuberosum* ssp. *andigena.* Despite 7 or more years of continuous culture of resistant potato cultivars on infested land, resistance-breaking races have not been detected (1,3) and thus the population is classified as Race R_1A. The internal response of resistant potatoes to invasion by *G. rostochiensis* was studied (4), but other mechanisms of resistance to the New York population of *G. rostochiensis* have not been identified. Nematodes may invade and leave a resistant host, but tend to remain in a susceptible host (2,7). In Scotland, more second-stage juveniles (J2) of potato-cyst nematodes (*Globodera* spp.) were reported to exit from resistant host roots than from susceptible ones, and smaller densities of *Globodera rostochiensis* (Woll.) Behrens adult males were found in resistant than susceptible potato (*Solanum tuberosum* L.) cultivars (2). A series of experiments was conducted, using susceptible and resistant potato cultivars and one population of *G. rostochiensis* from Steuben County, New York to obtain more information on possible mechanisms of expression of this resistance on J2 invasion and male density. The results of these experiments are reported here.

MATERIALS AND METHODS

The influence of the hosts on J2 egression and adult male density was determined using *Solanum tuberosum* ssp. *tuberosum* cvs. Katahdin and Rosa. Rosa bears the H_1 gene, derived from *Solanum tuberosum* ssp. *andigena.* Additionally, one-half of Rosa's pedigree consists of South American cultivated tetraploid potatoes, and one-quarter of its genome was derived from Katahdin. Rosa bears no other major resistance genes, as determined by progeny tests (R. L. Plaisted, pers. comm.). Tubers of Katahdin and Rosa were sprouted in the greenhouse at 20–25 C until shoots were 1–2 cm long. Tuber pieces, each 2 cm d and containing a single shoot, were dusted

Received for publication 9 March 1987.

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with the fungicide maneb and planted in 7.5-cm-d clay pots containing a 1:1 mixture of pasteurized loam and sand. The pots were placed in a greenhouse with a 16-hour photoperiod maintained at 20–25 C. To obtain inoculum, several thousand cysts were placed in potato root diffusate (PRD) that had been collected in advance and frozen until needed. Eggs were allowed to hatch from cysts over a 3-week period. Plants were inoculated 3 weeks after planting when they were ca. 5 cm tall. Inoculum densities of 4,200, 1,100, and 925 J2 per plant were used in experiments 1, 2, and 3, respectively, and were applied in three 1-ml aliquots via syringe into three depressions made in the soil about the stem.

Root systems were washed 24 hours (exp. 1, 2), or 16 days (exp. 3) after inoculation to remove soil and nematodes. Root systems of 20, 10, or 5 plants of each cultivar from the respective experiments were stained with acid fuchsin in hot lactophenol at that time. The remaining plants (20, 20, 14 replicates of both cultivars in exp. 1, 2, 3, respectively) were then transferred to 236-ml plastic containers containing full strength (exp. 1) or half strength (exp. 2, 3) Hoagland's nutrient solution. Plants were elevated on hardware cloth placed in the bottom of the container, and stems were supported by a square of black plastic fitted around the mouth of the container.

The solution in each container was removed daily for 28 days (exp. 1) or 14 days (exp. 2), beginning 24 hours after transfer to liquid culture. In experiment 3, the solution was removed at 4-day intervals over a 28-day period beginning at day 19. Plants were lifted out of the containers, and the solution was poured through nested 150-μm over 25-μm-pore sieves. Containers were rinsed twice, and the rinse solution was also passed through the sieves. Nematodes were collected from the 25-μm-pore sieve.

To determine the ability of egressed J2 to infect a second host, a 2 × 2 factorial design experiment was prepared with four treatments as follows: 1) J2 egressed from a susceptible host reinoculated to a susceptible host (S/S); 2) J2 from a susceptible host reinoculated to a resistant host (S/R); 3) J2 from a resistant host reinoculated to susceptible host (R/S); and 4) J2 from a resistant host reinoculated to a resistant host (R/R). Numbers of J2 that penetrated the second hosts were determined.

Resistant and susceptible plants were grown and inoculated (ca. 2,000 J2/plant) as in previous experiments. An additional 10 plants of each cultivar were grown to serve as second hosts. Twenty-four hours after inoculation of the first hosts, root systems were washed and plants were transferred to liquid culture as before. Egressed J2 were collected via sieving the culture solutions 3 days later. Active nematodes were separated from inactive ones by allowing them to migrate through a filter for 24 hours. Suspensions of ca. 175 active J2 from the susceptible or resistant hosts were applied around the stem of each of five susceptible or resistant second hosts. One week later, plants were harvested and a portion of each root system was stained with acid fuchsin in hot lactophenol. The numbers of juveniles in the roots were determined with the aid of a dissecting microscope.

Results and Discussion

The numbers of J2 that invaded the two cultivars did not differ significantly ($P = 0.05$) within any experiment (data not presented). Significantly ($P < 0.01$) more J2 egressed from the resistant Rosa than from the susceptible Katahdin (Table 1). Egression of J2 from both cultivars in both experiments was initially high, followed by a rapid decline (Figs. 1, 2). J2 egressed for the duration of both experiments, but their numbers differed significantly between cultivars only during the first 4 days of an experiment.

In experiment 1, 29% of the inoculum egressed from Rosa during the first 14 days of collection, whereas 45% egressed during the same period in experiment 2. In contrast, egression from Katahdin during this period was about 20% of the inoculum used in each experiment.

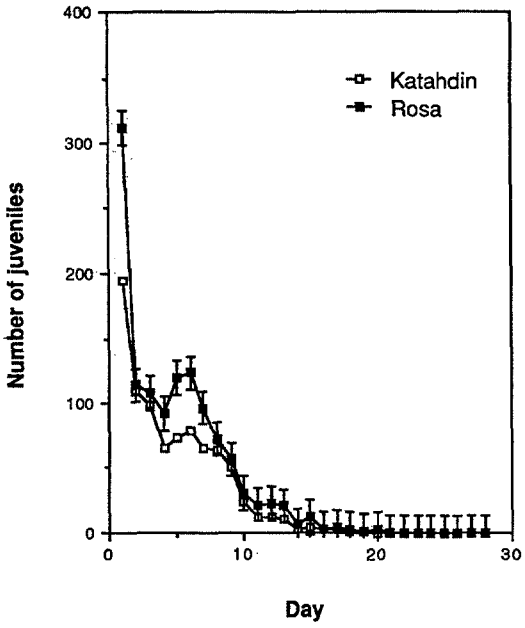

FIG. 1. Mean daily egression of *Globodera rostochiensis* juveniles from susceptible (Katahdin) or resistant (Rosa) plants for 28 days after inoculation with 4,200 juveniles/plant. Bars represent standard errors.

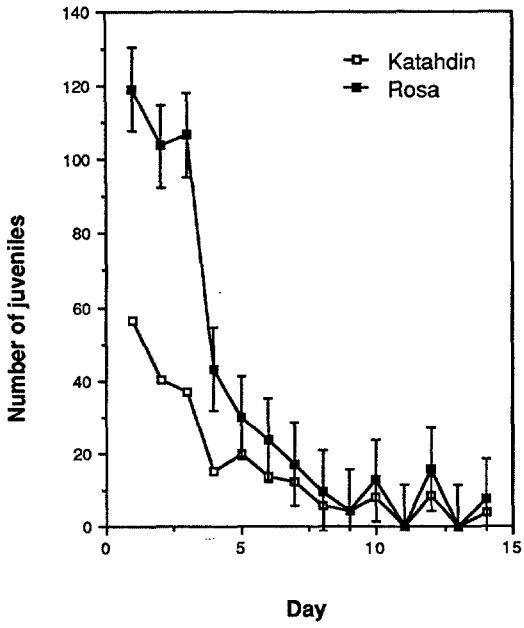

FIG. 2. Mean daily egression of *G. rostochiensis* juveniles from susceptible (Katahdin) and resistant (Rosa) plants for 14 days after inoculation with 1,100 juveniles/plant. Bars represent standard errors.

In all the experiments, adult males began to emerge from host roots about 15 days after inoculation. Significantly (P = 0.01) fewer males developed and emerged from Rosa than from Katahdin (Table 1). Few males emerged from Rosa in either experiment and emergence of males from Katahdin was variable. Male emergence from Katahdin peaked twice in experiment 1 (Fig. 2), whereas male emergence increased, peaked, and declined from Katahdin in experiment 3 (Fig. 3).

An equal number (6–8%) of J2 that egressed from the susceptible host Katahdin penetrated into a second resistant or susceptible host (P = 0.05); however, significantly (P = 0.01) fewer (1.5%) J2 that egressed from the resistant host Rosa, penetrated the second host (Table 2). A two-way analysis of variance (ANOVA) indicated a highly significant effect of the first host on the penetration of J2 into a second host (Table 3). The second host had no significant influence on J2 penetration, and there was no significant interaction of first host–second host combinations.

TABLE 1. Mean numbers of *Globodera rostochiensis* juveniles (J2) that egressed and males that emerged per plant from potato cultivars Katahdin (susceptible) and Rosa (resistant).

Experiment	Inoculum density (J2)	Exposure (collection)†	J2‡		Males§	
			Katahdin	Rosa	Katahdin	Rosa
1	4,200	1 (28)	872.5**	1,225.6	16.35**	0.75
2	1,100	1 (14)	224.0**	493.0		
3	975	16 (28)			5.25**	0.64

** Significant (P = 0.01) difference between Katahdin and Rosa.
† Number of days of exposure of plants to inoculum and number of days over which nematodes were collected from host roots (in parentheses).
‡ Average of 20 replications.
§ Average of 14 replications.

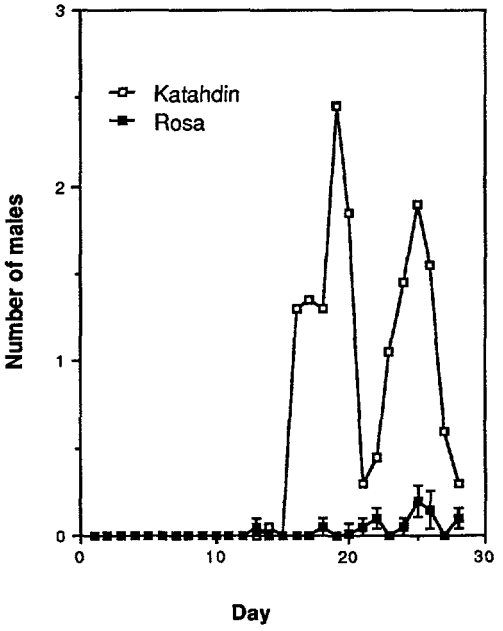

FIG. 3. Mean daily emergence of *G. rostochiensis* males from susceptible (Katahdin) and resistant (Rosa) plants for 28 days after inoculation with 4,200 juveniles/plant. Bars represent standard errors.

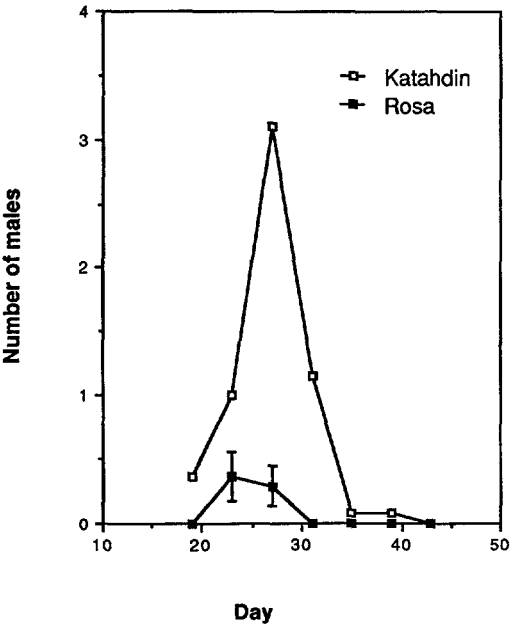

FIG. 4. Mean emergence of *G. rostochiensis* males from susceptible (Katahdin) and resistant (Rosa) plants at 4-day intervals beginning 19 days after inoculation with 925 juveniles/plant. Bars represent standard errors.

These data confirm the report of Forrest et al. (2) that host resistance influences the number of *G. rostochiensis* J2 egressing from potato roots soon after invasion. In our experiments, many J2 also egressed from susceptible host roots, suggesting that resistance is not the sole factor inducing egression. The cultivar Rosa might have been recognized by J2 as being unsuitable immediately following invasion, whereupon many J2 egressed; however, J2 that egressed from the resistant host invaded a second host, susceptible or resistant, less readily.

These experiments and those reported by Forrest et al. (2) indicate that resistance to *G. rostochiensis* associated with the H_1 gene suppresses male development. The high egression of J2 from the resistant cultivar Rosa reduced the total numbers of nematodes left in the roots; however, limited male development probably was not the result of J2 egression alone. The authors did not determine the numbers of females that developed in Rosa in these experiments, and hence sex ratios could

TABLE 2. Mean number of *Globodera rostochiensis* juveniles that penetrated into a second susceptible (S = Katahdin) or resistant (R = Rosa) host following egression.

Egressed from	Penetrated into	Symbol	Penetration per plant
Katahdin	Katahdin	S/S	10.714 A
Katahdin	Rosa	S/R	15.567 A
Rosa	Katahdin	R/S	2.561 B
Rosa	Rosa	R/R	2.730 B

Numbers followed by a different letter are significantly different at $P = 0.05$.

TABLE 3. Two-way analysis of variance of mean penetration of *Globodera rostochiensis* juveniles that egressed from a susceptible or resistant first host and penetrated a susceptible or resistant second host.

Source	df	SS	*F*
First host (emerged from)	1	550.8	11.64**
Second host (penetrated into)	1	31.5	0.67
Interaction	1	27.4	0.58
Error	16	757.6	
Total	19	1,367.3	

** Significant at $P < 0.01$.

not be calculated. Other experiments repeatedly show zero females developing in 80% of Rosa plants; 1–5 females may develop in the remaining 20% (Brodie, unpubl.). Resistance in Rosa is active against nematodes of either sex but may be less effective against males than females.

Absence of damage by *G. rostochiensis* has been associated with resistance conferred by the H_1 gene (5,6). Rosa yielded more than Katahdin under high densities of *G. rostochiensis* (5). Egression of J2 from resistant host roots may be a significant component of the mechanism for tolerance of potato to *G. rostochiensis*. If most J2 left host roots shortly after invasion, less damage would ensue than if most remained in the plant and began to feed, inducing the necrotic response.

Widespread use of a single source of resistance, when expressed as a single mode of action in a pathogen control program favors the selection of new races able to overcome the resistance. Despite the use of resistant potato cultivars with the H_1 gene for many years in infested research plots and in farmers' fields in New York, different races of *G. rostochiensis* have not been detected. These experiments imply that the H_1 gene may be simultaneously expressed in several ways, which might serve as an important factor in the apparent lack of development of new races in the United States population of *G. rostochiensis*. We suggest that single-gene resistance expressed as multiple mechanisms has essentially the same effect as that of polygenic resistance.

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