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Relationship between Root Growth of Potato, Root Diffusate Production, and Hatching of *Globodera rostochiensis*[1]

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Abstract: Hatching response of *Globodera rostochiensis* in potato root diffusate (PRD) collected by soaking individual potato, *Solanum tuberosum,* root systems in water for 2 hours was used to assess the relationship between root growth and PRD production. Resistant potato cultivars Hudson and Rosa were used as test plants. Maximum hatch occurred in PRD collected 3 weeks after plant emergence (AE) in the greenhouse, and declined after this time. Hatch was positively correlated with increased root weight only during the first 3 weeks AE. Hudson PRD was consistently more active than Rosa PRD in stimulating hatch, except when adjusted for root weight. Although the results indicated that cells at the root tip produced a more active PRD than cells located elsewhere, PRD appeared to be produced along the entire root. Differences in time length of the vegetative growth phase, extent of root growth, and volume of roots, rather than the production of a more active PRD per se, may explain why Hudson is more effective than Rosa in reducing *G. rostochiensis* population densities in soil.

Key words: hatch, *Globodera rostochiensis,* golden nematode, potato root growth, root diffusate, *Solanum tuberosum,* potato.

Although 17–53% of *Globodera rostochiensis* Behrens eggs may hatch spontaneously (5,14), they hatch primarily in response to an unidentified factor present in potato root diffusate (PRD) (7,19). Resistance to *G. rostochiensis* is not correlated with PRD production, as most resistant *Solanum* spp. produce PRD that stimulates hatch of eggs (11). The relationship between root biomass (plant age) and activity of the hatching factor in PRD has long been recognized (13). However, the procedure for PRD collection in previous studies (i.e., leaching of soil in pots with potatoes) prevented the quantitation of the relationships between root growth and PRD activity. Ellenby (3) and Forrest and Farrer (9) collected PRD by soaking potato root systems that were free of soil in a known volume of water for a standard time period. By this means PRD is standardized; the possible influence of soil microorganisms (4) and residual PRD in the soil (1,23) is removed. Furthermore, the potency of the diffusate collected from individual root systems can be measured and compared. Data from hatching tests are affected by variation in cyst contents (7) as well as variability in activity of the PRD used (8). Furthermore, Evans (6) and Turner and Stone (24) noted that interpretation of their data from pot plants may be dependent upon the root weights of the plants from which diffusate was collected.

The *Solanum tuberosum* L. cultivars Hudson and Rosa used in these experiments have resistance to *G. rostochiensis* (Rol) conferred by the H_1 gene (15,16). Under field conditions they differ in their ability to reduce nematode populations, Hudson being more efficient than Rosa (B. B. Brodie, unpubl.) On the basis of haulm growth, Hudson grows more vigorously than does Rosa (B. B. Brodie, R. L. Plaisted, unpubl.). If

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differences in haulm growth parallel differences in root growth and, hence, hatching activity of PRD, this difference may help to explain the differences in the effectiveness of these cultivars in reducing *G. rostochiensis* population densities in soil. Therefore, if hatching activity of PRD can be correlated to specific root growth parameters, it may be possible to breed plants with favorable characteristics. Our objective was to investigate the relationship between potato root system morphology and hatching factor activity in PRD produced by Hudson and Rosa.

Materials and Methods

Plant husbandry: Uniformly sized, single eye seed pieces taken with a melon baller from Hudson and Rosa tubers were treated with the fungicide mancozeb and planted in 10-cm-d pots containing sterile potting compost and sand (1:1) plus a slow release fertilizer. Plants were grown in a greenhouse maintained at a constant 20 C with supplemental lighting for a 12-hour photoperiod and were hand watered daily.

Hatching tests: PRD was obtained by soaking individual root systems that were free of soil in 300 ml distilled water at 22 C for 2 hours (3,9). The PRD was used immediately in a hatching test or frozen for later use. Twenty-five uniformly sized cysts (0.35–0.45 mm) with a mean viable egg content of 468.1 ± SE 12 were presoaked in distilled water for 1 week and then subjected to PRD for 3 weeks. Fresh PRD was provided weekly, and hatched juveniles that had emerged from cysts were counted on each occasion. Each PRD sample was replicated five times.

Root growth analysis: After PRD collection, root systems were weighed and preserved in FA 4:1 (21), and root lengths, numbers of root tips, and root diameters were determined. Root lengths were measured either by taking photocopies of displayed root systems and using a cartographer's map measurer (17) or, with larger root systems, by a line intersect method (22). Numbers of root tips were counted directly from root systems displayed for root length determinations. Root diameter was measured over the midpoints of 50 roots using an eye piece micrometer and stage micrometer. Assuming a cylindrical root shape, root volume was estimated from these figures using the equation $V = \pi r^2 h$ (where h = root length and r = mean root radius).

Plant age and PRD production: Five plants of each cultivar were harvested weekly after plant emergence (AE) for 6 weeks, and PRD was obtained as described for hatching tests. Shoot height and shoot and root fresh weights were recorded. Roots were then subjected to growth analysis.

Site of PRD production: Main roots that had either few primary laterals (main) or many primary laterals (main w/laterals) were collected from 2-week-old Hudson and Rosa plants. After collection, 1.4 g (fresh weight) of each type of root from each cultivar were soaked in 100 ml distilled water for 2 hours. The resulting diffusate was used in hatching tests. Roots from which diffusate was collected were then photocopied and parameters of root morphology were recorded.

Results

Plant age and PRD production: Percentage of hatch of encysted juveniles increased with time of PRD collection, peaking in PRD collected from both Hudson and Rosa plants 3 weeks AE (Table 1). The amount of hatch then decreased in PRD of both cultivars until the end of the test at 6 weeks AE. Hatch from cysts in water was 2.5% (11.7 eggs/cyst) over the same time period. Hudson PRD collected 2–6 weeks AE stimulated greater hatch than did Rosa PRD when measured directly in terms of percentage of hatch or eggs per cyst. However, when these figures were adjusted for root weight, Hudson PRD was significantly ($P = 0.05$) more effective than Rosa PRD only at the time of maximum hatch (i.e., 3 weeks AE) (Table 1). The relationship between root weight or length and PRD production (measured in terms of hatch) was linear for both cultivars during the first 3 weeks AE but not later (Figs. 1, 2).

Hudson plants were significantly ($P = 0.05$) taller and weighed more than Rosa plants from 2 weeks AE, but root fresh weights and total root lengths were not significantly different (Table 2). At 1 week AE, Rosa plants were significantly ($P = 0.05$) larger than Hudson. Rosa root sys-

TABLE 1. Hatch of *Globodera rostochiensis* in response to PRD collected from the potato cultivars Hudson and Rosa at weekly intervals after plant emergence.

	Cultivar	Weeks after emergence 1	2	3	4	5	6
Percentage of hatch							
	Hudson	2	36	50	35	46	31
	Rosa	4	21	38	23	22	18
LSD $P = 0.05$		NS	14	11	13	2	NS
$P = 0.01$						7	
Eggs per cyst							
	Hudson	9	158	227	162	212	142
	Rosa	16	102	180	112	103	82
LSD $P = 0.05$		NS	NS	44	26	15	NS
$P = 0.01$						34	
Eggs per cyst per g root							
	Hudson	18	55	51	21	27	16
	Rosa	22	42	44	15	17	11
LSD $P = 0.05$		NS	NS	7	NS	NS	NS

tems also had a greater number of main and primary lateral roots throughout the experimental period (Table 2).

Site of PRD production: This hatching test was repeated with three separate collections of PRD from Hudson and Rosa with similar results. Data on root characteristics of equivalent fresh weights of Hudson and Rosa roots from one experiment are presented here (Table 3). Compared with main roots with few laterals, main roots with many laterals, from both cultivars, had approximately four times as many root tips and twice the root length from one-fourth as many main roots, or fewer.

There were no significant differences in percentage of hatch, or hatch per cyst per g root, with PRD from either main roots with few laterals or main roots with many laterals (Table 4). However, when hatch was calculated in terms of root volume (eggs per cyst per cm^3 root), PRD from main roots with many laterals stimulated greater hatch than PRD from main roots with few

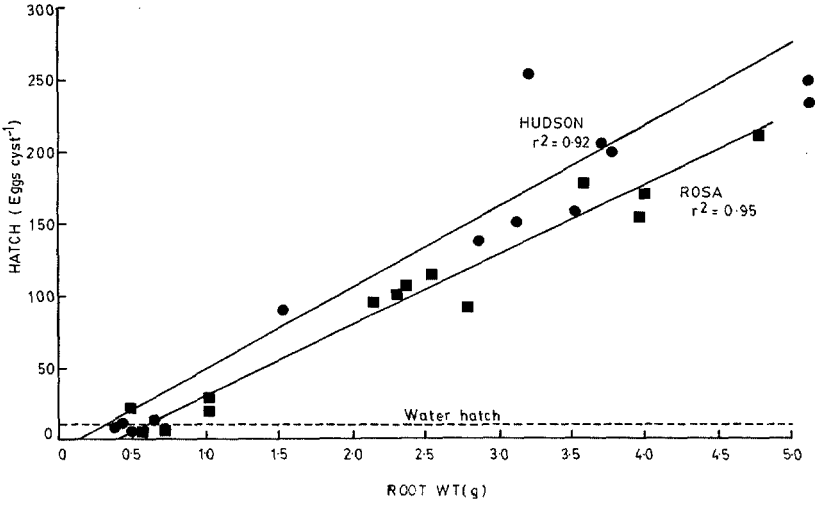

FIG. 1. Relationship between hatch of *Globodera rostochiensis* and root weight as related to hatching activity of potato root diffusate. ●, Hudson; ■, Rosa.

Hudson $y = 54.22x - 9.596$
Rosa $y = 49.36x - 21.747$

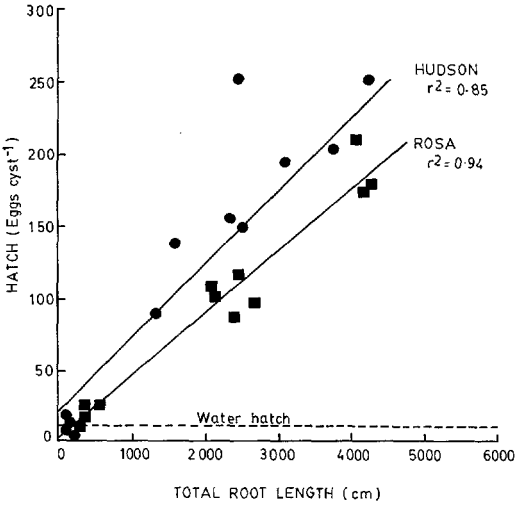

FIG. 2. Relationship between hatch of *Globodera rostochiensis* and root length as related to hatching activity of potato root diffusate. ●, Hudson; ■, Rosa.

Hudson $y = 0.05x + 24.84$
Rosa $y = 0.043x + 3.68$

laterals (Table 4) despite the smaller root volume (Table 3). Hudson PRD stimulated more hatch than Rosa PRD even though Rosa roots of both types were longer and had more primary laterals (Table 3). However, the volume of Hudson roots was greater ($P = 0.05$) than the volume of Rosa roots because the mean root diameters of main and lateral roots were, respectively, 0.87 ± 0.12 and 0.25 ± 0.04 mm for Hudson and 0.61 ± 0.08 and 0.19 ± 0.03 mm for Rosa (± standard deviation).

DISCUSSION

PRD production appears to be associated with the vegetative phase of plant growth. Under our experimental conditions, hatching activity of PRD peaked at 3 weeks AE, during which time it was directly correlated with root weight and length. After 3 weeks, PRD production declined. Low hatch could be related to either a decline in production or reduced potency of the hatching factor in the PRD. Also, onset of flowering and (or) tuber formation, and the subsequent change to reproductive development, may change photosynthate partitioning which may affect PRD production. Field data (D. Rawsthorne, unpubl.) indicate that Hudson remains vegetative longer than does Rosa (62 vs. 54 days to 50% flowering). A similar time interval was noted in the time taken to tuber set. The longer vegetative growth phase may therefore explain why Hudson produced a more active PRD for a longer time than Rosa. Potatoes that failed to flower when grown hydroponically produced active diffusate for many weeks (J. L. Riopel, pers. comm.). Widdowson (25) obtained similar results, but on a different time scale, because of cultivar and (or) environmental differences.

Specific differences in the hatching ac-

TABLE 2. Shoot and root growth analysis of Hudson (H) and Rosa (R) potato plants.

	Weeks after emergence							
	1		2		3		4	
Growth parameter	H	R	H	R	H	R	H	R
Shoot ht (cm)	3.2	3.6	15.0	11.2	27.3	20.4	30.2	21.3
LSD*	0.3		3.8		3.9		4.3	
Shoot fw (g)	0.21	0.24	2.81	1.29	4.94	3.61	7.65	4.77
LSD	0.01		0.48		NS		1.72	
Root fw (g)	0.48	0.78	2.87	2.2	0.46	4.08	7.63	7.69
LSD	0.25		NS		NS		NS	
Main roots (N)	8.4	15.4	9.4	16.8	12.8	20.0	12.8	17.8
LSD	2.6		4.1		5.5		2.8	
Total root length (cm)	83	227	2,078	2,318	4,233	4,112	5,160	5,689
LSD	53		NS		NS		NS	
Primary laterals (N)	24	108	672	1,199	1,064	1,346	1,200	1,397
LSD	44		208		281		387	

* LSD $P = 0.05$.

TABLE 3. Root characteristics of equivalent weights of 1.4 g of main roots with few laterals and main roots with many laterals of Hudson and Rosa potato plants.

Character	Hudson		Rosa	
	Main roots	Main roots + laterals	Main roots	Main roots + laterals
Main roots (N)	39	8	61	12
Root tips (N)	86	400	159	435
Main root length (cm)	356	95	493	164
Lateral root length (cm)	42	605	75	756
Root volume (cm^3)	2.1	0.8	1.5	0.7
Specific gravity	0.7	1.7	1.0	2.2

tivity of PRD produced by Hudson compared with Rosa occurred only at 3 weeks AE. Fenwick dilution curves (8) were not used to assess differences in activity of PRD because of problems with interpretation of results (19). Differences in root growth may account for differences in the ability of the two cultivars to reduce *G. rostochiensis* population densities in the field. Our data for root growth suggest that under field conditions Rosa would have a shallower more fibrous root system than Hudson, indicated by similar total root lengths but greater numbers of laterals in Rosa. This difference, coupled with the shorter vegetative growth phase of Rosa, may account for the differences in activity of PRD observed.

No definite conclusions could be drawn from the results of the study on the site of PRD production. PRD from similar weights of roots with different numbers of root tips from either cultivar did not differ in hatch stimulation, indicating that the number of actively growing roots was not an important factor in hatching activity of PRD. Similarly, root length was not related to hatching activity of PRD. Because of the larger diameters of main roots, the total root volume of equivalent weights of main roots was greater than main roots with many laterals for both cultivars. Hudson roots (both main and lateral) were thicker than Rosa roots; this may be related to hatching activity of PRD. If root volume is important, then all root cells may be capable of producing active PRD. When hatch was corrected for root volume, however, main roots with many laterals stimulated greater hatch than main roots with few laterals, suggesting that a large number of actively growing root tips is important. Clearly, these conflicting data indicate that more precise investigations with PRD collected from isolated portions of roots will be required to determine exactly where and in what quantity active PRD is produced. We recognize that the two types of roots collected may differ in physiological age and that when roots were cut from root systems open wounds and leakage of PRD probably occurred. Thus, where many main roots were used, there were many open wounds from which PRD could leak.

Assuming that differences observed are valid, there may be a basic difference between the root cells of Hudson and Rosa. Equivalent weights of roots of the two cultivars differed in volume. The greater root volume of Hudson suggests less dense cells, as indicated by root specific gravity (Table 3). Volume was determined, however, assuming cylindrical roots; therefore, the figures presented are not absolute values. The higher specific gravity of lateral roots, compared with main roots, may be due to a greater structural material content.

Screening for root volume would be difficult in a breeding program; therefore, root weight is suggested as an alternative

TABLE 4. Hatch of *Globodera rostochiensis* in response to potato root diffusate collected from main roots with few laterals and main roots with many laterals of Hudson and Rosa plants.

Hatch	Hudson		Rosa		LSD
	Main roots	Main roots + laterals	Main roots	Main roots + laterals	
Percentage of hatch	47.9	44.4	38.4	37.2	8.9*
Eggs per cyst per g root	157.7	145.8	126.2	122.3	57.2*
Eggs per cyst per cm^3 root	105	255	118	244	40.0**

* $P = 0.05$; ** $P = 0.01$.

for equating hatching test data. Furthermore, root weight could be used together with other root growth characteristics (e.g., extent of root growth through the soil profile) to assess possible efficiency of resistent cultivars in reducing *G. rostochiensis* population densities in the field.

Recent reports of density dependent decline in populations of *G. rostochiensis* (12,18) could be explained by nematode effects on root growth and, hence, activity of PRD produced. Nematode invasion causes extensive changes in root morphology, and reports of increased root weight and root growth in response to invasion by nematodes are common (2,10,20). Field growth of Hudson and Rosa during the first 4 weeks of growth AE in infested soil showed significantly greater root growth (root weight) compared with noninfested roots (D. Rawsthorne, unpubl.). The relationships among altered root morphology, hatching activity of PRD, and density dependent decline of *G. rostochiensis* populations need further investigation.

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