Journal of Nematology 27(3):382–386. 1995.

Hatch and Reproduction of *Globodera tabacum tabacum* in Response to Tobacco, Tomato, or Black Nightshade

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Abstract: The effects of broadleaf tobacco, tomato, and black nightshade on juvenile hatch and reproduction of *Globodera tabacum tabacum* were determined in laboratory and greenhouse experiments. Root exudates from nightshade stimulated greater egg hatch than those from either 'Rutgers' tomato or '86-4' tobacco. Hatch was greater at higher proportions of root exudates for all three plant species. Root exudates from plants greater than 3 weeks old stimulated more hatch than younger plants. No regression relationships existed between plant age and nematode hatch. In other experiments, hatch from eggs in cysts was higher for tomato and nightshade after 10 weeks in greenhouse pots compared to tobacco and bare soil. Numbers of second-stage juveniles in eggs in cysts produced from a previous generation on the same host were highest on nightshade and less on tomato and tobacco. Cysts of variable age recovered from field soil had increased hatch in both root exudates or water compared to recently produced cysts from plants in growth chambers. *Globodera t. tabacum* may be subject to both host and environmentally mediated diapause.

Key words: hatch stimulation, *Nicotiana tabacum, Lycopersicon esculentum,* nematode, root exudates, *Solanum nigrum,* tobacco cyst nematode.

The tobacco cyst nematode, *Globodera tabacum tabacum* (Lownsbery and Lownsbery) Behrens, is an important parasite of shade (9,13) and broadleaf (11) tobacco in the Connecticut River Valley of Connecticut and Massachusetts. While most important as a pathogen of tobacco, the host range of *G. t. tabacum* also includes tomato, eggplant, and solanaceous weeds such as black nightshade (7,12).

Hatch of many cyst nematodes may be stimulated to various degrees by root exudates of host plants (2,3,14,16,19) or, more uncommonly, by nonhost plants (19). Although *Globodera tabacum solanacearum* hatch was increased after exposure to tobacco roots in combination with soil microorganisms (6), the extent of *G. t. tabacum* hatch stimulation by root exudates of tobacco or other weed or crop plants is currently unknown. Approximately 80% of encysted second-stage juveniles in eggs hatched in response to resistant or susceptible tobacco in greenhouse pot experiments (8), but the influence of root exudates on hatch was not examined in these experiments. *G. t. tabacum,* however, can persist in soil for many years in the absence of a host (1), suggesting some form of dormancy with survival value.

The objectives of this research were to determine the i) effects of tobacco, tomato, and black nightshade on *G. t. tabacum* juvenile hatch stimulation; ii) effect of exudate dilution and plant age on hatch; iii) and relation of hatch stimulation to host efficiency for these plant species.

Materials and Methods

Root exudates were collected from '86-4' broadleaf tobacco (*Nicotiana tabacum* L.), 'Rutgers' tomato (*Lycopersicon esculentum* Mill.), and *Solanum nigrum* L. collected in Windsor, CT. Specimens of *S. nigrum* have been deposited at the Herbarium, Entomology Dept., CAES, P.O. Box 1106, New Haven, CT, 06504-1106, C. Lemmon, Director. Seeds were planted in 1:1 sand:potting mix (Sunshine mix no. 3, Fisons Western Corp., Downers Grove, IL) in 96-cavity seedling trays. Three weeks after emergence and weekly thereafter, roots were gently washed free of soil, and 2 g freshweight roots from three plants were soaked for 2 hours in 100 ml distilled water. Root exudate solutions were filtered through medium-porosity, slow flow-rate filter paper and frozen until experimental use at room temperature.

Received for publication 25 August 1994.

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The author thanks F. J. Ferrandino for assistance with statistical analyses, J. F. Ahrens for identification of *Solanum nigrum,* and Jane Canepa-Morrison for technical assistance.

Hatching tests were performed in 96-well trays using standardized cysts of known size and age (10). Ten uniformly sized (0.35–0.45 mm) *Globodera t. tabacum* cysts produced in pots in the greenhouse, with a mean viable egg content of 288, were placed in 10 replicate cells of 0.3 ml of the appropriate root exudate. Hatching trays were incubated in the dark at 20 C. Nematode hatch was counted weekly for 4 weeks, and the exudate solution was replaced with fresh exudate each week. Distilled water served as controls. Root exudates from each host source were added to separate hatching trays.

The effect of plant host on *G. t. tabacum* hatch was examined using root exudate solutions collected 3 weeks after emergence. Root exudate solution was used at full strength or serially diluted with distilled water to result in 1:1, 1:10, 1:100, or 1:1000 parts exudate to distilled water.

The effect of plant age on juvenile hatch from eggs was examined using exudates collected 3, 4, 5, 6, 7, or 8 weeks after seedling emergence. Root exudate solution was used at full strength.

Root exudate dilution and plant age effects on juvenile hatch from eggs in cysts were analyzed by stepwise polynomial regression up to a fourth-order polynomial with alpha 0.05 to enter and 0.10 to remove (15).

The influence of 86-4 tobacco, Rutgers tomato, or *S. nigrum* on *G. t. tabacum* hatch and reproduction in soil in greenhouse pots was also examined. Nylon mesh bags (8) (2 cm^2, mesh opening 210 μm) were placed in the center of 450-cm^3 pots containing a pasteurized Merrimac fine loamy sand field soil free of cyst nematodes (72.7% sand, 21.7% silt, 5.6% clay, pH = 6.8). Each nylon bag contained 25 *G. t. tabacum* cysts of uniform size and age (average 285 viable J2 in eggs per cyst). One bag containing cysts was placed in the center of each pot. Five-week-old transplants of 86-4 tobacco, Rutgers tomato, or *S. nigrum* were each transplanted to eight replicate pots so that the root ball was 1 cm above the nylon mesh bags. Pots containing soil and cysts in bags that were not planted served as bare soil or fallow controls. Pots were placed in the greenhouse on propagation mats at 22 C at 15-hour daylength. Cysts were recovered from the bags in pots after 10 weeks, crushed in water, and the number of unhatched J2 in eggs was counted. All of the soil in the pots was dried and washed using a modified Fenwick can to recover new cysts produced on the roots (18). Cysts were counted and crushed to determine the number of J2 in eggs per cyst. The experiments were performed twice.

Hatch from cysts recovered from field soil planted to tobacco for 6 consecutive years was compared to hatch of cysts recovered from tobacco plants grown in growth chambers at 24 C. Cysts were recovered from soil 2 weeks after tobacco harvest and after 8 weeks of tobacco growth. Five cysts of each type were exposed to tobacco root exudate or distilled water for 4 weeks in hatch chambers. Field cysts were of unknown age and contained fewer and more variable numbers of juveniles per cyst than growth chamber produced cysts. Cysts were crushed in water after 4 weeks, and hatch was expressed as percentage of total juveniles in cysts. There were five replicates of each treatment, and the data were analyzed after square-root transformation to standardize the variance.

Results

Globodera t. tabacum juvenile hatch was stimulated by root exudates of black nightshade, tomato, and tobacco (Figs. 1,2). There were no significant differences between constants (intercepts) in the nonlinear regression equations generated by stepwise polynomial regression. Paired t-tests between means indicated that root exudates from nightshade stimulated greater hatch than tomato or tobacco, which were not different. Hatch was greater at higher proportions of root exudates for all three plant species. While juvenile hatch was much lower in the second

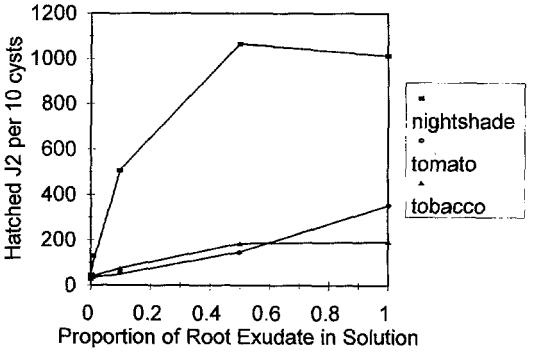

FIG. 1. Relationship between the proportion of nightshade, tobacco, or tomato root exudate in solution and *Globodera tabacum tabacum* hatch, experiment 1. Nightshade: $Y = 49.0 + 5513.1X - 9370.4X^2 + 4822.3X^3$; $R^2 = 0.998$, $P = 0.001$. Tomato: $Y = 33.6 + 137.6X + 181.3X^2$; $R^2 = 0.998$, $P = 0.001$. Tobacco: $Y = 34.8 + 440.7X - 282.5X^2$; $R^2 = 0.992$, $P = 0.001$.

Paired *t*-tests: Nightshade versus tomato ($P = 0.001$); Nightshade versus tobacco ($P = 0.001$); Tomato versus tobacco ($P = 0.41$).

set of experiments, the relation between hatch and the proportion of exudate in solution was similar.

Root exudates from 3-week-old plants did not stimulate as much juvenile hatch as older plants (Table 1). Hatch was greater in exudates from nightshade than from tomato or tobacco. Regression of plant age versus hatch (up to a fourth-order polynomial) indicated that all models were best described by the mean hatch, and that

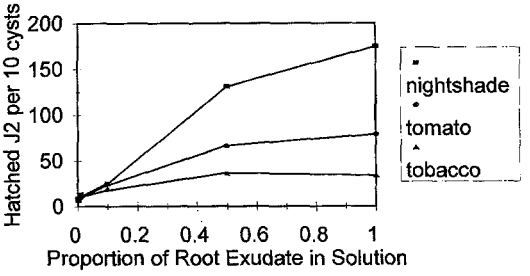

FIG. 2. Relationship between the proportion of nightshade, tobacco, or tomato root exudate in solution and *Globodera tabacum tabacum* hatch, experiment 2. Nightshade: $Y = 9.4 + 151.9X + 717.2X^3 - 703.5X^4$; $R^2 = 0.999$, $P = 0.001$. Tomato: $Y = 7.8 + 164.3X - 93.2X^2$; $R^2 = 0.998$, $P = 0.001$. Tobacco: $Y = 9.6 + 85.0X - 60.9X^2$; $R^2 = 0.970$, $P = 0.002$.

Paired t-tests: Nightshade versus tomato ($P = 0.04$); Nightshade versus tobacco ($P = 0.002$); Tomato versus tobacco ($P = 0.07$).

TABLE 1. Hatch stimulation of *Globodera tabacum tabacum* by root exudates of '86-4' tobacco, 'Rutgers' tomato, and black nightshade of different plant ages after 4 weeks.

	Experiment 1 Hatched J2 per 10 cysts†		
Plant age weeks	Nightshade	Tomato	Tobacco
3	839.7	289.2	108.1
4	1,515.8	330.1	340.9
5	1,628.4	406.0	444.2
6	1,394.4	504.7	571.7
7	1,555.6	971.1	454.2
8	1,666.6	157.6	245.0
LSD	259.0	151.9	174.4

Source of variation	df	Mean square	P
Host	2	21361836	0.001
Age	5	1131605	0.001
Interaction	10	441582	0.001
Error	162	29836	

	Experiment 2 Hatched J2 per 10 cysts†		
Plant age weeks	Nightshade	Tomato	Tobacco
3	96.5	26.7	20.3
4	239.3	24.7	21.8
5	359.2	97.7	34.6
6	214.2	86.1	48.3
7	285.5	93.5	101.2
8	232.4	36.7	48.5
LSD	166.1	50.2	37.9

Source of variation	df	Mean square	P
Host	2	684294.2	0.001
Age	5	56407.1	0.001
Interaction	10	19915.9	0.008
Error	162	8049.0	

† Initial mean content was 288 J2 in eggs per cyst.

there were no regression relationships between plant age and nematode hatch.

Hatch from inoculum cysts was highest, as evidenced by the low numbers of unhatched J2 per cyst, for tomato and nightshade after 10 weeks in greenhouse pots (Table 2). Percentage hatch under tobacco and bare soil were not significantly different. Mean numbers of juveniles within cysts produced on plant roots were highest for nightshade and less for tomato and tobacco. No new cysts were produced in soil without plants. Numbers of juveniles per cyst ranged from 141.5 in tobacco to 199.8 in nightshade.

Both cysts from plants in growth cham-

TABLE 2. Hatch stimulation of *Globodera tabacum tabacum* and reproduction in greenhouse pots after 10 weeks by '86-4' tobacco, 'Rutgers' tomato, black nightshade, or fallow.

	Unhatched J2 per cyst†	J2 per plant
Tobacco	35.5	20,165.6
Tomato	5.4	13,341.5
Nightshade	7.4	34,900.0
Bare soil	41.2	—
LSD	25.2	11,441.7

Source of variation	df	MS	P	MS	P
Host	3	5,546	0.001	1.9×10^9	0.001
Error	28	314		3.2×10^8	

† Initial mean content was 285 J2 in eggs per cyst.

bers and field cysts had increased hatch in tobacco root exudate compared to distilled water (Table 3). Percentage hatch was greater for field cysts than for newly produced cysts.

DISCUSSION

Quantitative comparisons of hatch stimulation by root exudates can be affected by factors other than exudate activity. Soil microbes (3), root weight (14), exudate dilution (5), and plant age (14) can all influence hatch of the potato cyst nematode in response to potato root exudate. Standardizing root exudate collection by standardizing the weight of roots, volume of water, and time of root exposure eliminates or reduces the effects of these variables as well as the role of soil microorganisms and residual exudates in soil. Comparisons of exudate activity can then be made between plants, especially when exudate dilutions are made and plant age is considered.

Tobacco, tomato, and nightshade root exudates all increased *Globodera t. tabacum* hatch in comparison to distilled water alone. Hatch stimulation declined as root exudates were diluted. Nightshade stimulated more hatch than tobacco or tomato. This is consistent with previous reports that showed nightshade was a more efficient host of *G. t. tabacum* than tobacco (7,12). Experimental approaches in these previous studies used juveniles in eggs in cysts as inoculum, so increased hatch stimulation may have been responsible for some of the increased nematode reproduction demonstrated on nightshade.

While the relationship between dilution of host exudates or plant age and hatch was similar, the magnitude of hatch was greatly reduced when the experiments were repeated. Cyst nematode hatch can be affected by dormancy or diapause caused by endogenous factors or by environmental conditions (2,20). Shepherd and Cox (17) reported that *G. rostochiensis* juveniles readily hatched from eggs in cysts when stimulated by potato root diffusate except during late autumn or early winter. Reduced hatch under conditions otherwise favorable for hatch was termed facultative diapause by Zheng and Ferris (20). Diapause has been reported for *G. rostochiensis* (4,17) and *H. schachtii* (20). In these experiments, cysts of variable age recovered from field soil had increased hatch in both root exudates or water compared to newly produced cysts from plants in growth chambers. Further research is needed to determine the types of diapause that occur in *G. t. tabacum* and, additionally, what conditions initiate and (or) end diapause.

Nonhosts or poor hosts do not stimulate hatch of *H. glycines* (16) or *G. rostochiensis* (14). Juvenile hatch from *G. t. tabacum* eggs

TABLE 3. Percentage hatch of *Globodera tabacum tabacum* as affected by cyst age and tobacco root exudate.

Cyst type†	Hatch solution	Percent emergence‡
Growth chamber	Distilled water	3.4
Growth chamber	Tobacco exudate	21.2
Field	Distilled water	25.1
Field	Tobacco exudate	56.1

Source of variation	df	MS	F	P
Cyst type	1	43.2	9.5	0.005
Hatch solution	1	31.3	6.9	0.016
Interaction	1	1.5	0.3	
Error	20	4.6		

† Cysts recently produced in a growth chamber at 24 C or field cysts of variable age.

‡ Data were analyzed after square root transformation.

in cysts in greenhouse pots without plants was much higher than that experienced in field soil, which has been about 40% annually (LaMondia, unpubl.). This difference may be due to temperature and moisture conditions in greenhouse pots. The dependence on a host plant to stimulate hatch of juveniles in high numbers may act as a survival mechanism (16).

Nightshade, the host resulting in the greatest *G. t. tabacum* cyst and egg production in these experiments and in field plots (LaMondia, unpubl.), results in the greatest level of *G. t. tabacum* hatch. This increased hatch stimulation may be useful as a means of nematode control by trap cropping. Shade tobacco growers generally fumigate soil or rotate into small grains for 2 or more years to manage *G. t. tabacum* below damaging levels. Transplantation and subsequent destruction of trap crops that stimulate a high proportion of juveniles in cysts to hatch could reduce the rotation time required for nematode management.

Literature Cited

1. Barker, K. R., and G. B. Lucas. 1984. Nematode parasites of tobacco. Pp. 213–242 *in* W. R. Nickle, ed. Plant and insect nematodes. Marcel Dekker, NY.

2. Clarke, A. J., and R. N. Perry. 1977. Hatching of cyst nematodes. Nematologica 23:350–368.

3. Ellenby, C. 1963. Stimulation of hatching of potato root eelworm by soil leachings. Nature 198:110.

4. Evans, A. A. F. 1987. Diapause in nematodes as a survival strategy. Pp. 180–187 *in* J. A. Veech and D. W. Dickson, eds. Vistas on nematology. Hyattsville, MD: Society of Nematologists.

5. Fenwick, D. W. 1952. The bio-assay of potato-root diffusate. Annals of Applied Biology 39:457–467.

6. Fox, J. A., and A. J. Webber. 1970. The hatching response of Osborne's cyst nematode to root leachates from germ-free tobacco plants. Journal of Parasitology 56:105. (Abstr.).

7. Harrison, M. B., and L. I. Miller. 1969. Additional hosts of the tobacco cyst nematode. Plant Disease Reporter 53:949–951.

8. LaMondia, J. A. 1988. Tobacco resistance to *Globodera tabacum*. Supplement to the Journal of Nematology 20:77–80.

9. LaMondia, J. A. 1990. Effect of oxamyl on *Globodera tabacum* population dynamics and shade tobacco growth and yield. Journal of Nematology 22: 654–657.

10. LaMondia, J. A., D. Rawsthorne, and B. B. Brodie. 1986. Methods for reducing experimental variation in *Globodera rostochiensis*. Journal of Nematology 18:415–418.

11. LaMondia, J. A., and G. S. Taylor. 1987. Influence of the tobacco cyst nematode *Globodera tabacum* on Fusarium wilt of Connecticut broadleaf tobacco. Plant Disease 71:1129–1132.

12. Lownsbery, B. F. 1953. Host preferences of the tobacco cyst nematode (*Heterodera* sp.). Phytopathology 43:106–107.

13. Lownsbery, B. F., and B. G. Peters. 1955. The relation of the tobacco cyst nematode to tobacco growth. Phytopathology 45:163–167.

14. Rawsthorne, D., and B. B. Brodie. 1986. Relationship between root growth of potato, root diffusate production, and hatching of *Globodera rostochiensis*. Journal of Nematology 18:379–384.

15. SAS Institute. 1988. SAS user's guide: Statistics. Cary, NC. SAS Institute.

16. Schmitt, D. P., and R. D. Riggs. 1991. Influence of selected plant species on hatching of eggs and development of juveniles of *Heterodera glycines*. Journal of Nematology 23:1–6.

17. Shepherd, A. M., and P. M. Cox. 1967. Observations on periodicity of hatching of eggs of the potato cyst nematode, *Heterodera rostochiensis Wollenweber*. Annals of Applied Biology 60:143–150.

18. Spears, J. P. 1968. The golden nematode handbook. USDA Handbook 353. Washington, D.C.: Government Printing Office.

19. Winslow, R. D. 1955. The hatching response of some root eelworms of the genus *Heterodera*. Annals of Applied Biology 43:19–36.

20. Zheng, L., and H. Ferris. 1991. Four types of dormancy exhibited by eggs of *Heterodera schachtii*. Revue de Nématologie 14:419–426.