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Host Status of Different Potato (*Solanum tuberosum*) Varieties and Hatching in Root Diffusates of *Globodera ellingtonae*

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Abstract: Globodera ellingtonae was detected in Oregon in 2008. In order to make decisions regarding the regulation of this nematode, knowledge of its biology is required. We determined the host status of a diversity of potato (*Solanum tuberosum*) varieties in soil-based experiments and identified hatching stimulants in in vitro hatching assays. 'Russet Burbank,' 'Desiree,' 'Modac,' 'Norland,' 'Umatilla,' and 'Yukon Gold' were good hosts (RF > 14) for *G. ellingtonae*. Potato varieties 'Maris Piper,' 'Atlantic,' and 'Satina,' all which contain the Ro1 gene that confers resistance to *G. rostochiensis*, were not hosts for *G. ellingtonae*. In in vitro hatching assays, *G. ellingtonae* hatched readily in the presence of diffusates from potato (PRD) and tomato (*Solanum lycopersicum*; TRD). Egg hatch occurred in an average of between 87% and 90% of exposed cysts, with an average of between 144 and 164 juveniles emerging per cyst, from PRD- and TRD-treated cysts, respectively. This nematode hatched rapidly in the presence of PRD and TRD, with at least 66% of total hatch occurring by day 3 of exposure. There was no dose-response of egg hatch to concentrations of PRD or TRD ranging from 1:5 to 1:100 diffusate to water. When *G. ellingtonae* was exposed to root diffusates from 21 different plants, hatch occurred in 0% to 70% of exposed cysts, with an average of between 0 to 27 juveniles emerging per cyst. When root diffusate-exposed cysts were subsequently transferred to PRD to test viability, root diffusates from arugula (*Eruca sativa*), sudangrass (*Sorghum bicolor* subsp. *drummondii*), and common vetch (*Vicia sativa*) continued to inhibit egg hatch compared with the other root diffusates or water in which hatch occurred readily (60 to 182 juveniles emerging per cyst). Previously known hatching stimulants of *G. rostochiensis* and *G. pallida*, sodium metavanadate, sodium orthovanadate, and sodium thiocyanate, stimulated some egg hatch. Although, *Globodera ellingtonae* hatched readily in PRD and TRD and reproduced on potato, the pathogenicity of this nematode on potato remains to be determined.

Key words: behavior, diffusates, *Globodera*, hatching, potato, resistance, tomato.

In 2008 an unusual population of *Globodera* was found in soil collected from Powell Butte, OR (Skantar et al., 2011). Based on morphological and molecular data, this nematode was described as a new species, *Globodera ellingtonae* n. sp. (Handoo et al., 2012). This cyst nematode is most similar to atypical *Globodera* populations from Argentina and Chile, and together these populations are distinct from *G. rostochiensis*, *G. tabacum*, *G. "mexicana,"* and *G. pallida*. Within the *Globodera*, *G. rostochiensis*, and *G. pallida* are quarantine pests in many countries, including the United States. Both of these nematodes are major pests to potato (*Solanum tuberosum*) (Trudgill and Cotes, 1983). Because of the close phylogenetic relationship of *G. ellingtonae* to *G. pallida* and *G. rostochiensis*, additional questions have arisen regarding its biology, pathogenicity, and ultimately its regulation.

The interaction of genera within the Heteroderinae with their hosts is complex and has been referred to as "the ultimate in evolutionary specialization" within the phylum Nematoda (Koenning and Sipes, 1988). For species within the genus *Globodera*, the need for specific hatching cues, narrow host ranges, synchrony of host and parasite life cycle, establishment of permanent feeding sites within a plant, and survival strategies demonstrate this specialization. Eggs of *G. rostochiensis* and *G. pallida* remain dormant in the absence of a host and hatch primarily in response to host plant root diffusates, specifically diffusates from Solanaceous plants. Exposure of *G. pallida* to potato root diffusate (PRD) for 5 min per wk for 4 wk was sufficient to induce hatch (Forrest and Perry, 1980). Only a 5-min exposure to PRD was needed to initiate the hatching process of *G. rostochiensis* (Perry et al., 1981), and *G. rostochiensis* juveniles started to move inside eggs 3 d after exposure to PRD (Doncaster and Shepherd, 1967). Multiple hatching factors in PRD have been detected (Devine et al., 1996); however, the specific role of these hatching factors has not been determined. It is unknown which types of root diffusates or compounds will stimulate hatch of *G. ellingtonae*.

Once the egg has hatched, the infective second-stage juvenile (J2) invades the root behind the zone of elongation and migrates through the root intercellularly before establishing a feeding site known as a syncytium (Turner and Evans, 1995). It has been shown that root diffusates from both resistant and susceptible potato varieties stimulate egg hatch, and that resistance is a function of the nematode not being able to establish a syncytium (Hooper et al., 1978). Whether potato varieties with resistance to *G. ellingtonae* are available is not known.

The overarching goal of our research program is to provide information on the biology, host range, and pathogenicity of *G. ellingtonae* upon which to make management and regulatory decisions. The objectives of the present investigation were to determine (i) if potato is a host for *G. ellingtonae*; (ii) which root diffusates

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stimulate/inhibit egg hatch of *G. ellingtonae*; and (iii) the hatching dynamics of *G. ellingtonae* in PRD and tomato root diffusate (TRD).

Materials and Methods

Host status of potato varieties: Soil was collected in spring 2011 from an infested field in Powell Butte, OR. This field had been planted to barley and Austrian winter pea (*Pisum sativum*) in 2007, potatoes in 2008, and was fallow in 2009–2011. For collection, a trowel attached to a length of PVC was used to scoop samples from the top 6 cm of soil. Collected soil was air-dried and thoroughly mixed prior to use in experiments. To determine initial cyst and egg densities in soil, cysts were extracted from ten 500-g dried soil subsamples using a USDA cyst extractor (Ayoub, 1980). Cysts were handpicked from washed samples and counted. The number of eggs/cysts was determined by crushing all collected cysts within a subsample with a rubber stopper on a 60- over a 500-mesh sieve. Eggs retained on the 500-mesh sieve were washed into a 50-ml polystyrene tube and the volume adjusted to 25 ml. Eggs were enumerated by counting two 1-ml aliquots using an inverted microscope.

In an experiment conducted twice, the host status of the nine diverse potato varieties 'Atlantic,' 'Russet Burbank,' 'Desiree,' 'Maris Piper,' 'Modoc,' 'Norland,' 'Satina,' 'Umatilla,' and 'Yukon Gold' were evaluated. 'Atlantic' is a chipper; 'Russet Burbank' and 'Umatilla' are russeted varieties used in processing and table stock; 'Desiree' (PCN susceptible standard), 'Modoc,' and 'Norland' are red table stock varieties; 'Satina' and 'Yukon Gold' are white and yellow table stock varieties, respectively; and 'Maris Piper' (PCN resistant standard) produces small white tubers used in specialty processing. Noninoculated controls of 'Russet Burbank' and 'Desiree' were also included to examine the effects of *G. ellingtonae* on root and tuber weight. Seed was obtained from certified seed growers. Tuber pieces, treated with the fungicide difenoconazole (Syngenta Crop Protection, Wilmington, DE), were sprouted in 36- by 36-cm flats containing perlite (Sun Gro Horticulture, Agawam, MA). Sprouted tubers, approximately 6 cm in height, were transplanted into 9.6-liter pots (Nursery Supplies Inc., Orange, CA) containing approximately 9 kg of a steam pasteurized 1:1 by volume washed sand and Willamette loam mix plus 1 kg of *G. ellingtonae*-infested Powell Butte soil containing 57.0 ± 3.0 cysts with 187.2 ± 16.3 eggs/cyst in trial 1 and 62.0 + 4.0 cysts with 199.4 ± 14.0 eggs/cyst in trial 2.

At planting, the Powell Butte soil was placed directly around the roots of the sprouted tubers. Pots were arranged in a randomized complete block design on greenhouse benches with five replications. Plants were grown under long-day conditions (16-h photoperiod) with 23/18°C day/night temperatures, and were fertilized two to three times a week with N-P-K: 20-20-20 (J.R. Peters, Allentown, PA). Plants were allowed to grow for at least 12 wk or until they naturally senesced. At harvest, the aboveground portion of the plant was removed and discarded. The content of the pot was spread onto a tray. Tubers were removed and weighed and roots were removed and then placed in a 70°C oven for 7 d before determining dry weight. The remaining soil was mixed and air dried. Once the soil was dry, two 500-g soil subsamples were collected and cysts were extracted, collected, counted, and egg density determined as described above. Reproductive factors (Rf = final egg density/initial egg density) were determine for each pot. Data were analyzed with Kruskal-Wallis and Duncan's multiple range test was used to separate means only when Kruskal-Wallis was significant at $P \leq 0.05$ using the computer software SAS (SAS Institute, Cary, NC).

Hatching assays: Soil and cysts were collected and processed in spring 2010 from a field in Powell Butte, OR, as described above. Cysts were handpicked from washed soil samples and placed in water until used in assays, usually within 24 to 48 hr. A subsample of cysts (n = 20) was crushed in water using a 7-ml tissue homogenizer (Pyrex, Lowell, MA) to determine the average number of eggs per cyst.

Plants commonly grown in the Pacific Northwest of the United States and plants previously evaluated for egg hatch of *Globodera* spp. (Franco et al., 1999) were included in experiments. Plants were grown in 15-cm pots containing potting soil (Sun Gro Horticulture) in a greenhouse under long-day conditions (16-h photoperiod) with 26/18°C day/night temperatures. Plants were fertilized with Osmocote Plus Multipurpose Plant Food (Scotts, Marysville, OH), and grown for 1 to 2 wk until the plants had sufficient root mass. At this time, the soil was saturated with deionized water and then another 50 to 100 ml of deionized water was added to the saturated soil and the resulting leachate collected. Root diffusates (Table 1) were kept at -20°C until used. All root diffusates were applied as 1:5 diffusate: water solutions unless otherwise noted. In addition to root diffusates, the following known stimulants of *G. rostochiensis* and *G. pallida* (Byrne et al., 2001) were tested: sodium metavanadate, sodium orthovanadate, and sodium thiocyanate. All were obtained from Sigma Aldrich (St. Louis, MO), prepared as 10 mg/ml water stock solutions, and diluted to concentrations of 1.0, 0.1, and 0.01 mg/ml.

A 96-well plate assay system modified from Byrne et al. (2001) and Twomey et al. (1995) was used. To each well, a 100-µl aliquot of the treatment solution (either root diffusate, inorganic hatching stimulant, or water) was added followed by a single *G. ellingtonae* cyst. The assay plates were sealed with parafilm, covered with aluminum foil to protect from light, and incubated at room temperature (~ 22°C). A water control was included on each plate. Cysts were incubated in test solutions for 3 d.

TABLE 1. Hatching of *Globodera ellingtonae* in root diffusates and water.[a]

Diffusate	Percentage (%) of cysts containing eggs exposed to diffusates in which hatch occurred over 24 d[b]	No. juveniles hatching per cysts containing eggs exposed to root diffusates for 24 d	Percentage (%) of diffusate-treated cysts containing eggs subsequently transferred to PRD 1:25 in which hatch occurred over 10 d[b]	No. juveniles hatching per diffusate-treated cysts containing eggs subsequently transferred to PRD 1:25 for 10 d
Water	15	1.6 (± 1.2)	80	77.3 (± 16.4)
Potato (*Solanum tuberosum*) (1:5)	90	143.5 (± 25.8)*	-	-
Tomato (*Solanum lycopersicum*) (1:5)	87	164.0 (± 27.1)*	-	-
Arugula (*Eruca sativa*)	20	0.4 (± 0.3)	30	7.0 (± 4.9)*
Canola (*Brassica napus*) 'Greenland'	70	1.8 (± 0.7)	90	67.4 (± 23.0)
Radish (*Raphanus sativus*)	40	0.8 (± 0.4)	90	98.8 (± 22.2)
White mustard (*Sinapis alba*) 'Achilles'	40	1.2 (± 0.9)	90	84.0 (± 25.5)
Yellow mustard (*Brassica juncea*) 'Pacific Gold'	20	0.4 (± 0.3)	100	90.7 (± 21.6)
White mustard (*Sinapis alba*) 'Martigena'	20	0.6 (± 0.4)	60	97.2 (± 31.8)
White mustard + yellow mustard (*S. alba* + *B. juncea*) 'Caliente'	20	0.5 (± 0.3)	90	124.7 (± 25.8)
Perennial ryegrass (*Lolium perenne*)	10	4.3 (± 4.3)	90	60.8 (± 23.1)
Sudangrass (*Sorghum bicolor* subsp. *drummondii*)	20	0.2 (± 0.1)	80	27.9 (± 9.7)*
Oats (*Avena sativa*) 'Monida'	40	0.9 (± 0.6)	100	182.1 (± 21.1)
Wheat (*Triticum aestivum*)	30	0.4 (± 0.2)	70	77.8 (± 23.5)
Rye (*Secale cereale*) 'Rhymin'	0	0	90	102.0 (± 19.2)
Redroot pigweed (*Amaranthus retroflexus*)	40	2.4 (± 1.3)	90	112.7 (± 31.5)
Barnyard grass (*Echinochloa crus-galli*)	20	0.4 (± 0.3)	100	115.4 (± 22.7)
Kochia (*Kochia scoparia*)	30	0.6 (± 0.3)	100	158.2 (± 23.7)
Green foxtail (*Setaria viridis*)	30	1.7 (± 1.1)	100	130.8 (± 20.2)
Lambsquarter (*Chenopodium album*)	30	0.7 (± 0.4)	90	136.4 (± 24.2)
Hairy vetch (*Vicia villosa*)	30	0.8 (± 0.4)	100	151.2 (± 23.1)
Quinoa (*Chenopodium quinoa*)	30	0.5 (± 0.3)	90	125.2 (± 22.6)
Common vetch (*Vicia sativa*)	20	27.7 (± 18.5)	60	40.1 (± 21.4)*
Lupin (*Lupinus mutabilis*)	50	2.7 (± 1.4)	100	137.7 (± 8.9)

[a] Individual cysts were exposed to treatments in 96-well plates containing 100 µl of each solution. The number of juveniles emerging from cysts containing eggs was counted and cysts were transferred to new wells containing fresh solutions at 3, 10, 17, and 24 d. At 24 d, cysts (except those initially exposed to potato or tomato root diffusates) were transferred to new wells containing 1:25 potato root diffusate and the number of juveniles emerging from cysts containing eggs was counted after 10 d.

[b] Values are the means of at least 10 observations.

* Indicate a significant difference in $\log_{10} + 1$ transformed count data from other values in the column, but not each other, by Tukey's Honestly Significant Difference test ($P = 0.05$). Values are presented as back-transformed means of the arithmetic mean ± standard error.

At this time, the number of J2 emerging from eggs in each well was enumerated at ×40 using an inverted compound microscope (Leica, Wetzlar, Germany). After counting, the cysts were moved to new wells containing 100-µl aliquots of fresh solutions. Any J2 inadvertently transferred with the cyst were noted at the time of transfer. Quantification of J2 and transfer of cysts to fresh solutions occurred again at day 10 and 17. Final J2 counts were made on day 24. After this, all cysts that were in solutions other than PRD or TRD were transferred to wells containing 100 µl of 1:25 PRD to check viability. Cysts were incubated in PRD for 10 d, at which time the number of J2 emerging from cysts were counted in each well.

Several different experiments were conducted in the 96-well assay system described above. First, a broad spectrum of root diffusates (Table 1) prepared as 1:5 diffusate: water solutions were screened against eggs contained in cysts. Second, cysts containing eggs were exposed to three inorganic hatching stimulants at 0.01, 0.1, 1.0, and 10 mg/ml. The third experiment evaluated egg hatch in PRD and TRD at a range of concentrations (1:100, 1:50, 1:25, 1:10, and 1:5 diffusate: water). In this experiment, J2 hatch from eggs was assessed more frequently over a 24-d period than described above to ascertain hatching dynamics. Regardless of the experiment, all treatments were replicated at least five times and experiments were conducted at least twice.

Data from hatching assays were analyzed by analysis of variance (ANOVA) and means were compared using Tukey's Honestly Significant Difference (HSD) test ($P = 0.05$). When necessary, transformations ($\log_{10} + 1$ or arcsine) of raw data were used to satisfy assumptions of variance. Data are presented as back-transformed means (when a transformation was used, as noted) of the arithmetic mean. All data were analyzed using the computer software JMP (SAS Institute, Cary, NC).

RESULTS

Host status of potato varieties: Data from the potato variety trials are summarized in Table 2. Results from both trials were similar. The most cysts were produced on 'Russet Burbank' and were significantly higher than all other varieties except 'Desiree,' the PCN susceptible standard. 'Umatilla,' 'Modoc,' 'Norland,' and 'Yukon

TABLE 2. Reproduction of *Globodera ellingtonae* on potato varieties.

Variety	Cysts/pot[a]	Eggs/cyst[b]	Rf (eggs)[c]
Trial 1			
Desiree	1,312 a[d]	238 c	29.8 b
Russet Burbank	1,404 a	378 a	50.0 a
Umatilla	766 b	347 a	25.2 b
Modoc	822 b	287 b	21.8 bc
Norland	790 b	245 bc	18.1 c
Yukon Gold	638 b	251 bc	14.6 c
Maris Piper	44 c	88 d	0.4 d
Atlantic	52 c	90 d	0.6 d
Satina	46 c	89 d	0.4 d
Trial 2			
Desiree	1,558 ab	292 a	36.4 b
Russet Burbank	2,300 a	314 a	57.9 a
Umatilla	1,100 c	331 a	29.1 c
Modoc	1,500 b	331 a	38.7 b
Norland	1,060 c	324 a	27.7 c
Yukon Gold	590 d	317 a	14.9 d
Maris Piper	60 e	150 b	0.7 e
Atlantic	54 e	110 b	0.5 e
Satina	60 e	92 b	0.5 e

[a] Initial cysts/pot = 57 in trial 1 and 62 in trial 2.
[b] Initial eggs/cyst = 187 in trial 1 and 199 in trial 2.
[c] Rf = Pf (number of eggs at harvest)/Pi (egg inoculum).
[d] Data are presented as back-transformed means of the arithmetic mean. Means within the same column under the same trial that are followed by the same letter are not significantly different according to Tukey's Honestly Significant Difference ($P \leq 0.05$), N = 5.

Gold' also produced high densities of cysts while 'Maris Piper,' 'Atlantic,' and 'Satina' had the same or slightly fewer cysts per pot than the number added at planting. In trial 1, the number of eggs per cyst were greater on 'Russet Burbank' and 'Umatilla,' and less on 'Maris Piper,' 'Atlantic,' and 'Satina' than all other varieties. In trial 2, the number of eggs per cyst separated into two groups; one consisting of 'Maris Piper,' 'Atlantic,' and 'Satina,' in which number of eggs per cyst were significantly less than in the other group that contained all other varieties. The highest Rf values were observed for 'Russet Burbank,' which were significantly higher than all other varieties in both trials. Rf values for 'Maris Piper,' 'Atlantic,' and 'Satina' were less than 1.0 and significantly less than all other varieties in both trials. Root weights and tuber weights were not different between inoculated and noninoculated 'Desiree' or 'Russet Burbank' in either trial (data not shown).

Hatching assays: The average number of *G. ellingtonae* eggs/cyst (n = 20) collected was 129.0 ± 13.0 eggs. Across all experiments, 40 cysts containing eggs were exposed to water for 24 d. Over this time an average of 1.0 ± 0.6 J2/cyst hatched. When these cysts were subsequently transferred to 1:25 PRD to test viability, hatch occurred in 82.5% of the cysts previously exposed to water at an average hatch rate of 90.5 ± 13.3 J2/cyst.

When cysts containing eggs were exposed to the root diffusates, both PRD and TRD at concentrations of 1:5 induced hatch in > 87% of exposed cysts as compared with 15% for water (Table 1). The percentage of exposed cysts in which hatch occurred in root diffusates other than PRD and TRD was ≤ 50%, except for canola root diffusate that stimulated egg hatch in 70% of the exposed cysts. In general, the percentage of exposed cysts from which hatch occurred in root diffusates was greater than that in water, except for perennial ryegrass and rye 'Rhymin' in which hatch occurred in ≤ 10% of exposed cysts. When cysts were transferred from water or root diffusates to 1:25 PRD to test viability, the percentage of exposed cysts with egg hatch increased to ≥ 60% except from those cysts exposed to arugula root diffusate.

PRD and TRD also induced a greater number of juveniles to emerge from cysts compared with water or other root diffusates ($P < 0.001$) (Table 1). In general, egg hatch after more than 24 d in diffusates from plants other than potato and tomato resulted in < 5 J2/cyst. There was no difference in egg hatch between the other diffusates and water ($P = 0.13$). Hatch of water- or diffusate-exposed eggs increased when transferred to 1:25 PRD. Eggs exposed to arugula, sudangrass, or common vetch diffusates hatched less readily when transferred to PRD than those exposed to water ($P < 0.001$). Common vetch resulted in the greatest, yet not significantly different hatch during the initial exposure period.

There was no difference in egg hatch between the tested concentrations of PRD ($P = 0.32$) and TRD ($P = 0.26$) (Fig. 1A,B). To compare hatching response between PRD and TRD, the results from all concentrations were combined, and cysts that were deemed nonviable (< 10 J2 emerging/cyst) were excluded from calculations. Regardless of concentration, eggs hatched in 75% and 80% of cysts exposed to PRD and TRD, respectively. However, PRD and TRD elicited different hatching dynamics (Fig. 2). At day 1, percentage egg hatch (J2 emerging each day/total J2 emerging over 24 d) was similar for TRD (14.8 ± 2.5%) and PRD (9.6 ± 1.8%) ($P = 0.08$). However, by day 2, cumulative percentage egg hatch had increased more in TRD (44.4 ± 3.4%) than PRD (8.6 ± 2.5%) ($P = 0.001$). Regardless of root diffusate type, there was a dramatic increase in percentage egg hatch on day 3. From day 3 onward there was no difference in percentage egg hatch between the two root diffusates. In addition to differences in hatching dynamics over time, a greater number of eggs hatched when exposed to TRD (173.8 ± 13.3 J2/cyst) compared with PRD (137.9 ± 11.7 J2/cyst) ($P < 0.04$).

After 24-d exposure to the inorganic hatching stimulants, only concentration was significant in the statistical model ($P < 0.001$). Hatch occurred in ≥ 60% of the cysts exposed to sodium metavanadate, sodium orthovanadate, and sodium thiocyanate at 0.01 and 0.1 mg/ml compared with ≤ 30% egg hatch from cysts exposed to water or hatching stimulants at 1.0 and 10.0 mg/ml. The only exception was when cysts were exposed to 1.0 mg/ml sodium metavanadate and hatch occurred in 70% of exposed cysts. When cysts containing eggs were transferred to 1:25 PRD after the initial 24-d exposure to

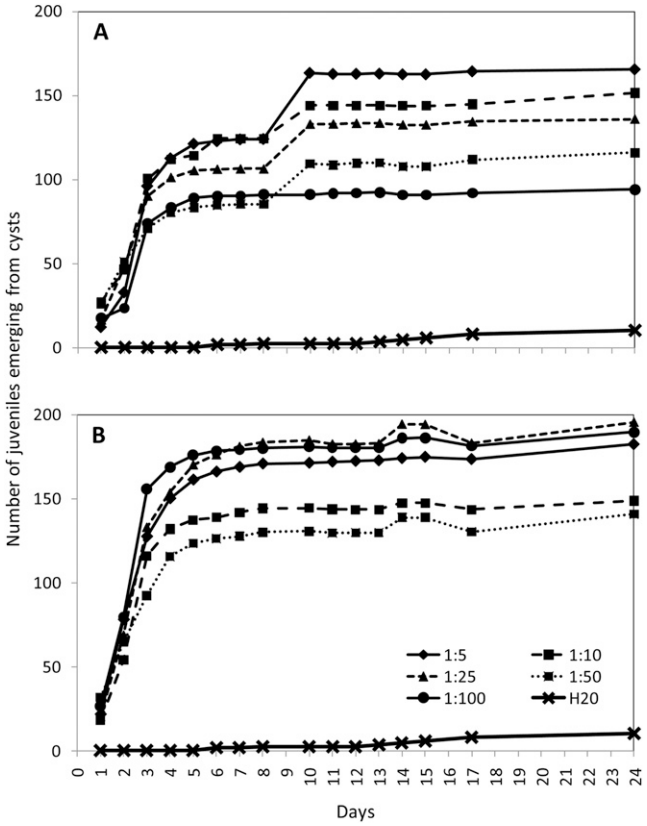

FIG. 1. Hatch of *Globodera ellingtonae* in a range of concentrations of (A) potato (PRD) and (B) tomato (TRD) root diffusates. Each data point is n = 10.

hatching stimulants, egg hatch was similar to cysts that had been in water for all hatching stimulants at 0.01 mg/ml, and sodium orthovanadate and sodium thiocyanate at 0.1 mg/ml. Exposure of cysts to sodium metavanadate at concentrations of 0.1, 1.0, and 10.0 mg/ml inhibited egg hatch even after transfer to PRD, as did sodium orthovanadate and sodium thiocyanate at 1.0 and 10.0 mg/ml compared with water.

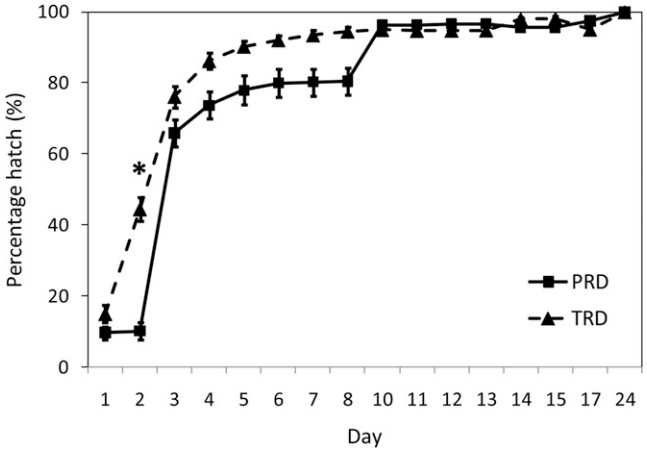

FIG. 2. Cumulative percentage egg hatch (number of J2 emerging from cysts each day/total number of J2 emerging after 24 d) of *Globodera ellingtonae* in potato (PRD) and tomato (TRD) root diffusates averaged over concentrations ranging from 1:100 to 1:5 (v/v) diffusate to water. A * above values on a given day indicates that there was a significant difference between these values according to Tukey's HSD test at $P < 0.05$. Each data point is n = 50.

DISCUSSION

We demonstrated that potato is a host for *G. ellingtonae*. In addition, we found that *G. ellingtonae* hatches readily in the presence of PRD and TRD and that none of the other tested root diffusates resulted in comparable hatch. These results are significant because they provide the first biological information about this recently described nematode species.

All potato varieties tested were highly susceptible to *G. ellingtonae* except for 'Atlantic,' 'Maris Piper,' and 'Satina.' Total number of cysts per pot at the end of the trials was nearly the same as the number added suggesting no new cysts were produced on these varieties. However, the number of eggs per cyst at the end was 47% less (averaged for the three varieties over both trials) than at planting indicating that these varieties did produce hatching factors that stimulated eggs to hatch but did not support nematode development. Resistance and susceptibility of the tested potato varieties for *G. ellingtonae*, *G. pallida*, and *G. rostochiensis* are summarized in Table 3. In this respect, *G. ellingtonae* behaved much like *G. rostochiensis* in that varieties with the gene for resistance to *G. rostochiensis* Ro1 (http://varieties.potato.org.uk) were also resistant to *G. ellingtonae*. Further research on potato growth and yield is necessary to determine if *G. ellingtonae* is a pathogen of susceptible potato varieties. In addition to confirming that potato is host, we have also demonstrated that tomato is a host (average Rf = 6.9; data not shown).

The hatching dynamics of *G. ellingtonae* was assessed across concentrations of PRD and TRD and over time. For both PRD and TRD, there was no statistically supported relationship between concentration and percentage hatch in this study. The chemical composition of the PRD and TRD used in these experiments was not characterized; therefore, it is hard to make direct comparisons with other studies. Devine et al., (1996)

TABLE 3. Resistance (R) and susceptibility (S) ratings of selected potato varieties for three *Globodera* species.

Potato variety	*G. ellingtonae*	*G. rostochiensis*	*G. pallida*
Desiree[a]	S	S	S
Russet Burbank[a]	S	S	S
Umatilla[b]	S	–	–
Modoc[b]	S	–	–
Norland[b]	S	–	–
Yukon Gold[a]	S	S	S
Maris Piper[a]	R	R	S
Atlantic[a]	R	R	S
Satina[c]	R	R	–

[a] British Potato Variety Database (http://varieties.potato.org.uk).
[b] No information on *G. pallida* and *G. rostochiensis* available.
[c] New York Certified Seed Potatoes 2010 Crop Directory (http://pppmb.cals.cornell.edu/about/facilities/upload/2010NYPOTATOCropDirectory.pdf).

demonstrated that there are multiple hatching factors in PRD that play a role in *G. rostochiensis* hatch. Hatching studies with *G. pallida* and *G. rostochiensis* demonstrated that hatch was related to PRD concentration. When *G. pallida* was exposed to undiluted and diluted (1:4) PRD, the percentage of juveniles hatched at the end of the experiment was approximately 30% less in diluted PRD compared with undiluted PRD (Arntzen et al., 1993). Conversely, high concentrations of PRD have been documented to inhibit hatch due to salts or natural hatching inhibitors (Perry and Beane, 1982), or by hatching factor-receptor interaction at high concentrations (Devine et al., 1996). It is possible that since we did not test full strength PRD or TRD that we never observed the full stimulation effect on hatching or hatch inhibition in these experiments.

Hatching rates of *G. ellingtonae* in PRD and TRD were similar and dissimilar to those previously reported for *G. pallida* and *G. rostochiensis*. Similar to our findings, the greatest daily emergence of juveniles from *G. pallida* cysts occurred 4 d after exposure to PRD (Forrest and Farrer, 1983). Doncaster and Shepherd (1967) also observed movement of *G. rostochiensis* juveniles 3 d after the application of PRD. The treatment of *G. pallida* and *G. rostochiensis* eggs with PRD for as little as 5 min was enough to stimulate a sequence of events culminating in the hatch of juveniles (Forrest and Perry, 1980; Perry et al., 1981). However, other studies have observed longer periods until juvenile emergence from PRD-treated cysts ranging from 7 d (McKenna and Winslow, 1972) to 10 to 14 d (Arntzen et al., 1993).

Initially, when eggs are placed in PRD there is little or no hatch, presumably because this is the period of time when the permeability of the lipoprotein membrane of the eggshell changes in response to the presence of PRD (Perry and Beane, 1982). With this change in eggshell permeability, solutes (trehalose) leave the egg allowing for rehydration of the unhatched juveniles (Ellenby and Perry, 1976). The second phase is a rapid, linear increase in juvenile emergence, when hydrated juveniles use their stylets to cut their way out of eggs. In the case of *G. ellingtonae*, more than 65% of juveniles emerged from eggs during this time period. Finally, hatch plateaus with a few juveniles emerging over time, and in this study 24% to 34% of total egg hatch occurred during the plateau phase.

None of the other tested root diffusates stimulated hatch of *G. ellingtonae*, to the extent observed by PRD and TRD. Root diffusates from arugula, sudangrass, and common vetch inhibited egg hatch when diffusate-exposed cysts were transferred to PRD. Sudangrass can be used as a green manure crop (Mojtahedi et al., 1993; Viaene and Abawi, 1998) and arugula as a trap crop (Melakeberhan et al., 2006) and both have been reported to suppress other plant-parasitic nematodes. There are no reports on the nematode-suppressive ability of common vetch. Whether the attributes of these plants that suppress nematodes in soil are similar to those in root diffusates is unknown.

Other root diffusates also appear to have a "neutralizing" effect on egg hatch. The effect of mustard root diffusate on *G. rostochiensis* hatch was first reported by Ellenby (1945). When *G. pallida* cysts were exposed to PRD then transferred to white mustard (*S. alba*) root diffusate hatch was inhibited, and hatch was reinitiated only when cysts were returned to PRD (Forrest and Farrer, 1983). However, it did not appear that white mustard root diffusate had any long-term consequences on hatch, with similar numbers of juveniles emerging from water- and mustard diffusate-exposed cysts when transferred to PRD. None of the Brassicaceae tested in our study inhibited hatch. It would be interesting to further test these root diffusates in an experimental setting similar to that of Forrest and Farrer (1983).

Lupinus mutabilis and *Cenopodium quinoa* were included in this study based on the findings of Franco et al. (1999). Cysts in muslin bags were exposed to common Bolivian crops in pots for 6 m after which hatching was either evaluated by exposing recovered cysts to PRD or by determining the unhatched eggs left within cysts. Genotypes of *L. mutabilis* varied from stimulating to permanently inhibiting egg hatch, while *C. quinoa* genotypes resulted in egg hatch similar to that observed for potato. Diffusates from these plants did not stimulate or inhibit egg hatch in this study. It is possible that the genotypes we included in our study were not the same as the genotypes tested in Bolivia.

We tested three known hatching stimulants, sodium metavanadate (Clarke and Shepherd, 1966; Greet, 1974; Byrne et al., 2001), sodium orthovanadate (Clarke and Shepherd, 1966; Byrne et al., 2001), and sodium thiocyanate (Byrne et al., 2001) against *G. ellingtonae*. Similar to *G. pallida* and *G. rostochiensis*, *G. ellingtonae* hatched in the presence of these stimulants when applied at 0.01 and 0.1 mg/ml. Vanadate ions were effective hatching chemicals for *G. rostochiensis* (Clarke and Shepherd, 1966). Byrne et al. (2001) demonstrated that *G. rostochiensis* hatched more readily than *G. pallida* in the presence of sodium thiocyanate, while there was no difference in hatching response between the two nematodes when exposed to sodium metavanadate and sodium orthovanadate. However, in another study (Greet, 1974), *G. rostochiensis* hatched more readily than *G. pallida* when exposed to 0.6 mM sodium metavanadate. In our study, higher concentrations (1.0 and 10.0 mg/ml) of all inorganic hatching stimulants inhibited hatch, and inhibition persisted even after transfer to PRD. Higher concentration of sodium metavandate inhibited hatch of *G. rostochiensis*, with maximum hatch occurring at 1 mM (Devine et al., 1996).

In conclusion, potato is a host to *G. ellingtonae* and this nematode hatched readily in PRD and TRD. The hatching dynamics of *G. ellingtonae* was very similar to that of *G. pallida* and *G. rostochiensis*, and this nematode

did hatch in the presence of inorganic hatching stimulants of *G. pallida* and *G. rostochiensis*. It appears that three plants, arugula, common vetch, and sudangrass, have the potential to inhibit egg hatch of *G. ellingtonae*. Additional information on the biology, host range, and pathogenicity of *G. ellingtonae* is still required in order to make the best possible management and regulatory decisions.

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