Developmental Dynamics of *Globodera ellingtonae* in Field-Grown Potato

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Abstract

Globodera ellingtonae is a recently described nematode parasite of potato, which is closely related to the economically significant potato cyst nematodes *G. rostochiensis* and *G. pallida*. Because of the close relationship of *G. ellingtonae* to the potato cyst nematodes, a greater understanding of its biology is critical. Two experiments were conducted in Oregon to explore the developmental biology of *G. ellingtonae* in field-grown potato. The first experiment was conducted in 2013 and 2014 to determine the developmental timing of *G. ellingtonae* life stages and reproduction by inoculating potato with soil containing cysts followed by weekly collection of soil and root samples. Life stages; second-stage juveniles (J2) in soil and roots, third-stage juveniles (J3) and fourth-stage (J4) females and males in roots, males and females or cysts in soil, and egg number and developmental stage were quantified. Normalizing across years using accumulated developmental degree days above 6°C (DD6), J2 of *G. ellingtonae* were found in soil from 41 to 588 DD6; two peaks of J2 invasion of roots were observed. The first adult females were observed at 387 and

449 DD6 in 2013 and 2014, respectively. The next generation of eggs was first observed from 675 to 854 DD6 and 50% egg development (containing a vermiform juvenile) occurred at approximately 920 DD6. A second J2 hatch was observed in both years at 927 to 1,073 DD6. The developmental dynamics of *G. ellingtonae* observed here are similar to those reported for *G. rostochiensis* and *G. pallida* from several geographical locations. In the second experiment, the effect of potato and bare soil on *G. ellingtonae* egg hatch was evaluated; in 2014 and 2015, packages containing cysts in soil were buried under potato or in bare soil at the time of planting and eggs per cyst determined weekly. Across years, a significant reduction in eggs per cysts under potato (>50%) was observed 35 days after planting (DAP) and, at 63 DAP, eggs per cyst were reduced by 76 to 96% compared with initial egg per cyst densities. In bare soil, the maximum reduction in densities of eggs per cyst was 55 to 73%. This annual reduction in egg numbers of *G. ellingtonae* in bare soil is similar to that reported for *G. pallida* and *G. rostochiensis*.

The potato cyst nematodes *Globodera pallida* and *G. rostochiensis* are globally important pathogens of potato. Both nematodes are found on every continent (except Antarctica) and are quarantine pests in many countries (CABI 2015). In the United Kingdom, it has been estimated that *G. pallida* and *G. rostochiensis* cause a 9% loss of potato yield annually (Moxnes and Hausken 2007). In the United States, *G. rostochiensis* was first reported in New York in the 1940s (Chitwood et al. 1942), whereas *G. pallida* was more recently found in Idaho in 2006 (Hafez et al. 2007). As part of a nationwide survey of potato cyst nematodes in the United States triggered by the find of *G. pallida* in Idaho, populations of unusual *Globodera* nematodes were found in two fields in Idaho with histories of potato production. Independent of these finds, another population of unusual *Globodera* nematodes was found at a research facility in Oregon. These populations were subsequently used to describe a new species of potato cyst nematode, *G. ellingtonae* (Handoo et al. 2012). Morphological and phylogenetic evidence indicate that *G. ellingtonae* also occurs in Chile and Argentina (Lax et al. 2014; Skantar et al. 2011). Because this nematode is biologically and phylogenetically closely related to the quarantine nematodes *G. pallida* and *G. rostochiensis* (Skantar et al. 2011; Zasada et al. 2013, 2015) a greater understanding of the biology of *G. ellingtonae* is critical.

Potato cyst nematodes are sedentary endoparasites with narrow host ranges, and their global success can be attributed to several unique survival strategies. Similar to other plant-parasitic nematodes, potato cyst nematodes have an egg, four juvenile, and adult stages (male and female). Hatch of the second-stage juvenile (J2) from an egg is stimulated by host root diffusates (Perry 1989). The J2 then invades the root, migrates in the root cortex, and establishes a permanent feeding site in the pericycle, cortex, or endodermis (Turner and

Evans 1995). Development to third-stage (J3) and fourth-stage (J4) juveniles continues in the root, with sex differentiation occurring at the J3 stage. Males emerge from the root after the final molt while females remain in the root and increase in size. Fertilized females eventually erupt from the root while filling with developing eggs. The cuticle of the female forms a tough protective cyst, containing 200 to 500 eggs that can survive in soil for up to 30 years (Turner 1996).

There is general agreement that *G. rostochiensis* can develop at higher temperatures than *G. pallida*, and *G. pallida* at lower temperatures than *G. rostochiensis* (Foot 1978; Kaczmarek et al. 2014; Mugniery 1978). We have demonstrated in experiments under controlled environment conditions that *G. ellingtonae* has developmental dynamics similar to those of *G. rostochiensis* for life cycle completion (Phillips et al. 2015). Setting a base temperature of 6°C, between 784 and 884 degree days were required for the completion of a complete life cycle (egg to egg). Understanding when key developmental events of *G. ellingtonae* occur in a field environment will allow for prediction of risk for this nematode to survive and complete its life cycle in other geographic locations and also will allow for the development of management practices aimed at disrupting the nematode's life cycle. For example, knowing when J2 hatch occurs and when J2 are present in soil may allow for the application of management strategies before the nematode enters the root, or targeted planting of trap crops to coincide with the nematode life cycle (Scholte and Vos 2000).

The goal of this research program is to provide biological data on *G. ellingtonae* upon which to make regulatory decisions and to develop management strategies against this new cyst nematode. In this study, we planted potato seed pieces with soil containing inoculum of *G. ellingtonae* and monitored nematode developmental stages in roots and soil to find key developmental time frames for management decisions. Additionally, to enable prediction of population dynamics of *G. ellingtonae* over a longer time frame, we tracked the reduction in egg numbers under potato and in bare soil.

Materials and Methods

Field environment and agronomic logistics. Field trials were carried out in 2013, 2014, and 2015 at the Oregon State University Central Oregon Agricultural Research Center, Powell Butte. The soil

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at Powell Butte, OR is a Redmond ashy sandy loam, and the climate is warm-summer Mediterranean, with average annual rainfall of 27 cm. Before planting potato, the entire area was treated with the preemergence herbicide S-ethyl dipropylthiocarbamate (Eptam; Gowan Company, Yuma, AZ) at a rate of 4.1 liters/ha, and triple-16 fertilizer (16-16-16, N-P-K) at 1,344 kg/ha prior to shallow cultivation. In 2013 and 2014 and 2015, 'Russet Burbank' and 'Russet Ranger' potato, respectively, were planted in furrows 86 cm apart in a section of field where *G. ellingtonae* was previously undetected. Cut potato seed pieces, approximately 56 g, were treated with mancozeb (Maxim MZ; Syngenta Crop Protection, Greensboro, NC) to protect seed pieces from soilborne pathogens prior to being placed in furrows at approximately 25 cm. Inoculation of seed pieces with *G. ellingtonae* varied with experiment and is described in detail below. After hand planting, the tubers were treated with the insecticide imidicloprid (Alias 4F; Adama, Aventura, FL) at 0.44 liter/ha and the fungicide azoxystrobin (Quadris; Syngenta Crop Protection) at 0.9 liter/ha applied in a 15-cm band over the seed, and then the rows were covered with additional soil using a tractor-mounted disk tiller. Potato seed pieces were planted 20 May, 19 May, and 14 May in 2013, 2014, and 2015, respectively. The trial area was overhead sprinkler irrigated starting approximately 4 to 5 weeks after planting, and potato was managed with cultural practices common in central Oregon. To control early blight (*Alternaria solani*), plants were treated with boscalid (Endura; BASF, Florham Park, NJ) at 0.33 liter/ha and azoxystrobin at 0.88 liter/ha based upon a growing-degree-day model used in the region (Gent and Schwartz 2003). Weeds were additionally managed postplant with pendimethalin (Prowl; BASF) at 2.34 liter/ha. During all trials, soil temperatures were recorded every 15 min with two WatchDog data loggers (Spectrum Technologies, Aurora, IL) buried at a depth of 15 cm in a row within the experimental trials.

***G. ellingtonae* development in potato.** Nematode inoculum for the trials was produced the corresponding previous field season by inoculating individual Russet Burbank potato pieces with *G. ellingtonae* cysts containing eggs in 22-liter pots (Grip Lip 2800; Nursery Supplies Inc., McMinnville, OR) buried in the field. The following spring, pots were removed from the ground and soil was emptied onto tarps, dried under shelter, thoroughly mixed, and sifted to remove rocks and large debris. Triplicate soil samples from the inoculum soil were tested for cyst and egg densities. Cysts with an average of 259 ± 27 and 307 ± 34 eggs/cyst were used for inoculum in 2013 and 2014, respectively.

Experiments were conducted in 2013 and 2014 in an area approximately 30 m long containing 16 rows of potato. At planting, a 250-cm³ scoop of field soil with cysts containing a total of approximately 2,000 to 3,000 eggs at an average of 10 cysts per 250 cm³ of soil was placed underneath each seed piece in the furrow. Sampling began 1 to 2 weeks after planting, with five plants collected each week from different rows within the 16-row area. Individual plants were removed from the ground with a shovel to enable collection of as much of the root system as possible while minimizing disturbance to the soil clinging to the roots. At the same time, soil was also collected from around the roots of each plant. Soil and roots were placed in a bag or bucket and returned to the laboratory the same day as collection and stored at 4°C until being processed the next day.

In the laboratory, the top portion of the plant was removed and discarded. Roots were carefully removed from collected soil so that disturbance to the surface of roots was minimized. To determine the number of adult males and J2 in bulk soil, a 250-g subsample was collected. Additionally, to account for nematodes in rhizosphere soil, the soil clinging to the roots was rinsed with water into a 2-liter plastic beaker. Both samples were then wet-sieve extracted by pouring the solutions over nested 250- and 25-μm sieves, with nematodes being collected on the 25-μm sieve, followed by sucrose gradient extraction (Byrd et al. 1966). Following sucrose centrifugation, nematodes were again collected on a 25-μm sieve and washed into a 50-ml tube to a volume of 10 ml, and duplicate 1-ml subsamples were counted using an inverted compound microscope (Leica, Buffalo Grove, IL). Once adult females were expected to have

developed, the 250-μm sieve fraction from the rhizosphere soil wash was saved and all females were collected and counted as described above. Eggs from females were enumerated by crushing females with a rubber stopper on a 125-μm sieve nested over a 25-μm sieve, washing the fraction collected on the 25-μm sieve with water into a 50-ml tube, and counting duplicate 1-ml samples. At the same time as enumerating eggs, the developmental state of the eggs was recorded, with the first 100 eggs counted categorized as either having a distinct vermiform-shaped juvenile within the egg ("developed") or not.

Collected roots, or a subsample of roots when samples were large, were washed, patted dry, weighed, stained with acid fuchsin, and stored in a 33% glycerol-33% lactic acid solution (Byrd et al. 1983). To extract stained nematodes from roots, roots were cut into approximately 3-mm pieces and processed with a blender in approximately 200 ml of water for 20 s on a high setting. The blended solution was poured over nested 250-, 125-, and 25-μm sieves and washed well with water, and the contents of the sieves were washed separately into 50-ml tubes and adjusted to 10- to 20-ml volumes. Nematode life stages (J2, J3, J4 female, J4 male, and female) were enumerated from duplicate 1-ml samples from the 25- and 125-μm sieve fractions using an inverted compound microscope and the entirety of the 250-μm sieve fraction using a dissecting microscope.

In 2013, cysts in soil collected from under plants were also characterized each week to track reduction in eggs per cyst due to hatch and increase in eggs per cyst due to reproduction. A subsample of bulk soil was dried on aluminum trays in a greenhouse. Cysts were then extracted from 500 g of the dried soil using a United States Department of Agriculture cyst extractor (Ayoub 1980). Both the number of cysts and eggs per cyst were determined. To enumerate eggs, cysts were crushed with a rubber stopper on a 250-μm sieve, with released eggs retained on a 25-μm sieve. Eggs were washed with water into a 50-ml tube to a volume of 20 ml and eggs were counted in duplicate 1-ml subsamples using an inverted microscope.

***G. ellingtonae* egg hatch under potato and in bare soil.** In a second set of experiments in 2014 and 2015, packets containing cysts were buried under potato and in bare ground to track reductions in egg numbers per cyst. To obtain soil with high cyst densities to place in packages, the floating fraction from dried soil was extracted by placing soil in a bucket, covering with water, and collecting float. This material was spread in a thin layer, allowed to dry for 2 to 3 days, and then mixed back into a portion of the microplot soil. Packets of this high-cyst soil were constructed by placing 35 g of the soil in a 10-cm square of 250-μm nylon mesh (McMaster-Carr, Santa Fe Springs, CA) folded into a triangle and heat sealed to close (Jores Technology, Sunrise, FL). In 2014, packets contained approximately 50 cysts, with a mean of 307 ± 34 eggs/cyst; and, in 2015, packets contained approximately 75 cysts, with a mean of 311 ± 26 eggs/cyst ($n = 5$). Potato seed pieces were planted as described above, with cyst packets being placed directly under the seed pieces. A furrow in bare soil, 30 m long, was also created 260 cm from the potato row in which cyst packets were placed at a spacing similar to that in the potato row, and the furrow was closed by disk.

Five cyst packets were collected weekly, beginning 1 week after planting and extending for 10 weeks, from randomly selected locations in potato and bare rows. The packets were transported to the laboratory the same day as collection and stored at 4°C until being processed within 2 days. To extract cysts, the packets were cut open and all contents were placed in a 2-liter beaker and vigorously mixed with approximately 1 liter of water. The solution was allowed to settle for 15 s before being poured over nested 250- and 25-μm sieves, with sediment retained in the beaker. The sediment was suspended and the suspension was decanted two more times, each time taking care to flush out cysts clinging to the sides of the beaker. For collection of cysts, the 250-μm sieve contents were further size differentiated for the collection of slightly larger cyst sizes, while removing excess duff by passing through a nested 1,000- over 354-μm sieve stack. The 354-μm sieve contents were washed onto filter paper, dried, and stored at room temperature until further processing. Cysts from each sample were collected and eggs per cyst were enumerated as described above.

Statistical analyses. Accumulated degree days above 6°C (DD6) were calculated using daily mean temperatures. Eggs per cyst data from cysts collected underneath potato in 2013 and from packages under potato and in bare ground in 2014 and 2015 were square root transformed prior to analysis to correct for heteroscedasticity. The data were then analyzed for effects of time, treatment (potato versus bare), time–treatment interaction, and replication using a mixed linear model analysis of variance, with time and treatment as fixed factors and replication as a random factor. Treatment means within and across time were separated using Tukey’s test ($P = 0.05$). All analyses were performed using JMP 9.1 (SAS Institute, Cary, NC).

Results

Yearly climate conditions. A large shift in mean soil temperature occurred early in the 2013 growing season, with temperatures increasing from 10°C at planting to 20°C 2 weeks later (Fig. 1). Soil temperatures were more constant during the first 14 days after planting (DAP) in 2014 and 2015, ranging from 14.7 to 18.6 and 12.7 to 19.8°C, respectively. Maximum soil temperatures recorded in 2013, 2014, and 2015 were 27.0, 23.5, and 25.0°C, respectively.

***G. ellingtonae* development in potato.** The timing of J2 hatch varied between years. In 2013, the highest density of J2 in soil was recorded at the first sampling day, 14 DAP (129 DD6), and J2 were present in soil until 42 DAP (491 DD6) (Fig. 2A). In 2014, J2 appeared in soil at 21 DAP (239 DD6), with peak presence of J2 in soil at 35 DAP (381 DD6) and J2 remaining in soil until 49 DAP (533 DD6) (Fig. 2A). As another measure of hatching dynamics, the numbers of eggs in cysts were tracked in 2013 (Table 1). There was a significant decrease ($P < 0.001$) in eggs per cyst between 28 DAP (321 DD6) and 35 DAP (387 DD6), which corresponded with an increase in J2 in roots at 35 DAP (Fig. 3A).

The dynamics of the invasion of roots by J2 generally followed those of J2 in soil, with peak invasions occurring the week of or the week following peaks of J2 in soil. There were two peaks of initial J2 invasion of roots in 2013, the first peak at 14 DAP (129 DD6) and the higher, second peak at 35 DAP (387 DD6) (Fig. 3A). In 2014, there was an initial invasion of roots at approximately 21 DAP (239 DD6) to 28 DAP (301 DD6) but a much higher peak J2 population in roots occurred at 42 DAP (533 DD6) (Fig. 3B). In both years, advancement to the next juvenile stage appeared to occur at weekly intervals (Fig. 3A and B). In 2013, substantial root populations of J2 were already present at 14 DAP (129 DD6), J3 at 21 DAP (234 DD6), and J4 at 28 DAP (321 DD6), and males appeared in soil at 35 DAP (387 DD6) (Figs. 2B and 3A). Similarly, a second series of developmental stages appeared at weekly intervals following the second J2 root invasion in 2013 at 35 DAP (387 DAP), with peaks of J3 at 42 DAP (491 DD6) and J4 at 49 to 56 DAP (588 to 675 DD6), and a second peak of males in soil at 63 DAP (762 DD6). There was a similar timing for the progression of developmental stages in 2014 (Figs. 2B and 3B).

Fig. 1. Average daily soil temperature from planting to harvest of potato (*Solanum tuberosum*) for 3 years at Powell Butte, OR. Soil temperatures were measured at a depth of 15 cm. Data points for each day are the average of two data loggers, each recording temperatures at 15-min intervals.

The first adult females were observed at 36 DAP (387 DD6) in 2013 and 42 DAP (449 DD6) in 2014 (data not shown). Similar to the development of other life stages, eggs in females were seen earlier in 2013, at 56 DAP (675 DD6), than in 2014, when eggs were not recorded until 77 DAP (854 DD6) (Fig. 4A). The number of eggs per female at the end of the season in 2013 was much higher than in 2014 (Fig. 4A). By 77 and 84 DAP (916 and 927 DD6) in 2013 and 2014, respectively, over 50% of eggs contained vermiform juveniles (developed eggs) (Fig. 4B). At 98 DAP (1,147 and 1,065 DD6 in 2013 and 2014, respectively), over 80% of eggs were developed in both years, despite the number of eggs per female nearly doubling between 98 and 112 DAP (1,147 and 1,306 DD6) in 2013 (Fig. 4A). In 2013, there was a late-season hatch of J2, beginning at 91 DAP (1,073 DD6) (Fig. 2A). A late-season hatch also was observed in 2014 starting at 84 DAP (927 DD6).

***G. ellingtonae* egg hatch under potato and in bare soil.** Across years, in general, a significant ($P < 0.001$) reduction (>50%) in number of eggs per cyst occurred at 35 DAP (Table 1) under potato. In 2015, the number of eggs per cyst under potato initially dropped at 21 DAP (218 DD6) compared with 14 DAP (125 DD6) but the larger reduction (61%) compared with initial eggs per cyst occurred at 35 DAP (392 DD6). In 2013, number of eggs per cyst was monitored for a longer period of time under potato compared with 2014 and 2015. In 2013, after the initial reduction in eggs per cysts through 63 DAP (761 DD6), new eggs (next generation) were observed starting at 70 DAP (843 DD6). In 2014 and 2015, when number of eggs per cyst under potato versus in bare soil was considered, numbers of eggs per cysts were similar under these treatments through 28 DAP, after which there were significantly fewer eggs per cyst found under potato compared with in bare soil ($P < 0.001$; Table 1). There was a general decline in eggs per cyst in bare soil over the timeframe of the experiment (70 DAP), with an approximately 50% reduction in eggs per cyst during both years in bare soil.

Discussion

This is the first detailed description of the life cycle of *G. ellingtonae* in a field environment. J2 hatched from eggs at between 7 and 49 days, corresponding to 41 and 588 DD6, after planting of potato, with the development to subsequent life stages (J3, J4, and adults) occurring at roughly weekly increments after invasion of roots by J2.

Fig. 2. Occurrence of *Globodera ellingtonae* **A**, second-stage juveniles and **B**, males in soil under potato (*Solanum tuberosum*) over time during two growing seasons at Powell Butte, OR. Each data point is the mean of five replicates. Error bars designate the standard errors of means.

The time to at least 50% development of eggs occurred after the same amount of time in both years (916 and 927 DD6). Also in both years, a second flush of J2 in soil was observed late in the growing season, presumably from new eggs. Although it is difficult to make direct comparisons of *G. ellingtonae* development to the data on *G. rostochiensis* and *G. pallida* in the literature because of difference in biotic and abiotic conditions at experimental sites, some generalizations can be made. A summary of the developmental dynamics of *Globodera* spp. reported in the literature is presented in Table 2. Only

studies where development was tracked in a field environment and samples were collected weekly were considered (Alonso et al. 2011; Bačić et al. 2011; Ebrahimi et al. 2014; Greco et al. 1988; Jiménez-Pérez et al. 2009; Mimee et al. 2015; Renčo 2007). This table will be referenced throughout the discussion to aid in comparing and contrasting *G. ellingtonae* development to that of *G. pallida* and *G. rostochiensis*.

Hatching factors, compounds produced by roots in the rhizosphere, are required to initiate hatch of *Globodera* spp. (Perry

Table 1. Change in densities of *Globodera ellingtonae* eggs per cyst over time under potato and in bare ground during 3 years at Powell Butte, OR^y

DAP ^z	2013		2014				2015			
	Potato		Potato		Bare ground		Potato		Bare ground	
	Eggs/cyst	%	Eggs/cyst	%	Eggs/cyst	%	Eggs/cyst	%	Eggs/cyst	%
7	...	...	311 (±40) a	0	269 (±23) ab	0	353 (±20) ab	0	320 (±42) a-c	0
14	189 (±8) a	26	305 (±29) a	0	278 (±22) a	0	367 (±23) a	0	286 (±15) a-d	8
21	231 (±43) a	11	202 (±16) a-c	30	211 (±23) ab	18	247 (±12) b-f	20	298 (±16) a-d	4
28	205 (±17) a	21	242 (±38) ab	8	198 (±20) abc	28	258 (±18) b-e	17	318 (±32) a-d	0
35	64 (±12) bc	75	112 (±26) c-e	52	211 (±16) ab	34	122 (±11) h-k	61	237 (±15) c-g	24
42	46 (±6) bc	82	39 (±7) ef	85	152 (±22) b-d	44	82 (±3) i-l	74	235 (±30) c-g	24
49	57 (±3) bc	77	27 (±4) f	89	160 (±17) b-d	53	85 (±9) i-l	73	211 (±6) d-h	32
56	24 (±8) c	90	16 (±2) f	94	93 (±9) de	73	39 (±3) j-l	87	169 (±12) e-i	46
63	61 (±19) bc	76	13 (±2) f	95	111 (±21) c-e	63	11 (±3) l	96	139 (±35) g-j	55
70	108 (±14) ab	58	28 (±3) f	89	147 (±11) b-d	55	23 (±12) kl	92	145 (±20) f-j	53
77	203 (±27) a	21	...	...	...	...	...	...	...	...
84	197 (±21) a	23	...	...	...	...	...	...	...	...
91	209 (±19) a	19	...	...	...	...	...	...	...	...

^y Values are means of five (2013 and 2014) and four (2015) replicates followed by standard errors. Values within a column followed by the same letter are not significantly different according to Tukey's adjustment for multiple comparisons ($P \geq 0.05$). Percent (%) indicates percentage reduction in eggs per cysts from original number of eggs per cyst of 259 (±27), 307 (±34), and 311 (±26) in 2013, 2014 and 2015, respectively ($n = 5$).

^z Days after planting.

Fig. 3. Population dynamics of *Globodera ellingtonae* second-stage (J2) and third-stage (J3) juveniles and fourth-stage (J4) juvenile males and females in roots of potato (*Solanum tuberosum*) in **A**, 2013 and **B**, 2014 at Powell Butte, OR. Each data point is the mean number of each stage in the total root system of one plant from five replicates. Error bars designate the standard errors of means.

Fig. 4. A, Density of eggs per cyst in *Globodera ellingtonae* females associated with the roots of potato (*Solanum tuberosum*), and **B**, proportion of developed eggs (containing vermiform juveniles) in adult *G. ellingtonae* females. Both variables were measured over time in 2013 and 2014 at Powell Butte, OR. Each data point is the mean of five replicates. Error bars designate the standard errors of means.

1989). We previously demonstrated in laboratory assays that *G. ellingtonae* hatches vary rapidly after exposure to potato root diffuse, with 66% of eggs hatching within 3 days of exposure (Zasada et al. 2013). In addition to hatching factors, some have proposed that a specific number of degree days must accumulate before hatch and invasion (Jones and Perry 1978). Soil temperatures at and after planting (10 to 22°C) were well within the range where *G. ellingtonae* hatch would be expected to occur (Phillips et al. 2015). In another study (Renčo 2007), the presence of J2 of *G. rostochiensis* in soil ranged from 21 to 28 DAP (Table 2). At two locations in Canada, *G. rostochiensis* J2 were found in soil 14 to 28 DAP (Mimee et al. 2015).

Once hatched, *G. ellingtonae* J2 invaded roots quickly, similar to what has been observed for *G. rostochiensis*, and for *G. pallida* in Belgium (Table 2). In 2013, samples were not collected until 14 DAP, and the initial invasion of roots by J2 was potentially not captured. In 2014, we began sampling at 7 DAP but J2 were not observed in roots until 21 DAP. The number of DAP reported for *G. rostochiensis* J2 to be observed in roots after planting ranged from 7 to 35 days (Bačić et al. 2011; Ebrahimi et al. 2014; Greco et al. 1988; Jiménez-Pérez et al. 2009; Mimee et al. 2015; Renčo 2007). For *G. pallida*, Alonso et al. (2011) reported invasion occurring 56 to 57 DAP; however, plants did not emerge until that same time. In our study, it appeared that there were two waves of initial J2 invasion roughly separated by 14 to 21 days. This has not been widely reported in other studies examining *Globodera* developmental dynamics. Our combined growth chamber (Phillips et al. 2015) and field data indicate that multiple punctuated hatches within a growing season from the same generation of cysts may be common in *G. ellingtonae*.

We previously performed growth chamber studies comparing *G. ellingtonae* development at constant temperatures of 10 to 26.5°C, where days to developmental stages varied greatly between temperatures but DD6 at which developmental stages were observed remained fairly consistent (Phillips et al. 2015). The DD6 to first presence of developmental stages were very similar between the growth chamber studies and the field in 2013: J2 in roots were initially seen approximately 100 DD6, J3 at 200 to 230 DD6, and males in soil at approximately 400 DD6. Due to delayed hatch, the DD6 from planting to developmental stages were later in 2014 but the DD6 between stages was similar to that observed in 2013. Development of the next generation of eggs occurred slightly earlier under growth chamber conditions, with approximately 10% developed eggs at approximately 650 DD6 and 50% developed eggs at 784 to 884 DD6, as compared with approximately 700 and 900 DD6, respectively, for both years in the field. In studies with *G. rostochiensis* and *G. pallida*, reports of the first sign of eggs range from 42 to 68 DAP (Ebrahimi et al. 2014; Greco et al. 1988; Jiménez-Pérez et al. 2009; Mimee et al. 2015; Renčo 2007), similar to what we report here

for *G. ellingtonae* and also what is reported for *G. pallida* in Belgium (Ebrahimi et al. 2014).

Unfortunately, the base temperatures used for accumulated degree day calculations have been inconsistent between *Globodera* studies. For example, in studies considering the development of *G. rostochiensis*, base temperatures of 5.9 to 6°C (Ebrahimi et al. 2014; Mimee et al. 2015) and 10°C (Greco et al. 1988; Greco and Moreno 1992) have been used. Based upon our growth chamber experiments (Phillips et al. 2015), 6°C was chosen as the base developmental temperature for *G. ellingtonae*. As noted in our growth chamber study, the developmental progress we observed for *G. ellingtonae* in roots was very similar to that observed for *G. rostochiensis* and *G. pallida* in Belgium (Ebrahimi et al. 2014). The DD6 to first appearance of each developmental stage in the 2013 field trial also was similar to that observed in Belgium for *G. rostochiensis*.

During both years, we observed a second flush of *G. ellingtonae* J2 in soil beginning at approximately 77 to 84 DAP. This hatching event was of greater magnitude in 2013 compared with 2014. Others have reported a second J2 hatch within a growing season. There was a second J2 hatch and invasion of roots of *G. rostochiensis* at 91 to 112 DAP in Slovakia (Renčo 2007), at approximately 95 DAP in Italy (Greco et al. 1988), and at 75 to 100 DAP in Canada (Mimee et al. 2015). In Central Chile, where potato crops can be cultivated for most of the year, a second J2 hatch of *G. rostochiensis* only occurred in spring-planted potato, not in summer- and winter-planted potato (Greco and Moreno 1992). Similar to our finding that there was no evidence of high levels of successful invasion of roots or subsequent development from the secondary J2 hatch in Oregon, Greco and Moreno (1992) also reported a lack of development of *G. rostochiensis* J2 at the end of the spring potato season in Chile, suggesting a single generation in both of these environments. However, in southern Italy and in Venezuela, *G. rostochiensis* has been reported to be able to complete a second generation within a growing season (Greco et al. 1988; Jiménez-Pérez et al. 2009). *Globodera* spp. are native to South America (Grenier et al. 2010), and this second J2 hatch may be a relic of origins in warmer South American environments where more than one generation per year is possible. The risk associated with a second J2 hatch in warmer environments, even when potato is not grown back to back, is the potential for solanaceous weeds that are hosts for *Globodera* spp. (Boydston et al. 2010; Mimee et al. 2015) to allow for the continued development of the nematode beyond a single generation. For *G. ellingtonae*, an alternative host that has the potential to support invasion of a second generation is hairy nightshade (W. S. Phillips and I. A. Zasada, unpublished data).

An understanding of the natural decline in *Globodera* eggs is an important component of developing rotational schemes in the absence of a host. We observed an approximately 50% reduction in number of *G. ellingtonae* eggs per cyst in bare soil over the course

Table 2. Comparison of the developmental dynamics of *Globodera ellingtonae* at Powell Butte, OR with that of *G. rostochiensis* and *G. pallida* at various locations reported in the literature^x

Stage ^z	Days after planting (degree days) ^y								
	<i>G. ellingtonae</i>	<i>G. rostochiensis</i>						<i>G. pallida</i>	
	Oregon	Serbia	Slov	Canada	Ven	Italy	Belgium	Belgium	Spain
J2 soil	7–21 (129–239)	7	14–21	14–28 (122)	14	16	24	21 (87)	29–35 (211–234)
J2 root	14–21 (129–239)	7	27	...	14	16	35	24	56–57 (348–411)
J3	21–28 (234–300)	21–28	34	...	21	26	35	35	...
J4	28–35 (321–381)	28	40	...	28	34	49	49	...
Male soil	28–42 (387–449)	28	46	35–42 (299)	35	46	59 (391)	59 (391)	77 (541–548)
Female	36–42 (387–449)	35	43	28–42 (299)	35	46	59 (391)	59 (391)	77 (541–548)
Egg	56–77 (675–854)	...	55	42–63 (579)	42	56	68 (401)	65 (463)	...

^x Literature considered from left to right: *G. rostochiensis* in Serbia (Bačić et al. 2011), Slovakia (Slov) (Renčo 2007), Canada (Mimee et al. 2015), Venezuela (Ven) (Jiménez-Pérez et al. 2009), Italy (Greco et al. 1988), and Belgium (Ebrahimi et al. 2014); and *G. pallida* in Belgium (Ebrahimi et al. 2014) and Spain (Alonso et al. 2011).

^y Degree days presented when available. Degree days for *G. ellingtonae* calculated using a base temperature of 6°C (Phillips et al. 2015), for *G. rostochiensis* calculated using a base temperature of 5.9°C, and for *G. pallida* calculated using a base temperature of 3.9°C. Values presented are the days after planting or degree days of first occurrence of a life stage. If a range is presented, then data from multiple years were included.

^z *Globodera* life stages: J2 = second stage juvenile, J3 = third-stage juvenile, and J4 = fourth-stage juvenile.

of 10 weeks. This reduction is similar to that which has been reported for *G. pallida* and *G. rostochiensis*. The annual rate of egg decline varied with soil type in New Zealand, with annual attrition rates of 30 to 70% for *G. pallida* and 45 to 61% for *G. rostochiensis* (Marshall 1998). In another study comparing *G. pallida* and *G. rostochiensis* hatch in the absence of a host (Ryan and Devine 2005), initially there was a difference in the rate of spontaneous hatch between the two species, with *G. rostochiensis* hatching in greater numbers than *G. pallida*; however, by the end of the study, the reduction in egg numbers was similar between the two species (37%). In a 2-year study, the decline in egg numbers of *G. rostochiensis* was 57% in the first year and 40% in the second year in the absence of a host (Devine et al. 1999). In this same study, it was discovered that hatch accounted for approximately 75% of this decline and egg mortality for an additional 10%. Many factors, acting alone or together, can influence the decline in *Globodera* egg numbers in a field environment, including temperature and egg-degrading microorganisms (Devine et al. 1999). The amount of time required for the complete hatch or death of *G. ellingtonae* eggs in the absence of a host is unknown.

Our data provide key insights into the developmental biology of *G. ellingtonae*. From a practical perspective, these data provide evidence for potential avenues to pursue from both management and regulatory perspectives. For example, our field data plus our previous observations on egg hatch of *G. ellingtonae* (Phillips et al. 2015; Zasada et al. 2013) indicate that *G. ellingtonae* J2 hatch and invade roots rapidly under suitable conditions. Understanding the time frame at which J2 are expected in soil will allow for the targeted application of nematicides. Our data also indicate that approximately 600 DD6 after planting is an optimal time to terminate a trap crop to achieve greatest reduction of old eggs before new eggs develop.

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