Life cycle and population dynamics of *Rhabditolaimus ulmi* (Nematoda: Rhabditida: Diplogastridae) *in vitro*

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Summary. The life cycle and population cycle of bacteriophagous nematodes *Rhabditolaimus ulmi* were studied *in vitro*. The nematodes are commensals of the bark beetle *Scolytus multistriatus* feeding on the elm *Ulmus glabra*. They use beetles as vectors and the tree as a habitat for the propagative generation. The individual nematode development cycle (G) takes approximately 8 days. From the inoculation date, female start oviposition after 1.07 (1-2) days; second-stage juveniles hatch after 2.6 (2-4) days; third-stage juveniles (J3) develop in 3.7 (3-5) days; fourth-stage juveniles develop in 6.01 (5-9) days; and adults of the new generation appear in 8 (7-10) days. In laboratory cultures, the total nematode abundance reached peaks on day 28 (3.5G); the population cycle equals 9 generations (9G) finishing with 100% of J3 (day 70). Adult individuals are osmotically sensitive and die in tap water in 30 min; only the J3 may be a resistant stage. The host and vector presumably provide osmotic protection for adult nematodes. The average fecundity rate of females is 2.80 ± 1.82 eggs day^{-1} during the first generation, and 1.54 ± 0.94 on the first day after inoculation. The average nematode lifespan equals 57 (56-59) days (7G) at 21-23°C. Formulae developed for the exponential growth of the total nematode population were (number of individuals = $63.2e^{0.2253T}$; $R^2 = 0.8859$) and abundance of females (number of individuals = $14.262e^{0.1759T}$; $R^2 = 0.9093$), where T = number of days after inoculation.

Key words: agar culture, biological model, detrital horticulture, diapause, exponential growth formula, fecundity, food web, microbiome, oviposition, population dynamics.

When discussing the problems of the origin of plant-parasitic nematodes with complex polyxenous cycles that include the insect vectors and tree hosts, the question of the evolutionary sequence of the inclusion of parasite hosts in the nematode life cycle is raised: whether the vector or the plant host first formed the dixenous life cycle (Paramonov, 1970; Ryss, 2009, 2016; Ryss & Subbotin, 2017; Polyanina *et al.*, 2019). Proponents of insect vector primacy point to a narrower specificity to the insect than to the plant host. Researchers agree that the ancestor of phytoparasitic nematodes was a free-living detritophage, *i.e.*, a bacteriophage or mycophage that later shifted to feeding on the host or vector tissues; in the phylogenetic tree of the phylum Nematoda, the lines leading to parasitic taxa contain basal clades of detritophages (Holterman *et al.*, 2006; Megen *et al.*, 2009). A search for a saprophage nematode species with a specific vector and associated with dead trees will allow us to understand the advantages for the nematode to use two different habitat-forming partners in the trixenous life cycle.

Dead wood (wood and bark debris) is now the popular research area among forest ecologists, forest entomologists and mycologists who consider the dead wood as the unique environment of the Red Book species: fungi and insects and other invertebrates. Dead wood decomposition (decay) is divided to several conventional stages, and every stage has its own species indicators and biodiversity protection value (Stokland *et al.*, 2012; Cuff *et al.*, 2021; Wainhouse & Boddy, 2022). A future study of such a biological model will make it possible to evaluate the contribution of entomochoric nematodes to wood decomposition using a population growth model (Polyanina & Ryss, 2021).

The bacteriophagous nematode *Rhabditolaimus ulmi*, an associate of the dead wood of elms *Ulmus laevis*, *U. glabra* and *Ulmus* spp. killed by the Dutch elm disease (DED) in Russia (Ryss *et al.*, 2021), was chosen as the object in this study. This

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nematode was found in St. Petersburg and in Leningrad, Moscow, and Voronezh regions, as well as in Dagestan (Ryss *et al.*, 2021; Ryss unpublished), where it was collected together with *Bursaphelenchus ulmophilus* (Ryss *et al.*, 2015). The nematode has specific vectors, the beetles *Scolytus multistriatus* and *S. scolytus* (Rühm, 1956; Ryss *et al.*, 2021).

The research is aimed: (*i*) to calculate the parameters of the individual life cycle of *R. ulmi*; (*ii*) to construct a population growth model *in vitro* under the conditions of initial food abundance and an inoculum of adult males and females; and (*iii*) to determine the number of generations in the population cycle, the lifespan of females, and the final population structure when food resources are exhausted.

MATERIAL AND METHODS

Taxonomy. *Rhabditolaimus ulmi* (Goodey, 1930) Susoy and Herrmann, 2012. Phylum Nematoda. Class Chromodorea. Order Rhabditida. Family Diplogastridae.

Isolate origin. The nematodes were collected in September 2019, in park of the State Forest Technical University, St. Petersburg, Russia, GIS: 59.991923°N, 30.342697°E, from old elm *Ulmus glabra* Huds., killed by DED. The Baermann funnel modification technique (Ryss, 2017a) was used for extraction from bark, phloem and sapwood with larvae and imagoes of the smaller European elm bark beetle *Scolytus multistriatus* (Marsham, 1802). The nematode isolate was propagated in potato sugar agar media (PSA) with an annual passage *via* multiplication in fresh elm branch pieces of 5-8 mm diam. It is maintained in the Nematode Living Cultures Collection of the Zoological Institute of the Russian Academy of Sciences.

Preparation of the agar media. Forty g of peeled potatoes were cut into 1 cm^3 cubes, boiled for 40 min in 200 ml of tap water and mashed with a press; the solution was filtered through two layers of gauze and poured into a heat-resistant container. Ten ml of glycerol, 4 g of sucrose and 4 g of agar were added to the hot solution, stirred until the reagents were dissolved, tap water was added to give a final volume of 200 ml, and sterilised for 40 min in an autoclave at 130°C and pressure of 354 kPa (3.5 atm). The autoclaved solution was poured at 80°C into 6 cm diam. Petri dishes in a sterile microbiological box. The agar layer was very thin (1-2 mm) for subsequent observation of nematodes. Observations were carried out at room temperature of 22 (21-23)°C.

Petri dishes with 2% PSA were kept until the bacterial feed mixture (BFM) was sown. The mixture was collected with an inoculation loop from the nematode standard source culture and spread evenly over the surface of the PSA with an addition of 40 µl of distilled water. The seeded dishes were placed in an incubator at 38°C for 3 days. During this time, the grown bacterial mixture covered the surface of the agar.

The BFM cultures consisted of nematode ectosymbiont bacteria and they gave the best results for reproduction of *R. ulmi*. The standard *Escherichia coli* cultures on LB medium (Lysogeny broth, Sambrook *et al.*, 1989), and direct seeding of nematodes on sterile PSA with the assumption that ectosymbiont bacteria will propagate and provide nematodes with food, as has been shown for cultures of *Panagrolaimus detritophagus* (Polyanina & Ryss, 2021), were also tested.

Identification of nematode stages. Stages were identified by size, corresponding to the morphometric data for juveniles at different stages (second- (J2), third- (J3) and fourth-stage (J4) juveniles) (Table 1). The morphology of juveniles during their moults was studied initially; the first moult occurred inside the eggshell, the three subsequent moults took place after hatching, in PSA medium (Ryss *et al.*, 2021). In addition to differences in body length, juveniles differ in the size of the genital primordia. The adult individuals are the largest, with external copulatory organs that are absent in juveniles, namely the spicules in males and the vulva in females.

Inoculation. Three days before an inoculation date, Petri dishes with 2% PSA medium were seeded with a bacterial feed mixture (BFM) and placed in an incubator at 38°C and on the day of inoculation the dishes were transferred to the room temperature. Fifty actively moving adult individuals were picked out with an entomological needle from the source nematode culture in a ratio 30 females: 20 males. Nematodes were picked out directly from the source culture to avoid contact with tap water, because the nematodes were immobilised and died in tap water in 5-30 min. The method of collecting of nematodes before inoculation in a 20 µl drop of tap water was also tested.

The study of individual development. During the 3 days nematodes produced many eggs; 5-20 eggs in a drop of tap water were transferred to a new Petri dish with PSA medium to study moults and individual development. There were 10 replicates.

The study of population dynamics. Initially, during the first generation, there was no difficulty to count numbers of nematodes and their eggs for the

whole Petri dish area, and to sort the nematodes into stages. However, precise counting of subsequent generations was impossible without extraction, which would disturb the microbiome in a Petri dish. Thus, nematodes were counted without removal from the medium, in a random sample of 20 light fields (each 3 mm^2) using the 10× objective of a compound microscope (Mikmed-6 var-7, LOMO-Microsystems; lomo-microsystems.ru). The nematode distribution pattern was not aggregated but random, differing from the agglomeration spots of *P. detritophagus* (Polyanina & Ryss, 2021).

The number of nematodes grouped by life-cycle stages was summarised for 20 light fields and these values were recalculated for the entire Petri dish area. Population dynamics were monitored in 15 Petri dishes at the following days after inoculation: 1, 2, 3, 4, 5, 6, 7, 8, 10, 14, 21, 27, 35, 42, 49, 56, 63, 70, 77, 84 and 91 days.

Monitoring during the first generation. An inoculation of nematodes synchronised at the adult stage makes it possible to calculate the time of one generation by the date of the first noticeable increase in the number of adults. We used the moment of doubling the number of adult females (60 or more for the inoculum of 30 females) as the time of one generation. During this time, the inoculated females, on average, laid at least one egg per female from which a young female of the new generation developed. A count of juvenile stages on the date preceding the appearance of adults of the second generation makes it possible to calculate the total number of eggs produced by the 30 inoculated females during the first generation time.

We used the same approach to find out the timing of the developmental phases (juveniles and males) in the population. When the number of juvenile stages (J2, J3 or J4) in a Petri dish first exceeded the number of inoculum females (30) and the juveniles were evenly distributed throughout the dish area, we considered this date to be the average time of a transformation to a given juvenile stage.

Female fecundity definition. Two methods were used to calculate female fecundity: *i*) 1 day after inoculation, the number of eggs laid in the Petri dish was counted and divided by the number of females in an inoculum (30); *ii*) the day before emergence of the second generation females (G-1), the total number of eggs and juveniles of different stages were divided by the number of females in the inoculum (30) and the number of days, G-1. The juvenile stages were determined using the body size groups, and size and position of the genital primordium (Table 1).

The following formula was used to calculate female fecundity:

$F(egg+jj) = [N(Egg)+N(J2)+ N(J3)+ N(J4)]/N(f)\times T$; where F(egg+jj) – an average fecundity per day; N(Egg) – number of eggs counted in the final day of T; N(J2) … N(J4) – number of juveniles of each stage counted in a day; N(f) – number of inoculated females (30); T – number of days from the date of inoculation until the day of counting, *i.e.*, a day before the emergence of the second generation females (T = G-1).

Determining the time of the end of the population cycle. When the total abundance of nematodes and the total number of juveniles ceased to increase, and the eggs did not contain live juveniles, we considered the population cycle was completed due to the exhaustion of food resources by the increased population.

Table 1. *Rhabditolaimus ulmi*. Developmental stages. Measurements (in μm) and indices. Values are expressed as mean ± standard deviation (range). J2, J3, J4 = second-, third- and fourth-stage juveniles, respectively (after Ryss *et al.*, 2021).

Character/stage	J2	J3	J4	Male	Female
n	10	11	10	20	20
Body length (L)	290.2 ± 31.4 (246-352)	354.4 ± 38.6 (298-423)	526.4 ± 66.8 (409-666)	715.0 ± 148.0 (557-1007)	804.6 ± 139.2 (633-1012)
Body diam.	7.8 ± 1.2 (5.5-10.5)	9.6 ± 1 (7.5-12.0)	12.6 ± 1.4 (9.5-16.5)	20.7 ± 5.2 (15.5-28.0)	27.0 ± 7.0 (19.0-36.0)
Genital primordium, length (GPL)	7.7 ± 1.7 (5.5-10.5)	13.4 ± 6.8 (6.0-29.0)	100.8 ± 63.0 (11.0-170.5)	411.0 ± 100.0 (295-586)	403.0 ± 172.7 (204-760)
Genital primordium, width (GPW)	3.5 ± 0.8 (2.0-4.5)	5.1 ± 1.5 (3.0-7.0)	6.9 ± 1.3 (4.0-10.5)	19.0 ± 8.8 (9.5-35.5)	27.6 ± 9.0 (15.5-39.0)
Ratio: body length/body diam. (a)	37.5 ± 2.7 (33.4-43.1)	37.3 ± 3.8 (27.9-43.0)	41.7 ± 2.9 (36.4-44.5)	35.1 ± 4.4 (27.8-39.9)	30.6 ± 4.6 (26.1-38.0)
GPL/GPW	2.3 ± 0.5 (1.4-3.1)	2.5 ± 0.7 (1.7-4.4)	14.4 ± 8.9 (2.7-30.0)	24.8 ± 10.8 (13.5-41.4)	14.7 ± 4.4 (10.8-23.3)
GPL/L, %	3 ± 1 (2-4)	4 ± 2 (2-7)	19 ± 12 (3-37)	60 ± 10 (40-70)	50 ± 20 (30-90)

After counting in the light fields, we rinsed each Petri dish with tap water five times and poured the nematode suspension from a Petri dish into a 1.5 ml Eppendorf tube, then centrifuged the tube in a Microspin FV-2400 centrifuge-vortex (Biosan) at 500 *g*; the supernatant was withdrawn with a syringe, the nematode suspension sediment was washed 5 times with tap water and centrifuged as described above, then fixed with hot TAF (4% formaldehyde with the addition of 2 ml triethanolamine (100 ml solution)$^{-1}$ as a buffer; in water bath with boiling water for 30 min) (Ryss, 2017a).

Two days after the fixation date (time of hardening of the morphological structures of fixed worms), nematodes grouped by stage were counted in 10 drops of 20 µl volume, as a control to compare with the last count in the light fields in a Petri dish. The number of nematodes detected in the 20 µl subsample was recalculated to the entire 1.5 ml microtube volume, which corresponded to the number of nematodes in the Petri dish at the last count. Permanent collection slides were made from the nematode suspension using the express method of Ryss (2017b).

Statistical calculations were performed using the MS Excel software. Calculations of the mean and standard deviation of the sample were used; Student's test (module 'Student.test') was used to assess the statistical significance values of difference between the samples.

To determine the formula of the exponential population growth (total abundance of the active worm-like stages and the abundance of females) the MS Excel graph template modules were used as well as the build-in MS Excel functions for the exponential coefficients, *a* and *b*.

The assumption of unlimited population growth according to the exponential model was used to determine the time when the saprophagous nematodes would be able to fill completely the dead tree volume. Mean elm wood volume of 1.5 m^3 was taken from 'Table for calculating cubic meters of wood by tree species' (Anonymous, 2021).

Parameters and indices. G is the duration of ontogeny (generation), time of individual development from egg to egg. Here an equivalent parameter was calculated: the average developmental time from a female of the previous generation to a female of a subsequent generation, G (fem-fem). This is the time when the number of adult females begins to exceed the number of inoculated females twice or more. The development time to the corresponding stages (J2, J3 and J4) was determined as the period when the number of juveniles of a given stage becomes equal to or exceeds the number of inoculated females.

The inoculum is the number of active adult females and males used to start the experimental culture in agar medium. Here 30 females and 20 males were used as the inoculum.

The start of oviposition is the time (day) of the first egg laying by a group of inoculated females.

Fecundity (F) is the average number of eggs laid by single female per day calculated for the first day after inoculation and separately for the whole first generation of the population.

In the population of *R. ulmi* accumulation of eggs with arrested hatching of invasive juveniles (in diapause) was not detected, so the proportion of diapausing eggs, as determined for *P. detritophagus* (Polyanina & Ryss, 2021), was not calculated.

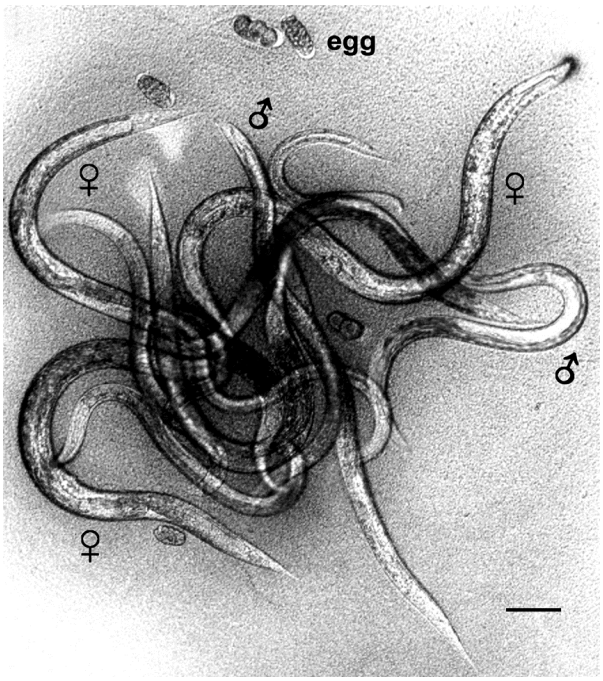

Fig. 1. *Rhabditolaimus ulmi*. Swarming (mating group of males and females), with eggs produced by females of the group. Scale 50 µm.

RESULTS

Preliminary testing of the culture media and an inoculum sampling methods from the source culture. The source culture was started using the extracted suspension of *R. ulmi* from a dying elm tree. The culture was propagated on the fungus *Botrytis cinerea*; during culturing the fungus was replaced by the bacterial ectosymbionts of *R. ulmi*. The 2% PSA medium with the fungus *B. cinerea* allows the successful and sustainable reproduction

of *R. ulmi*; however, it does not allow clear observation of the reproduction stages of the population, because the mycelium of the fungus is opaque. The use of standard *E. coli* on LB agar with addition of trypsin proved to be unsuccessful, since population development in most Petri dishes with this medium ceased after 7-12 days.

The inoculation of undefined remaining bacterial mixture from the old most successful source cultures of *R. ulmi* on sterile 2% PSA medium proved to be a productive and sustainable technique. When this medium was placed in an incubator (38°C, for 3 days), the bacterial mixture of ectosymbiont bacteria formed a transparent film. If some nematodes from the source medium got into the mixture, they died within 2-4 h at 38°C in the thermostat and only bacteria suitable for nematode feeding and active growth remained in the medium; these were used for the BFM culture.

A real problem was that inoculated adult females and males, as well as J2 and J4 died from osmotic shock within 5-30 min when placed in a drop of tap water. Females burst during oviposition. Only the J3 remained active. Replacing the tap water with Prescott and James medium (PJ; https:// www.ccap.ac.uk/wp-content/ uploads/ MR_PJ. pdf) kept the nematodes alive, but the nematode females did not lay eggs after the use of this medium. Ringer solution (RS) was also tested in two modifications: for humans and for invertebrates. However, in both RS modifications the nematodes also died in 30 min. The use of 2% PSA prepared on the RS for invertebrates was also unsuccessful: BFM did not grow on this medium. Therefore, picking out the adult females and males directly from the medium of source culture and the direct transfer into 2% PSA medium with the previously grown BFM was used.

Additionally, the individual development could not be traced from the egg to adult nematode using the water drop method. All observations were made here at the population level. The timing of the hatching process on the agar slice coincided with the population growth data and was combined with the latter (Table 2).

Parameters of individual development. The correspondence of juvenile stages to their size groups was used for population observations (Table 1). The first moult takes place within the egg; after hatching the J2 emerges from the egg, further developing in a series of moults into J3, J4 and male and female adults. The latter lays eggs from which the next generation juveniles hatch.

The mean development time from egg to a new generation egg was 8 days (Table 2). The starting point is the introduction of nematode inoculum into the culture. The initial delay period of 1.07 ± 0.25 days is associated with the need for mating and the subsequent maturation of the egg in the female body (Fig. 1). Although the developmental time ranges of different stages overlap, they are significantly different ($P < 0.05$; t-test).

Table 2. *Rhabditolaimus ulmi*. Scenario of individual development (days). Values are expressed as: mean ± standard deviation (range). J2, J3, J4 = second-, third- and fourth-stage juveniles, respectively. G – number of days of in one generation; timing of a population development after inoculation of 50 adult individuals: 30 females and 20 males; n = number of individuals during monitoring. F(1d) and F(G) are a fecundity, *i.e.*, the number of eggs per day laid by one female during the first day after inoculation of adults and a mean fecundity calculated for a whole period of first generation, respectively.

Character	n	Population studies
Egg laying start	693	1.07 ± 0.25 (1-2)
J2	399	2.56 ± 0.53 (2-4)
J3	290	3.70 ± 0.96 (3-5)
J4	237	6.01 ± 0.67 (5-9)
G-Females	89	7.83 ± 1.32 (7-10)
G-Males	82	7.98 ± 1.38 (7-10)
F (1d)	10	1.54 ± 0.94 (0.37-3.33)
F(G)	10	2.80 ± 1.82 (1.11-6.06)

The average fecundity per female also differs in two different estimates: 1.54 ± 0.94 days when counted on the first day after inoculation of females and males, and 2.80 ± 1.82 days for the whole period of first generation. This difference was expected, because in a new medium the establishment of mating structure and egg maturation within a female body need some time.

Population dynamics. These nematodes rarely swarm to form mobile balls in which the females mate with males and lay eggs (Fig. 1). Usually the worms are evenly distributed on the agar plate in the Petri dish and quickly move across the surface. However, some groups of 1-2 males and 3-5 females were detected. Clusters of 3-10 eggs may be found because some females prefer to lay eggs in groups of 3-5 individuals.

After the first generation period (G = 8 days), an exponential increase in the total number of nematodes was observed until 28th day, when total abundance reached its maximum and then decreased, first slowly (28-49 days) and then rapidly (49-70 days) (Fig. 2). By day 35, the nematodes left the agar media *en masse* and emerged on the top lid of the Petri dish, where they died. From the diagram in Figures 2 and 3, we can conclude that there are no more than nine generations in the microbiome (70 days of total population cycle equals 9G, while generation period G equals 8 days); the decrease in the number of mobile stages after 28 days can be explained by cessation of egg development, a decrease in the number of females and their proportion in the population (Fig. 3), while the proportion of J3 in the population increased to 100% with a decrease in their numbers (Fig. 4; Table 3). The J3 is the only stage of the life cycle capable of active movements in tap water, enabling their survival and transmission. By the 42nd day, the eggs contained no live invasive juveniles and no J2 hatch occurred when eggs were transferred to a new 2% PSA plate.

For the period of 1-28 days, the formula for exponential growth of the abundance of total mobile stages was determined: $N = 62.543e^{0,2267T}$; $R^2 = 0.890$; N = total abundance of worm-like individuals, T = number of days after inoculation (Fig. 2). The formula for the growth of number of females (period of 1-28 days): $N = 14.582e^{0,1729T}$; $R^2 = 0.899$.

Lifespan of females. Figure 3 shows that the majority of females are present in the population from 14 to 63 days. Therefore, the female lifespan in adult state is 63-14 = 49 days, another 8 (7-10) days is time of the individual development from egg to adult state. Consequently, female longevity is presumed to be 57 (56-59) days.

Population growth formula applied to calculate the time to decompose the already dead wood with the nematode contribution. Elm tree volume (mean) = 1.50 m^3 (Anonymous, 2021), while the PSA media in a Petri dish equals 14 cm^3. 1.5 $m^3 = 1.5\times10^6$ cm^3, which equals approximately 10^5 volumes of the agar media in a Petri dish, the latter reaches its maximum of 15×10^3 nematodes at 28th day after inoculation.

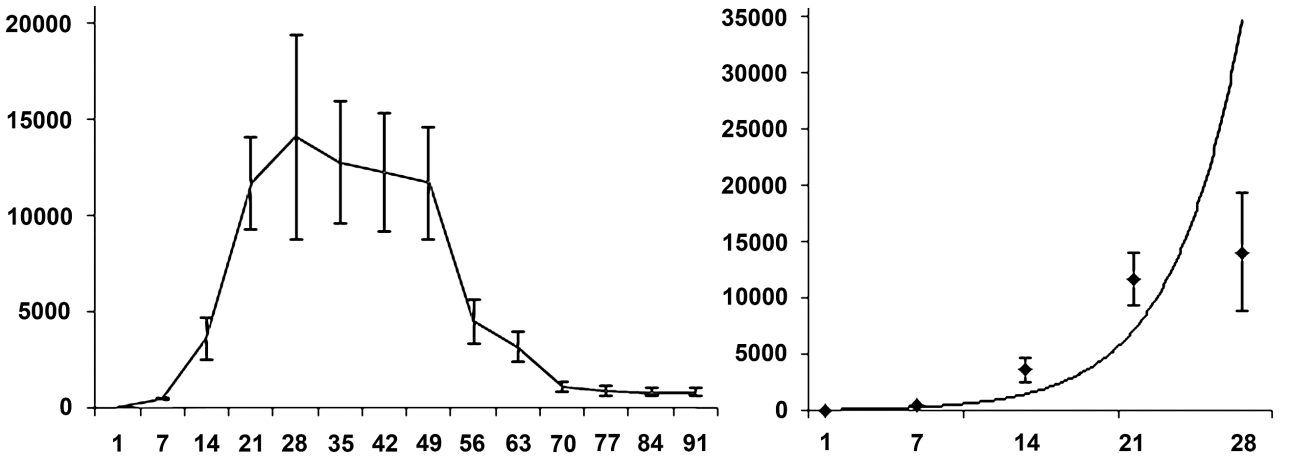

Fig. 2. *Rhabditolaimus ulmi*. Changes in the total abundance of worm stages (juveniles, males and females) during the population cycle in a laboratory culture. A: During the total duration of the experiment; B: During the exponential growth phase (1-28 days). Ordinate axis: abundance, abscissa axis: number of days after inoculation with 50 adult nematodes. Error bars denote standard deviation; the smooth curve in B serves as an approximation of the exponential growth in the period up to 28 days after inoculation. Formula of the exponential growth: $N = 63.2e^{0.2253T}$; $R^2 = 0.8859$; where N = number of specimens and T = number of days after inoculation.

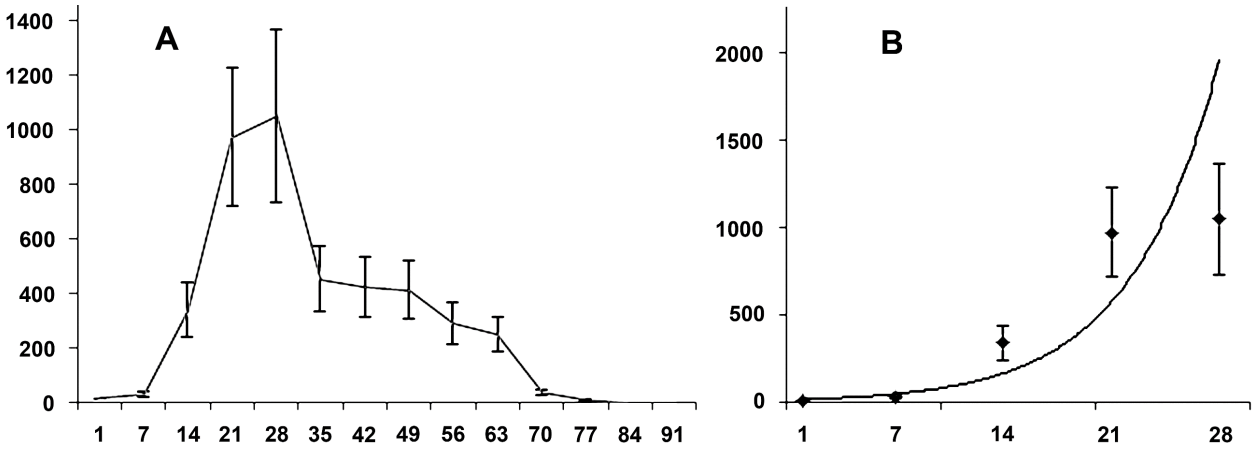

Fig. 3. *Rhabditolaimus ulmi*. Number of females in the population cycle in a laboratory culture. A: During population cycle; B: During the phase of exponential growth. Ordinate axis: abundance, abscissa axis: number of days after inoculation with 50 adult nematodes. Error bars denote standard deviation; in B the smooth curve serves as an approximation of the exponential growth in the period up to 28 days after inoculation. Formula of exponential growth: $N = 14.262e^{0.1759T}$; $R^2 = 0.9093$; where N = number of specimens; T = number of days after inoculation.

Table 3. *Rhabditolaimus ulmi*. Relative abundances (%) of the life cycle stages (eggs, juveniles, adults) in different phases of population dynamics. Values are expressed as mean ± s.d.

Character	Inoculation	8 days (G)	28 days	70-91 days (the end of a population cycle)
n	15	15	15	15
eggs	0	24.4 ± 12.4	8.3 ± 3.3	0
J2	0	22.7 ± 11.7	21.2 ± 6.0	0
J3	0	20.3 ± 5.8	47.3 ± 13.5	100%
J4	0	9.2 ± 2.9	10.8 ± 5.2	0
Females	60	11.8 ± 4.8	6.2 ± 2.2	0
Males	40	11.7 ± 5.4	6.2 ± 3.2	0

J2, J3, J4 = second-, third- and fourth-stage juveniles, respectively.

The quantity of nematodes which may fill the tree in case of non-arrested reproduction: 15×10^3 individuals $\times 10^5 = 15 \times 10^8 = 1.5 \times 10^9$ individuals.

Using the formula of the exponential population growth: $N = 63.2e^{0.2253T}$; $R^2 = 0.8859$ it is possible to calculate the time that is necessary for the nematode population to fill the tree when the nematode multiplication continues according to the exponential formula, not being restricted by food limitation.

$T(\text{days}) = LN(N/K)/k = LN(1.5\times10^9/63.2)/0.2253 = 75.4$ days, or approximately 2.5 months of the warmest season when the average day temperature is higher than 21°C. While the warmest period with such temperature in the temperate climate zone is about 1 month per year, it means that nematodes and their symbionts need 2-3 years before the dead elm can be utilised by the nematodes.

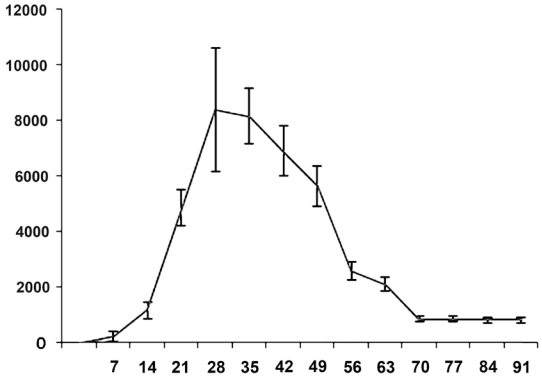

Fig. 4. *Rhabditolaimus ulmi*. Changes in the number of third-stage juveniles during population cycle in a laboratory culture. Ordinate axis: abundance, abscissa axis: number of days after inoculation with 50 adult nematodes. Error bars denote standard deviation.

DISCUSSION

The nematode life cycle (G) averages 8 days. The start date of the cycle is considered here as the date of completion of the final moult of a young female or colonisation of a new substrate. From the inoculation date, the female starts oviposition in 1.07 (1-2) days; J2 hatch in 2.6 (2-4) days; J3 appear in 3.7 (3-5) days; J4 appear in 6.01 (5-9) days; and adult males and females of the new generation appear after 8 (7-10) days.

The intensive population growth lasts for 28 days (4 weeks) *in vitro* at 21-23ºC, which corresponds to 3.5-4.0 generations. By day 35, the nematodes begin to leave the agar medium *en masse* and die; after nine generations (70 days), the population stops its development and declines. For the phase of initial population growth, the formulae of exponential growth of the total abundance of active stages and the number of females were developed.

The population cycle was completed when the population comprised 100% J3, probably the true diapause (survival) stage. Since only J3 are resistant to osmotic stress, they can be considered as the resistant stages for transmission and diapause, which is consistent with previous studies (Ryss *et al.*, 2021). The mature individuals die in tap water within 30 min, and the eggs do not contain invasive J2 and are dead. Consequently, both dwelling inside the tree bark and on the surface of the vector body provides the nematodes with an osmotic protection from an outer environment. Completion of the cycle by an increase in the number of resistant juvenile stages was found earlier for *Panagrolaimus* and *Ditylenchus* species (Perry, 1977, 1999; Perry & Moens, 2013; Polyanina & Ryss, 2021).

The fecundity of females on the first day of oviposition (one day later than inoculation to a new substrate) is 1.5 ± 0.9 eggs per day; it is almost half that of the fecundity during the whole first generation (2.8 ± 1.8). The increase in fecundity occurred probably due to the formation of temporary swarming groups, which were the sustainable mating and oviposition aggregations.

The average lifespan of the *Rhabditolaimus* female in an adult phase is the difference between the period of maximum population abundance (14-63 days) and the time of reaching the high number of individuals (14 days). Taking into account the additional time of development (one more generation), the average lifespan of the nematode female is approximately equal to seven generations, *i.e.*, 57 (56-59) days at 21-23°C.

Compared to the *Panagrolaimus* population cycle *in vitro*, the *Rhabditolaimus* cycle is longer (9G *vs* 4G) and its final stages are different: in *Rhabditolaimus* these are J3, while the *Panagrolaimus* final population structure includes the diapausing eggs and J3 (Polyanina & Ryss, 2021). These differences are due to the relationships of *Rhabditolaimus* with the vector and host plant, which presumably provides an osmotic protection for the adult individuals of the commensal nematode species.

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А.Ю. Рысс и К.С. Полянина. Цикл развития и динамика численности популяции *Rhabditolaimus ulmi* (Nematoda: Rhabditida: Diplogastridae) *in vitro*.

Резюме. Исследованы цикл индивидуального развития и цикл популяции *in vitro* нематод бактериофагов *Rhabditolaimus ulmi*, комменсалов короеда *Scolytus multistriatus* из вяза *Ulmus glabra*, нематоды используют жука как переносчика, а дерево как среду обитания пропагативного поколения. Цикл индивидуального развития (G) в среднем составляет 8 дней. Стартом цикла удобно считать дату завершения финальной линьки молодой самки или колонизации нового субстрата. От стартовой даты самка приступает к яйцекладке через 1.07 (1-2) дня; личинки J2 вылупляются через 2.6 (2-4) дней, личинки J3 – 3.7 (3-5) дней; J4 – 6.01 (5-9) дней; новые половозрелые особи появляются через 8 (7-10) дней. В лабораторных культурах пик численности наблюдается на 28 день (3.5G); популяционный цикл равен 9 поколениям (9G) и завершается структурой с 100% личинок J3 (70-91 день). Половозрелые особи осмотически чувствительны и гибнут в пресной воде, поэтому лишь личинки J3 могут быть резистентными стадиями. Хозяин и переносчик предположительно обеспечивает взрослым особям осмотическую защиту. Средняя скорость яйцекладки самки 2.80 ± 1.82 яиц/сут., а в первые сутки после инокуляции – 1.54 ± 0.94. Средняя продолжительность жизни нематод при температуре 22 (21-23)°C приблизительно равна 57 (56-59) сут. (7 генераций). Разработаны формулы экспоненциального роста общей популяции нематод ($N = 63.2e^{0.2253T}$; $R^2 = 0.8859$) и числа самок ($N = 14.262e^{0.1759T}$; $R^2 = 0.9093$), где N – численность, а T – время в сут.