The development and population dynamics of *Aphelenchoides* sp. (Rhabditida: Aphelenchoididae) from *Quercus robur in vitro*

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Summary. The ontogeny parameters and population dynamics of nematodes are important for modelling the forest ecosystem functioning. Parameters of ontogeny of *Aphelenchoides* sp. found from oak wood were determined in cultures of the fungus *Botrytis cinerea*. Oviposition began 4.6 (3-5) DAI with adult nematodes. Timelines of juvenile formation were: second-stage juveniles – 6.6 (6-8) days, third-stage juveniles – 7.5 (7-9) days, fourth-stage juveniles – 8.8 (8-10) days; first generation (1G) ended with a J4 moulting to adults after 10.5 (9-11) days. After 1-2 days from the formation of the first generation, nematodes left the 2% potato-sugar agar (PSA) medium with the fungus food and formed a migratory group (MG) on the lid. In PSA the total nematode abundance reached peaks on day 21 (2G), in the MG on day 28 (2.8G). The fecundity of females during the population growth period was determined as 1.3 eggs day^{-1} and a model of exponential population growth was developed for total nematodes $N = 87.877e^{0.1723T}$, $R^2 = 0.9389$ and for females $N = 84.918e^{0.1187T}$, $R^2 = 0.9255$, where T is a number of days. The average lifespan of nematodes was 33-44 days at 22 (21-23)°C. Under the conditions of unrestricted reproduction and temperature of 21-23°C, the nematode and fungus can colonise 1 m^3 of PSA-like medium in 92 days. The survival stage in the propagative generation was presumably the adult inseminated female. The female lifespan consists of a feeding and reproductive phase in the PSA (10-11 days) and a survival phase in the MG (3 weeks to 1 year). In MG, nematodes formed swarming aggregations of 10-80 individuals.

Key words: fecundity, forest ecosystem, lifespan, moults, mycophagy, population modelling, propagative generation, survival stage, swarming.

In studies of the successions of soil nematode communities, the ratios of 'guilds' are widely used. The guild is an ecological group of nematode genera that have similar feeding type, life span, generation time, fecundity, and place in the 5-8 year succession of soil recovery starting after destructive influences, such as pollution or drought, when the community re-establishes from the basal state along two food web trajectories – the structural and enrichment (Ferris *et al.*, 2001; Ferris, 2010; Du Preez *et al.*, 2022).

Compared to soil nematodes, the successions of dead wood nematode communities are less studied, although Northern European countries have adopted programmes for the conservation of dead wood since rare species of fungi and invertebrates inhabit the decomposing dead wood, including species listed in The International Union for Conservation of Nature Red List of Threatened Species (IUCN, 2022; https://www.iucnredlist.org/). Nematodes are important participants in the 5-stage decomposing of dead wood, and have associations with arthropods, fungi and bacteria involved in dead wood biological utilisation (Stokland *et al.*, 2012; Cuff *et al.*, 2021; Wainhouse & Boddy, 2022). A search for the model nematode species to quantify wood succession is a contribution to the development of predictive technology for biodiversity conservation.

This study is a part of research of life cycles *in vitro* of four species of the order Rhabditida (*Panagrolaimus detritophagus*, *Rhabditolaimus ulmi*, *Aphelenchoides* sp. and *Bursaphelenchus willibaldi*), belonging to two trophic groups: bacterial feeders, and plant and fungal feeders (Polyanina & Ryss, 2021; Ryss & Polyanina, 2022a, b). Although the species belong to the same phylogenetic line (Paramonov, 1970; Holterman *et al.*, 2006; van Megen *et al.*, 2009), parameters of their life cycles may be used for the comparative

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analysis of diversity and origin of life cycles, from the monoxenic bacterial feeders to polyxenic parasites (Ryss, 2009, 2016).

The current research deals with the nematode *Aphelenchoides* sp., which combines mycophagy and phytoparasitism on the oak *Quercus robur* L. The study is aimed: *i*) to determine the duration of one generation, the timing of hatching and moulting between juvenile stages; *ii*) to determine the oviposition, the juvenile stage at hatching and the sex-age proportions during the population cycle; *iii*) to monitor the population dynamics under initial food abundance and develop a mathematical model of initial exponential population growth phase *in vitro*; and *iv*) to identify the survival stage that prevails at the end of the population cycle and the age-sex pyramid of the migration fraction of the population.

MATERIAL AND METHODS

Nematode population. Specimens of *Aphelenchoides* sp. (order Rhabditida, family Aphelenchoididae) were collected in August 2021 from decaying wood of oak *Quercus robur* in the park zone, Bolshoi prospect VO, building 25, St. Petersburg, Russia. GIS coordinates were 59.93922, 30.28179. The species was characterised using morphological and molecular approaches (Ryss *et al.*, in preparation) and indicated 86.6% of similarity in the D2-D3 of 28S rRNA gene sequence with that of *Aphelenchoides eldaricus* (LC191272).

Morphological and molecular characteristics of the nematode isolate did not match. The species *Aphelenchoides eldaricus* belongs to the *A. fragariae* species group (*Aphelenchoides*-2 clade according to Ryss *et al.*, 2013); the group is diagnosed by the needle like single mucro, and excretory pore posterior to nerve ring. However, according to the morphological database of 150 *Aphelenchoides* species and 27 characters (Ryss *et al.*, 2008, the computerised key was updated in 2022) *Aphelenchoides* sp. isolated from oak is most close to *Aphelenchoides brevistylus* Jain & Singh, 1984 belonging to *Aphelenchoides*-1 clade *sensu* Ryss *et al.* (2013) and Kanzaki and Giblin-Davis (2012) with the species *A. variacaudatus* and *A. xui*. *Aphelenchoides* sp. from oak is characterised by the mucron in the form of a step-like projection with many tiny nodular protuberances, and the excretory pore at or anterior to the nerve ring. According to the *Aphelenchoides* spp. morphological grouping by Shahina (1996), *Aphelenchoides* sp. isolated from oak belongs to the Group 2.

Due to differences from *A. eldaricus* in spermatheca, mucron and spicule morphology, and from *A. brevistylus* in the lateral field structure and the position of the excretory pore, the isolate is characterised here as *Aphelenchoides* sp. and will be described as a new species in a future publication. However, the morphological characters distinguishing juvenile stages were defined while it was necessary to study life cycle and population composition at different phases of population dynamics.

The species is maintained in a laboratory agar culture of the fungus *Botrytis cinerea* Pers. and in sterile oak bark in the Nematode Collection of the Zoological Institute of Russian Academy of Sciences ('UFK ZIN RAS'): Living Nematode Culture Collection; Nematode Preparation Collection; Nematode Genetic Material Collection. Other isolates of this species in the oak wood and bark were collected in parks of the following districts of St. Petersburg, Russia: Admiralteisky, Vasileostrovsky, Moskovsky, Petrogradsky, and Tsentralny.

Extraction and cultivation. Nematodes were extracted using the modified Baermann funnel method according to Ryss (2017a) and maintained on *B. cinerea* growing on potato-sugar agar (PSA) medium. For PSA preparation, 40 g of peeled potatoes were grated, boiled for 60 min in 400 ml of tap water, and filtered through a double layer of gauze. Ten ml of glycerol, 4 g of sucrose, and 4 g of agar were added to the hot solution, and stirred until the reagents were dissolved. The mixture was filled with tap water to give a final volume of 200 ml, and sterilised for 30 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 up to a 2-3 mm layer, cooled, and Petri dishes were sealed with Parafilm. Before nematode inoculation, two sterilised pieces of oak bark (20×10×1 mm) were placed on the agar surface of each Petri dish. A 2 mm wide tape of mycelium from the upper layer of the *B. cinerea* culture was introduced with a sterile needle. A ring with a 15 mm diam. was formed from the tape in the centre of Petri dish and a suspension of adult individuals was inoculated, with a common average ratio of females:males of 7:1.

For the first and second experimental set (the population cycle monitoring, 20 Petri dishes) the inoculum of 150 adult individuals was used (131 females: 19 males), and for the third set (the life cycle study, 10 Petri dishes) 50 adult individuals (44

Table 1. Measurements (in µm) and indices of *Aphelenchoides* sp. developmental stages.
Values are expressed as mean ± standard deviation (range). J2, J3, J4 – second-, third- and fourth-stage juveniles, respectively.

Character/stage	J2	J3	J4	Male	Female
n	10	10	10	20	20
Body length (L)	161 ± 24 (129-188)	267 ± 15 (247-285)	391 ± 11 (373-402)	564 ± 62 (457-649)	623 ± 69 (438-729)
Body diam.	8.8 ± 0.7 (8-10)	10.5 ± 0.8 (9-11)	12.7 ± 1.1 (11-14)	15.0 ± 1.2 (13-17)	17.1 ± 1.7 (12.5-19.5)
Genital primordium, or gonad length (GPL)	8.6 ± 1.1 (7-10)	20.9 ± 2.1 (18-23)	83.4 ± 1.7 (80-85)	250.7 ± 40.4 (202-323)	319 ± 41 (248-393)
Genital primordium, width (GPW)	4.7 ± 0.9 (3.5-6.0)	4.9 ± 1.0 (4.0-6.0)	5.5 ± 0.2 (5.0-6.0)	9.7 ± 0.3 (9.0-10.5)	10.0 ± 2.0 (6.0-13.0)
Ratio: body length/body diam. (a)	18.4 ± 2.4 (14.8-21.7)	25.7 ± 2.1 (22.1-27.9)	31.0 ± 1.8 (28.8-33.1)	37.4 ± 1.9 (34.1-39.9)	36.5 ± 1.9 (33.3-40.6)
GPL/GPW	1.9 ± 0.4 (1.3-2.5)	4.4 ± 1.0 (3.1-5.5)	15.1 ± 0.7 (14.1-16.2)	25.7 ± 3.2 (22.0-31.4)	34.0 ± 7.2 (24.4-54.7)
GPL/L, %	5.4 ± 0.9 (4.7-7.0)	7.8 ± 1.0 (6.1-8.9)	21.4 ± 0.9 (19.9-22.8)	44.3 ± 3.5 (38.7-49.8)	53.0 ± 4.0 (43.0-59.0)

females and 6 males) were inoculated. The PSA without host bark was not a good medium for the active and stable nematode reproduction; presumably, the nematodes needed some unidentified compounds from the host bark. With the addition of oak bark, the reproduction of this nematode species was most stable in every set of 10 Petri dishes, as verified in a preliminary study. The bark forms a brown film on the surface of the agar that is probably important for stable nematode reproduction. Observations were carried out at room temperature of 22 (21-23)°C.

Identification of the juvenile stages and adult individuals. Juvenile stages were identified by the body size of live and fixed specimens (Table 1). Nematodes were fixed by a hot TAF solution (10 ml of 39% formaldehyde, 90 ml of distilled water and 2 ml of triethanolamine) at 85-90°C and processed in the glycerol-water mixture to anhydrous glycerol using the 'cocktail method' (Ryss, 2017a, b) and mounted in permanent slides. Stages of juveniles differed in body size and genital primordium structure and size; males and females were identified by copulatory organs. Moulting specimens are assigned to the oldest stage (into which the juvenile is transformed during a moult); for every juvenile stage the proportion of moulting individuals was also evaluated. If the juvenile was inside the egg shell the individual was considered as an egg; any egg with a partially emerged juvenile was classified as a second-stage juvenile (J2).

Population dynamics experiment. Experiment series 1 and 2. Estimation of abundance in different phases of population dynamics. The experiment was performed in two series, using 10 Petri dishes in each series. Adult individuals (150; 131 females and 19 males, sex ratio 7:1) were inoculated in every Petri dish, as described above. The large inoculum was used to approximate the experimental conditions to the natural infestation and to improve the statistics, because large numbers of nematodes are easier to detect and count in the opaque *B. cinerea* mycelium and such inoculum was less susceptible to random influences and mortality during the initial period of a culture reproduction.

The purpose of the experiment was to determine the mean value for each date and to find out statistical differences between count dates (dynamics); the spatial variability of sampling spots in each Petri dish was not evaluated as it was not in scope of the experiment. The possible spatial variability was reduced by combining the 10 samples in each of the two series into a single container, from which 10 samples of equal volume (100 µl) were then taken; in each the number of nematodes was counted. A recalculation was made to obtain the mean value for the Petri dish in two series with 10 Petri dishes in each.

Four discs were cut from the PSA medium in each of ten Petri dishes using an interchangeable micropipette tip for 20-200 µl volumes, trimmed to a 5 mm diam. Forty discs (total volume of 3-4 ml) were placed into a 10 ml tube. The tube was filled with sterilised tap water to 6 ml and the PSA clumps were carefully crushed with tweezers, and dispersed in a Microspin FV-2400 centrifuge-vortex (Biosan) at 500 *g* using a shaking mode. The suspension was dispensed into four 1.5 ml tubes. Tubes were placed in the shaker again. From the obtained suspension, 10 sub-volumes of 100 µl were taken by

micropipette and the number of females, males, and juvenile stages (J2, third-stage [J3] and fourth-stage juveniles [J4]), and eggs, as well as their proportions and total abundance, were determined. Data for sub-volumes (n = 10) were recalculated first for the entire 6 ml volume test tube volume and then for a single average Petri dish. This count (eggs, J2, J3, J4, males and females) was a sampling element; there were 10 such counts for each time date (n = 10). There were two such series of experiments, using the same protocol, with an interval of 35 days between the series. Thus, the number of counts (sampling elements) on each date from the time of inoculation was, n = 20. To avoid a decrease in the nematode individuals in the Petri dish due to withdrawal of nematodes, the suspension (600 µl each) was returned to Petri dishes after counting. Nematode abundances were counted for 7, 11, 13, 15, 21, 28, 35, 42, 48, 55 and 63 days after inoculation (DAI).

At the mature stages of the population development, adult nematodes moved to the underside of the lid of the Petri dish, where they continued mating and oviposition in water condensate droplets. This nematode fraction (migration group; MG) was counted separately using a similar method and intervals as described above for the PSA fraction. Nematodes from 10 dishes were flushed with tap water into a 1.5 ml tube, shaken in a microcentrifuge (Microspin) and independently counted in 10 sub-samples of 100 µl taken from the tube; the values were recalculated as a total for one dish at a count date. As stated above for PSA, there were two such series of experiments of 10 counts for each date (n = 20 counts combined in one sample). In the case of MG, the suspensions were not returned to Petri dishes after counting because these nematodes did not return to the PSA layer, but survived without feeding.

Mathematical modelling of the population cycle. A graph of population dynamics, namely the phase of rapid initial growth of the population increase, was used to develop the mathematical model. We assumed that the population growth at the beginning reflected the abundance of food, and the subsequent decrease in the growth rate was due to the gradual depletion of food. To determine model parameters the exponential growth module of the MS Excel software was used. The time required for the nematode reproductive population to colonise the fungus-infected PSA substrate was estimated using a reverse recalculation, from the exponential growth time-dependent model formula to the natural logarithm of the final nematode abundance that would fill PSA-like substrate at its peak of population propagation.

Determination of the survival stage in a population at the end of the population cycle. We assumed that due to food exhaustion at the end of the population cycle, the population goes into a state of survival adapted to the long wait for either a new food source, or the appearance of a host or vector. Therefore, the structure of the population was analysed during the period of the greatest decrease in population size with the identification of the predominant (survival) stage in population.

Estimation of the proportions of ontogenesis stages during the population cycle (PC). The shares of ontogenesis stages were analysed at the main dates of the population cycle: *i*) at the end of the first generation (G); *ii*) when the abundance in the agar medium reached its maximum; *iii*) at the peak of the weekly abundance of the nematode migrating group (MG); and *iv*) at the end of the population cycle (PC).

Estimation of the oviposition rate per day using the population dynamics curve. In an isolated small group of nematodes in water and on the surface of 2% agar, mating and oviposition conditions can be very different from those occurring inside a nematode culture on mycelium. Therefore, it is important to make calculations of oviposition rates from the population dynamic plots using abundances of all nematodes and the female fraction. The initial period of culture is difficult to monitor due to the low number of nematodes, the opacity of the mycelium, and the fact that the eggs of the first generation themselves are poorly visible within the mycelium, and there are still few juveniles. However, comparisons of the culture composition from when the number of females doubles to when the number of females reaches its peak enables the egg production to be calculated by dividing the increment in the total population in a selected count period by the number of females at the start of the count period. Thus, the oviposition rate per day and per female (F) is given by:

$$F = [(N_{fin}-N_{start})/N_{fem\text{-}s}]/T \qquad \text{(Equation 1)},$$

where N_{fin} = total number of nematodes at the end of the count period; N_{start} = total number of nematodes at the beginning of the count period; $N_{fem\text{-}s}$ = number of females at the beginning of the count period; T = number of days of the count period.

A method to estimate daily oviposition rate by the number of eggs and nematode juveniles produced by females on the lid. In a mature culture, adults and J4 migrated to the lid of a Petri dish where there is no fungus for food. They formed ball-like swarming aggregations (MG) (Perry, 1999; Perry & Moens, 2013; Polyanina & Ryss, 2021) and

produced eggs. During the first day after exit to the lid, the females are well fed and presumably produce eggs at the same rate as the females in the PSA medium feeding on the fungus. Therefore, the daily flush makes it possible to estimate the rate of egg production. Hatching and the second moult also occur during the first day, as seen in nematode suspensions inside condensate droplets on the underside of lid; however, the small J2 and J3 are unable to reach the lid surface. Therefore, to calculate the number of eggs laid per day [N(egg × d^{-1})] was determined by the formula:

$$N(egg \times d^{-1}) = [N(egg) + N(J2) + N(J3)]/N(fem) \quad \text{(Equation 2)},$$

where N(egg) = egg number; N(J2) and N(J3) = number of second- and third-stages juveniles, respectively, in a daily flush from the lid. Total number of eggs, J2 and J3 was divided by the number of females, N(fem).

Evaluation of the life cycle parameters during the first generation. Experiment series 3. Evaluating the generation time. An additional third series of population dynamics assessment was performed with an inoculum of 50 adults (44 females and 6 males) with counts at 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 and 13 DAI (Petri dishes, n = 10). The count days were planned from results of the first two experimental series. The frequency of dates aimed to determine accurately the time of every juvenile stage formation and to evaluate the total time of the first generation.

One generation time (G) is the most important parameter for a mathematical model of population dynamics. Observation under ideal conditions of a single female or a group of 1-5 individuals may differ from the average parameters of a mass population. In the mass population a detection of female nematodes is often erroneous because the real number can be larger than the counted data after extraction due to the death of nematodes during extraction and opacity of the PSA-fungus medium. Therefore, the time when the number of females was doubled compared to the inoculum was taken as the G-date for the total population. The doubling of the number of females meant that, on average, each inoculated female produced at least one adult female of the second generation, which then also produces the next generation. The calculation of the G-date was made on the basis of females, because females are the producers of the eggs that form the next generation.

To identify the duration of one generation and the timeline of ontogenesis stages (divided by moults), a series of 10 Petri dishes (6 cm diam.) were filled with a 2-3 mm PSA medium layer. In the centre of each dish a 15 mm ring was formed from the 2 mm wide band of the fungus *B. cinerea*. Fifty adult nematodes (44 females and 6 males) were inoculated into a mycelium ring. Over 2 days, mycelium grew faster than nematodes, thus providing the nematodes with fresh food for rapid development. Every day of a 2-13 days period, four discs of PSA were excised from each dish using a pipette tip cut at the end to 5 mm diam. Discs were processed as detailed above for the first series of experiments: they were dispersed in tap water, and the number of eggs and mobile nematodes of different ontogeny stages in ten 100 µl sub-samples were counted separately. The obtained values were recalculated to the total area of PSA in one average dish.

The duration of one generation was identified using three methods: *i*) by the date when the number of females exceeded the inoculum (32 females) twofold, which meant that on average every inoculated female produced at least one new female; *ii*) by the average date of appearance of moulting females; and *iii*) according to the date when the maximum number of moults of J4 to adult was observed. The average dates of mass hatching and moults for every juvenile stage were calculated in a similar way.

Evaluation of hatching and moulting times. The average date of hatching was considered to be the day when the number of J2 first exceeded the number of inoculated females. Using a similar approach, the average dates of emergence of J3, J4 and adult females were determined: the date when the number of individuals of the corresponding stage first exceeded the number of inoculated females was considered as the date of the development of the corresponding stage. The date when the number of females exceeded the number of inoculated females by twofold was considered as the end of the first generation (1G).

We used Student's test in MS Excel to assess the significance of differences in the timing of the formation of juvenile stages in the ontogeny series.

Evaluating of the life cycle parameters in the individual age groups: determination of hatching and moulting. The timing of hatching and moulting can be determined by direct observation of hatching in a culture, using an isolated group of eggs. Hatching was observed in a group of 5-10 eggs on 2% agar for 2 days at 2 h intervals. For observation of moulting, a thin layer of mycelium of the *B. cinerea* (1 cm diam.) was placed on 2% agar in a 6 cm diam. Petri dish and juveniles were grouped by age (J2, J3 or J4), with at least 20 specimens per group. Observation was made daily by upending the Petri dish and identifying the number of different stages visible through the agar layer at the bottom of Petri dish.

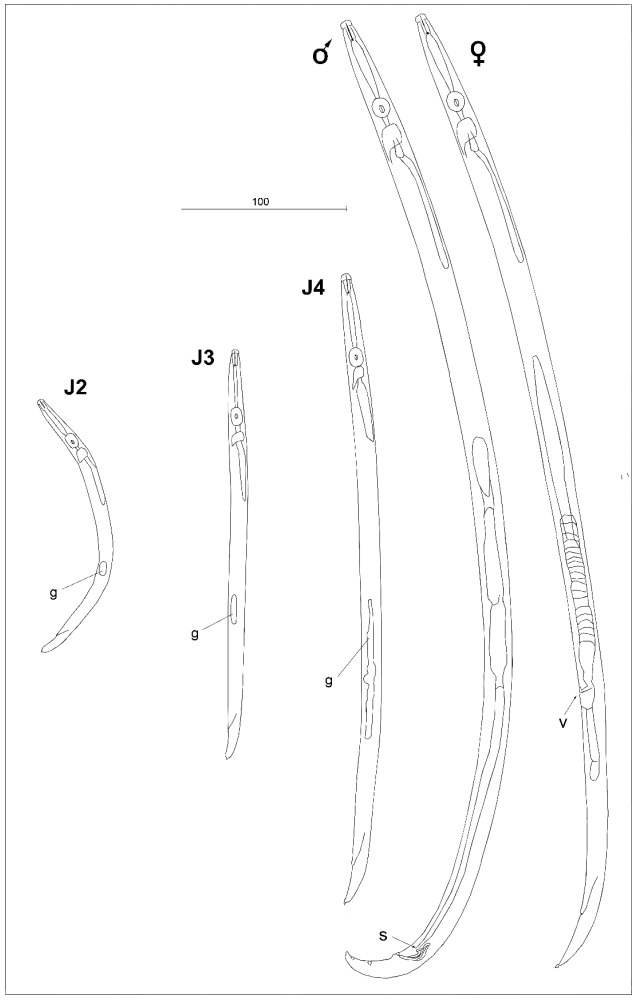

Fig. 1. *Aphelenchoides* sp. J2, J3, J4 – juveniles of 2, 3 and 4 stages, respectively. Adult male and female are marked by gender symbols. Abbreviations: g – genital primordium, s – spicules of male, v – vulva of female. Scale 100 μm.

RESULTS

Individual development, timing of the first generation. Diagnosis of juvenile stages by the body size and genital structures is presented in Table 1 and Figure 1. Measurements were made on permanent collection preparations in glycerol (hot TAF fixation; Ryss, 2017a); the measurements did not differ from that of live nematodes on microphotographs ($P < 0.05$).

The diagram of population dynamics during the first generation and the timelines of developmental stages are given in Table 2 and Figure 2. Dates for the ontogeny stages were significantly different from each other ($P < 0.05$).

Population dynamics in *B. cinerea* cultures. The population dynamics are illustrated in Figure 3. The peak of female and the total population abundance was reached on 21 DAI (2G), and then there was a sharp decrease until the day 35, which was followed by a smooth decrease until the end of the observations. The decrease was due to the mass migration of nematodes to the Petri dish lids, because the loss in of nematode abundance in the PSA medium between days 21 and 28 corresponded to the weekly increase in the number of nematodes in the MG (Figs 3 & 4). The emergence of nematodes in the MG occurred after 12-13 DAI (approximately 1G + 1 day); the peak of MG abundance was reached on day 28 (about 3G), which was 7 days (*i.e.*, less than 1G) later than the peak of abundance in the PSA medium.

In Table 3 the proportions of ontogeny stages are presented. In the PSA medium the egg production stopped after 21 days (2G, date of population peak in the PSA); in the MG females also almost stopped oviposition by day 28 (2.5G, date of population peak in the MG). Throughout the population cycle, the J2 proportion increased in the PSA. By day 63 (the completion of population cycle, 6G), the proportion of mature inseminated females increased significantly in the MG and PSA compared to earlier dates, their proportion in the population being about 92% and 33%, respectively. The data indicate the predominance of females as a possible specific survival stage at the end of PC. On the day of population peak (day 21), J4 become the dominant stage in PSA.

Calculation of oviposition rate from the graph of population dynamics (until the maximum population size reached). Experiments with observation of oviposition in transparent agar or on a lid (females, n = 50, from the daily flush of MG left in tap water for an additional one day) did not yield results of the egg production. In the absence of food, nematodes stopped oviposition; females laid eggs only within the first 6 h of entering the MG. Therefore, the real oviposition rate can only be estimated for nematodes in the PSA with fungal food source, during the period from 1G to 2G (*i.e.*, during a phase of an active exponential growth of the population). We can assume that the population increment (δ) in this period t_1-t_2, $\delta = N_{all}(t_2)-N_{all}(t_1)$, is the result of the production of the number of females at the beginning of the period, *i.e.*, at time t_1. Accordingly, the daily egg production (F, fecundity) can be estimated by the equation:

$$F = [\delta / N_{Fem}(t_1)]/T \quad \text{(Equation 3)},$$

F was 1.3 eggs day^{-1} for 10 measurements per female per day (Table 2). As the population approached to the abundance peak (from day 11 to day 21), the oviposition rate decreased from 2.3 to 0.5 eggs day^{-1} per female.

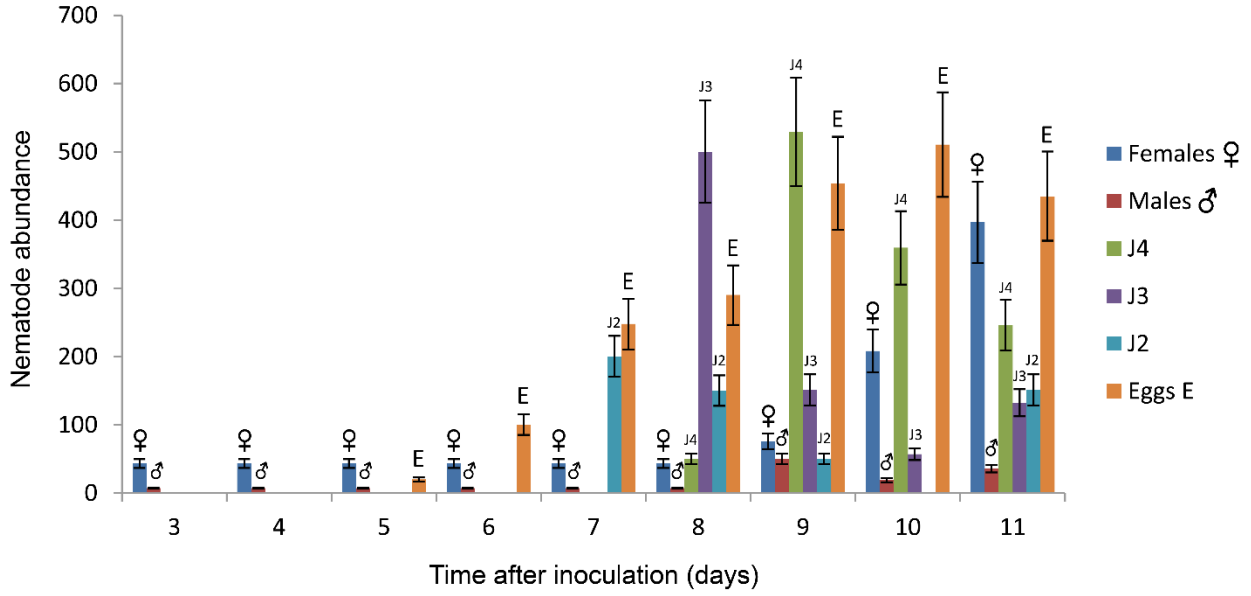

Fig. 2. Dynamics of the population development of *Aphelenchoides* sp. during the first generation. Inoculum: 50 adult nematodes (ratio 88%:12%, 44 females and 6 males). J2, J3, J4 – second, third, and fourth stage juveniles, respectively. n = 10 (number of Petri dishes).

Table 2. Scenario of individual development (days) of *Aphelenchoides* sp.

Character	n	Population studies	Time 2 × N (F_i)	Moult & hatching peaks
Egg laying start	368	4.6 ± 0.9 (3-5)	5	5
J2	400	6.6 ± 1.0 (6-8)	6,7	6,7
J3	707	7.5 ± 1 (7-9)	8	7,8
J4	1184	8.8 ± 1.3 (8-10)	9	9
G-Males	105	9.5 ± 1.2 (9-11)	9	9,10
G-Females	681	10.5 ± 1.5 (9-11)	11	9,10,11
F(G)	10	1.32 ± 0.63 (0.51-2.28)	–	–

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: 44 females and 6 males; n = number of individuals during monitoring. F(G) is a fecundity, *i.e.*, the number of eggs per day laid by one female calculated for a whole period of first generation.

Table 3. Proportions of different stages of *Aphelenchoides* sp. ontogenesis in the most important dates of population dynamics. The data are given as mean ± standard deviation per Petri dish; for the migration group (MG) the abundances of 7 days flushes are given.

Character	PSA medium					MG (lid of Petri dish)			
	Inoculation	11 days (G)	21 days*	28 days	63 days	14 days***	21 days	28 days*	63 days
n	**20**	**20**	**20**	**20**	**20**	**20**	**20**	**20**	**20**
eggs	0	35.8±10.6	0	1.4±0.6	0	6.8±2.5	5.7±1.5	0.9±0.5	0
J2**	0	3.3±1.1	2.8±1.5	35.5±8.1	21.8±5.2	9.9±1.4	16.5±1.8	9.0±3.3	2.0±0.5
J3	0	1.7±0.8	0	22.8±4.7	0	9.7±2.1	1.1±0.5	3.2±0.7	0
J4	0	13.3±4.1	64.9±11.6	16.9±4.8	10.4±4.0	23.2±6.4	2.5±1.5	5.5±1.7	2.4±0.8
Females	88	42.5±10.1	28.0±7.1	17.7±8.0	33.0±10.1	45±17.3	68.3±2.5	75.4±4.7	91.9±7.5
Males	12	3.3±1.4	4.3+1.3	5.6±2.1	1.9±0.7	5.2±1.8	5.8±2.3	6.3±1.3	3.8±1.3

* Peak of density.

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

*** First appearance of nematodes on a top lid of Petri dish.

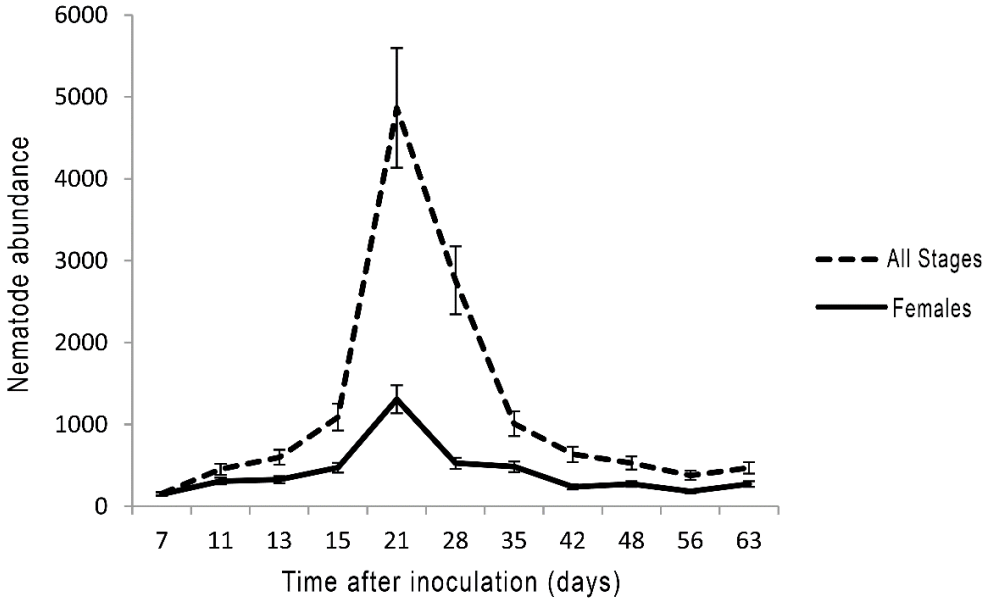

Fig. 3. *Aphelenchoides* sp. population dynamics in PSA medium. Inoculum of 150 adult individuals (131 females and 19 males). n = 20 (number of Petri dishes).

Mathematical model of population dynamics. During the period of active population growth (up to day 21, 2G), the population dynamics of females and total abundance in the PSA medium can be expressed by an exponential mathematical model (Fig. 5).

For total abundance:

$N = 87.877e^{0.1723T}$; $R^2 = 0.9389$; $F = 61.4793$; $P = 0.0014$; $df = 5$;

for female population dynamics:

$N = 84.918e^{0.1187T}$; $R^2 = 0.9255$; $F = 49.7001$; $P = 0.0021$; $df = 5$.

The exponential model is also applicable for the MG fraction, both for females and total nematode abundance, but with a shift of 7 days (less than 1G) (Fig. 6).

For the total MG population dynamics:

$N = 121.3e^{0.1072T}$; $R^2 = 0.956$; $F = 43.4232$; $P = 0.0223$; $df = 3$;

and for the female fraction dynamics in the MG:

$N = 100.57e^{0.1034T}$; $R^2 = 0.9317$; $F = 27.2838$; $P = 0.0348$; $df = 3$.

Calculation of the time of the substrate colonisation for 1 m^3 (T). Exponential models are applicable for determining the time when the association of nematodes and fungi reach the capacity to fill the PSA volume of 1 m^3 in environmental conditions similar to that of the experiment. The calculation can be done by a simple inverse transformation of the exponential formula of abundance into a formula for calculating the time T.

The volume of the PSA medium in a Petri dish was 7 cm^3 (inner diam. of Petri dish, d = 5.5 cm, thickness of agar layer, h = 3 mm, according to the formula volume of PSA layer in the dish $\pi d^2/4 = 7$ cm^3). Substrate 1.0 m^3 = 1.0×10^6 cm^3, which corresponds to 1.4×10^5 volumes of the PSA layer per Petri dish. On day 21 after inoculation, the population peaked at an average of 4.86×10^3 nematode individuals (Fig. 3); at this time the PSA medium is fully consumed by the nematodes.

Consequently, a nematode population of $(4.86 \times 10^3) \times (1.4 \times 10^5) = 6.8 \times 10^8$ individuals are required to fill a 1 m^3 of the PSA-like substrate. From the formula $N = 87.877e^{0.1723T}$ we obtain the number of days which are necessary to fill 1 m^3 of the PSA-like substrate with nematodes: $T(\text{days}) = LN(6.8\ 10^8/87.877)/0.1723 = 92.1$ days.

For MS Excel, the formula template looks like: = LN((6,8*10^8)/87,877)/0,1723.

Thus, under favourable temperature conditions (not less than 21°C) and the presence of fungal food, the nematode-fungus association is able to occupy 1 m^3 of the PSA-like medium for *ca* 92 days. Considering that the number of days a year with an average temperature above 21°C in a temperate climate zone in Russia varies from 25 to 31 days (according to the calculation of the warmest 30-day period in July-August for last years), the nematode-fungus association is able to fill oak wood (1 m3) approximately for 3 years.

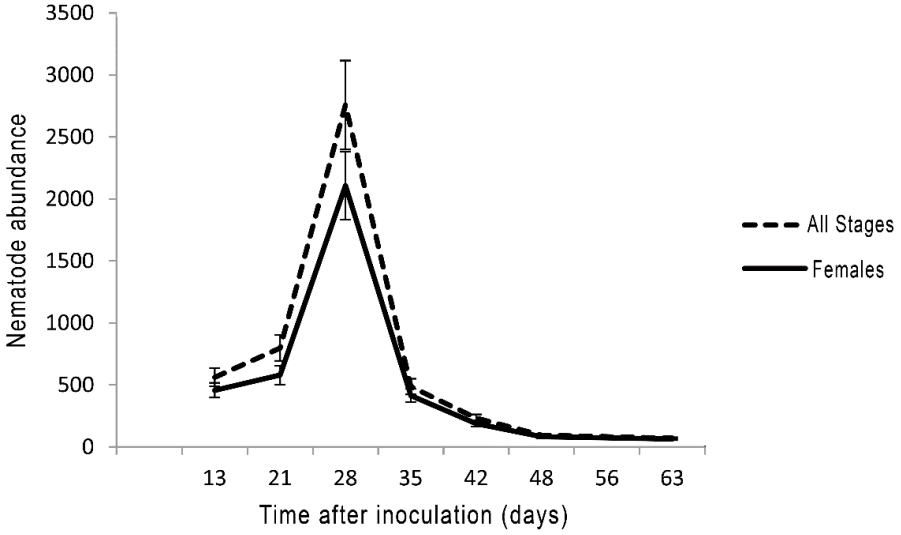

Fig. 4. *Aphelenchoides* sp. Population dynamics in a migratory group (MG) on the underside of the Petri dish lid). Inoculum of 150 adults (131 females and 19 males). n= 20 (number of Petri dishes).

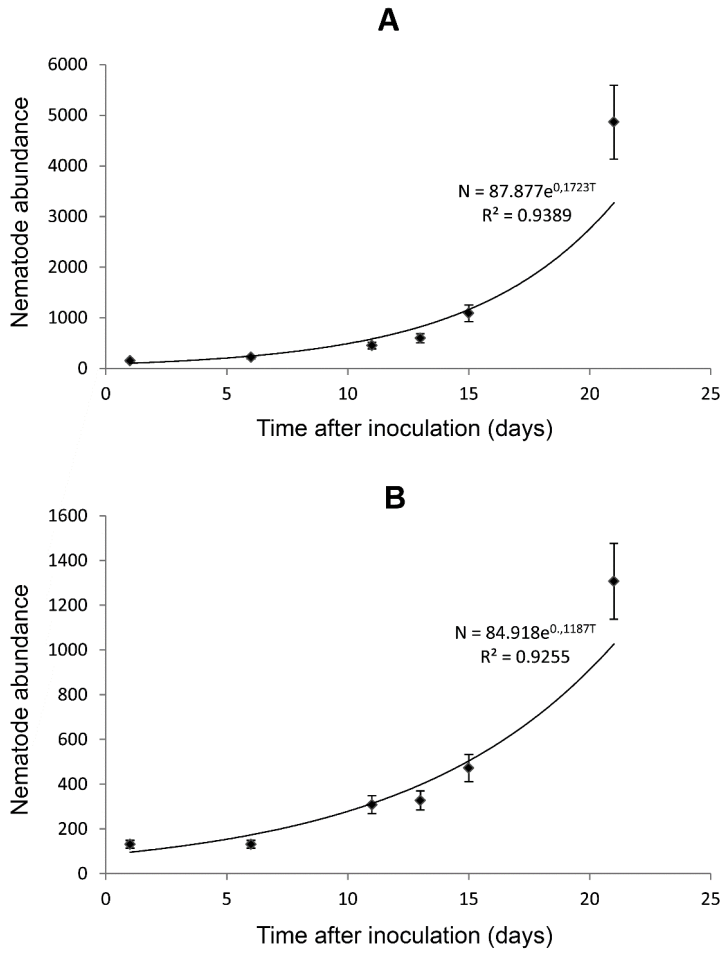

Fig. 5. *Aphelenchoides* sp. Population dynamics in PSA medium (feeding group of nematodes). A – dynamics of total abundance $N = 87.877e^{0,1723T}$, $R^2 = 0.9389$; $F = 61.4793$; $P = 0.0014$; $df = 5$. B – dynamics of females' abundance $N = 84.918e^{0,1187T}$, $R^2 = 0.9255$; $F = 49.7001$; $P = 0.0021$; $df = 5$. Error bars expressed standard deviation.

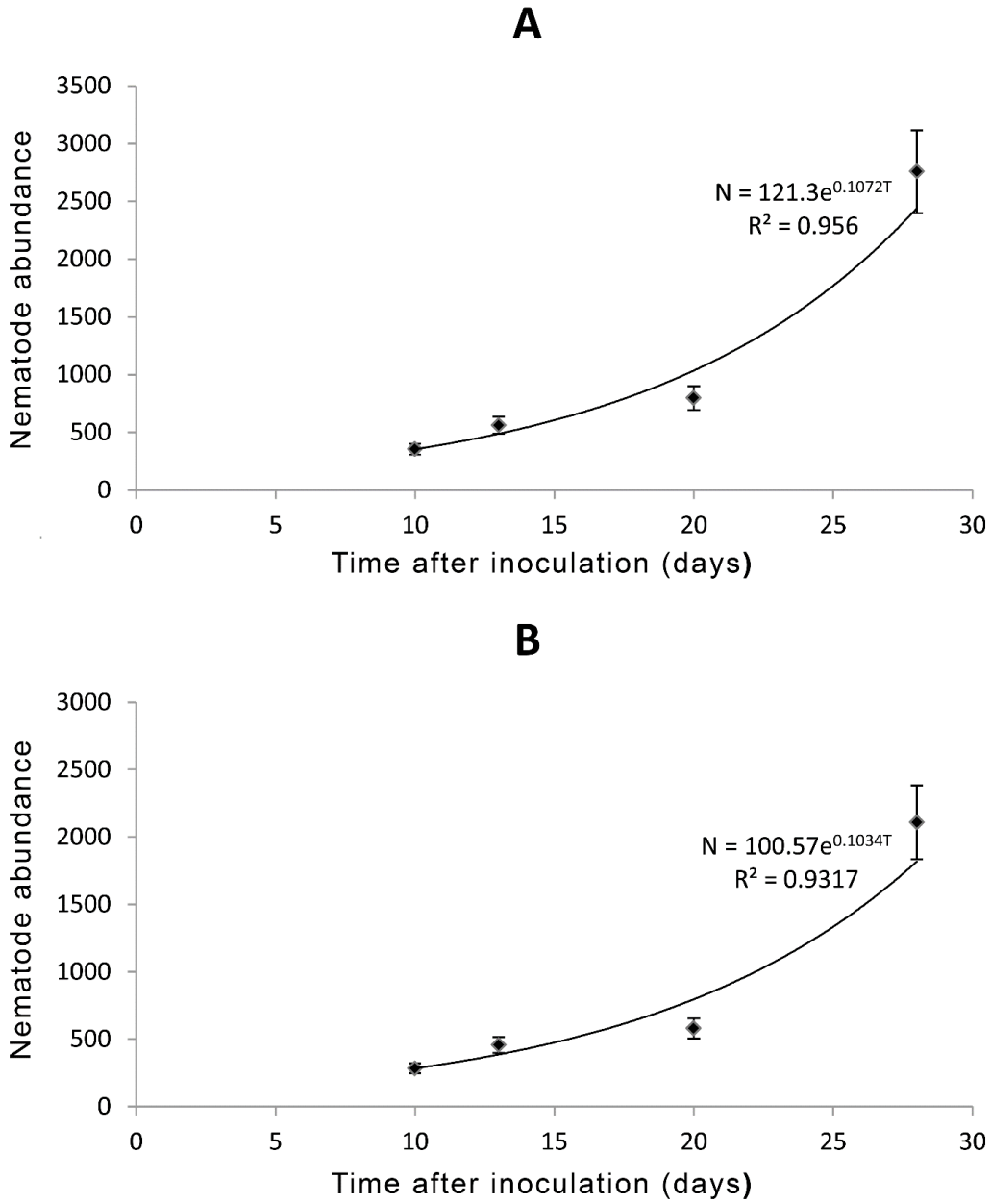

Fig. 6. *Aphelenchoides* sp. migration group (MG) on the underside of the Petri dish lid. A – total abundance dynamics $N = 121.3e^{0.1072T}$, $R^2 = 0.956$; F = 43.4232; *P* = 0.0223; df = 3. B – female abundance dynamics, $N = 100.57e^{0.1034T}$, $R^2 = 0.9317$. F = 27.2838; *P* = 0.0348; df = 3. Error bars expressed standard deviation.

Swarming. The movement of nematodes from the PSA medium to MG occurred in groups along pathways marked on the surface of Petri dishes with water condensation spots. On the surface of the lid, the nematodes form moving ball-shaped swarming groups of 10-80 individuals. We did not study the migration stimuli, but migration itself started at the end of the 1G period and continued to the end of the population cycle. Migration occurred along common pathways for groups of nematodes, and swarming was evident in which mating and oviposition occurred during the first 5-6 h after upward migration of nematodes.

Nematode lifespan. The most important parameter for the population model is the lifespan of females, and especially the duration of active oviposition of females. Figures 3 and 4 show that the nematode spent part of its ontogenesis (1G) from egg to adult in the fungus-seeded PSA, and then the bulk of the adult nematodes and J4 migrated

upwards and females stopped oviposition during the first day after migration; nematodes have stopped feeding in the MG.

The duration of life in the migration phase depended on the temperature. In a refrigerator at +4°C, nematodes could survive and serve as inoculum for a new culture for 6-12 months. At room temperature, the migration 'starvation' phase of ontogenesis lasts approximately 2-4 weeks (3 weeks or 2G, on average). It is important that the suspension of live nematodes occurred under conditions of oxygen access: in a Petri dish there is a water film on the lid, and in a tube in a minimum volume of water, no more than 250 µl for 1.5 ml tube. In a tube completely filled with water, the nematodes died within 2 days both at +4°C as well as at 21-23°C. For long-term survival, the nematodes must be without agar medium or organics, simply in clean, well oxygenated tap water.

Figure 3 can be used to calculate the duration of the first (oviposition) phase of the female ontogeny: the female abundance peaked on day 21, *i.e.*, 2G. It means that most of egg-producing females in *B. cinerea*-PSA culture were the females of the second generation; they stopped their oviposition on day 21 (2G; Table 3) indicating the maximum individual reproduction period of 21-11 = 10 days (1G). Therefore, at the room temperature (21-23°C) the female lifespan was limited by 1G + 1G + 2G = 4G, or 44 days. The female lifespan was the shortest when a nematode migrated upwards from the PSA medium just after the last moult (1G) and then survived in the MG for only 3 weeks (2G), with the minimum life duration of 1G + 2G = 3G, *i.e.*, 33 days.

DISCUSSION

Ontogeny and oviposition. The basic parameters of the *Aphelenchoides* sp. life cycle *in vitro* were revealed; these parameters were used in modelling of the colonisation of the PSA-like substrate with fungus and nematodes association. The total life cycle duration of 10.5 ± 1.5 (9-11) days is longer as compared to the previously studied life cycles *in vitro* at 21-23°C of bacteriophages: *Panagrolaimus detritophagus* (7.3 ± 0.8 days), *Rhabditolaimus ulmi* (7.8 ± 1.3 days) and *Bursaphelenchus willibaldi* (5.8 ± 0.5 days) (Polyanina & Ryss, 2021; Ryss & Polyanina 2022a, b). In the initial 3 days of the ten-day life cycle, feeding of inoculated nematode adults occurred but oviposition did not start. This conclusion was proved in an additional experiment with oviposition in absence of food where MG females stopped egg production during the first 6 h without nutrition.

The rate of oviposition varied from 2.3 to 0.5 eggs day^{-1} female^{-1} as the population approached the abundance peak and the corresponding exhaustion of food resources. This result further confirmed the importance of continuous nematode feeding for reproduction. The average oviposition rate was 1.3 ± 0.6 eggs day^{-1} female^{-1}, *i.e.*, *ca* 4 eggs per 3 days; this rate is very slow compared to this parameter of other studied xylobiont nematodes: 4.5 ± 1.3 in *Panagrolaimus detritophagus*; 2.8 ± 1.8 in *Rhabditolaimus ulmi*; and 3.4 ± 1.5 in *Bursaphelenchus willibaldi* (Polyanina & Ryss, 2021; Ryss & Polyanina, 2022 a, b), and in combination with the long ontogeny it provides the slowest population growth among the species we have studied.

Lifespan evaluation. Since most nematodes joined the MG after development to adults or to the J4 stage, the minimum lifespan was the sum of 1G (a development from egg to adult female), plus reproductive period (equals 1G), plus the survival time as a part of the MG (equals 2G). In the MG the females were in a starvation state and the oviposition ceased during 6 h after migration. Survival of the MG group dominated by females depended on temperature: at 21-23°C the female survived *ca* 3 weeks (2G) and at 4°C – 6-12 months. Thus, a female reproductive period lasted 1G, followed by a period of dispersal and survival of at least 2G. The total female lifespan was limited by a sum of phases: 1G + 1G + 2G = 4G, *i.e.*, 44 days or more at the longest; if the young female immediately joined the MG after the last moult, the minimum lifespan may be 1G + 2G = 3G, or 33 days as the shortest time, *i.e.*, between 33 and 44 days. It is possible that males had a similar lifespan, although due to the small number of males this parameter was difficult to calculate accurately. The *Aphelenchoides* female lifespan is moderate among other studied species: the female life was the shortest in *Panagrolaimus detritophagus* (13-20 days), the longest in *Rhabditolaimus ulmi* (56-59 days), and 18-40 days in *Bursaphelenchus willibaldi* (Polyanina & Ryss, 2021; Ryss & Polyanina, 2022a, b). However, the oviposition period (10 days, on average) of the *Aphelenchoides* sp. was one of the shortest among others: 10 days in *P. detritophagus*, 16 days in *B. willibaldi* and 49 days in *R. ulmi*.

Migration as a phase of life cycle. Active migration (MG) is the feature distinguishing the cycles of fungal and plant feeders *Aphelenchoides* spp. and *Bursaphelenchus* spp. from the previously studied life cycles of bacteriophagous xylobiont nematodes belonging to the *Panagrolaimus* and *Rhabditolaimus* genera (Polyanina & Ryss, 2021, Ryss *et al.*, 2021b; Ryss & Polyanina, 2022a).

Nematode migration is probably related to the search for a new food source or a vector. It began from G + 1 DAI and reached its maximum at about 3G, and then gradually decreased in one month after the peak of the population in the PSA medium and until the end of the experiment. There is a distinct dominant stage at the end of the population cycle – these are adult females with giant spermathecae filled with mature elongated bipolar sperm. The MG females were not actively moving and survived for 1 month at room temperature and up to 1 year at 4°C. The MG females are second- and subsequent-generation females; since their number significantly exceeds the number of inoculum and they appear after the completion of the first generation in the fungal environment, they can be characterised as a potential survival and dispersal phase of the parasitic life cycle.

If we consider the saprobiont life cycle as the basal ontogeny type in Nematoda, it is likely that there is a migratory phase of the secondary life cycles, which originated with adaptation to parasitic dispersal, both entomochore, airborne or in water drops. Such specialisation through the use of a non-feeding resistant stage is less advanced than the presence of a specialised transmissive generation in *Bursaphelenchus* spp. (Ryss & Subbotin, 2017; Ryss *et al.*, 2021a). It can be characterised as the first step in the evolution of a parasitic cycle.

Dispersal and survival stages. Taking into account the equal duration of the two phases of the cycle, the first phase, which is in the PSA medium with the fungus, can be termed the propagative phase, and the second phase in the MG, can be called the dispersal phase. A specialised transmissive phase of the life cycle was not detected since we studied only the propagative generation in culture. From the predominance of the mature inseminated females at the end of population cycle, presumably the females are most resistant among all stages. This conclusion follows from the dominance of mature females in the MG during the whole period of migration and until the end of the experiment, and in the PSA (2 months).

It is not recommended to mix up the entomochoric dauers of the transmission generation and resistant stages of the propagative generation studied here. Different species of the family Aphelenchoididae have different survival stages; for each species this stage has to be defined separately *in vitro*. In this study, *Aphelenchoides* sp. from oak wood, the survival stage was an inseminated adult female; in our study on *B. willibaldi* the J4 propagative juveniles survived best (Ryss & Polyanina, 2022b). The J4 is probably the supplementary dispersal stage of *B. willibaldi*, but it is a stage within the propagative (rather than transmission) generation of the polyxenic life cycle, while its entomophilic transmissive dauers is still unknown, as well as for *Aphelenchoides* sp. from oak wood. The role of the J4 as a resistant stage of the life cycle is already known for other tylenchs, such as *Ditylenchus dipsaci* (Perry, 1977, 1999; Perry & Moens, 2013). The dispersal role of adult inseminated females is known for other Aphelenchoididae, *e.g.*, for the nematodes of subfamilies Acugutturinae and Entaphelenchinae (Wachek, 1955; Hunt, 1980, 1993). The survival stages in *P. detritophagus* are J3 and eggs, and in *R. ulmi* only J3 (Polyanina & Ryss, 2021; Ryss & Polyanina, 2022a). The non-specific dispersal *via* the survival stage of the propagative generation may be adaptation of the Aphelenchoididae cycles that are devoid of the entomochoric transmission (Hunt, 1993).

Swarming groups. After reaching a peak in abundance in PSA, adult nematodes began to leave the PSA medium and they formed ball-like and filamentous swarming aggregations of 10-80 individuals, where they continued to mate and lay eggs during 5-6 h. Presumably, the migration of nematodes was associated with the onset of unfavourable conditions stimulating nematodes to unite in groups. The movement along the lid occurred along the filamentous strands consisting of nematode bodies. It is possible that grouping of nematodes is an adaptation to survive adverse conditions in starvation for more than 2 months. It is a part of the survival strategy known as the behavioural response to food exhaustion (Perry, 1999; Perry & Moens, 2013). It is also a possible role of swarming groups as a sticky trapping net for contact of 10-80 nematodes with the arthropod vector (Ryss & Polyanina, 2022b).

The use of population growth model to calculate the time of substrate colonisation. The formula used here to calculate the growth rate of nematode population in 1 m^3 of substrate similar to the fungus-seeded PSA medium may be used as a model for similar substrates. The 2% PSA contained water, 2% agar, *ca* 2% soluble sugar and *ca* 20% potato starch (in 200 ml of medium: 4 g sucrose, 40 g potato with a routine starch concentration of 90%; see Chow *et al.*, 2004). Dead and living wood are similar to the PSA in starch and sugar concentrations (Chow *et al.*, 2004), but have a more complex composition, with lignins, cellulose, terpenoids, *etc*. In experiments we inoculated fungus and nematodes simultaneously at 21-23°C. The fungus occupied the Petri dish after 3-4 days, then the mycelium was consumed by

nematodes after 28 days when the MG abundance reached its peak. Thus, nutrients (sugar and starch) of the medium were first consumed by the mycelium, then the latter was replaced by nematode biomass. This is a basal stage of decomposition of dead wood, commonly divided into five stages (Stokland *et al.*, 2012). Nematodes of the family Aphelenchoididae are plant and fungal feeders (the guild Fu2); they are classified in the *cp-2* class of the MI and the basal stage of the enrichment and structure trajectories of the food web succession (Ferris *et al.*, 2001).

The long cycle and low fecundity cause a slow colonisation rate according to our model, which is many times longer than the colonisation time calculated for previously studied species, both bacteriophages and mycophages (Polyanina & Ryss, 2021; Ryss & Polyanina, 2022a, b). A weak pathogen may nevertheless be more resilient in an ecosystem because its population is provided with food for a long time.

To estimate the degree-days required to complete the life cycle and the substrate colonisation, it would be necessary to know the lowest temperature limit of reproduction. To calculate the biomass and production values the sizes of juveniles and adults and their abundances during colonisation and migration can be used in the future ecological research of wood-inhabiting nematodes. Such approach has been demonstrated on the estuarine nematode species *Diplolaimella dievengatensis* (family Monhysteridae) (Oliveira *et al.*, 2021). The use of *Aphelenchoides* sp. as a biomodel for ecological studies has the advantage that the population of this species turns entirely into a migratory flux (MG), and this flux can be estimated as biomass and energy gain for the population as a whole.

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А.Ю. Рысс и К.С. Полянина. Цикл развития и динамика численности популяции *Aphelenchoides* sp. (Rhabditida: Aphelenchoididae) из *Quercus robur in vitro*.

Резюме. Параметры онтогенеза и динамика популяции стволовых нематод были использованы для моделирования биоразложения мертвой древесины и сравнительного анализа жизненных циклов паразитических нематод. Параметры онтогенеза *Aphelenchoides* sp. из древесины дуба определяли в культуре *Botrytis cinerea* на картофельно-сахарном агаре (PSA) с добавлением коры дуба. Самки начинали яйцекладку через 4.6 (3-5) суток от даты инокуляции; сроки формирования личинок: J2 – 6.6 (6-8) суток, J3 – 7.5 (7-9) суток, J4 – 8.8 (8-10) суток; первое поколение (1G) завершилось линькой с превращением во взрослых особей через 10.5 (9-11) дней. Через 1-2 суток после формирования первого поколения нематоды массово покидали агаровую среду PSA и перемещались вверх, образуя миграционную группу (МГ). В PSA общая численность нематод достигала пика на 21 сутки (2G), в МГ – на 28 сутки (2.8G). Была определена плодовитость самок в период роста популяции (1.3 яйца/сутки) и разработана модель экспоненциального роста популяции для общего числа нематод $N = 87.877e^{0.1723T}$, $R^2 = 0.9389$ и для самок $N = 84.918e^{0.1187T}$, $R^2 = 0.9255$, где T – число дней. Средняя продолжительность жизни нематод при температуре 22 (21-23)°C равна 33-44 суток. Ассоциация нематоды и мицелия гриба может заполнить 1 $м^3$ PSA подобного субстрата за 92 дня. Стадией выживания и дисперсии, вероятно, служит зрелая осемененная самка. Жизнь самки состоит из репродуктивной фазы в PSA (10-11 суток) и фазы выживания в МГ (в среднем от 3 недель до года). В МГ нематоды формировали сворминги (клубки-агрегации) из 10-80 особей.