Effects of Oxygen and Temperature on the Activity and Survival of Nothanguina phyllobia

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Abstract: The effects of oxygen and temperature on the activity and survival of infective forth-stage juveniles of *Nothanguina phyllobia* Thorne were examined in aqueous suspension. Rate of movement was not affected by a wide range of O_2 concentration (0.8–8.6 ppm). Activity decreased below 0.8 ppm O_2, and at 0.15 ppm O_2 nematodes became motionless. Activity increased as a linear function of temperature up to a thermal optimum of 24 C; beyond 24 C activity decreased. Survival was greatly prolonged at low temperature. At 23 C, 50% mortality occurred within 7 d, whereas at 4 C, 70% survived after 98 d. *Key words:* oxygen, temperature, activity, survival, *Nothanguina phyllobia,* biological control of weeds.

Orr et al. (9) proposed the use of *Nothanguina phyllobia* Thorne for biological control of the agronomically important perennial weed *Solanum elaeagnifolium* Cav. The virulence, host specificity, anhydrobiotic capability (10), histopathology, and high biotic potential (12) of the nematode identify it as a good biological control agent. More information is needed concerning the effects of edaphic variables on survival and transmission. We describe here the effects in vitro of dissolved oxygen and temperature on the activity and survival of infective L4 juveniles.

MATERIALS AND METHODS

Nothanguina phyllobia infective juveniles were obtained from foliar galls of *S. elaeagnifolium.* Galled leaves were air-dried (24 C) to 11% moisture, broken into pieces < 1-cm-d, thoroughly stirred, randomly partitioned, and stored at −20 C. When experimental organisms were required, 12 g of broken leaves were soaked in oxygen-saturated deionized water (23 C) for 6 h and nematodes that egressed from plant tissue were sieve-separated from it. Further separation on a Baermann funnel

yielded 100% viable juveniles (ca. 50,000 nematodes) which were stored 24 h at 4 C prior to experimental use. Nematodes for all experiments were extracted from plant material that was collected and processed for storage at the same time. Deionized water was used as the experimental substrate.

In order to quantify activity, the activity index (AI) of a nematode was defined to be the number of dorsoventral undulations (or waves) of the body observed within 15 sec, one undulation being one complete cycle of events at the anterior end. Except where noted, the AI of each treatment was determined for 50 randomly selected nematodes and a confidence interval was generated about their mean with Student's-t distribution. Where less precision was required, activity was measured for 20 nematodes/treatment.

Variation between and within nematode extractions was evaluated by measuring the average AI's of nematodes from six randomly selected aliquots of processed plant material. The consistency through time in the rate of movement of nematodes was evaluated by measuring 10 consecutive AI's for each of 50 randomly selected individuals.

Three experiments were designed to investigate the effects of oxygen on nematode activity and survival. In the first experiment we examined the effects of various O_2 concentrations on AI. Approximately 300 nematodes were placed in sealed watchglasses containing water at 10 O_2 concentrations from 0.1 to 8.6 ppm; after 3 and 6 h, AI's were measured. Dissolved oxygen was read to within 0.1 ppm at the beginnings and ends of incubations with a $NaSO_3$-calibrated silver/lead galvanic cell.

In the second oxygen experiment we examined the effects of anoxia on AI during the first 24 h of exposure. Nematodes were observed after passing N_2 through nematode suspensions for 0.75, 1.5, 3, 6, 12, and 24 h; O_2 concentration after 0.75 h was < 0.15 ppm. Air was then passed through each suspension for 24 h and AI's were determined. In the third experiment we examined longer exposures to anoxia and subsequent survival in the presence of oxygen. Nematodes were exposed to N_2 for

0, 1, 2, 3, 4, and 6 d, at which times nematodes were transferred to oxygenated water; AI's were read 1, 3, 6, 9, and 12 d after each transfer. The first oxygen experiment was conducted twice at an ambient temperature of 19 ± 1 C. The remaining oxygen experiments were conducted at 23 ± 0.5 C.

Four experiments were designed to investigate the effects of temperature on nematode activity and survival. In the first experiment we varied the rate of temperature change when nematodes were removed from cold storage. Juveniles stored at 4 C for 24 h were transferred to a 23 C environment instantaneously, or gradually, via a linear rate of temperature increase (0.3 C/min). Instantaneous temperature change was achieved by pipetting five drops (0.13 ml) of concentrated nematode suspension (50 juveniles/drop) at 4 C into a temperature-controlled observation well (11) containing 25 ml water at 23 C. Gradual change was achieved by transferring nematodes from the same 4-C source to 25 ml water at 4 C and raising the well temperature manually. Temperature, which was recorded at 60-sec intervals, deviated no more than 0.2 C from a 0.3 C/min control line. Activity indices were measured 0.5, 1.5, 3, 4.5, and 6 h after the attainment of 23 C. In the second experiment we examined activity stabilization after various temperature changes. Nematodes which had been stored at 4 C for 24 h were subjected to instantaneous changes from 4 C to 14, 23, or 32 C; AI's were measured 0.3, 0.75, 1.5, 3, and 6 h after transfers.

In the third temperature experiment we compared stabilized activity at various temperatures. Nematodes stored at 4 C for 24 h were held for 6 h at 4, 8, 12, 16, 20, 24, 29, 32, 36, and 40 C and AI's were read; nematodes were then held for 24 h at 24 C and AI's were redetermined.

In the fourth experiment we examined nematode survival. Activity indices were measured after 0, 1, 2, 4, 9, 17, 30, 48, and 98 d of storage at 4 and 23 C. Dissolved oxygen readings verified that significant oxygen depletion did not occur. The remaining survival potential of nematodes stored at 4 C for 98 d was determined by transferring to 23 C and measuring AI's after 0.17, 0.33, 1, 2, 3, 5, and 7 d. The first
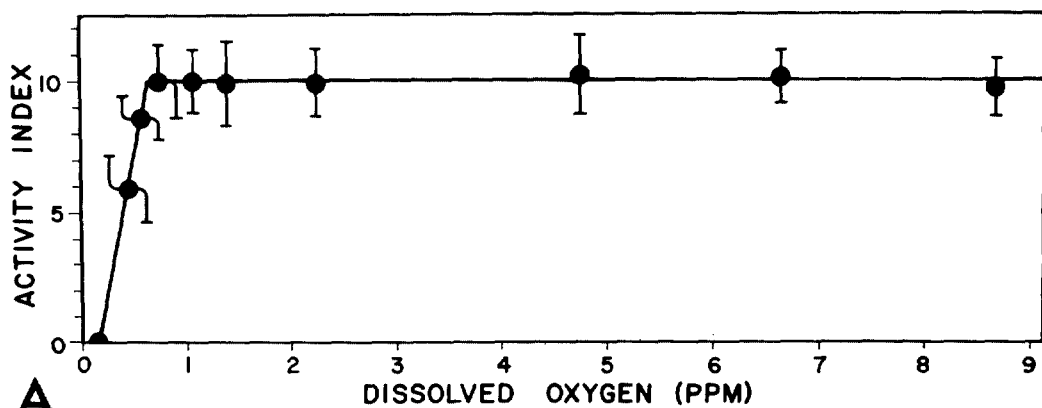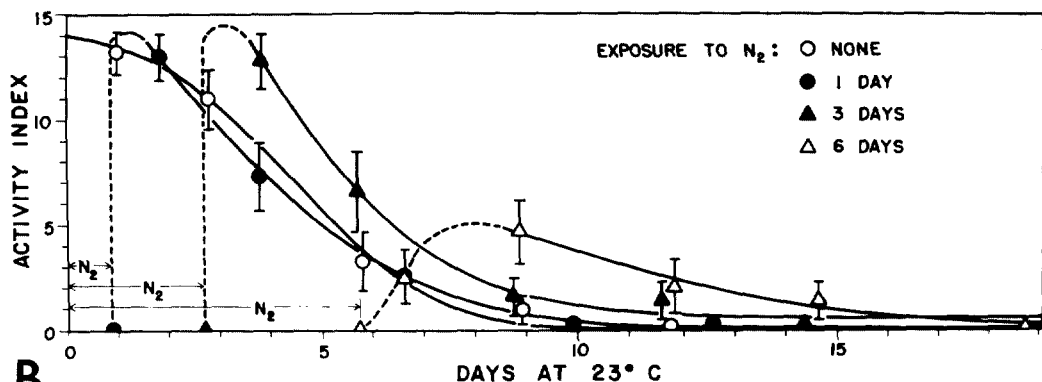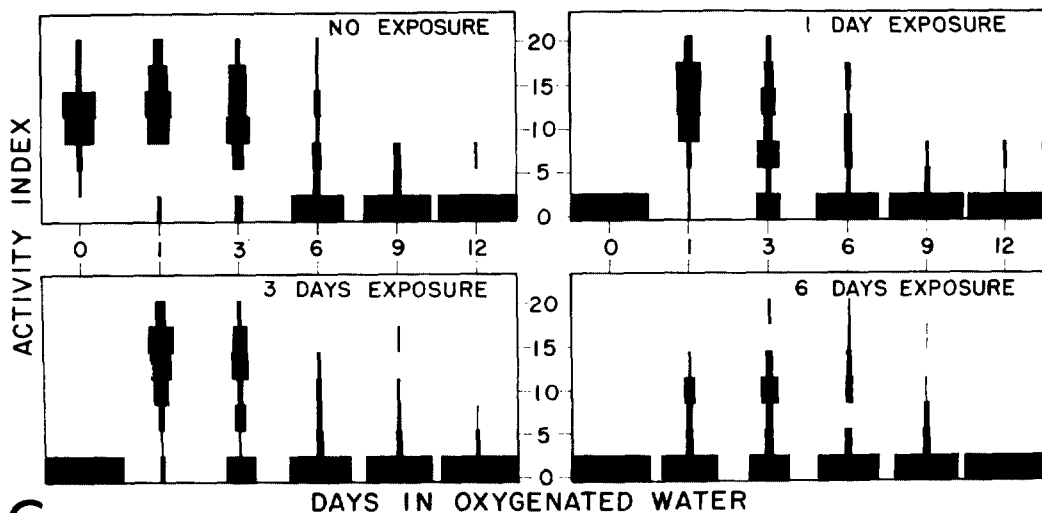
Fig. 1. The activity index (AI = undulations/15 sec) measured for *Nothanguina phyllobia* (50 infective juveniles/datum) under various oxygen regimes. Brackets indicate confidence limits at $P = 0.05$. (A) After 6-h exposure to various O_2 concentrations at 19 C. Lines fitted to data by least squares. (B) In aerated water after exposure to N_2 for 0, 1, 3, and 6 d at 23 C. Solid curves represent least squares fits to inverse cumulative normal distributions using numerical approximation methods. Broken curves are fitted visually. (C) Size class distributions for the 50 nematodes/datum whose means are given in B.

and fourth temperature experiments were conducted twice. Temperature was controlled in all experiments to $\pm$ 0.5 C with the multi-temperature observation well apparatus described previously (11).

RESULTS

The average AI for nematodes from six standardized extractions was 12.7 waves/15 sec with a standard deviation about extractions 5% of the mean. Controls in all subsequent experiments verified comparable activity. Variation between individual nematodes within extractions was much greater, with standard deviation 25% of the mean and AI's ranging from 5 to 20 waves/15 sec. Variation through time for individual nematodes was small ($\pm$ 2 waves/15 sec).

The activity of *N. phyllobia* was not changed by changes in O_2 concentration from 0.8 to 8.6 ppm; all AI's measured in this range were within 3% of the O_2-saturated control (Fig. 1). Below 0.8 ppm O_2, activity dropped sharply with O_2 depletion and reached zero between 0.4 and 0.1 ppm. When nematodes were placed in water at < 0.15 ppm O_2, they became motionless within seconds and did not recover until oxygen was replaced. After reacclimation in oxygenated water, nematodes from all N_2 time exposures up to 24 h yielded AI's within 5% of the control. Examination of survival in aerated water after the longer N_2 exposures of 2–6 d yielded additional information concerning the effects of anoxia (Fig. 1). Exposure to N_2 for 2, 3, and 4 d unquestionably prolonged survival. The amount by which survival was prolonged was ca. 1 day shorter than could be accounted for by the duration of anoxybiosis. The same effect occurred for nematodes exposed to N_2 for 1 d, so that the survival of nematodes following 1-d exposure to anoxia was essentially indistinguishable from the survival of nematodes from the same source, but held continuously in aerated water. After aeration the average activity of nematodes exposed to N_2 for 6 d was markedly reduced, compared with other exposure times. Examination of AI size class distribution changes during 12 d in aerated water after exposure to N_2 for 0, 1, 2, 3, 4, and 6 d

(Fig. 1) showed that 6-d reduction in average activity was due to an irreversible cessation of movement by ca. 70% of the population. The remaining 30% conformed to an AI distribution time sequence similar to that of the control.

The rate of temperature change from 4 to 23 C did not have a reproducibly measurable effect on final activity. A small effect (11%) was measured in one of the two experiments conducted ($P = 0.01$). In the other experiment, AI's for the two methods of temperature transition differed by < 5% at $P = 0.001$. In our opinion, the magnitude of the maximum effect detected was small enough to justify the use of rapid temperature change in subsequent experiments.

Activity completely adjusted to increased temperature within 90 min; from 1.5 to 6 h after transfer to 14, 23 and 32 C, the activity of nematodes previously stored 24 h at 4 C varied by < 10% (Fig. 2). In the comparison of AI's at 10 temperatures, activity from 8–24 C increased as a strict linear function of temperature; regression analysis of AI = f (temperature) yielded a linear correlation coefficient ($r = 0.998$) significant at $P = 0.001$ (Fig. 3). Variation as measured by standard deviations also increased linearly from 0 to 24 C ($P = 0.001$). Above 24 C, variation increased exponentially and AI dropped sharply; by 40 C activity ceased. Activity indices read after 24 h reacclimation at 24 C showed that at 40 C much permanent damage had occurred (Fig. 3). By contast, temperatures < 40 C had measurable but comparatively small permanent effects. Regression analysis of AI's read after 24 h at 24 C yielded a negative linear correlation between AI and test temperature (4–36 C; $P = 0.01$) with a slope of only –0.04 waves/15 sec/degree.

The survival of *N. phyllobia* infective larvae at 23 C was short. Although a small proportion (5–10%) of nematodes consistently maintained a high level of activity (AI $\geq$ 12 waves/sec) for several days, only 6.5 d were required for a 50% reduction in AI, overall (Fig. 4). The same rate of decrease in mean AI (ca. 50% reduction/6 d) was seen in the second survival experiment (data not shown), and in the control data for the N_2 recovery experiment (Fig. 1);
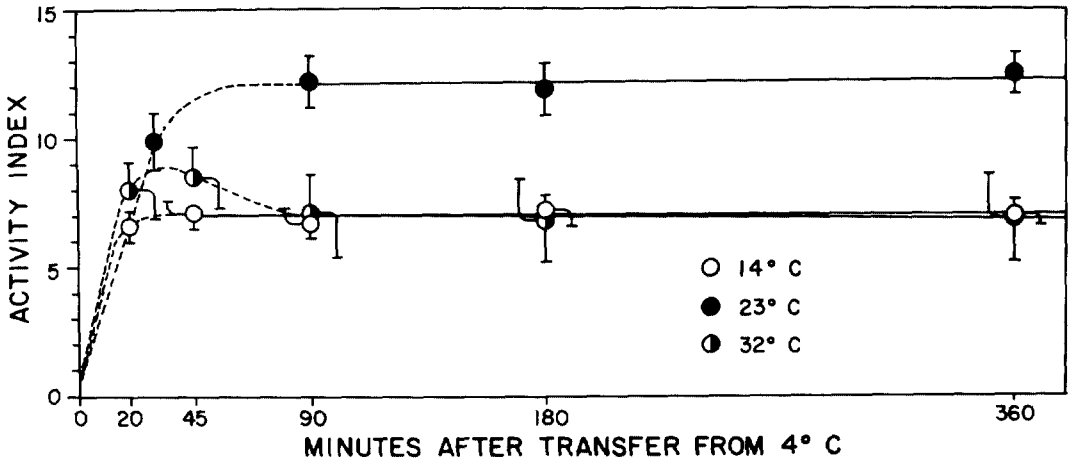
Fig. 2. Stabilization of *Nothanguina phyllobia* activity index (undulations/15 sec, 50 infective juveniles/datum) at three temperatures after 24 h at 4 C. Solid lines fitted to data by least squares and broken lines fitted visually. Brackets indicate confidence limits at $P = 0.05$.

the 24 h at 23 C which was required for hydration, extraction, and separation must be added to the time coordinate of the data given. Survival at 4 C was greatly prolonged. Activity index size class distributions of nematodes held continuously at 23 C from the time of hydration were compared with those of nematodes held continuously at 23 C after 98 d at 4 C (Fig. 4).

Reacclimation to 23 C after 98 d at 4 C required a long time (8–24 h) compared with the 90 min sufficient for the complete adjustment of activity by nematodes stored only 24 h at 4 C. Some worms never recovered while others reachieved and sustained for 4 d a level of activity comparable to that of freshly extracted nematodes.
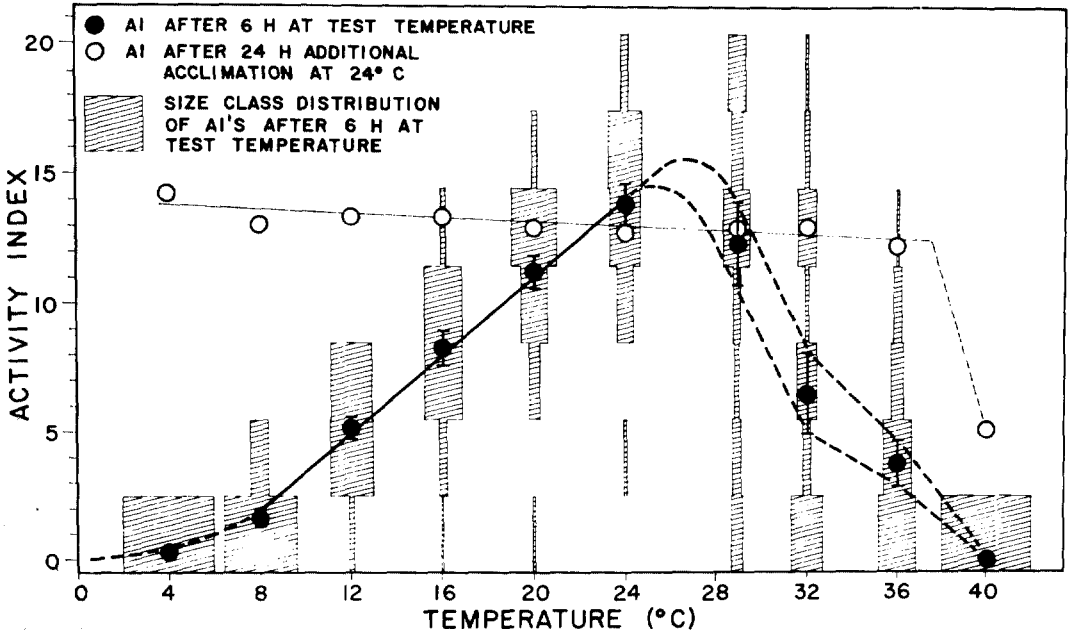
The following qualitative observations

Fig. 3. The activity index (AI = undulations/15 sec) of *Nothanguina phyllobia* after 6-h exposure to each of 10 temperatures (50 infective juveniles/datum) and after 24-h additional acclimation at 24 C (20 infective juveniles/datum). Solid lines fitted by least squares and broken lines fitted visually. Brackets indicate confidence limits at $P = 0.05$.
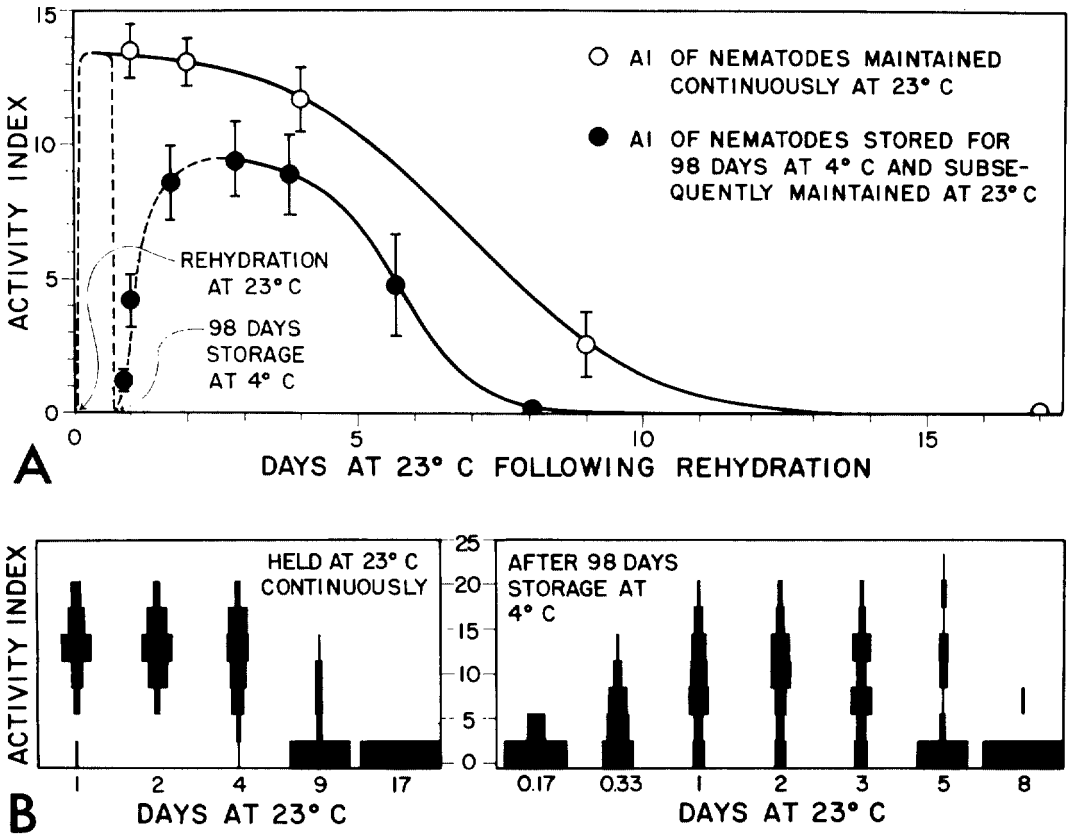
Fig. 4. The comparative survial of *Nothanguina phyllobia* maintained continuously at 23 C during and following rehydration, and maintained continuously at 23 C after 98-d storage at 4 C. Note slow reacclimation (24–48 h) after 4-C storage and subsequent 2–3-d negative shift in survival curve. (A) Mean activity index (AI = undulations/15 sec) for 50 infective juveniles. (B) Size class distributions for the 50 nematodes/datum whose means are given in A.

concern changes in the appearance of nematodes. On day 1 of survival experiments the interior of most nematodes was highly refractive and appeared granular. As days passed, control nematodes lost optical refractivity. The transition to translucence coincided with the drop through time of the mean AI for the population, but was not directly relatable to the AI of any given nematode; numerous highly translucent, highly active nematodes were observed. Exposure to N_2 preserved the refractive appearance of newly extracted nematodes until oxygen was replaced, at which time it disappeared at a rate similar to that observed in the control, with the following exception: nematodes which never regained mobility after 6-d exposure to N_2 remained highly refractive for the duration of the experiment; nematodes that regained activity became translucent in the usual fashion.

DISCUSSION

Above 0.8 ppm O_2 (= 14 Torr pO_2 or 2% O_2 in air at STP), variation in oxygen concentration has no effect on the rate of movement of infective juveniles of *N. phyllobia*. This oxygen concentration compares with critical oxygen tensions (< 40 Torr) for reproduction, activity, and survival of other soil-inhabiting nematodes (1,3,4,7) and suggests that the activity of *N. phyllobia* is adversely affected as soon as oxygen availability begins to limit respiration, a phenomenon that characterizes invertebrates in general (13).

The survival time of *N. phyllobia* juveniles in anoxic water (4–6 d) was similar to those reported for other nematodes, includ-

ing free-living species (2,3,6), plant parasites (4), and the infective stages of animal parasites (5,7). Among free-living and plant-parasitic nematodes, significantly longer anaerobic survival (60 d) has been reported only for the myceliophagous *Aphelenchus avenae* Bastian (4) and several salt marsh species (6). The latter were examined at 7 C, which may explain their greater longevity. For *N. phyllobia*, an inverse relationship was obtained between the duration of exposure to anoxia and the speed of recovery. The same phenomenon was observed for the soil nematode *Caernorhabditis elegans* (Maupas) Dougherty by Anderson (2) and for the marine nematode *Enoplus communis* Bastian by Wieser and Kanwisher (14), who attributed it to the accumulation of metabolic waste products. Because the infective juveniles of *N. phyllobia* are influenced by anaerobic conditions in vitro in a fashion similar to other nematodes, generalizations concerning the oxygen-related adverse effects on nematodes of flooding, heavy clay soils, and organic soil amendments apply equally.

Energy was conserved by *N. phyllobia* juveniles during the first 4 d of anaerobiosis, as evidenced by subsequent aerobic survival; optical refractivity similarly was retained until oxygen was made available. The loss of optical refractivity under aerobic conditions very likely resulted from lipid depletion. If so, the death of *N. phyllobia* juveniles after 6-d exposure to N_2 did not result from the exhaustion of lipid reserves; the nematodes killed by anoxia were in fact the ones that never lost their refractive appearance when oxygen was replaced. Similar results have been obtained by other workers. Histochemical techniques were used to compare lipid utilization during the aerobic and anaerobic survival of *A. avenae, C. elegans* (3), L2 *Meloidogyne naasi* Franklin (8), and L3 *Ancyclostoma tubaeforme* (7). In each case, lipids were utilized only under aerobic conditions.

The linear relationship obtained for *N. phyllobia* between temperature and activity (8–24 C) was not surprising for a poikilotherm. What we did not expect, however, was the wide variation in the susceptibility of nematodes to completely reversible high temperature-induced immobilization. This phenomenon occurred over a temperature range (29–36 C) characteristic of the nematode's natural habitat and may be of adaptive significance.

The prolonged aerobic survival of *N. phyllobia* at 4 C (> 98 d) indicates that in temperate latitudes overwintering may be possible in the continuously hydrated condition, something we had not suspected. The short survival at 23 C (< 12 d) emphasizes the importance of host availability during warm, moist conditions. The optimum osmolality, pH, and specific ion ratios for the survival of hydrated *N. phyllobia* have not been determined, and the survival curves obtained under our experimental conditions may fall short of actual values in the soil.

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