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MOISTURE AND TEMPERATURE EFFECTS ON SURVIVAL AND INFECTIVITY OF FIRST-STAGE LARVAE OF *PARELAPHOSTRONGYLUS ODOCOILEI* AND *P. TENUIS* (NEMATODA: METASTRONGYLOIDEA)

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ABSTRACT: The survival of first-stage larvae (L_1) of *Parelaphostrongylus odocoilei* and *P. tenuis* (Nematoda: Metastrongyloidea) and their infectivity to the snail *Triodopsis multilineata* were determined experimentally in a variety of temperature and moisture conditions. Survival of larvae of *P. odocoilei* increased with decreasing temperature. Survival of larvae in water was similar to survival in air at 17 and 45% RH; survival of larvae in air at 75, 85, and 95% RH was considerably lower at corresponding temperatures. The infectivity of larvae of *P. odocoilei* that survived desiccation was greatly reduced. Repeated freezing or repeated desiccation resulted in reduced survival of *P. odocoilei* and *P. tenuis*. Larvae of *Parelaphostrongylus odocoilei* from mule deer of Jasper National Park, Canada, were better able to resist the effects of freezing, but less able to resist the effects of desiccation, than were larvae of *P. tenuis* from white-tailed deer of Pennsylvania, USA.

Nematodes of the genus *Parelaphostrongylus* (Metastrongyloidea: Protostrongylidae) have been reported from numerous localities in the United States and Canada. Two species occur as adults in the musculature of their definitive hosts: *P. odocoilei* (Hobmaier and Hobmaier, 1934) in mule and black-tailed deer *Odocoileus hemionus,* and *P. andersoni* (Prestwood, 1972) in white-tailed deer, *Odocoileus virginianus.* A third species, *P. tenuis* (Dougherty, 1945), is found as adult in the central nervous system of *O. virginianus.*

Parelaphostrongylus odocoilei has been reported from the Pacific coastal mountain ranges of California (Brunetti, 1969), Vancouver Island (Pybus et al., 1984), and western Alberta (Platt and Samuel, 1978). *Parelaphostrongylus andersoni* is known in white-tailed deer from the southeastern United States, New Jersey (see review of Anderson and Prestwood, 1981), and British Columbia (Pybus and Samuel, 1981). *Parelaphostrongylus tenuis* has been reported in white-tailed deer throughout deciduous forests of eastern North America (Anderson and Prestwood, 1981) and, in Canada, is known as far west as western Manitoba (Bindernagel and Anderson, 1972).

The distribution of a parasite requires, in part, the ability of its free-living stages to survive in variable environmental conditions. For *Parelaphostrongylus* spp. the free-living stage is the first-stage larva (L_1) that is shed in the feces of the definitive host and penetrates the gastropod host (Platt and Samuel, 1984).

The importance of environmental influences on the free-living stages of parasitic nematodes has long been recognized in epizootiological studies of parasites in domestic animals (Gordon, 1948; Levine, 1963; Rogers and Sommerville, 1963; Kates, 1965; Gibbs, 1973). However, only Lankester and Anderson (1968) provide information on environmental resistance of *Parelaphostrongylus* free-living larvae. Their study, which considered primarily the intermediate hosts of *P. tenuis,* established that L_1s of *P. tenuis* are somewhat resistant to desiccation and freezing.

Acknowledging the importance of the genus *Parelaphostrongylus* for wildlife of North America (see review of Anderson and Prestwood, 1981; Pybus, 1983) and the suggestion of possible westward spread of *P. tenuis* in Canada (Bindernagel and Anderson, 1972), the present study was initiated to expand upon the work of Lankester and Anderson (1968). It was designed to provide baseline survival characteristics for the L_1 of *P. odocoilei* and *P. tenuis* in a range of temperatures and moistures that probably occur in nature. Survival of larvae and infectivity to snails were used to test responses.

METHODS

First-stage larvae of *P. odocoilei* were of two sources: from mule deer (*Odocoileus hemionus hemionus*) ex-

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perimentally infected with *P. odocoilei* originating from fresh frozen feces of mule deer in Jasper National Park, Alberta (MD source) and from black-tailed deer (*O. hemonius columbianus*) experimentally infected with *P. odocoilei* originating from fresh frozen feces of black-tailed deer on Vancouver Island, British Columbia (BTD source). Captive deer were housed indoors at the University Biomedical Animal Centre. First-stage larvae of *P. tenuis* were obtained from feces collected in the winter from white-tailed deer (*Odocoileus virginianus borealis*) of the Rachelwood Wildlife Research Preserve in Pennsylvania. They were kept frozen until needed. Some larvae of *P. tenuis* were passaged through snails in the laboratory, and resulting third-stage larvae (L_3s) were used to infect a white-tailed deer (*O. v. dacotensis*) from Alberta to provide an additional source of L_1s. Freshly-collected and previously-frozen larvae of all three origins (*P. odocoilei* MD and BTD; *P. tenuis*) were available. In experimentation involving larvae of more than one origin, larvae having similar, prior, storage and handling procedures were used. Identity of larvae was confirmed by recovery of adult helminths from experimental infections of deer with each source.

First-stage larvae were recovered from the feces of the host by a modified Baermann technique (Samuel and Gray, 1982). They were repeatedly washed in distilled water (used in all experiments) until the water was clear, and were stored at 8 C until required.

Relative humidities (RH) were maintained by use of salt solutions (O'Brien, 1948; Winston and Bates, 1960). Small chambers, 13 × 11.5 × 13 cm plywood sealed in polyethylene to reduce moisture transfer, were used to house larvae. Samples of larvae were held on four shelves of 5-mm mesh, acrylic-coated galvanized metal and 50 ml of saturated salt solution was placed on the bottom of the chamber. Chambers provided a gradient from low to high RH with no overlap between ranges. Five ranges of RH were tested: 17 ± 5 (LiCl); 45 ± 5 (K_2CO_3); 75 ± 5 (NaCl); 85 ± 5 (KCl); and 95 ± 5 (H_2O). (The variances given encompass the theoretical deviations of RH over the temperature range used in this study, because the RH controlled by specific solutions remains constant at a given temperature, but varies slightly as temperatures change.) Ambient RH was measured as 35 ± 2%.

Temperatures were maintained within ± 1 C by a variety of controlled environment facilities. Availability of these facilities for other purposes placed minor constraints on choice of temperature. Room temperature was 18 C.

Prior to each experiment, aliquots from suspensions of the appropriate L_1s were placed in 60-mm plastic Petri dishes. Water was allowed to evaporate until only a thin film remained. The samples were then placed in appropriate experimental conditions.

Survival of larvae in each sample was assessed only once, at the end of its experimental treatment. Only larvae exhibiting spontaneous motion, or which responded to prodding with a sharp probe, were considered living. Survival of larvae in air was assessed after rehydration in water for a minimum of 3 hr; survival of frozen larvae was assessed no sooner than 1 hr after thawing.

Infectivity of larvae following experimental treatment was assayed by determining the proportion of surviving L_1s that infected *Triodopsis multilineata* (Gastropoda) (12–17 mm diameter = approximately 6 wk of age) and developed to the second or third stage. An aqueous suspension of L_1s was placed on filter paper in a finger bowl; snails were added and allowed to crawl on the paper for several hours. Snails climbing the walls of the bowl were periodically returned to the filter paper. The variables standardized for each series of assays were: number of snails per bowl, number of live L_1s, and duration of exposure. Following a minimum 4-wk incubation period at 18 C, snails were digested individually in artificial pepsin solution (6 g pepsin, 7 ml concentrated HCl/1 liter H_2O) and larvae recovered.

Data were analyzed statistically using procedures outlined by Snedecor and Cochran (1967), Sokal and Rohlf (1969), and Finney (1971). Unless otherwise indicated, data are represented as mean ± 1 SD. Probability values of less than 5% were considered significant.

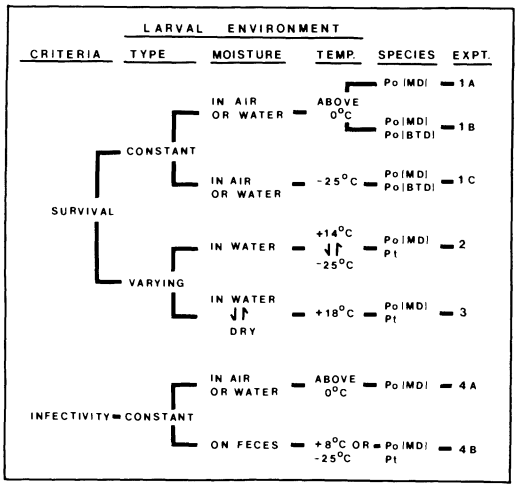

FIGURE 1. Nested nature of the experimental design. Double arrows indicate cycling between conditions. Species codes are: Po(MD)—*P. odocoilei* of mule deer source; Po(BTD)—*P. odocoilei* of black-tailed deer source; Pt—*P. tenuis* of white-tailed deer source. Experiment numbers correspond to those in text.

EXPERIMENTAL DESIGN

(Fig. 1)

Survival under constant conditions

Experiment 1A: Freshly collected larvae of *P. odocoilei* (MD) were stored under combinations of six conditions of moisture and of five temperatures. Conditions were maintained constant over the course of the experiment. At various intervals several samples (usually 4) were removed from storage in each treatment condition and the proportion of L_1s surviving in each was determined. Mean number of larvae per sample was 78 ± 28 (n = 529 samples).

Experiment 1B: Survival of freshly collected *P. odocoilei* from MD and BTD sources was compared in two experiments (1B, 1C). Six samples of larvae of each

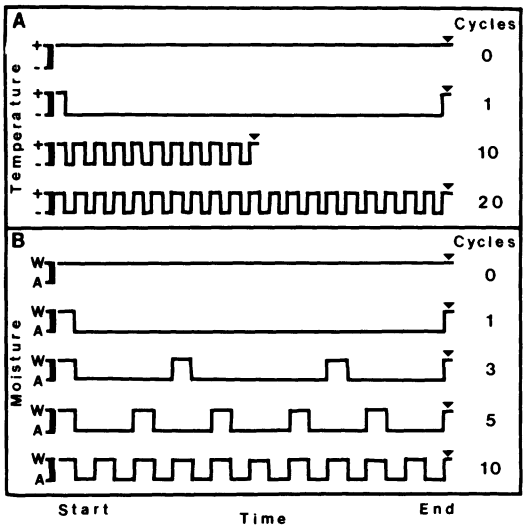

FIGURE 2. Schematic diagram of transfer sequences of larvae of *P. odocoilei* and *P. tenuis* in varying-condition experiments. **A.** Larvae in water transferred between 14 C (+) and −25 C (−) (Experiment 2). **B.** Larvae in water (W) at 18 C allowed to dry in air (A), followed by rewetting (Experiment 3). Arrow heads (▼) indicate when survival was assessed. Groups undergoing 0 or 1 cycle between the two conditions were controls.

source were placed in each of four treatments at 25 C: 12 days hydrated (= covered with water); desiccated (= in air) for 5 days at 95% RH, 7 days at 75% RH, and 19 days at 45% RH. Times were chosen to allow for moderate mortality of larvae in each moisture condition. Mean number of larvae per sample was 68 ± 21 (MD) and 83 ± 24 (BTD).

Experiment 1C: Larvae of MD and BTD sources were collected fresh and stored at −25 C for up to 280 days. Two moisture regimes were used: larvae submerged in water when frozen, and larvae dried at room temperature and ambient RH overnight prior to freezing. From one to three samples per source and treatment were examined for larval survival at three intervals (100, 190, and 280 days) in this experiment. Both sources of larvae had been frozen on feces for 1 wk prior to use. Mean number of larvae per sample of MD source was 91 ± 26 (n = 12); of BTD source, 86 ± 23 (n = 11).

Survival under varying temperatures

Experiment 2: To assess effects of repeated freezing and thawing, larvae of *P. odocoilei* (MD) and *P. tenuis* in water were repeatedly transferred between 14 and −25 C (Fig. 2A) at a rate of two cycles per day. Controls were stored continuously at the two temperatures. Mean number of larvae per sample of *P. odocoilei* was 156 ± 26 (n = 12); of *P. tenuis* 102 ± 22 (n = 12).

Survival under varying moisture conditions

Experiment 3: To assess effects of repeated desiccation, larvae of *P. odocoilei* (MD) and *P. tenuis* in water were repeatedly allowed to dry by evaporation under ambient conditions, then rewetted with 5.0 ml distilled water (Fig. 2B) a maximum of one cycle per day. Controls were stored continuously in water, or were allowed to dry and continuously exposed to air. Mean numbers of *P. odocoilei* per dish were 127 ± 67 (n = 65); of *P. tenuis,* 89 ± 20 (n = 34).

Infectivity following storage under constant conditions

Retention of infectivity of L_1s to the intermediate host was assessed following desiccation and high temperature storage of *P. odocoilei* (MD), and following freezing of *P. odocoilei* (MD) and *P. tenuis.*

Experiment 4A: A single stock of several hundred thousand *P. odocoilei* was subdivided and allocated to 12 treatments in a factorial design. Treatments comprised all combinations of two temperatures (20 and 26 C), three moisture conditions (in water, in air at 95% RH, in air at 45% RH), and two treatment durations (2 days, 10 days). These conditions were chosen such that even under the harshest of them there would be sufficient numbers of surviving larvae to expose to snails. Following treatments, larvae were rehydrated if necessary. From each treatment, 10 snails were exposed to an aliquot estimated to contain 10,000 live larvae for 6 hr.

Experiment 4B: Infectivity of L_1s following freezing was assessed in the following manner. For each of *P. odocoilei* and *P. tenuis,* two sources of larvae were used: one collected from fresh feces; the other collected from the same host individuals 30 days prior, and stored in the feces since then at −25 C. These four groups of larvae were recovered from the feces and repeatedly washed in distilled water at 8 C on the same schedule over 7 days. From each of the four groups, two replicate exposures of five snails to 5,000 live larvae for 4 hr were done. One replicate was digested 4 wk postexposure, the other at 5.5 wk.

RESULTS

Survival under constant conditions

Experiment 1A: Fifty-percent survival times for larvae of *P. odocoilei* (MD) (Table I) were determined using a probit analysis (Finney, 1971) for 20 of the 27 temperature-moisture combinations tested. Larval mortality was so rapid for the remaining seven that insufficient data points were available for probit analysis. Generally, larval survival varied inversely with temperature, and survival of larvae in air varied inversely with relative humidity. However, larvae that remained in water had the same high survival of larvae in air at low RH, at corresponding temperatures.

Experiment 1B: Larvae of *P. odocoilei* from mule deer (Jasper National Park) and black-tailed deer (Vancouver Island) responded similarly to four moisture regimes at 25 C (Table II). Analysis of variance (ANOVA) indicated no significant

TABLE I. *Survival of first-stage larvae of P. odocoilei (MD-source) in constant temperature and moisture conditions (Experiment 1A).*

Moisture conditions	Temp. (C)	Number of samples*	50% Survival time in days (95% fiducial limits)
In water	5	10	232.0 (119.0–995.0)
	14	22	73.5 (49.3–195.0)
	26	23	23.0 (16.9–38.1)
	36	20	4.3 (3.5–5.0)
	48	8	1.5†
In air	5	32	50.5 (37.7–68.5)
95% RH	14	32	52.1 (44.6–60.5)
	26	16	4.3 (2.8–5.5)
	36	8	2.0†
	48	9	1.0†
85% RH	5	32	42.2 (32.6–52.3)
	36	8	2.0†
	48	9	1.0†
75% RH	5	28	110.0 (80.8–156.0)
	14	28	74.7 (60.0–91.8)
	26	32	9.3 (6.6–11.8)
	36	8	2.0†
	48	9	1.0†
46% RH	5	19	306.0 (251.0–441.0)
	14	24	211.0 (197.0–226.0)
	26	28	24.8 (19.7–31.4)
	36	20	3.9 (3.3–4.4)
	48	16	2.4 (2.1–2.6)
17% RH	14	24	213.0 (204.0–223.0)
	26	28	31.8 (27.3–38.0)
	36	16	6.5 (5.6–7.4)
	48	20	2.8 (2.3–3.1)

* A sample is a dish containing first-stage larvae (see text for numbers).
† Maximum survival time in days. Data insufficient for determination of 50% time or fiducial limits.

difference attributable to larval source ($F_{1,40} = 1.99$, $P > 0.10$).

Experiment 1C: Larvae of the two sources responded similarly to freezing (Fig. 3). Those frozen in water had minimal mortality after 280 days, whereas there was a strong decrease in survival over time for larvae that were dried prior to freezing.

TABLE II. *Survival of first-stage larvae of P. odocoilei from mule deer and black-tailed deer under various moisture conditions at 25 C (Experiment 1B).*

Moisture conditions*	Duration (days)	Mean % survival ± 1 SD: *P. odocoilei* (MD)	Mean % survival ± 1 SD: *P. odocoilei* (BTD)
In water	12	71.3 ± 5.9	78.9 ± 3.1
In air			
45% RH	19	78.8 ± 7.3	78.0 ± 5.1
75% RH	7	17.2 ± 9.6	14.8 ± 7.9
95% RH	5	7.3 ± 3.6	12.2 ± 8.2

* The number of samples (defined in Table I) was six in each instance.

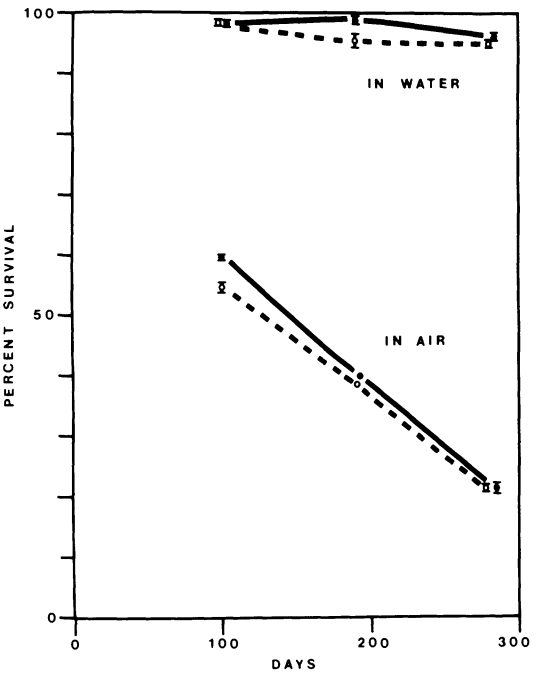

FIGURE 3. Mean percent survival of first-stage larvae of *P. odocoilei* from mule deer (open circles and dashed lines) and black-tailed deer (solid circles and lines) stored at −25 C. Top groups were larvae in water when placed at −25 C; bottom groups were dry (Experiment 1C).

Survival under varying temperatures

Experiment 2: Survival of L_1s of *P. odocoilei* (MD) and *P. tenuis* following repeated freezing is shown in Table III. For both species, survival of larvae decreased with number of freeze–thaw cycles. In the two experimental groups (10 and 20 freeze–thaw cycles) survival was lower than in either control group. Survival of *P. tenuis* was significantly lower than *P. odocoilei* following 1, 10, and 20 freeze–thaw cycles.

Survival under varying moisture

Experiment 3: Survival of larvae of *P. odocoilei* (MD) and *P. tenuis* decreased with number of desiccation–rehydration cycles (Table IV). In all experimental groups (3–11 desiccations), survival was lower than in either control group. Survival of *P. odocoilei* was similar to *P. tenuis* from zero to five desiccations, but was lower from six to 11 desiccations.

Infectivity following storage under constant conditions

Two measures of larval recovery from *T. multilineata* were used. The first, recovery per snail,

TABLE III. *Survival of first-stage larvae of P. odocoilei and P. tenuis following repeated freezing (Experiment 2).**

Number of freeze–thaw cycles	*P. odocoilei*		*P. tenuis*		*t*-test on means		
	Mean % survival ± 1 SD	Survival as % of 14 C control	Mean % survival ± 1 SD	Survival as % of 14 C control	df	*t*	*P*
0 (14 C control)	98.0 ± 2.6	100.0	99.7 ± 0.6	100.0	4	1.10	NS
1 (−25 C control)	98.5 ± 0.5	100.5	95.9 ± 1.4	96.2	4	3.03	< 0.05
10	79.5 ± 4.5	81.1	66.4 ± 4.7	66.6	4	3.49	< 0.05
20	11.8 ± 2.4	12.0	4.5 ± 0.7	4.5	4	5.06	< 0.01

* The number of samples (defined in Table I) was three in each instance.

was useful in assessing larval events that occurred after acquisition by the snail, such as their developmental rate. Because snails in the same exposure dish encountered a common supply of larvae, larval recoveries per snail did not provide independent measures of infectivity. Thus a second measure, total larval recovery from the snails in each exposure dish, was used to assess infectivity of the larval source.

Experiment 4A: Recovery of larvae from snails in the factorial-design experiment is given in Table V. Drying of larvae, and increased temperature or duration of storage, resulted in reduced infectivity of larvae that survived. Most larvae recovered from snails that acquired an infection, had developed to the third stage.

Experiment 4B: Recovery of larvae of *P. odocoilei* (MD) and *P. tenuis* is given in Table VI. Prior freezing of L_1s resulted in greatly reduced recovery of *P. tenuis,* but little change in recovery of the already low numbers of *P. odocoilei,* compared to the corresponding nonfrozen groups. The ANOVA on total larval recoveries indicated that this interaction between species and temperature was significant ($F_{1,4} = 15.4$, $P < 0.05$). The nonfrozen larvae of both species had similar developmental rates. After 4 wk, most larvae recovered were second stage, and after 5.5 wk, most were in the third stage. After 4 wk, a similar proportion of the larvae of *P. tenuis* were in the third stage whether frozen previously (4/33, 12%) or not (77/399, 20%) ($\chi^2 = 0.62$, $P > 0.50$). However, after 5.5 wk, a significantly lower proportion of *P. tenuis* reached the third stage when previously frozen (21/38, 55%) than when not (846/998, 85%) ($\chi^2 = 21.2$, $P < 0.01$). Larval recoveries of *P. odocoilei* were too low to permit a similar analysis.

DISCUSSION

Survival of first-stage larvae of *P. odocoilei* (MD) within the temperature range −25 C to 48 C was similar to that of first-stage larvae of other metastrongyloid nematodes (Rose, 1957; Forrester and Senger, 1963; Mitskevich, 1964; Morev, 1966; Lankester and Anderson, 1968; Hansson,

TABLE IV. *Survival of first-stage larvae of P. odocoilei and P. tenuis following repeated desiccation (Experiment 3).*

Number of desiccation–rehydration cycles	*P. odocoilei*			*P. tenuis*			*t*-test on means		
	Number of samples*	Mean % survival ± 1 SD	Survival as % of hydrated control	Number of samples*	Mean % survival ± 1 SD	Survival as % of hydrated control	df	*t*	*P*
0 (control in water)	14	97.9 ± 2.9	100.0	7	99.7 ± 0.4	100.0	19	1.87	NS
1 (control in air)	12	94.8 ± 3.6	96.8	5	96.7 ± 1.8	97.0	15	1.11	NS
3–5	15	73.2 ± 19.9	74.8	8	83.8 ± 14.8	84.1	21	1.32	NS
6–8	10	20.2 ± 9.4	20.6	6	58.3 ± 8.1	58.5	14	8.24	< 0.01
9–11	12	7.9 ± 2.9	8.1	8	12.9 ± 4.4	12.9	18	3.08	< 0.01

* Defined in Table I.

TABLE V. *Numbers of second-stage (L_2) and third-stage (L_3) larvae of P. odocoilei recovered from T. multilineata 5 wk after exposure of groups of 10 snails to 10,000 live first-stage larvae (L_1) for 6 hr (Experiment 4A).*

L_1 storage conditions			Mean larval recovery per snail ± SD			Total larval recovery
Moisture	Temperature (C)	Duration (days)	L_2	L_3	$L_2 + L_3$	
In water	+20	2	4.2 ± 3.9	69.0 ± 21.6	73.2 ± 23.5	732
	+20	10	0.3 ± 0.5	5.9 ± 3.9	6.2 ± 4.0	62*
	+26	2	0.9 ± 1.1	23.6 ± 9.6	24.5 ± 9.4	245
	+26	10	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0
In air						
45% RH	+20	2	0.3 ± 0.5	10.0 ± 5.9	10.3 ± 5.8	103
	+20	10	0.0 ± 0.0	0.1 ± 0.3	0.1 ± 0.3	1*
	+26	2	0.3 ± 0.5	2.3 ± 1.8	2.6 ± 2.0	26
	+26	10	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0
75% RH	+20	2	0.0 ± 0.0	0.1 ± 0.3	0.1 ± 0.3	1*
	+20	10	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0*
	+26	2	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0
	+26	10	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0

* Where fewer than the initial 10 snails survived ($n = 9$ in all cases), total larval recovery per exposure of 10 snails was estimated from mean recovery per snail and number of snails remaining.

1974). Whereas the magnitude of response differs between species, survival in all was promoted by cooler temperatures.

The survival of desiccated, first-stage larvae of *P. odocoilei* in relation to relative humidity differed qualitatively from the characteristics displayed by free living forms of other nematodes (Fig. 4). To emphasize qualitative differences in survival resulting from RH when larvae are stored in air, we standardized data from the literature in the following manner. For each species, the maximum reported survival when in air was given a value of 1. Survival at all other RH's (at the same temperature) was calculated as the proportion of maximum survival, and given a value of less than one. This method minimized variables between studies, such as temperatures used, survival criteria employed, and the absolute durations of survival of the different species studied. It maximized differences in survival of each species relative to the RH at which they were stored. Although the nine species selected from the literature differed in their sensitivity to level of RH (*Strongyloides papillosus* was most sensitive, *Trichostrongylus retortaeformis* the least), all were qualitatively similar in having greatest survival at higher RH. *Parelaphostrongylus odocoilei* stood alone in having greatest survival at low RH, and least at high RH. As our methodology was fundamentally similar to that in the other eight studies selected, we conclude that methodology was not likely responsible for this unusual observation. Further investigation of the mechanism of desiccation resistance in *P. odocoilei* should prove fruitful.

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TABLE VI. *Numbers of second-stage (L_2) and third-stage (L_3) larvae of P. odocoilei and P. tenuis recovered from T. multilineata 4 wk (Exposure 1) and 5.5 wk (Exposure 2) after exposure of groups of 5 snails to 5,000 live first-stage larvae (L_1) for 4 hr (Experiment 4B).*

Species	L_1 storage conditions		Exposure no.	Mean larval recovery per snail ± 1 SD			Total larval recovery
	Temperature (C)	Duration (days)		L_2	L_3	$L_2 + L_3$	
P. odocoilei	+8	7	1	1.2 ± 1.3	0.0 ± 0.0	1.2 ± 1.3	6
			2	0.0 ± 0.0	0.4 ± 0.5	0.4 ± 0.5	2
	−25	30	1	0.6 ± 0.9	0.0 ± 0.0	0.6 ± 0.9	3
	and +8	7	2	0.2 ± 0.4	0.6 ± 0.9	0.8 ± 1.1	4
P. tenuis	+8	7	1	64.4 ± 13.3	15.4 ± 19.7	79.8 ± 20.2	399
			2	30.4 ± 21.2	169.2 ± 67.5	199.6 ± 87.3	997
	−25	30	1	5.8 ± 4.2	0.8 ± 0.8	6.6 ± 4.8	33
	and +8	7	2	3.4 ± 2.5	4.2 ± 2.2	7.6 ± 4.3	38

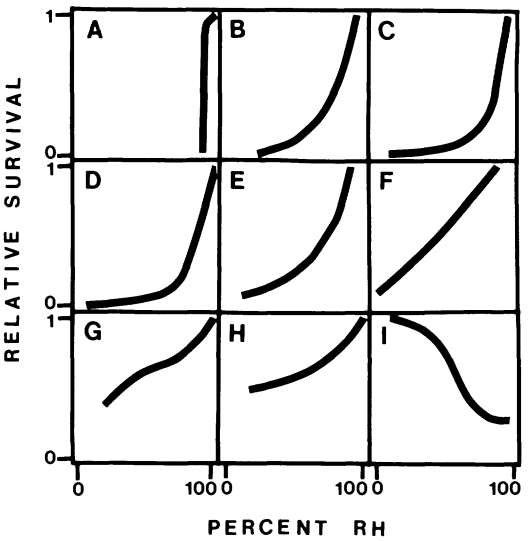

FIGURE 4. Relative lengths of survival of a variety of plant- and animal-parasitic nematodes at a variety of relative humidities. See text for explanation of relative survival lengths. **A.** *Strongyloides papillosus* (after Nath, 1978). **B.** *Ditylenchus myceliophagus* (after Perry, 1977). **C.** *Skrjabingylus nasicola* (after Hansson, 1974). **D.** *Bunostomum trigonocephalum* (after Belle, 1959). **E.** *Heterodera schachtii* and *H. rostochiensis* (after Ellenby, 1968). **F.** *Ditylenchus dipsaci* (after Perry, 1977). **G.** *Muellerius capillaris* (after Rose, 1957). **H.** *Trichostrongylus retortaeformis* (after Prasad, 1959). **I.** *Parelaphostrongylus odocoilei* (present study).

between survival and infectivity of first-stage larvae of metastrongyloid nematodes. The infectivity of stocks of first-stage larvae decreases following freezing (Lankester and Anderson, 1968) or with the passage of time (Arroyo and Morera, 1978; Bullick and Ubelaker, 1978; Cabaret, 1980), but these studies did not eliminate larval mortality as the cause of decreased infectivity. In the present study we controlled for larval mortality by standardizing the number of live larvae exposed to snails within each experiment, and have demonstrated that a proportion of the first-stage larvae that survived certain temperature and moisture stresses lost their infectivity to the intermediate host. This has important implications for the transmission of the parasite. Although the data of Experiment 1A show that survival of larvae in air at 45% RH is as lengthy as survival of larvae in water, it is apparent from Experiment 4A that drying produces a major loss of infectivity in those larvae that revive following wetting. The loss of infectivity over time may result in part from the depletion of energy reserves necessary for penetration of the intermediate host.

Parelaphostrongylus odocoilei and *P. tenuis* survived a limited number of repeated desiccations and freezings. This was observed for first-stage larvae of *Elaphostrongylus rangiferi* (Mitskevich, 1964), and for third-stage larvae of numerous trichostrongyloid nematodes (Turner, 1953; Andersen and Levine, 1968; Todd et al., 1970, 1976). Todd et al. (1970) postulated that accumulated impurities from successive drying–rewetting cycles are responsible for reduced survival. Whereas this has merit, the reduced infectivity of *P. odocoilei* followed a single drying and rewetting (where accumulation of impurities would not likely be significant), and the reduced infectivity and altered developmental rate of *P. tenuis* following a single freeze–thaw cycle suggest that physiological stress may be involved in the process of drying or freezing.

We have assumed that the use of previously frozen larvae, which was necessary in some experiments, did not substantially alter subsequent behavior of the larvae. Indeed, there were only minimal differences in survival for L_1s that experienced no or one freeze–thaw episode (Table III). Comparison of our data with that from similar experiments in the literature shows no major inconsistencies (see previous discussion). It should be emphasized that the unusual observations of Experiment 1A occurred despite use of only freshly collected larvae in that experiment. Within each experiment we used only larvae with similar prior handling (including temperature of storage) to minimize this potential problem.

Many parasites distributed over a wide geographic range have local variants that differ in biological characteristics, even though these variants may not be detectable morphologically (Thompson, 1982). We examined *P. odocoilei* from two geographic regions 1,500 km apart, and occurring in a different subspecies of host in each area, yet could detect no difference in survival responses between them. We had only one source of *P. tenuis* available, and it was more resistant to moisture stress, but less resistant to temperature stress, than *P. odocoilei* from Jasper National Park.

Admittedly, feces of black-tailed deer from Vancouver Island were stored frozen after collection, thus exposing *P. odocoilei* L_1s of this source to conditions not common on the Island. When we conducted experiments later we could

have been selecting larvae tolerant to freezing; that would account for the similar responses of the mule and black-tailed deer *P. odocoilei* (ex Fig. 3). However, if selection for freezing resistance resulted in the similarity between the two sources of *P. odocoilei,* then the differences between *P. odocoilei* and *P. tenuis* become more significant biologically. Differences in survival and infectivity of larval *P. odocoilei* and *P. tenuis* would have great importance in defining the geographic limits of the two species, and determining the possibility for range extensions or coexistence of the two species in a given habitat.

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