Temperature Effects on Survival of *Xiphinema bakeri* in Fallow Soil

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Abstract: Numbers of *Xiphinema bakeri* increased during the first month of storage at temperatures 5–30 C in naturally-infested soil sealed in polyethylene bags. From 1 to 6 months, populations trended toward later developmental stages, and total numbers declined, especially at the higher temperatures. Similarly-packaged *X. bakeri* eggs, larvae and adults were killed by –18 C for 48 hr or –34 C for 12 hr. *Key Words:* Freezing.

We have recently studied the ecology, biology and pathogenicity of *Xiphinema bakeri* Williams on forest nursery seedlings in British Columbia (7, 8, 9). We were unable to rear *X. bakeri* on seedlings in soil sterilized with steam, methyl bromide or propylene oxide, even when other soil microorganisms were introduced into sterilized soils at various times before or after *X. bakeri*. The observed decline and disappearance of *X. bakeri* within a few days after introduction into these soils led to the hypothesis that greenhouse or growth room temperatures were responsible. The purpose of this study was to determine the effects of a wide range of soil temperatures on the survival of *X. bakeri*.

MATERIALS AND METHODS

ABOVE FREEZING TEMPERATURES: Koksilah sandy loam infested with *X. bakeri* was collected from Douglas-fir [*Pseudotsuga menziesii* (Mirb.) Franco] seedbeds at the B. C. Forest Service nursery (at Duncan, B.C.) in December. This soil (at *p*F 2.53 it contained 38% moisture; oven-dry basis) containing 31% moisture, was thoroughly mixed with a 400-g sample placed in each polyethylene bag; the top was tied, and 30 randomly selected samples for each temperature (5 C intervals from 0–30 C) were enclosed in a large polyethylene bag. Soils at all temperatures were sampled monthly for 6 months. Soil moisture was determined at each sampling period. Nematodes were extracted from five replicate samples by a modified (final screen, 325-mesh) Christie and Perry method (2) and counted. At 1, 3 and 6 months the nematodes were categorized into early (L_1 to L_3) and late (L_4 and females) developmental stages (10) and each group expressed as a percent of the total population. Males were rare (<1% of total) and thus were not recorded. Treatment means were compared by Duncan's new multiple range test (6); arcsin transformation was used to analyze the percentage data (6).

SUBFREEZING TEMPERATURE: The nematode-infested sandy loam soil, collected in December from the B. C. Forest Service nursery (at Nanaimo, B. C.) contained 17% moisture (at *p*F 2.53 it contained 29% moisture; oven-dry basis) and the treatment temperatures were 0, –10, –18 and –34 C. The latter three fluctuated 3 to 5 C each day, but never fell below the specified levels. Preliminary study results showed that sub-freezing temperatures quickly killed most of the *X. bakeri*, thus the exposure periods were 12, 24 and 48 hr. Each treatment was replicated 10 times. Samples were thawed overnight at 4 C before the nematodes were extracted. Duncan's new multiple range test (6) was used to compare treatment means.

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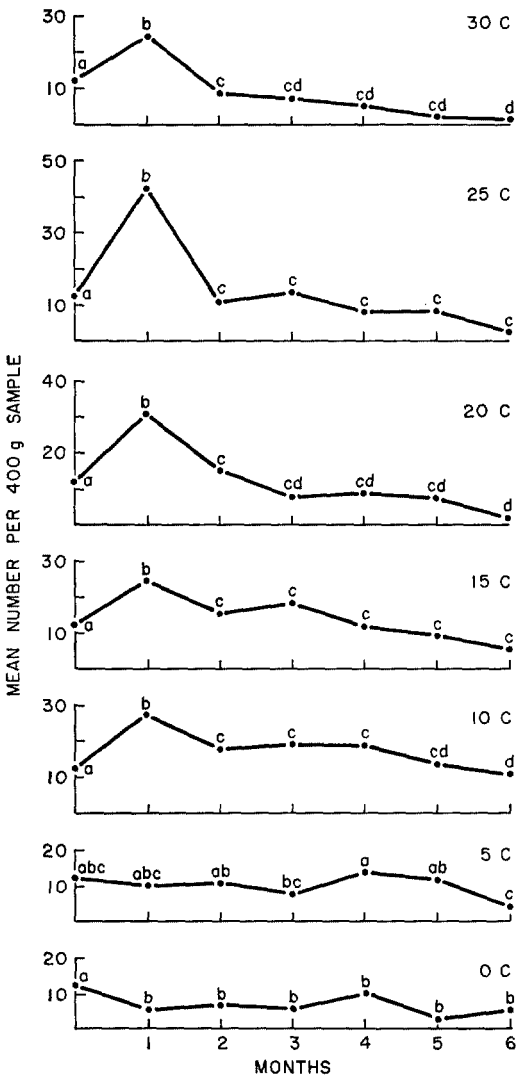

FIG. 1. Changes in *Xiphinema bakeri* populations in fallow soil stored at different temperatures for 6 months. Points (means of five replicates) with a letter in common are not significantly different ($P = .05$).

TABLE 1. Time and temperature effects on the developmental stages of *Xiphinema bakeri* stored in fallow soil.

Length of storage (months)	Developmental stage groups[a]	
	Early (L_1 to L_3) %	Late (L_4 plus females) %
0	75.8 a	24.2 a
1	43.6 b	56.3 b
3	30.1 bc	69.9 bc
6	27.2 c	72.9 c
Temperature (C)		
0	61.5 x	38.5 x
5	44.7 xy	55.3 xy
10	43.9 xy	56.1 xy
15	26.7 yz	73.3 yz
20	29.2 yz	70.7 yz
25	16.5 z	83.5 z
30	13.2 z	86.8 z

[a] Numbers are means of five replicates; numbers in a column followed by a letter in common are not significantly different ($P = .01$).

RESULTS

ABOVE-FREEZING TEMPERATURES: Total numbers of *X. bakeri* increased significantly ($P = .05$) during the first month of storage in soils kept above 5 C (Fig. 1), followed by a gradual decline that continued to the end of the experiment. Populations stored at 0 and 5 C declined throughout the experiment. Final populations were lowest at temperatures above 15 C, but at least some live *X. bakeri* were present at all temperatures when the experiment was terminated. As storage time and temperature increased, the percentage of nematodes in the early developmental stage group (L_1 to L_3) declined and the late group (L_4 and females) increased (Table 1). Both time and temperature significantly ($P = .01$) affected the ratio between the two groups, but the time × temperature interaction was not significant. The numbers of gravid females or males did not increase at any temperature. There was no evidence that predaceous nematodes or fungi caused the population declines. Soil moisture levels in the bags decreased less than 2% over the 6 months.

SUBFREEZING TEMPERATURES: Table 2 shows that the total numbers of *X. bakeri* were not significantly affected by storage at 0 C for 48 hr and that at −10 C there was no effect until the samples had been stored

TABLE 2. Influence of freezing temperatures on *Xiphinema bakeri* survival in fallow soil.

Temperature (C)	Nematodes in 400 g of soil[a]			
	Exposure time (hr)			
	0	12	24	48
0	32.0 a	32.6 a	28.8 a	32.6 a
−10	32.0 a	24.8 a	14.4 b	8.2 b
−18	32.0 a	3.6 b	0.4 b	0 b
−34	32.0 a	0 b	0 b	0 b

[a] Numbers are means of 10 replicates; reading across, means followed by a letter in common are not significantly different ($P = .05$).

24 hr. Storage at −18 and −34 C caused a significant ($P = .05$) reduction in the numbers of *X. bakeri* after 12 hr of exposure, but treatments of 48 and 12 hr, respectively, were needed to kill all the *X. bakeri* stored at these temperatures.

DISCUSSION

The initial increases in the numbers of *X. bakeri* (Fig. 1) in soils stored above 5 C resembles the trend Griffin and Barker (3) found for *X. americanum*. We do not know why this increase occurs, but apparently it is not due to hatching of eggs because the early developmental stage group (L_1 to L_3) of *X. bakeri* decreased rather than increased (Table 1). It may be caused by more efficient extraction resulting from increased nematode activity during the first month of storage, e.g. soils for Griffin and Barker's study (3) and ours were collected during cold weather, thus nematode activity likely increased when these soils were placed at warmer temperatures. Failure of *X. bakeri* populations to increase in soils stored at 0 and 5 C supports this hypothesis. A second possibility is that increased nematode activity and extraction efficiency resulted from better soil aeration due to prestorage mixing of the soil to obtain a homogeneous distribution of the nematodes. In either case, after 1-month storage nematode mortality probably becomes large enough to cause the populations to decline. The shift in the population composition from the early to the late developmental stages (Table 1) indicates that mortality is greatest among the early larval stages; this becomes more pronounced as storage time and temperature increase.

Soil temperature was not the major factor responsible for *X. bakeri* population decline in sterilized soils because the nematodes survived at least 6 months in fallow soil at 0 to 30 C. We now feel that soil moisture fluctuations (4) are involved; this problem is currently being investigated. The 6-month or more survival period of *X. bakeri* in fallow soil agrees with that for several other nematodes (11).

Lethal effects of subfreezing temperatures on *X. bakeri* are similar to those for *X. americanum* (5). Low soil temperatures may be responsible for limiting the distribution of *X. bakeri* to the warmer coastal areas of British Columbia (1). Data for two interior locations, Prince George, B. C. (1) and Williams Lake, B. C. (J. R. Marshall, personal communication), show that temperatures in the upper 10 cm of soil may average −1 to −2 C during the most severe winter months. Unfortunately, we were unable to test temperatures in the 0 to −10 C range.

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