Time, Temperature, and Soil Moisture Effects on Xiphinema bakeri Nematode Survival in Fallow Soil

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ABSTRACT

Survival of *Xiphinema bakeri* nematodes in soils stored at various combinations of time, temp and soil moisture tensions was studied to determine why nematodes decline in bare-fallowed, forest nursery soils. In soil stored from 1 to 32 wk at 4, 15, or 30 C at moisture tensions of pF 2.5, 3.5, or 4.2, nematode survival was significantly affected by time and moisture tension, but not by temp. *X. bakeri* survived best for the entire 32-wk period in soil kept at 4 C and pF 2.4; survival was poorest (only 16 wk) at 30 C and pF 4.2. Nematodes in early developmental stages were more susceptible to adverse temp and soil moisture conditions than were pre-adult and adult nematodes. In soil at pF 3.8, all *X. bakeri* nematodes were killed by exposure to 46 C for 15 min, and at 20 C, all nematodes were killed at pF 5.14. To determine levels at which the single or combined effect of time, temp and soil moisture tension were lethal to *X. bakeri*, nematode-infested soil was stored for 0 to 8 h at 36, 39 or 42 C at moisture tensions of pF 3.5, 4.2, 4.5 or 4.8. Overall, nematode survival decreased most rapidly between 0 and 4 h storage, when temp exceeded 39 C. It decreased linearly with increasing soil moisture tension. It was concluded that *X. bakeri* nematode longevity in hot, dry, fallow soil depends upon the combined effect of time, temp and soil moisture.

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Additional key words: Douglas-fir nursery, corky root control.

RÉSUMÉ

L'auteur étudia la survie de Nématodes (*Xiphinema bakeri*) dans des sols, lorsque les Nématodes furent placés durant différentes périodes de temps, à des températures et hudidités du sol différentes, afin de trouver pourquoi les populations de nématodes diminuent en sol de pépinière forestière en jachère à nu. Lorsque les sols furent entreposés pour 1, 2, 4, 8, 16, et 32 semaines à 4, 15, et 30 C lorsque la tension d'humidité était de pF 2.5, 3.5, et 4.2, la survie des Nématodes fut affectée significativement par le temps et la tension d'humidité, mais non par la température. *X. bakeri* survécut le mieux durant toute la période de 32 semaines en sol tenu à 4 C et au pF de 2.4; la survie la moindre se produisit à 30 C et au pF de 4.2 lorsque les Nématodes vécurent seulement 16 semaines. Les Nématodes aux premiers stades de leur développement étaient plus vulnérables à des conditions mauvaises de température et d'humidité que les pré-adultes et les adultes. En sol contenant suffisamment d'humidité pour leur permettre de survivre, les Nématodes furent tués lorsqu'exposés à 46 C durant 15 mn; à température non mortelle (20 C), les Nématodes furent tués lorsque la tension d'humidité était au pF de 5.14. Afin de trouver les niveaux auxquels l'effet individuel ou combiné de temps, température et humidité du sol peuvent tuer *X. bakeri*, le sol infesté de Nématodes fut placé pour 0, 0.5, 1, 2, 4, 6 et 8 h à 36, 39 et 42 C sous tension d'humidité au pF de 4.2, 4.5 et 4.8. En tout, la survie des nématodes diminua le plus rapidement lors de l'entreposage de 0 à 4 h après que la température excédait 39 C, et linéairement à mesure que la tension d'humidité du sol augmentait. L'auteur conclut que la longévité d'*X. bakeri* en sol sec chaud, en jachère, dépend des effets combinés de temps, température et degré d'humidité du sol.

Since 1963, corky root disease has ruined about 1.5 million Douglas-fir [*Pseudotsuga menziesii* (Mirb.) Franco] seedlings in coastal British Columbia forest nurseries (13). A recent study (2) gave the history, phenology, and distribution of the disease and concluded that the nematode *Xiphinema bakeri* Williams was the "primary" pathogen. Corky root can be controlled by preplant application of D-D nematicide (1), but we recently found that excellent disease control was obtained if nematode-infested soils were fallowed for 1 yr between seedling crops and frequently disked during the hot, dry part of late summer. This control method is practical because it fits in with a similar practice used to control weeds. Presumably a combination of starvation, heat and desiccation kills the nematodes but, because the precise lethal conditions were undefined, we studied the effects of time, temp and moisture on *X. bakeri* survival in fallow soil.

METHODS AND RESULTS.—*Soils, moisture desorption curves, and nematode extraction.*—Soil samples were taken from the Duncan and Brannen Lake (near Nanaimo), B.C. nurseries. The soils were naturally infested with large *X. bakeri* populations, which were sometimes augmented by sowing the soils with Douglas-fir. A mechanical analysis (3) showed both soils to be sandy loams. Their moisture desorption curves (moisture characteristics) are given in Fig. 1, where the pF (10) values (logarithm$_{10}$ of the numerical value of the negative pressure of the soil moisture expressed in cm of water) were obtained using suction-plate and pressure-membrane apparatus (6), and by drying the soils to equilibrium over saturated salt solutions. "Since there is no unique relationship between moisture content and suction for different soils, it is almost essential to give the moisture characteristic in experiments where soil and nematodes are involved" (19). Live nematodes were extracted from soils by a modified (final screen 325 - mesh) Christie and Perry method (5).

Xiphinema bakeri survival in Brannen Lake soil at temp below 30 C and at moisture tensions up to pF 4.2.—We first determined the survival of *X. bakeri* in several combinations of soil temp and moisture tensions which prevail in fallow soil during most of the year. *Xiphinema bakeri*-infested soil (about 40 nematodes/100 g soil) was saturated with water, allowed to drain to pF 2.53 moisture tension, then spread thinly onto a plastic sheet and air-dried at about 20 C to moisture tensions of pF 3.4 and 4.2. The soil was turned each hour during drying and every 2 h the moisture levels were monitored gravimetrically. After sufficient drying, 100-g aliquots of soil of each moisture tension were placed in sealed polyethylene bags which were put into moisture-proof boxes and stored at 4, 15, or 30 C. Each treatment was replicated six times, with an additional replicate included to monitor any changes in moisture content. After 0, 1, 2, 4, 8, 16, or 32 wk of storage, the *X. bakeri* nematodes were extracted, counted, and categorized into early (L_1 to L_3) and late (L_4 and adults) developmental stages (15), and each group was expressed as a percentage of the total population. The results of this factorial experiment (three temp × three soil moisture tensions × 7 sampling periods) were subjected to analysis of variance (14), and treatment means were compared using the Newman-Keuls test (11). For the total population data, nematode survival was expressed as a percentage of the number of nematodes in the control samples and for statistical analysis, the data were transformed to $\sqrt{\%\ \text{survival}}$ to correct for heterogeneous error variances. An arcsin transformation was used for the statistical analysis of the developmental stage data.

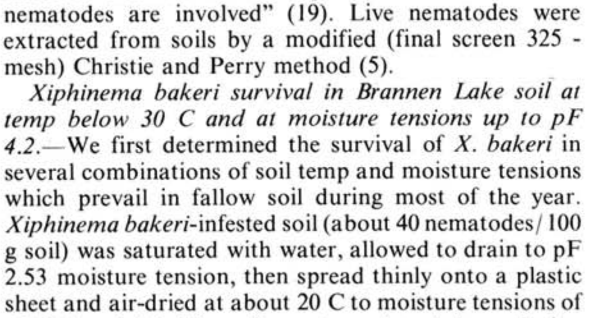

Fig. 1. Soil moisture desorption curves for Brannen Lake and Duncan nursery soils.

Experimental results are given in Fig. 2 and 3. Fig. 2 shows the influence of time, temp and soil moisture tension, their two-factor interactions (e.g., time × temp) and the triple interaction (time × temp × moisture) on *X. bakeri* survival. Nematode survival decreased steadily during the first 8 wk of storage (Fig. 2-A), then leveled off, and the populations remained stable until the end of the 32-wk storage period. Storage temp did not have a simple effect on nematode survival (Fig. 2-B), and although soil moisture significantly affected survival, the trend was not consistent (Fig. 2-C). Figure 2-D gives the time-temp interaction; survival was best and steadiest at 4 C, next best at 15 C, and poorest at 30 C. The time-moisture interaction (Fig. 2-E) showed that after 32-wk of storage, nematode survival was similar at the three moisture tensions. In the temp-moisture interaction (Fig. 2-F), *X. bakeri* survived best at all storage temp in soil with the lowest moisture tension (pF 2.5). The triple interaction of time × temp × soil moisture (Fig. 2-G to 2-O) shows that *X. bakeri* survival was favored by low storage temp, especially 4 C, and moist soil (pF 2.5); conversely, high temp and dry (pF 4.2) soil (Fig. 2-I, -L and -O) shortened nematode longevity. Although it is difficult to interpret this triple interaction, we assume that it is an interaction of time, soil moisture and temp.

The main effects of time, temp and soil moisture tension on survival of the early (L_1 to L_3) and late (L_4 and adult) developmental stage groups of *X. bakeri* are depicted in Fig. 3; the two-factor interactions and the triple-interaction were not significant and are not given. The data show that the decline in the percentage survival of nematodes in the early developmental stage group was confined to the first 8 wk of storage (Fig. 3-A), to soil stored at 30 C (Fig. 3-B), and to soil kept at pF 4.2 moisture tension (Fig. 3-C).

Thermal and soil moisture tension death points of X. bakeri.—Results of the preceding experiment indicated that rapid eradication of *X. bakeri* nematodes by fallowing soils depends upon temp and moisture tensions,

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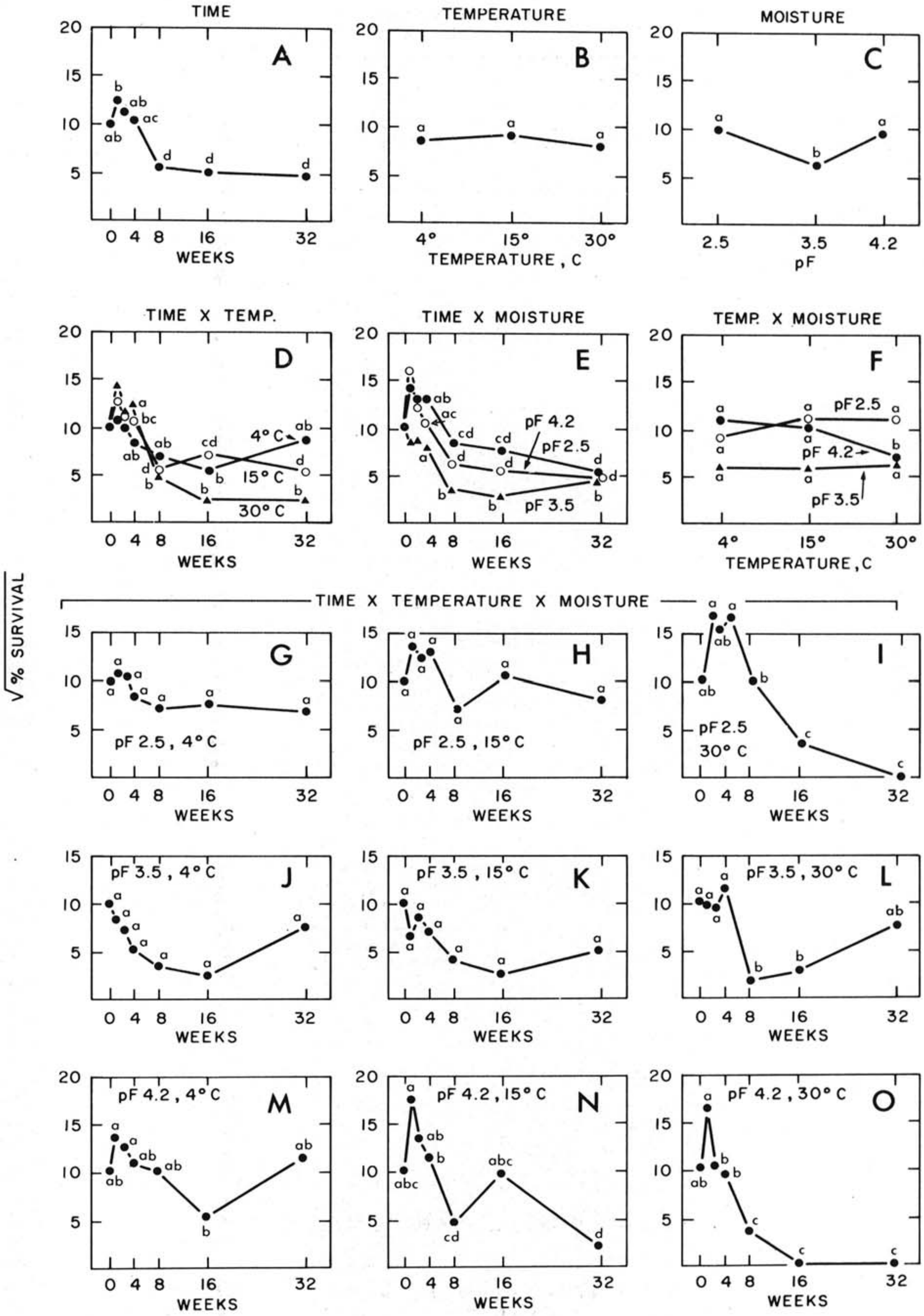
Fig. 2-(A to O). *Xiphinema bakeri* survival in Brannen Lake nursery soil at temp below 30 C and moisture tensions up to pF 4.2. **A, B, C**) Main effects of time, temp, and moisture. **D, E, F**) Effects of two-factor interactions. **G to O**) Effect of the triple interaction of time × temp × moisture. All F-values were significant ($P = 0.01$) except the main effect of temp; all points on the graphs are means of five replicates and points followed by a letter in common are not significantly different ($P = 0.01$) according to the Newman-Keuls test (11).


TIME
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A
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TIME X TEMP.
TIME X MOISTURE
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4° C
15° C
30° C
pF 4.2
pF 2.5
pF 3.5
TEMPERATURE, C
√% SURVIVAL
TIME X TEMPERATURE X MOISTURE
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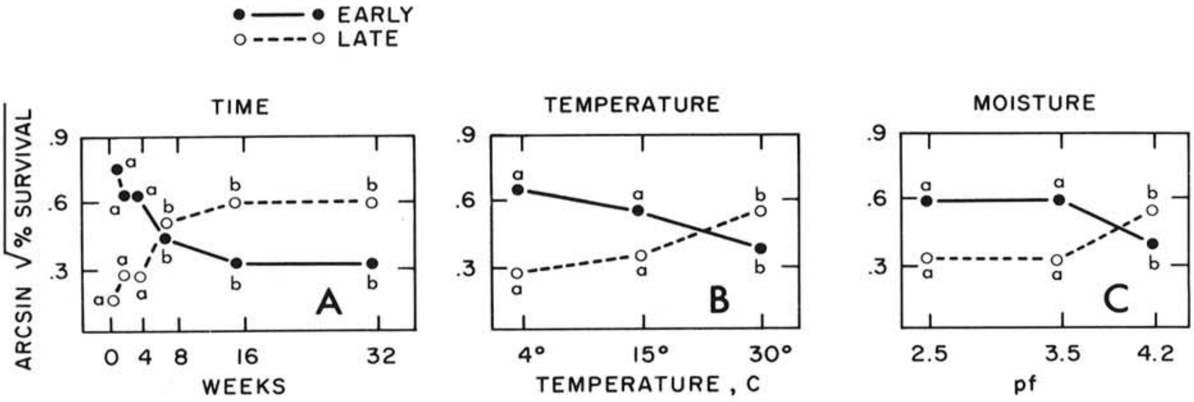

Fig. 3-(A to C). Survival of the early (L_1 to L_3) and late (L_4 and adult) developmental stage groups of *Xiphinema bakeri* nematodes in Brannen Lake nursery soil at temp below 30 C and moisture tensions up to pF 4.2. **A, B, C)** Main effects of time, temp, and moisture; all F-values were significant ($P = 0.01$). The two-factor interactions and the triple interaction were not significant and are not shown. All points on the graphs are means of five replicates, and points followed by a letter in common are not significantly different ($P = 0.01$) according to the Newman-Keuls test (11).

or their combinations, exceeding 30 C and pF 4.2. Thus, we felt that under summer fallow conditions the combined effects of higher temp and moisture tensions were killing the nematodes, but before testing this hypothesis we needed to know the lethal limits of the individual factors. To determine the nematode's thermal death points, Duncan soil (pF 2.8) was mixed and 50 g, containing about 25 nematodes, was packed lightly into each 50-ml stoppered test tube, and five replicates of each treatment were randomly assigned to water baths. Preliminary results demonstrated that temp lethal to *X. bakeri* were in the 39 to 46 C range; consequently, treatments were 40, 42, 44 or 46 C. Storage times ranged from 15 min to 3 days, after which the nematodes were extracted from the soils and counted. Nematode survival time decreased steadily with increasing temp and when kept at 40, 42, 44, and 46 C the nematodes were killed after 25, 4, 1, and 0.25 h exposure, respectively.

To determine the soil moisture tension death point of *X. bakeri*, an 18-g sample of Duncan soil (at pF 3.28) was put into each 30-ml plastic cup; each 18-g sample contained about 24 *X. bakeri* nematodes, and 90 samples were placed on perforated Plexiglas shelves over saturated potassium acetate solution (pF 6.36) in a desiccator at 20 C. Each day the cups were weighed to two-place accuracy; the soil samples which had dried to the desired moisture tension were removed, and the nematodes were extracted and counted. A wide range of soil moisture tensions was obtained because of variation in drying rate among samples. The moisture tension of each sample was determined by calculating a regression equation for that part of the moisture desorption curve between pF 4.0 and 6.0. Twenty pairs of values (pF vs. soil moisture) were obtained from the desorption curve and the equation was calculated, using pF as the dependent variable (Y) and the soil moisture content as the independent variable (X). By using the mean percent moisture content of 10 randomly selected samples, the oven-dry wt of the soil in each cup was calculated. Knowing the oven-dry wt, the sample wet wt, the cup wt, and the regression equation ($pF = 7.67 - \sqrt{1.19X}$), a computer program was written to calculate pF from the combined cup and soil wt within 1 h of weighing the samples. To prevent further drying during this time, the samples were kept over water in a desiccator. The results were subjected to linear regression analysis which showed that *X. bakeri* survival decreased as soil moisture tensions increased beyond pF 4.2. The regression line, $Y (\log_{10} \text{ of } n + 1) = 6.12 - 1.19 X$, intercepted the X axis at pF 5.14, which is the theoretical pF value at which all *X. bakeri* nematodes were killed. The coefficient of determination (r^2) indicated that 84.3% of the variation in the percent nematode survival was attributable to soil moisture tension.

Xiphinema bakeri survival in Duncan soil at temp above 36 C and moisture tensions exceeding pF 3.5.—Knowing the results of our thermal and dryness death point experiments, we then designed an experiment to determine the combined effects of near-lethal temp and soil moisture tensions on nematode survival. Soil moisture tensions of pF 3.5 (control), 4.2, 4.5, and 4.8 were obtained by the same procedure as for the first experiment and 20-g soil samples, containing about 10 *X. bakeri* nematodes, were put into 50-ml stoppered test tubes. Seven randomly selected samples for each treatment were then stored in water baths at 36 (control), 39, or 42 C for 0-8 h, whereupon the *X. bakeri* nematodes were extracted and counted. During the 5-day processing period, the 504 samples were stored at 25 C and randomly selected for evaluation. For statistical analysis, the nematode count data were transformed to the log (natural) of n + 1. Survival based on a percentage of the controls was not practical because the combined effects of low, initial nematode numbers at high moisture tensions and experimental error sometimes indicated that survival was greater in treated than in control soils.

Figure 4 shows the effect of time, temp and soil moisture tension (main effects), the two-factor interactions (e.g., time × temp) and the three-factor interaction (time × temp × moisture) on *X. bakeri* survival. Because nematode mortality occurred when the soil moisture tensions were adjusted, the initial nematode

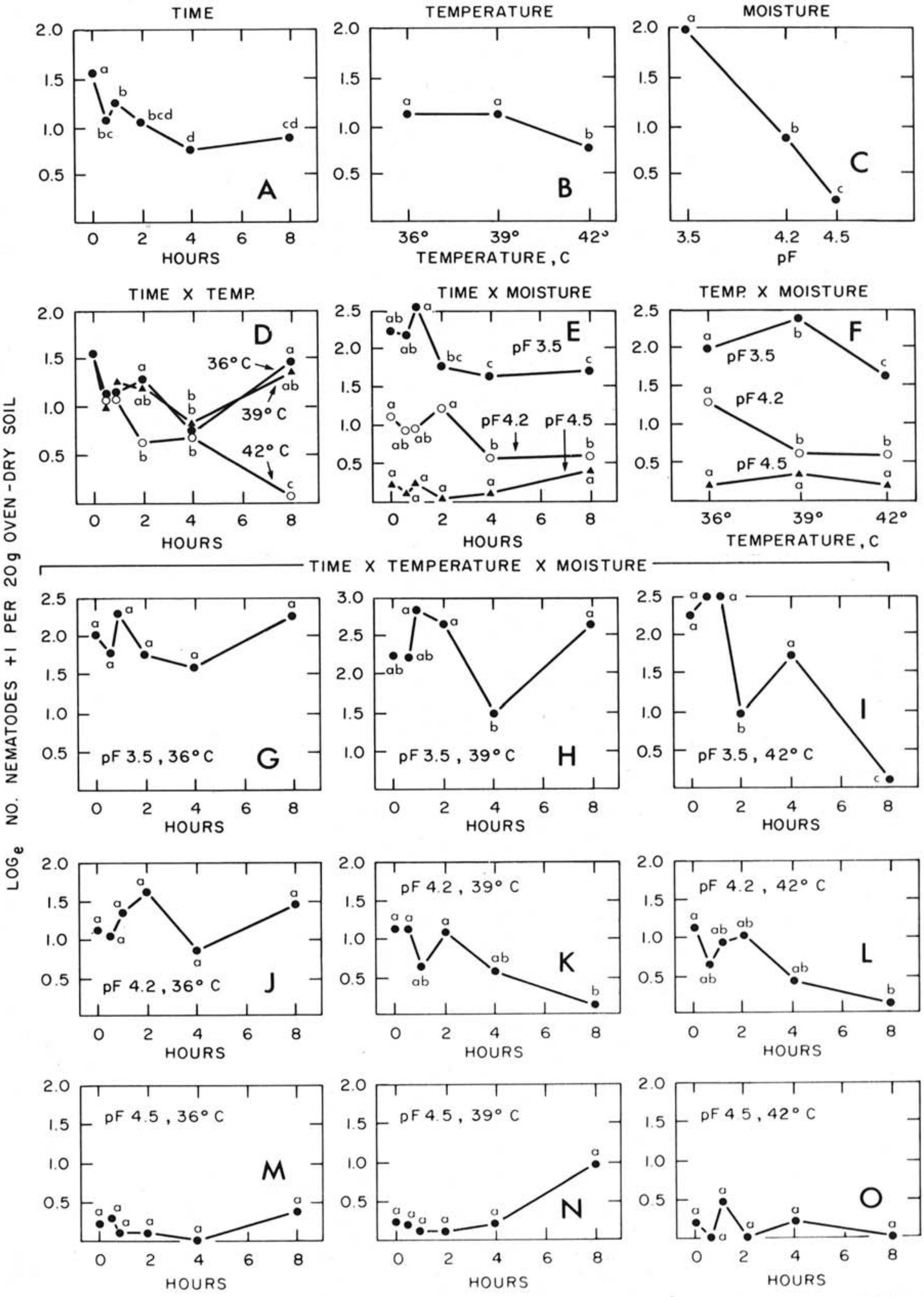

Fig. 4-(A to O). *Xiphinema bakeri* survival in Duncan nursery soil at temp above 36 C and moisture tensions exceeding pF 3.5. **A, B, C**) Main effects of time, temp, and moisture. **D, E, F**) Effects of the two-factor interactions. **G to O**) Effect of the triple interaction of time × temp × moisture. All F-values were significant ($P = 0.01$); all points on the graphs are means of seven replicates and points followed by a letter in common are not significantly different ($P = 0.01$) according to the Newman-Keuls test (11).

population levels differed for each pF level; consequently, horizontal (but not vertical) comparisons can be made in graphs where pF is one of the variables. No data are given for pF 4.8 because, when soil moisture was adjusted to this tension, the *X. bakeri* numbers were too low for analysis. Overall, *X. bakeri* numbers decreased as storage time, temp and soil moisture tension increased, with nematode survival declining most rapidly between 0 and 0.5 h storage (Fig. 4-A) and when storage temp exceeded 39 C (Fig. 4-B). However, no such threshold levels existed between survival and soil moisture tension, as this relationship was linear and negative (Fig. 4-C). All of the two-factor interactions (Fig. 4-D to -F) significantly affected nematode survival. The time-temp interaction (Fig. 4-D) showed that *X. bakeri* numbers declined steadily with increased storage time at 42 C, but no nematode mortality occurred in soils kept at 36 or 39 C. In the time-moisture interaction (Fig. 4-E), the detrimental effect of lengthening the storage period was annulled as soil moisture tension increased; i.e., nematode numbers declined after 2 h of storage at pF 3.5 and 4.2, respectively, while at pF 4.5, initial and final (8 h of storage) populations were the same. The situation was similar in the temp-moisture interaction (Fig. 4-F). Figure 4-G to 4-O depicts the effect of the triple interaction time, temp and moisture on *X. bakeri* survival. Based on the data in Fig. 4-A to -F, we interpret this as an interaction of time × temp × moisture rather than, e.g., an interaction of time-temp × moisture. In general, these triple interaction results show that nematode survival decreases as time, temp and soil moisture tension increase, and that these time and temp effects become less pronounced and are eventually nullified with increasing soil moisture tension.

DISCUSSION.—This study has shown that survival of *X. bakeri* nematodes in cool (4 and 15 C), moist (pF 2.5 and 3.5) fallow soil is mainly affected by length of storage, and that nematode longevity is shortened when temp and dryness are increased to 30 C and pF 4.2 (Fig. 2). Even then, at least 90% of the nematodes survived 1 mo of storage and about 10% were alive after 2 mo (Fig. 2-O). Consequently, for nursery practice, where a high nematode mortality is desired during the summer fallow period, soil temp and moisture tension should exceed 30 C and pF 4.2 during fallowing. The shift in population composition from early (L_1 to L_3) to late (L_4 and female) developmental stages with increasing storage time, temp and soil moisture tension (Fig. 3) agrees with earlier observations for *X. bakeri* (16) and *X. americanum* (7), and is probably due to their increased food reserves (17) from root feeding during their L_1 to L_3 stages. The longevity of *X. bakeri* is not known, but we feel that nematode mortality in our experiments was attributable to starvation rather than to aging.

Studies on the single effects of temp and moisture tension demonstrated that increases in either factor adversely affected nematode survival; e.g., *X. bakeri* longevity decreased from 2 h at 44 C to less than 15 min at 46 C, and nematode mortality increased linearly with increasing moisture tension. The thermal death point (46 C) for *X. bakeri* agrees with data on nine other plant-parasitic nematode species (18), and the maximum soil moisture tension (pF 5.14) for nematode survival is almost identical to that for *Pratylenchus penetrans* (9).

When the effects of near-lethal soil temp (>36 C) and soil moisture tensions (>pF 3.5) on *X. bakeri* nematode were studied, we found that the nematode can be killed by either the individual or combined effect of time, temp and soil moisture (Fig. 4), especially when the latter exceeds pF 4.2 (Fig. 4-C). Thus, in the upper 2.5 cm of disked, fallow, coastal British Columbia forest nursery soils, where summer temp frequently reach 35 to 55 C for 2-4 h each day and where moisture tension can exceed pF 6.0 (J. R. Sutherland and L. J. Sluggett, *unpublished*), *X. bakeri* nematodes are certainly killed by the combined effects of time, heat, and desiccation. The desiccation effect is undoubtedly attributable to an increase in the matrix potential of the soil water, and not an osmotic effect, because the latter was insignificant [only 0.354 and 0.613 atm calculated from conductance readings using a saturated soil paste (4)] at pF 4.18 and 5.18, respectively. Frequent disking of fallow soils ensures that most of the upper 16 to 25 cm of soil is eventually turned up into the 2.5-cm surface layer, where conditions lethal to nematode survival exist. We obtained some evidence that disking may also mechanically kill large nematodes such as *X. bakeri* (adults = 4,040 × 58 μ), which were killed at pF 5.18 when soil was dried over a desiccant, and at pF 4.8 when the soil moisture tension was adjusted by air-drying and turning (Fig. 4-C).

Most of the disadvantages which have been listed (8) for controlling nematodes by fallowing do not exist in coastal British Columbia forest nurseries, where nematode control is integrated with the weed control program. The latter encourages weed germination and growth during the moist spring and early summer (the weeds are killed by infrequent disking); during the dry, late summer and fall, *X. bakeri*-infested soils are disked daily for nematode control. Fallowing and disking reduce the nematode populations to nondamaging levels but, because some nematodes may survive the practice, it would probably not be useful for controlling nematodes that serve as virus reservoirs (12).

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