

The effect of soil moisture and soil particle size on the survival and population increase of *Xiphinema index*

Sufian A. SULTAN and Howard FERRIS

*Department of Agricultural Sciences, An Najah National University, West Bank, Israel,
and Department of Nematology, University of California, Davis, CA 95616, USA.*

SUMMARY

The interaction of soil moisture and incubation time on the survival and subsequent reproductive potential of *Xiphinema index*, and the effect of sand particle size on population increase, were studied under greenhouse conditions. In the absence of a host, fewer than 10 % of the nematodes survived for 60 days even under favorable (intermediate) moisture conditions. Survival was very low in both saturated and dry soils. Reproduction of *X. index* following incubation increased for those individuals that survived incubation in dry soil and decreased for those incubated under biologically favorable conditions. In the presence of a host, population increase of the nematode was highest in sandy loam and in fine sands of 250 µm particle size. Population increase was low in coarse sand of particle sizes 534 µm and larger. Root damage to host plants was directly related to the increase in nematode population.

RÉSUMÉ

Effets de l'humidité et de la taille des particules du sol sur la survie et l'accroissement de population de Xiphinema index

L'effet de l'interaction entre l'humidité du sol et le temps d'incubation sur la survie et la fécondité de *Xiphinema index*, ainsi que l'influence de la granulométrie du sable utilisé ont été étudiés en serre. En l'absence de plante-hôte, moins de 10 % des nématodes ont survécu pendant 60 jours, même en conditions favorables (intermédiaires) d'humidité du sol. La survie est très faible en sol sec ou saturé en eau. La fécondité de *X. index* après incubation est accrue pour les individus qui survivent en sol sec, et diminuée pour ceux incubés en conditions d'humidité favorable. En présence de plante-hôte, l'accroissement de population est le plus important sur sol sablo-limoneux et sur sable ayant des particules de 250 µm. L'augmentation de population sur sable grossier est uniformément faible. Les dommages infligés aux racines sont directement liés à l'accroissement de population du nématode.

Xiphinema index Thorne & Allen is a large (3 mm length), soil-inhabiting, plant-parasitic nematode. It reproduces parthenogenetically, and males are extremely rare (Siddiqi, 1974). Estimates of the length of the life cycle vary from 27 days to 9 months (Radewald & Raski, 1962; Cohn & Mordechai, 1969). The nematode is associated primarily with woody perennial plants, including grapevine, fig, rose, mulberry and pistachio (Weiner & Raski, 1966; Raski, Hewitt & Schmitt, 1971). The world-wide distribution of *X. index* is closely related to that of grapevine. The nematode causes direct damage to vineyards (Radewald & Raski, 1962; Van Gundy *et al.*, 1962) and is also a vector of grapevine fanleaf virus (Hewitt, Raski & Goheen, 1958).

Although *X. index* is found in a range of soil textures (Prota, 1970; Cohn & Mordechai, 1970), the aeration and pore size of sandy soils increase nematode viability (Cohn, 1969). When soil moisture is not limiting, coarse-textured soils promote the reproduction of *X. index*; under conditions of limited moisture, nematode reproduction increases in fine-textured soil with a greater moisture holding capacity (Harris, 1979). *X. index*

survived for 5 months in moist, sterile sandy loam, and for 35 days in the same soil when dry; it survived 9 months in moist soil in the field in the absence of a host (Raski & Hewitt, 1960; Taylor & Raski, 1964). Survival of the nematode stored for 69 days in a range of relative humidities was low in both dry and saturated soils (Harris, 1979). The lack of definitive time course studies on the survival of *X. index* at different soil moisture levels suggested a need for the present study. Also, the effect of different soil textures on the survival and population increase of *X. index* are reported only from field observations (Cohn, 1969; Harris, 1979).

The primary objectives of this study were two-fold : first, to determine the effects of the interaction of soil moisture and time on the survival of *X. index* and on the subsequent reproductive potential of the nematode and, second, to determine the effect of soil texture on survival and population increase of *X. index* under controlled laboratory conditions. A secondary objective of the study was to improve and standardize laboratory techniques for culture of *X. index* to provide a dependable supply of nematodes for experimental purposes and to stan-

standardize plant screening techniques for resistance to *X. index* and to fanleaf virus.

Materials and methods

EFFECT OF SOIL MOISTURE ON SURVIVAL

X. index was extracted by wet-screening soil from the root zone of a healthy fig (*Ficus carica* L.) grown in a greenhouse. One hundred g (62.5 cm³) of washed, oven-dried, sandy loam were placed in each of 210 styrofoam cups (150 cm³ capacity). Two hundred and fifty *X. index* in 3 ml water were added to the soil in each cup. The cups were divided into six groups. The six groups were adjusted to nominal 0, 5, 10, 15, 20 and 25 % soil moisture on a gravimetric basis; however, the nominal 0 % moisture level was initially 3 % due to the water added with the nematodes. The soil was fully saturated at 25 % moisture.

The styrofoam cups were covered with plastic Petri dishes to reduce water evaporation and were placed on a greenhouse bench maintained at 23 ± 3 °C. At 3-day intervals, each cup was weighed and water added as necessary to maintain the required soil moisture level; a constraint of this method is that a moisture gradient may have developed at moisture levels drier than field capacity. At intervals of 10 days for 60 days, nematodes were extracted and counted by wet-screening the soil from five replicates in each group. The dry series (ca 0 % moisture) was discontinued after 40 days as few nematodes were recovered in that treatment beyond 20 days. The surviving nematodes at each incubation interval, where sufficient in number, were used as the test population in the subsequent experiment.

Nematode count data were treated as a factorially designed experiment; the direct effects of time and soil moisture, and the interaction of time and moisture were evaluated by analysis of variance. Response surfaces were fitted to data sets to illustrate the nature of the relationships. Although means separation tests are not generally applied to data where independent variables are continuous, this approach was selected because of the non-linear nature of the models describing the responses. The Student-Newman-Keuls test was used to indicate the degree of change in response to prescribed increments of time and temperature.

EFFECT OF INCUBATION TIME AND SOIL MOISTURE LEVELS ON POPULATION INCREASE OF *X. INDEX*

At each sample interval, 100 *X. index* were hand-picked from the nematodes surviving at each moisture level established in the experiment described above. Because there were insufficient nematodes surviving beyond 40 days at all soil moisture levels and beyond 20 days at ca 0 % soil moisture, these treatments were excluded from the population increase experiment. For

each time interval and moisture level, 100 *X. index* were divided into groups of 20 to create five replicates. Nematodes were inoculated around the root zone of a single-bud rooting of *Vitis vinifera* L. cv. St. George in sandy loam in a 150 cm³ styrofoam cup. Control plants were also established. All plants were maintained in a greenhouse at 23 ± 3 °C, watered daily, and supplied with nutrient solution at 2-week intervals. After 5 months, nematodes were wet-screened from the soil in each cup and counted. Root weights were determined for plants inoculated with nematodes and for non-inoculated control plants.

The direct effects and interactions of soil moisture level and incubation time on nematode population increase were determined by analysis of variance as a factorial experiment. Since only two incubation periods were represented at ca 0 % moisture (10 and 20 days) and four at all other moisture levels (10, 20, 30 and 40 days), the experiment was not balanced. Consequently, the factorial comparisons were conducted in two sets so that main effect means of one factor would span all levels of the other factor in each set and so be unbiased. The data for all soil moisture levels at 10 and 20 days were analyzed as one set, and those for moisture levels 5, 10, 15, 20 and 25 % for incubation periods 10 through 40 days as the other set.

EFFECT OF SOIL PARTICLE SIZE ON *X. INDEX* POPULATION INCREASE

Sands of particle size 2360, 850, 534, 373 and 250 µm were washed and steam sterilized. Two kg of sand of each particle size and 2 kg of steam-sterilized sandy loam containing 85 % sand, 13 % silt, and 2 % organic matter were placed in 15-cm diameter clay pots. Single-bud cuttings of *V. vinifera* cv. St. George from virus-free plants were grown in sterile sandy loam and selected for top and root uniformity. The roots were washed free of soil and transplanted into the prepared pots of sand and sandy loam. For each soil texture, five pots were inoculated with 100 hand-picked *X. index* obtained from a greenhouse-grown fig plant (*F. carica*) and five pots served as non-inoculated controls.

All plants were maintained in a greenhouse at 20 to 26 °C for 5 months, and were watered daily with tap water and weekly with nutrient solution. Nematodes were extracted from the soil by wet screening and were counted to determine population increase. In all treatments, plant roots were weighed. Nematode counts and plant root weights were subjected to analysis of variance to evaluate the differences between particle size treatments.

RELATIONSHIP BETWEEN PARTICLE SIZE AND SUCTION POTENTIAL

To provide a basis for interpretation of the particle size experiment, moisture release curves for each sand

particle size and the sandy loam were developed with tension funnels (Kulte, 1986; Vrain, 1986). Soil water content is related to suction by $\theta_e = (\theta - \theta_r)/(\theta_m - \theta_r) = (h_d - h_c)^a$ where θ is the volumetric water content of the soil (cm^3/cm^3), θ_e is the effective water content, θ_m the maximum water content, θ_r the residual water content, h_d the displacement pressure head (cm), h_c the capillary pressure head (cm) and a the pore size distribution index (from 1 to 5) (Corey, 1977). Linear regression was used to obtain parameter values for the equation (Corey, 1977; Kulte, 1986).

Results

EFFECT OF SOIL MOISTURE ON *X. INDEX* SURVIVAL

Factorial analysis of variance, where the independent variables were treated as classes, indicated highly significant ($P < 0.01$) direct effects and interaction of soil moisture and time of incubation on nematode survival. Greatest survival across all time intervals occurred at 10 and 15 % soil moisture; survival was intermediate at 5 and 20 % moisture, less at 25 and least at 0 % soil moisture, indicating an optimum survival zone in the 10 to 15 % moisture range (Table 1). Nematode survival decreased significantly at all moisture levels over time and reached a uniform low on days 50 and 60 (Table 1).

Data on *X. index* survival relative to soil moisture and time indicated a quadratic effect of both independent variables. The relationship was well described by the equation $y = 70.25 + 1.59m - 0.06m^2 - 4.48t + 0.09t^2 - 0.0006t^3$ for $t \geq 10$, where y is percent survival, m is percent gravimetric soil moisture content and t is time in days ($r^2 = 0.89$, $n = 180$) (Fig. 1).

Table 1

Main effects of soil moisture level and time of incubation on survival of *X. index*.

Soil Moisture (%)	Survival (%)	Time (Days)	Survival (%)	
0	18.4 <i>d</i>	0	100.0	<i>a</i>
5	24.8 <i>b</i>	10	40.2	<i>b</i>
10	28.0 <i>a</i>	20	17.0	<i>c</i>
15	27.3 <i>a</i>	30	0.07	<i>d</i>
20	24.0 <i>b</i>	40	0.03	<i>e</i>
25	21.1 <i>c</i>	50	0.003	<i>f</i>
		60	0.0008	<i>f</i>

Data are means of five replications. In each column, means followed by the same letter do not differ significantly from each other at the 5 % level according to a Student-Newman-Keuls test.

EFFECT OF INCUBATION TIME AND SOIL MOISTURE LEVEL ON POPULATION INCREASE OF *X. INDEX*

For the population data obtained at 10 and 20 days, across all moisture levels, the main effects of soil moisture and incubation time were highly significant ($P < 0.01$) in their effect on *X. index* population increase; however, the interaction of soil moisture and incubation time was not significant for these data. Factorial analysis of variance and a means separation test indicated that the main effect of soil moisture across all incubation periods on population increase was greatest at 0.5 and 10 % soil moisture, less at 15 % and least at 20 and 25 % (Table 2). The main effect of incubation time was a significant decline in population increase at each 10 day interval (Table 2).

Table 2

Effect of incubation time and soil moisture on subsequent population increase of twenty *X. index* over 5 months.

Soil Moisture (%)	Final Population	Time (Days)	Final Population	Soil Moisture (%)	Final Population	Time (Days)	Final Population
0	355 <i>ab</i>	10	376 <i>a</i>	5	318 <i>a</i>	10	361 <i>a</i>
5	432 <i>a</i>	20	244 <i>b</i>	10	285 <i>ab</i>	20	241 <i>b</i>
10	396 <i>ab</i>			15	256 <i>b</i>	30	159 <i>c</i>
15	362 <i>b</i>			20	142 <i>c</i>	40	129 <i>c</i>
20	198 <i>c</i>			25	113 <i>c</i>		
25	153 <i>c</i>						

Data are means of five replications of one factor averaged over all levels of the other factor. In each column, means followed by the same letter do not differ significantly from each other at the 5 % level according to a Student-Newman-Keuls test. Since all moisture levels were not represented at every time interval, two separate analyses were conducted.

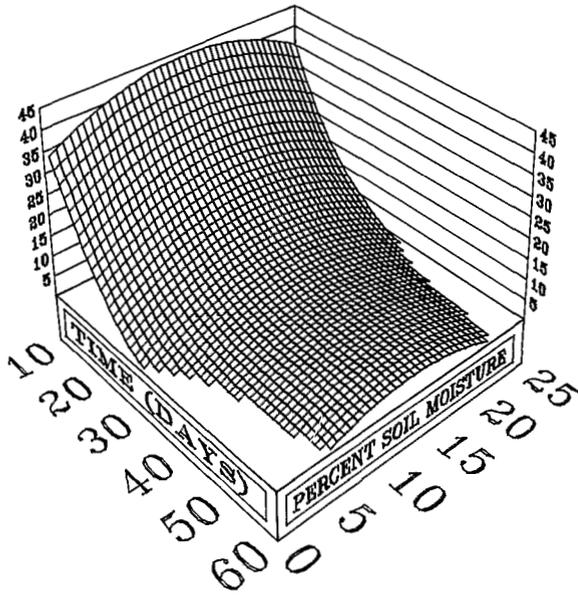


Fig. 1. The relationship between percent survival (y) of *Xiphinema index* and soil moisture (m) for incubation periods (t) of 10 to 60 days, $y = 70.25 + 1.59 m - 0.06 m^2 - 4.48 t + 0.09 t^2 - 0.0006 t^3$ ($r^2 = 0.89$).

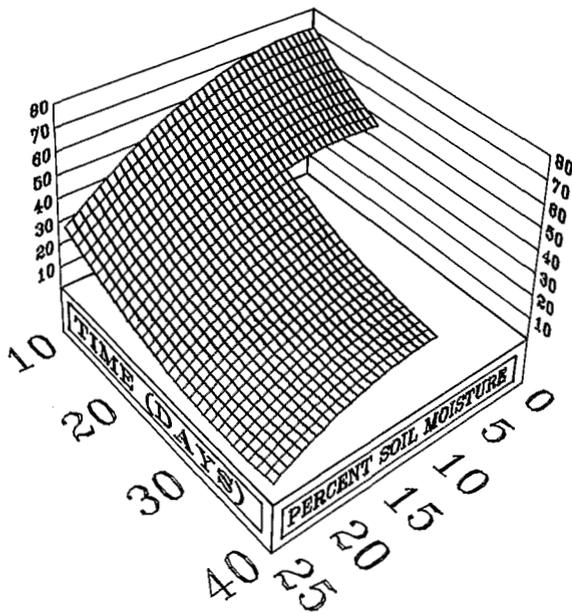


Fig. 2. The effect of period of incubation (t) at various soil moisture levels (m) on population increase of *Xiphinema index* as a percentage of the population increase of non-incubated nematodes (y). The relationship is described by $y = 104.3 - 0.06 m - 0.09 m^2 - 3.54 t + 0.034 t^2 + 0.044 mt$ ($r^2 = 0.78$).

For the data obtained at soil moisture levels 5 through 25 %, across all time intervals, both the main effects and interactions of soil moisture and incubation time were highly significant ($P < 0.01$) (Table 2). Population increase was again greatest after incubation at 5 and 10 % soil moisture, less at 15 % and least at 20 and 25 % soil moisture. A significant decline in population increase occurred over time; the lowest population increases were observed on days 30 and 40 (Table 2).

The interaction of incubation time and soil moisture on the population increase of *X. index*, expressed as a percentage of the population increase of non-incubated nematodes, was well described by the equation $y = 104.3 - 0.06 m - 0.09 m^2 - 3.54 t + 0.034 t^2 + 0.044 mt$ for $t \geq 10$ ($r^2 = 0.78$, $n = 110$) (Fig. 2), where y is the final nematode population as an indicator of population increase, m is the percent soil moisture and t is time in days. At all time intervals, population increase was lower for the surviving nematodes incubated at higher moisture levels.

There was a linear relationship between root weight of *X. index* host plants and the final nematode population (Fig. 3). The relationship was described by the equation $y = 15.91 - 0.0215 x$ ($r^2 = 0.77$, $n = 110$), where y is root weight and x is the final population level of *X. index*.

EFFECT OF SAND PARTICLE SIZE ON POPULATION INCREASE OF *X. INDEX*

Differences in population increase of *X. index* in sands of different particle size were highly significant ($P < 0.01$). Highest levels of *X. index* population increase were observed in the sandy loam control; however, the population increase in sand of 250 μm particle size was not significantly lower than the control (Table 3).

Table 3

Effect of sand particle size on population increase of 100 *X. index* and on root weight of host plants and root weight of nematode-inoculated plants relative to non-inoculated plants after 5 months.

Particle Size (μm)	Final Population	Root Weight (g)	Relative Root Weight
2 360	139 c	19.5 a	1.08 a
850	179 c	16.2 b	0.92 ab
534	291 c	15.7 b	0.91 ab
373	1 020 b	11.9 c	0.76 b
250	1 174 ab	11.7 c	0.72 b
Sandy loam	1 590 a	11.1 c	0.72 b

Data are means of five replications of one factor averaged over all levels of the other factor. In each column, means followed by the same letter do not differ significantly from each other at the 5 % level according to a Student-Newman-Keuls test.

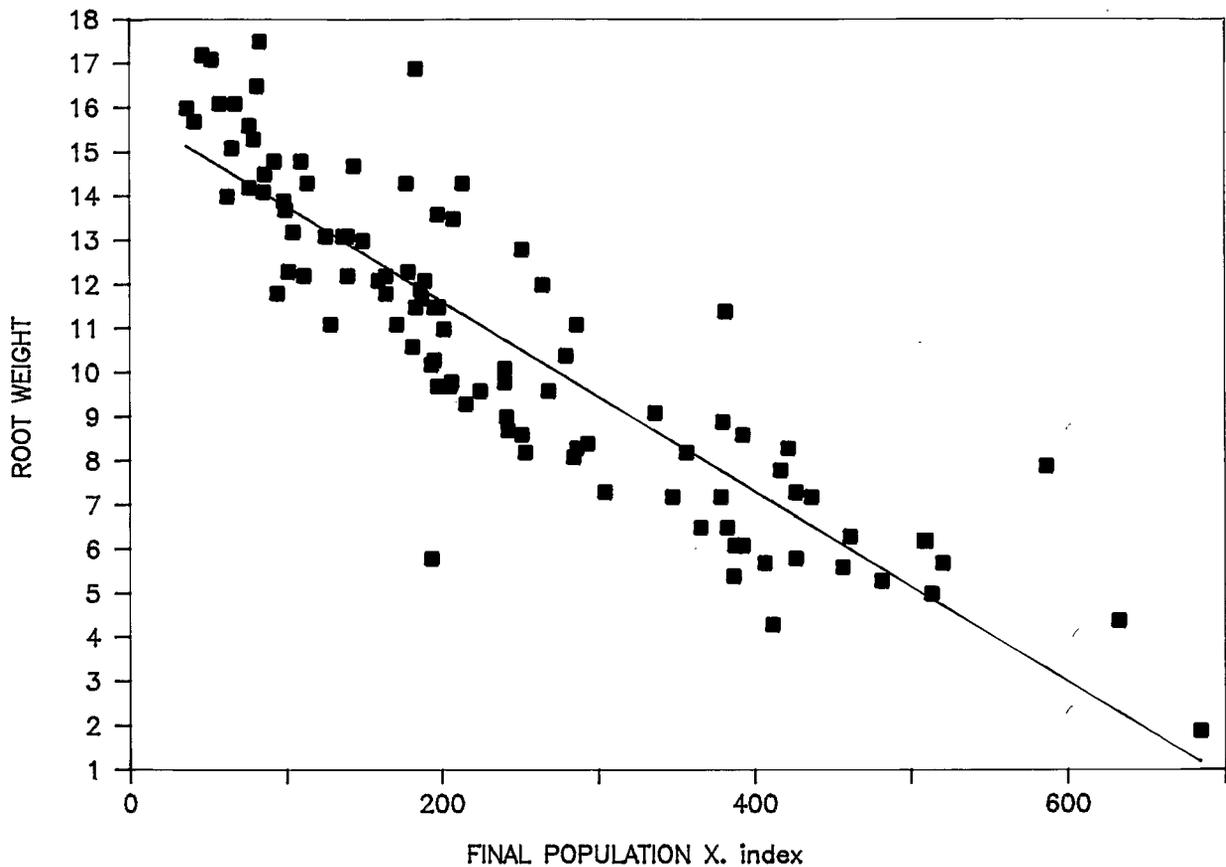


Fig. 3. The relationship between grape root weight (grams, y) and final population density (x) of *Xiphinema index*, $y = 15.91 - 0.0215x$ ($r^2 = 0.77$), after 5 months of exposure.

Population increase in 373 μm sand was not significantly lower than in 250 μm sand, while population increase in coarser sands of particle size 2 360, 850 and 534 μm was significantly lower than in sands of finer particle size (Table 3). Relative plant root weight (inoculated/control, for each particle size) was greatest in 2 360 μm sand, less in sands of 850 and 534 μm , and least in sands of 373 and 250 μm particle size and in sandy loam. As root weight decreased, nematode reproduction increased in sand of all particle sizes and in sandy loam (Table 3). The interaction between nematode population and particle size on root weight was significant ($P < 0.05$) and was related to reduced root growth in sands of finer particle size when nematodes were present.

MOISTURE RELEASE CURVES

Moisture release curves for coarse-textured sands of 2360 and 850 μm were virtually identical to each other and nearly identical to the curve for 534 μm sand (Fig. 4). Similarly, the moisture release curves for the two sands of finer particle size (373 and 250 μm) were

almost identical, but differed substantially from the curves for coarse-textured sand. The greatest water retention occurred in sandy loam (Fig. 4).

Discussion

In the absence of a host for 60 days, the low survival of *X. index* at intermediate soil moisture levels (10 and 15 %) and very low survival in dry and saturated soils (Fig. 1) are qualitatively similar to previous studies. Harris (1979) observed survival of less than 50 % over 69 days under favorable soil moisture conditions and low survival in both dry and saturated soil. Low survival in dry soil may reflect an inability of *X. index* to withstand desiccation (Harris, 1979), while low survival in wet soil may result from oxygen deprivation (Van Gundy *et al.*, 1962).

Temperatures maintained in these studies were favorable for nematode metabolic activity, development and movement, and we assume that the nematode population declined even under optimum moisture conditions

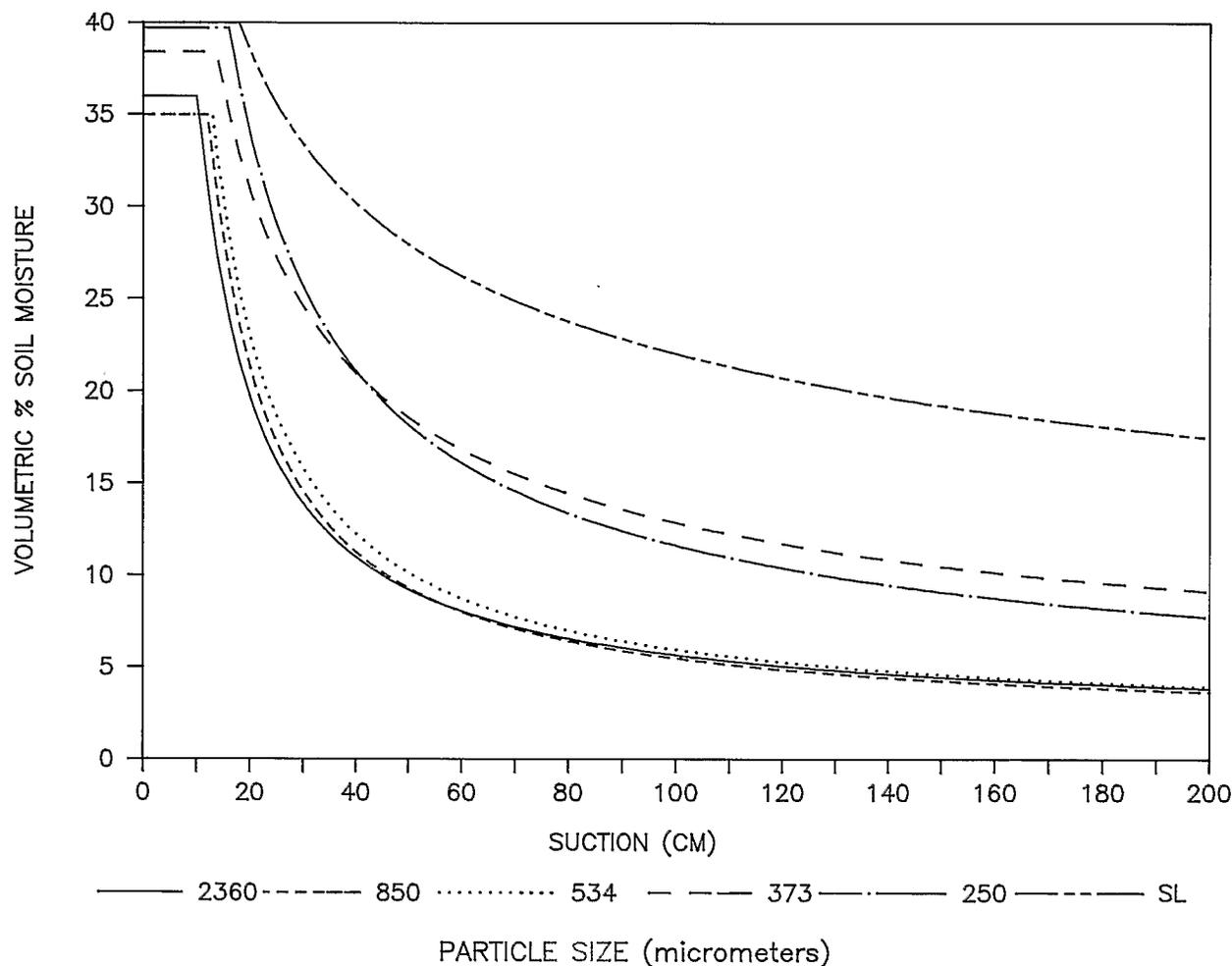


Fig. 4. Moisture release curves for sands of different particle size and sandy loam used in these experiments.

because internal food reserves were depleted and nematodes aged. Population increase of *X. index* observed in this study was greatest following incubation in intermediate to dry soil (Fig. 2). The reproductive potential was lower after incubation in moist soil, possibly reflecting increased utilization of food reserves associated with greater metabolic and muscular activity in moist environments. The relationship between root weight of host plants and final population densities of *X. index* indicates direct damage to the plants caused by the feeding and population growth of the nematode (Fig. 3).

These studies suggest possibilities for eliminating or reducing populations of *X. index* between crops by prolonging the host-free period, by soil flooding, or by maintaining the soil in a dry state. Each of these options, however, has limitations. Host-free periods can be difficult to attain in woody perennials as nematodes may feed on root fragments remaining in the soil; soil flooding requires level land, adequate availability of water and

may not be effective (Meagher *et al.*, 1976); maintaining field soil in a dry state to a substantial depth is difficult to achieve and is affected by climate.

Nematode population increase was greatest in both sandy loam and fine-textured sand. Population increase in coarse-textured sand of particle sizes $\geq 534 \mu\text{m}$ was uniformly low although root weight was highest in 2360 μm sand and less in sands of finer particle size and in sandy loam (Table 3). When root weights are compared with those of non-inoculated control plants or expressed on a relative basis (Table 3) for each particle size, the direct damage caused by feeding and reproduction of *X. index* is apparent. Root growth was significantly negatively correlated with final population level of *X. index* ($r^2 = 0.73$).

The moisture release curves for sands of different particle size separated clearly into two groups (Fig. 4) indicating two categories of environment affecting nematode viability and host plant growth. Sands of 2360,

850 and 534 μm particle size had similar, low water retention capacities; *X. index* population increase was uniformly low in these sands. Sands of particle size 373 and 250 μm had higher, similar moisture holding capacities and supported greater, uniform levels of population increase and resultant damage to host plant roots. Sandy loam exhibited the highest moisture retention and supported the greatest nematode population increase; however, root damage in sandy loam and in fine-textured sands did not differ substantially. Similar results have been observed for reproduction of *Belonolaimus longicaudatus* (Rau) in sands of comparable particle size (Robbins & Barker, 1974). Greater root growth in coarse sands occurred both in the presence and absence of nematodes (Table 3) and may indicate better tolerance for low moisture conditions by grape roots than that of *X. index*, or greater availability of space for root growth. Vrain (1986) noted that when low soil moisture levels inhibit reproduction of plant-parasitic nematodes, susceptible crops may grow as well in infested soils as in uninfested soil.

Although large nematodes are potentially limited by pore space in fine-textured soil, *X. index* survives in both fine sands and sandy loam at intermediate moisture levels. Jones, Larbey and Parrot (1969) observed that *X. diversicaudatum* (Micoletzky) Thorne thrives in well-structured, fine-textured soils by inhabiting the macropores between peds. In structureless sands, space and aeration are adequate for *X. index*, but moisture availability may become limiting, as noted elsewhere (Wallace, 1971; Harris, 1979; Vrain, 1986).

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