Meloidogyne hapla in Organic Soil: Effects of Environment on Hatch, Movement and Root Invasion[1]

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Abstract: Using new techniques, hatch and movement of *Meloidogyne hapla* and nematode invasion of lettuce roots growing in organic soil were studied under controlled soil conditions of temperature, moisture, O_2 and CO_2. When O_2 levels of 2.7, 5, 10, 21 and 40% with CO_2 maintained at 0.03% were used, O_2 below 21% or at 40% reduced nematode activities compared with those at 21%. When CO_2 levels of 0.03, 0.33, 2.8, 10 and 30% with O_2 maintained at 21% were used, all levels above 0.03% CO_2 resulted in less activity than at 0.03% except for more invasion at 0.33% than at 0.03%. Results suggested *M. hapla* was tolerant of CO_2 below 10% but adversely affected by 30% CO_2. Effect of O_2 was influenced by the level of CO_2 present. No larvae invaded roots at 3.2% O_2 and 18.6% CO_2 but hatch and movement occurred. Night and day temperatures of 21.1 and 26.7 C were more favorable for movement and invasion than 15.5 and 21.1 C, 26.7 and 32.2 C or 26.7 and 32.2 C. Optimum moisture for movement was 80 cm suction and for invasion was 100 cm. *Key Words:* Soil atmosphere, soil moisture, soil temperature, lettuce.

Hatch, movement and invasion are influenced by the soil environment (9, 16) and are important in determining the number of nematodes which will eventually become successful parasites.

Hatch of *Meloidogyne javanica* eggs was unaffected by soil moistures from pF 0 - 3.6 (pF is log of suction in cm of water) but decreased at higher pF values probably because eggs lose water and shrink. This reversible loss of water inhibiting hatch may be a survival mechanism during moisture stress (1). *M. hapla* eggs hatched in water at temperatures of 12 - 27 C but the optimum temperature varied with the hatching period, being 21 ± 1 C after 30 days and 27 ± 1 C after 3 days (18). The temperature optima for hatch of eggs of *M. javanica* and *M. hapla* were 30 C and 25 C, respectively (4). Low oxygen reduced hatch of *M. javanica* eggs while absence of O_2, inhibited hatch and may be lethal to eggs in some stages of embryonic development (2, 15). Although the rate of hatch was lower at low O_2 the final hatch after 20 days did not differ from that at higher O_2 concentrations. Subsequent hatch in aerated water was reduced when length of exposure to an oxygen-free atmosphere was increased (15).

Field capacity was reported by Wallace (14) to be more favorable for movement of *M. javanica* larvae than either a higher or lower moisture content. The optimum temperature for movement of *M. javanica* was 25 - 30 C and was 20 C for *M. hapla* (4). Van Gundy and Stolzy (13) reported a linear relationship between movement of *M. javanica* and oxygen diffusion rate in cellulose sponges.

Some data have been published on the effect of moisture on nematode invasion of plant roots. *M. hapla* (6), *Pratylenchus penetrans* (7) and *M. javanica* (14) invaded roots in larger numbers at field capacity than at higher moisture contents. At high suctions of pF 3 few *P. penetrans* larvae invaded alfalfa roots (7). *M. javanica* invaded tomato roots over a wide temperature range but for *M. hapla* 15 C (8 hr) - 20 C (16 hr) was optimum (4). Kinloch and Allen (8) found 20 C to be suitable for invasion of tomato roots by both *M. hapla* and *M. javanica*.

Very few studies of environmental influences on hatch, movement and invasion have been conducted in organic soil. Although the effect of O_2 on hatch and movement has been studied no published data on invasion has been noted. Little is known about the effect of CO_2 on nematode activities in soil.

This study was made to determine the influence of temperature and soil moisture on movement and invasion of lettuce roots by *M. hapla* larvae in organic soil and the influence of O_2 and CO_2 on hatch of eggs, movement of larvae and invasion of lettuce roots by *M. hapla* larvae in organic soil.

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MATERIALS AND METHODS

Second-stage infective larvae were obtained from a greenhouse culture established from egg masses taken from 'Minetto' lettuce (*Lactuca sativa* L.) growing in organic soil (Histosol or soil of plant and animal origin) in Oswego County, New York, and maintained by periodic transfer to 'Rutgers' tomato (*Lycopersicon esculentum* Mill.). Eggs were obtained from egg masses which were broken open and placed in 20 ml of 1.2% sodium hypochlorite solution and agitated with a 'Vibromixer'® for 3 min. The resultant suspension was poured through two 325-mesh sieves to remove larvae and the eggs were collected in a 0.45 μ Millipore filter after rinsing several times with water. Eggs then were washed into several counting dishes where additional larvae were removed, recollected on a Millipore filter and washed into a beaker to make a suspension of the desired concentration.

Control of Moisture, Oxygen and carbon dioxide in organic soil: A Büchner funnel with a fine porous plate (pore size 4 - 5.5 μ) connected to a water manometer was used to regulate and maintain soil moisture. The funnel was filled with 4 cm of organic soil and plastic tubes or hollow straws were pushed into the soil so that they rested on the porous plate. The soil was saturated by raising the side-arm of the manometer above the soil. Subsequently, the side-arm was lowered creating a suction equal to the vertical distance from the meniscus in the manometer to the soil surface. By keeping the meniscus at a constant level the moisture of the soil in the funnel was maintained.

Levels of O_2 and CO_2 in organic soil were obtained by controlling the composition of the gas maintained over the soil in the funnel. Mixtures of O_2, CO_2 and N_2 were prepared by metering the required partial pressure of each component gas into an evacuated cylinder and then rotating the cylinder horizontally to blend the gases (11). To check the gas composition, each cylinder of gas was analysed with an Orsat gas analyser or a gas chromatograph. A continuous flow of the gas mixture from the cylinder was maintained at 80 ml/min through color-coded tygon tubes to polyethylene bags fitted over the funnels containing organic soil (Fig. 1). The polyethylene bags were held securely with a wire twisted on top of a strip of rubber sponge stretched around the bag and the Büchner funnel. The outlet from the bag was placed in 100 ml of water contained in a 250-ml Erlenmeyer flask. The water in the flask prevented a back flow of atmospheric air into the bag and served as an indication of the flow rate. The soil was subjected to the gas mixture for 24 hr prior to each experiment.

The gas in the soil was sampled daily from a sampling-well buried in the soil (Fig. 1). The reservoir was made from a sawed-off disposable plastic syringe barrel with the cut end covered with a 325-mesh sieve to keep out the soil. The other end of the reservoir was fitted into a 20-gauge needle which was connected to capillary polyethylene tubing (internal diam 0.085 cm). The capillary tubing passed outside the bag through a rubber septum and was attached to a 2.5 ml disposable syringe fitted with a 20-gauge needle. During sampling 1 ml of the soil gas was withdrawn by the syringe outside the bag thereby causing minimum disturbance to the soil within the bag. The needle of the syringe was removed and inserted in a rubber stopper to prevent leaking of the soil gas until it was analysed. The capillary tubing was immediately sealed by plugging it with a wire of the same diameter.

Soil air was analyzed in a 'Varian 90-P' gas chromatograph connected to a 'Varian G-1000' recorder (Varian aerograph, 2700 Mitchell Drive, Walnut Creek, California 94598). The gas chromatograph was fitted with two columns, a molecular sieve and a silica gel column for analysing oxygen and carbon dioxide, respectively. The molecular sieve column lying outside the instrument was maintained at room temperature while the silica gel column was maintained at 85 C (10). The carrier gas, helium, was maintained at 40 ml/min and the filament current was maintained at 200 mA for all measurements except for 0.33% and 0.03% CO_2 when it was at 275 mA.

A 1-ml sample of soil air was injected swiftly into the gas chromatograph through a rubber septum. Each analysis took approximately 3 min. The flow rate of 80 ml/min maintained the soil air at the composition of that in the gas mixture over the soil. The sensitivity of the instrument at 0.03% partial pressure of CO_2 was poor so that no peak was registered on the recorder. Judging from the stability of the other measurable levels of CO_2, the concentration of CO_2 at atmospheric level was assumed to be unchanged.

Invasion: The effect of temperature and levels of soil moisture, O_2 and CO_2 on invasion

was studied. All treatments had five replications and all experiments were repeated at least once. In each treatment, five butyl acetate plastic tubes, 1-cm diam and 5-cm long, were placed in organic soil contained in a 9-cm Büchner funnel. The soil was saturated and then adjusted to 100 cm suction after which a germinated 'Minetto' lettuce seed was planted in the soil enclosed in each plastic tubing. Prior to the day of inoculation, the soil in the funnels

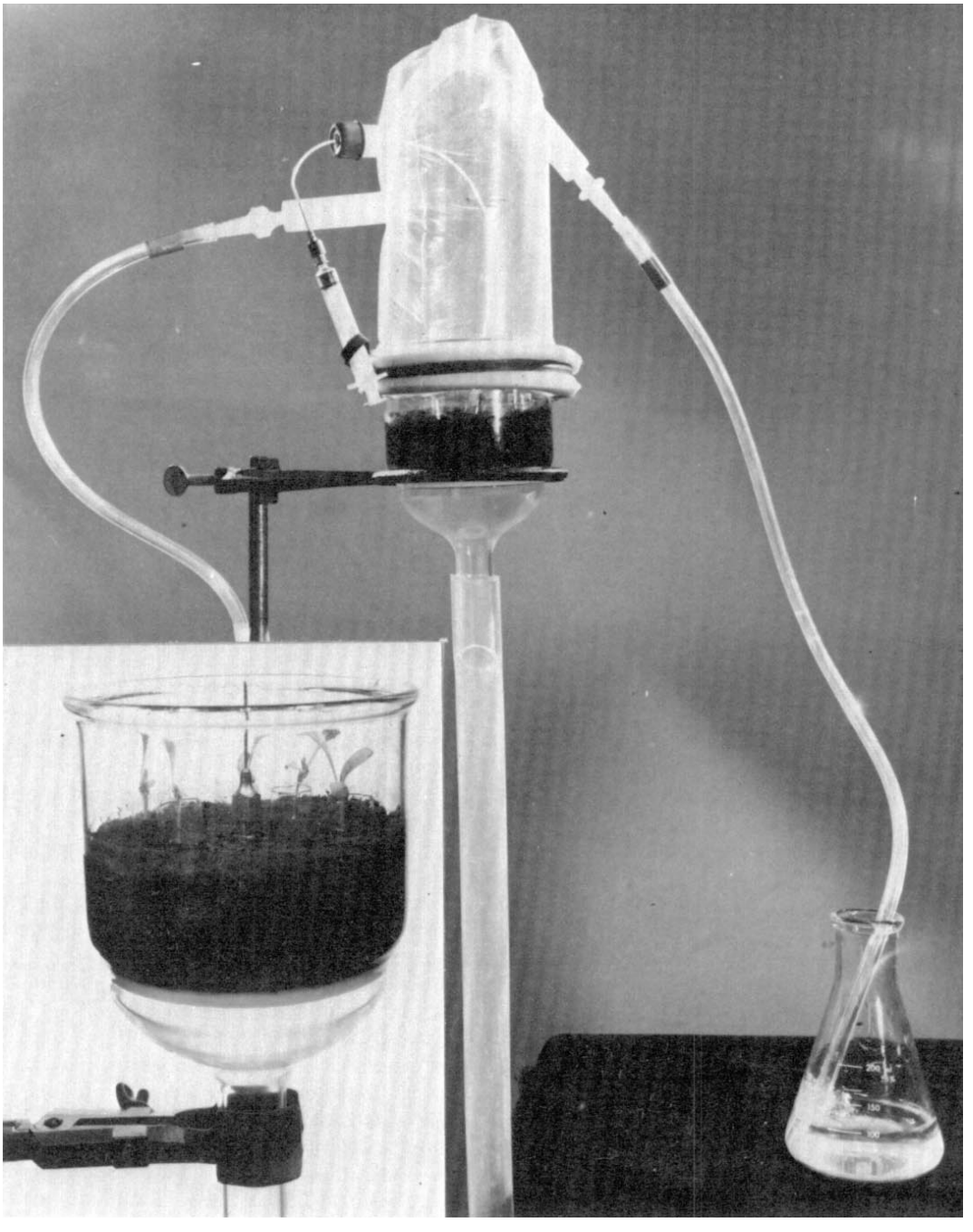

FIG. 1. Apparatus for maintaining known levels of soil moisture, O_2 and CO_2 in organic soil. Insert shows lettuce seedlings growing in organic soil and well for sampling soil air.

was saturated and then adjusted to suctions of 0, 20, 40, 60, 80, 100 and 120 cm of water. Each 1-week-old lettuce seedling was inoculated with 300-400 nematodes. Four days after inoculation, the roots were washed and stained with 0.1% acid fuchsin in lactophenol. After clearing overnight in lactophenol the roots were pressed between two glass slides and the nematodes inside the roots counted.

Temperature effect on invasion was studied at 100 cm suction in growth chambers at the respective night and day temperatures of 15.5 and 21.1 C (low), 21.1 and 26.7 C (intermediate) and 26.7 and 32.2 C (high). Each chamber received 12 hr of light at 2000 ft-c.

Oxygen was studied at 2.7, 5, 10, 21 and 40% with CO_2 maintained at the atmospheric level of 0.03%. The effect of CO_2 was studied at 0.03, 0.33, 2.8, 10 and 30% with O_2 maintained at 21%. After studying the effect of O_2 and CO_2 individually their combined effect was investigated at the following combinations of O_2 and CO_2: 21.0% O_2 and 0.03% CO_2; 14.8% O_2 and 6.1% CO_2; 10.2% O_2 and 10.7% CO_2; 4.9% O_2 and 17.1% CO_2; 3.2% O_2 and 18.6% CO_2; and 1.7% O_2 and 21.2% CO_2. Air with atmospheric levels of O_2 and CO_2 were supplied by an aquarium pump. The soil and the 1-week-old seedlings were exposed to each premixed gas for 24 hr prior to inoculation. All experiments were carried out in a growth chamber at 20 and 25.5 C night and day temperature, respectively, and provided with 12 hr of light at 2000 ft-c. Each day after inoculation, 1 ml of soil air was sampled and analyzed. Four days after inoculation, roots of inoculated seedlings were washed, stained and the larvae within counted.

Movement: A Büchner funnel was filled to a depth of about 1 cm with organic soil and five pieces of hollow plastic drinking straw, 0.5-cm diam and 5-cm long, were arranged vertically in a row along the diameter of the funnel with their ends resting on the porous plate of the funnel. The straws and the funnel then were gradually filled with soil. The soil was saturated and adjusted to the desired suction. On the following day, 400-500 nematodes suspended in 0.05 ml of water were pipetted onto the surface of the soil within the straw. After 10 min the funnel was turned so that the straws in the funnel were horizontal. Two days later, the straws were removed, cut into 1-cm segments and the soil inside each segment pushed out with a glass rod onto a small modified pie-pan made from a plastic counting dish and a piece of milk filter held in a plastic ring. Water was added until it just covered the soil. Nematodes emerging into the water were counted after 24 hr and those recovered more than 1 cm from the soil surface to which they had been added were considered to have moved. The number of nematodes moving more than 1 cm was expressed as a percentage of the number of nematodes recovered from all the segments in the straw.

Temperature effect on movement was studied at low, intermediate and high temperature regimes in the same growth chambers as were used for invasion studies. The soil was maintained at 80 cm suction which was most favorable for movement in preliminary experiments.

The effect of O_2 and CO_2 on movement was studied at 80 cm suction at the same levels and in the same growth chamber as was used for invasion studies. Soil was exposed to premixed gases for 24 hr and soil air was analyzed daily.

Hatch: The effect of O_2 and CO_2 on hatch was investigated at the same levels and in the same growth chamber as was used in invasion studies. The effect of soil moisture and temperature on hatch of *M. hapla* eggs was not studied.

Eggs suspended in 0.05 ml water were pipetted onto the surface of organic soil held in butyl acetate tubes. The soil was maintained at 100 cm suction and was exposed to the premixed gas for 24 hr before the eggs were added. Soil air was sampled and analyzed daily. Five days later, the soil in each butyl acetate tube was washed into 50-ml centrifuge tubes with a sugar solution containing 30 g sucrose and 70 ml water and after shaking thoroughly was centrifuged for 5 min. Eggs and larvae in the supernatant were collected in a 500-mesh sieve, washed into a counting dish and counted. The number of hatched larvae was expressed as a percentage of the number of eggs and larvae recovered.

RESULTS

Invasion: No nematodes entered lettuce roots at zero suction (Fig. 2A) but as suction was increased, more nematodes entered lettuce roots. Significantly more nematodes ($P = 0.05$) entered lettuce roots at the intermediate than at either the high or low temperature regimes (Table 1).

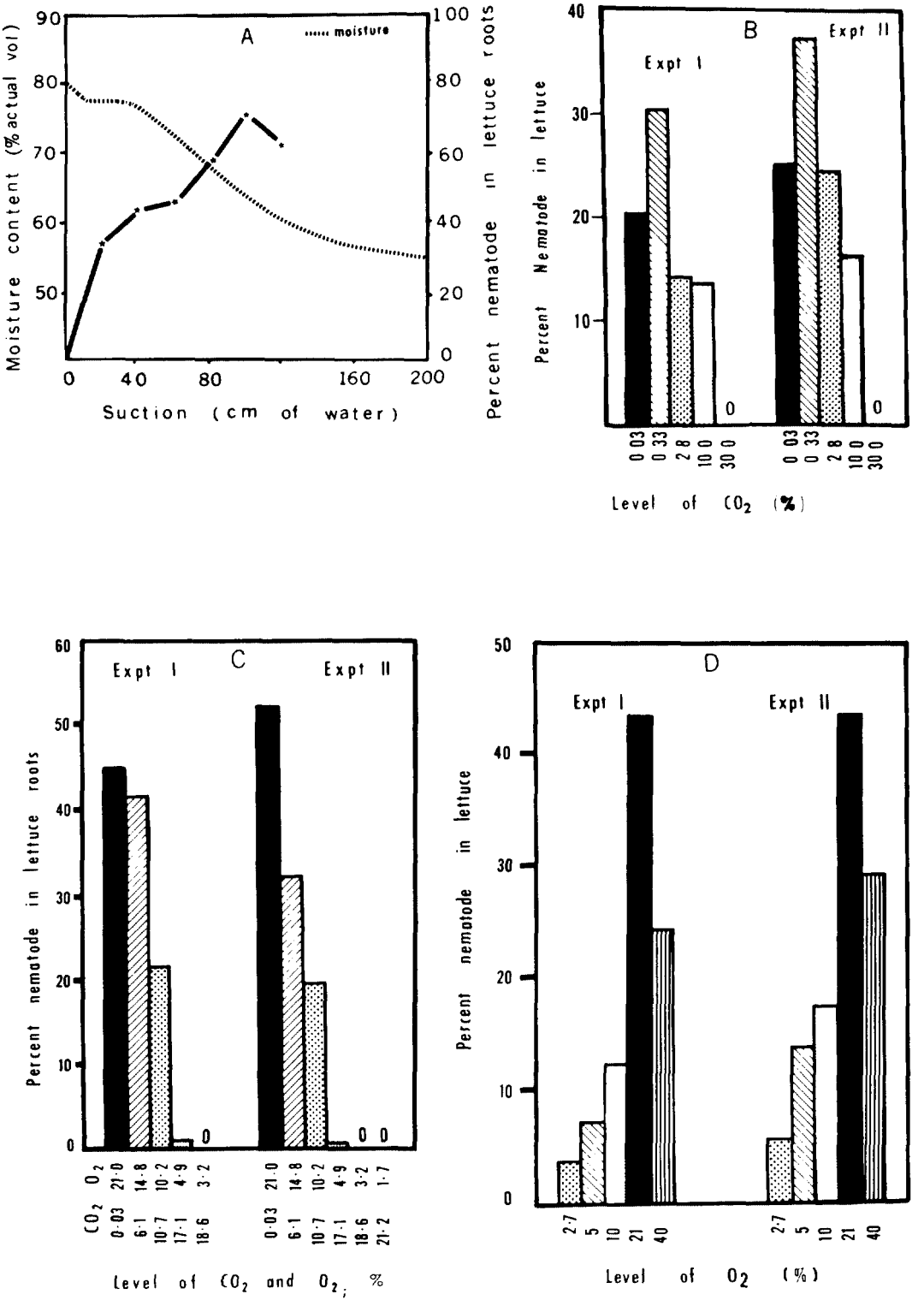

FIG. 2. The influence of soil moisture, O_2 and CO_2 levels on invasion of 'Minetto' lettuce roots by *Meloidogyne hapla* in organic soil. **A)** Influence of soil moisture; **B)** influence of CO_2; **C)** influence of various combinations of levels of O_2 and CO_2; **D)** influence of O_2.

With CO_2 held at atmospheric level, highest numbers of *M. hapla* larvae invaded lettuce at 21% O_2 (Fig. 2D). When O_2 was lowered to 10%, larvae entering roots decreased from 43.6% in Experiment I and 43.9% in Experiment II to 12.3% and 17.6%, respectively, indicating that low oxygen decreased invasion by *M. hapla* larvae. At 2.7% O_2, invasion was not completely inhibited as 3.7% of larvae in Experiment I and 5.4% in Experiment II still invaded roots. Oxygen at 40% was less favorable for invasion than at 21%.

With O_2 maintained at atmospheric level, more *M. hapla* larvae invaded lettuce roots at 0.33% CO_2 than at 0.03% CO_2 (Fig. 2B). However, CO_2 levels above 0.33% were less favorable for invasion than atmospheric CO_2. When CO_2 was increased from 0.03% to 10%, the percentage of larvae invading roots decreased from 20.3% in Experiment I and 25.5% in Experiment II to 18.6% and 15.7%, respectively. CO_2 at 30% completely inhibited invasion and also caused seedlings to become chlorotic and stunted.

Comparison of the effect of various combinations of O_2 and CO_2 on invasion indicates maximum invasion occurred when soil was maintained at atmospheric levels of O_2 and CO_2 (Fig. 2C). At 4.9% O_2 and 17.1% CO_2, only about 1% of the nematodes invaded roots while at 3.2% O_2 and 18.6% CO_2 no invasion occurred.

Movement: Movement of *M. hapla* in organic soil was influenced by temperature and moisture similar to the way invasion was influenced by these factors. Fewer larvae moved beyond 1 cm at lower suctions than at higher suctions (Fig. 3A). The intermediate temperature regime was the most favorable one for movement at 80 cm suction (Table 2).

Larvae moved in greatest numbers at 21% O_2 (Fig. 3C) and when O_2 was lowered from 21% to 10%, movement was reduced from 53.7% and 56.7% in Experiment I and Experiment II to 19.0% and 20.4%, respectively. Oxygen at 40% was less favorable for movement than at 21%.

Unlike invasion, movement was not higher at 0.33% than at 0.03% (Fig. 3B). Fewer larvae moved when CO_2 levels were increased from 0.03%. At 10% CO_2, 24.8% of the larvae moved in Experiment I and 16.5% in Experiment II as compared to 52.2% and 43.9%, respectively, at 0.03% CO_2. Very few nematodes moved at 30% CO_2.

Movement was highest in soil maintained at 21% O_2 and 0.03% CO_2 (Fig. 3D). Invasion was decreased more than movement at combinations of low O_2 and high CO_2 since up to 2.9% of the nematodes moved at the combination of 1.7% O_2 and 21.2% CO_2 but no invasion occurred at this level.

Hatch: At 2.7% and 40% O_2 hatch was approximately one-half that at 21% O_2 (Fig. 4A). Although differences were noted, similar trends of hatch were evident in both experiments.

Increasing the level of CO_2 did not significantly change the rate of hatch until CO_2 reached 30% (Fig. 4B). Maximum hatch occurred at 21% O_2 and 0.03% CO_2, but as the O_2 was lowered in various combinations of O_2 and CO_2 levels, hatch decreased. Like movement, hatch was not completely inhibited at the combination with the lowest O_2 tested (Fig. 4C).

TABLE 1. Influence of temperature on invasion of 'Minetto' lettuce by *Meloidogyne hapla* at 100 cm suction in organic soil after 4 days.

Temperature (C)		Percent nematodes invading lettuce	
Night	Day	Experiment I	Experiment II
15.5	21.1	15.6	21.5
21.1	26.7	33.9	44.9
26.7	32.2	18.2	25.9
LSD 0.05		5.5	6.4

DISCUSSION

Lack of O_2 or its limited supply may be responsible for the decline of nematode activity in water-logged soil. Invasion, movement and hatch differ in the extent to which each is affected by low O_2. Invasion appears to be the most affected and hatch the least. For *M. javanica* eggs, Wallace (15) reported an initial decrease in hatch at low $O_2 : CO_2$ ratio but total hatch figure after 20 days showed no decrease. Evidence to support the relatively small effect of high soil moisture, and thus most likely aeration, on hatch was also reported by Baxter and Blake (1) who found hatch to be independent of suction between pF 0 and pF 3.6. The complete absence of oxygen, however, inhibited hatch (15). Movement appears to be affected more than hatch by low oxygen. Movement was almost inhibited by the mixture

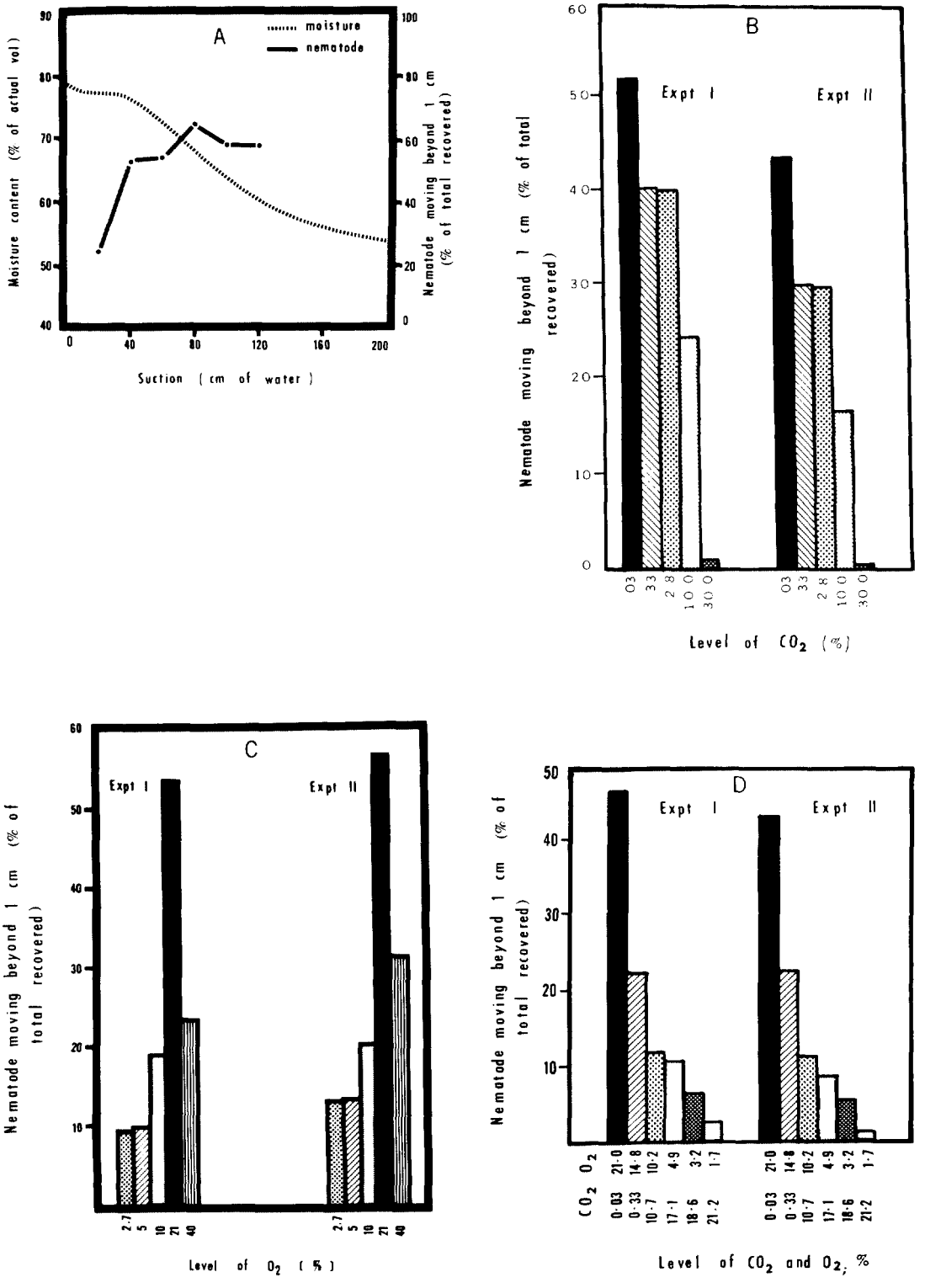

FIG. 3. The influence of soil moisture and O_2 and CO_2 levels on movement of *Meloidogyne hapla* in organic soil after 2 days. A) Influence of soil moisture; B) influence of CO_2; C) influence of O_2; D) influence of various combinations of levels of O_2 and CO_2.

with the lowest O_2 but occurred at 3.2% O_2 and 18.6% CO_2 when invasion was inhibited completely.

Our results suggest *M. hapla* is tolerant to CO_2 up to 10% since reductions of hatch, movement and invasion were not very great until 30% CO_2 where all activities were completely inhibited. Under field conditions the level of CO_2 in the root zone is usually above that of the atmosphere and has been found to be as high as 10%. Adaptation to high CO_2 levels present in the soil has been suggested to explain the higher respiration rate of *Ditylenchus dipsaci* at 1% CO_2 as compared with that at 0.03%. A foliar pathogen, *Aphelenchoides ritzemabosi*, did not have a higher respiration rate at the 1% CO_2 level (3).

The effect of O_2 on invasion, movement and hatch of *M. hapla* in organic soil appears to be modified by the level of CO_2 present. Our results suggest that nematode activity at each level of O_2 is partially influenced by the level of CO_2 present.

The importance of moisture and oxygen in influencing activities of plant parasitic nematodes is well known (9, 16). Results of this study show that low oxygen limits invasion, movement and hatch. During early spring, cool and wet conditions usually occur in the organic soil of New York State. At this time the temperature, moisture and levels of O_2 and

TABLE 2. Influence of temperature on movement of *Meloidogyne hapla* in organic soil at 80 cm suction after 2 days.

Temperature (C)		Percent nematodes moving beyond 1 cm (of nematodes recovered)	
Night	Day	Experiment I	Experiment II
15.5	21.1	33.9	22.7
21.1	26.7	53.4	34.9
26.7	32.2	34.7	28.7
LSD 0.05		8.8	10.1

CO_2 in the soil possibly do not prevent hatch, since hatch is least affected by low oxygen and can occur over a wide range of temperature (18) and moisture conditions (1), but the rate of hatch may be reduced. Because movement is affected more by unfavorable temperature, moisture and oxygen, movement may be reduced at this time. Nematodes which remain quiescent and inactive under unfavorable conditions can prolong their infectivity (12) but second-stage infective larvae will eventually die if host tissue is not available. Therefore, before lettuce is sown the inoculum may already be present in the infective stage. After infested fields are sown, the seedling roots growing in the soil eventually encounter the infective larvae. Invasion, as indicated in this

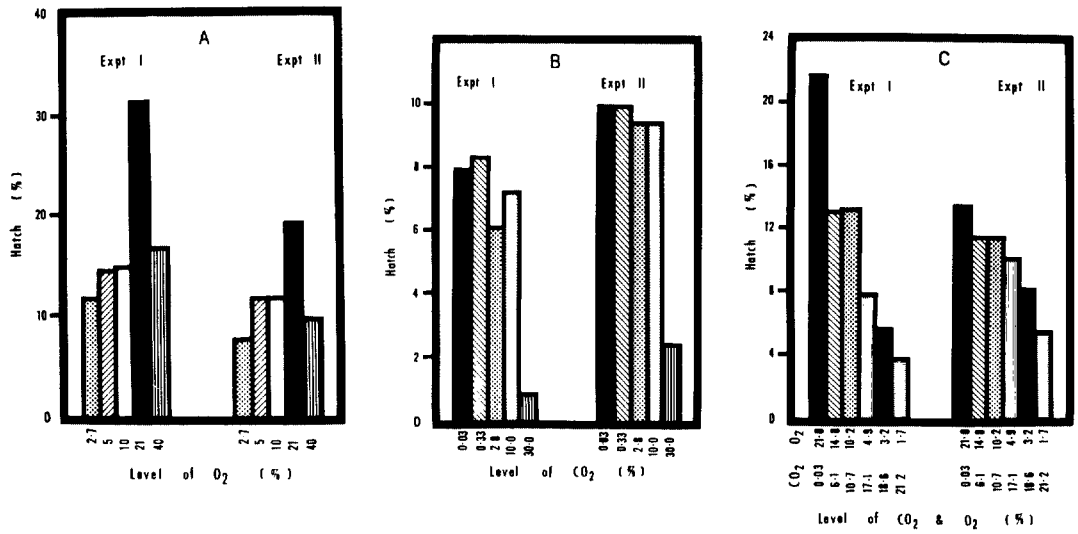

FIG. 4. The influence of O_2 and CO_2 on hatch of *Meloidogyne hapla* eggs in organic soil at 80 cm suction after 5 days. **A)** Influence of O_2; **B)** influence of CO_2; **C)** influence of various combinations of levels of O_2 and CO_2.

study, can take place over a range of temperature conditions but is limited greatly by soil moisture and oxygen. In wet soil low O_2 may inhibit invasion completely or may limit it so that only a small number invade lettuce roots. If these conditions continue for 2 weeks or more without seriously hampering crop growth, only a light infection of lettuce may result. By the end of this period, the crop would have grown substantially and have become more tolerant to infection (17). However, if the soil is warm and moist after seeding, conditions for invasion are favorable so that within a day or two invasion may be considerable. Larvae of *Meloidogyne* sp. enter roots within 6 hr after inoculation (5). Large numbers of larvae may, therefore, invade lettuce roots soon after germination, reducing top growth and root weight so severely that heads are prevented from attaining marketable size at harvest. Although environmental conditions during the remainder of the growing season markedly influence the host-pathogen interaction, these data indicate that activities of the nematode during the early part of the growing season may also influence disease severity.

Because water relations of organic soil differ markedly from those of mineral soils, results with mineral soils may be entirely different from those reported in this paper.

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