Nematode Reproduction in Environments of Fluctuating Aeration[1]

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Abstract: Reproduction of *Aphelenchus avenae*, reared on *Rhizoctonia solani* growing on steamed wheat seeds and *Caenorhabditis* sp., reared on a mixed bacterial culture grown on oatmeal, was significantly reduced at 5% oxygen and inhibited at 4% oxygen and below. Aeration ranging from atmospheric air (21%) to 10% oxygen had no effect on reproduction. Close interval (5 days or less) fluctuations, between high and low oxygen concentrations, inhibited population buildup of *Hemicycliophora arenaria* on tomato in soil, and of *A. avenae* and *Caenorhabditis* sp. *in vitro*. In soil tests with *H. arenaria* exposed to 12 hr of nitrogen every three days (in air) inhibited the rate of buildup compared to controls maintained in continuous air. With the *in vitro* studies, as little as 4 hr nitrogen every 3 days (stored in air) significantly influenced the population numbers. *Key Words: Hemicycliophora arenaria, Aphelenchus avenae, Caenorhabditis* sp., Oxygen, Nitrogen, Aeration, Reproduction, Population.

The influence of soil aeration on nematodes has been reported in numerous studies (12, 14, 15, 17, 19, 20); all of these, however, evaluated the effects of constant oxygen levels which are probably a rarity in the natural soil environment (7).

McElroy (9) observed field populations of *Hemicycliophora arenaria* Raski were reduced in proportion to the frequency and duration of irrigation and postulated reduced aeration might be the primary factor.

Van Gundy *et al.* (16) investigated the phenomenon in terms of measured oxygen diffusion rates (O.D.R.) in irrigated field soil. They found only trace amounts of oxygen in the liquid phase down to a depth of 61 cm immediately following irrigation. After 12 hr, oxygen had diffused to a depth of 15 cm but 7 days were required for restoration of normal O.D.R. to the entire depth. A profile of the O.D.R.'s following irrigation was determined. It was hypothesized that irrigations, even though of short duration, were frequent enough to interrupt or slow down nematode activity and reduce reproduction.

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The purpose of the present study was to test the *in vivo* responses of a plant parasitic nematode (*Hemicycliophora arenaria*), and the *in vitro* behavior of a mycophagous nematode (*Aphelenchus avenae*) and a microphagous nematode (*Caenorhabditis* sp.) exposed to interrupted aeration under controlled conditions.

Materials and Methods

The *in vivo* effect of interrupted aeration on reproduction of *H. arenaria* was studied on tomato plants (*Lycopersicon esculentum* Mill.) transplanted into Ramona sandy loam in sealed plastic containers as described by Stolzy *et al.* (15). Ten days after transplanting, 600 *H. arenaria* larvae and adults were introduced around the roots of the tomato seedlings. The low oxygen treatments of short duration were imposed on the plant roots and nematodes by flushing the containers with nitrogen 12 or 24 hr every 3 or 5 days over a period of 30 days. Nitrogen delivery was regulated using the technique of Van Gundy and Stolzy (19). Each treatment was replicated four times. The plants were watered three times daily with full strength Hoagland's solution.

Illumination was provided by cool-white fluorescent lamps supplemented with incan-

descent lamps giving a total average intensity of 2,500 ± 250 foot-candles. Initially, the intensity was 1,700 fc, but as the plants grew, the tops of the plant received 3,500 fc. A photoperiod of 16 hr was employed. No direct temperature control was used, and the mean air temperature was 31 ± 1 C with the lights on, decreasing to 26 ± 1 C in darkness. The relative humidity ranged from 10–30% during this test.

At the end of the treatment period each container was individually harvested; root systems were washed and placed on mist chamber extraction while the nematodes and broken fine roots, separated from the soil on 32 and 325-mesh screens, were Baermann-extracted, all for 48 hr.

Reproduction of *Aphelenchus avenae* Bastian and *Caenorhabditis* sp. was studied *in vitro*. Three hundred Aretan-sterilized fourth-stage larvae and adult *A. avenae* were introduced into a 3-day-old pure culture of *Rhizoctonia solani* Kühn, growing on 30 ml of 5% potato dextrose agar (PDA) supplemented with 1% yeast extract in a 90 × 15 mm petri dish. Aseptic techniques were used throughout. Twenty third-stage larvae of *Caenorhabditis* sp. were introduced onto a 20-day-old mixed bacterial culture growing on 6 g of oatmeal (Quaker Oats Co.) in a 90 × 15 mm petri dish.

The *Caenorhabditis* sp. used in these studies, obtained from Dr. R. Mankau, U. C. Riverside, had been isolated from soil on the Riverside campus. Comparisons were made with specimens and illustrations of described *Caenorhabditis* species and no specific identification could be made. It is the opinion of the authors that this nematode is an undescribed species. Specimens and permanent slides have been placed on deposit at the Department of Nematology, University of California, Riverside 92502 for further reference and identification.

Continuous aerobic, microaerobic and anaerobic environments were achieved by flushing commercially prepared (Matheson) gaseous mixtures of 90% N_2 + 10% O_2, 95% N_2 + 5% O_2, 96% N_2 + 4% O_2, 98% N_2 + 2% O_2, 85% N_2 +10% O_2 + 5% CO_2, N_2, or air (which served as control) over 1-day-old cultures of *A. avenae* and *Caenorhabditis* sp. for 30 days. The cultures were stored in 100 × 150 mm sealed plastic containers, with one petri dish per container. In the lid of each plastic container were placed two No. 30 syringe needles, one for gas intake and another for gas exhaust. A manifold system supplied all replicates simultaneously. The flow rates were monitored at the exhaust vent where a flow of 20 ± 2 ml/min was maintained. The cultures were stored in the dark at 27 C. Six replicates were used for each treatment and the test was repeated once.

Alternating aerobic and microaerobic or anaerobic environments were achieved by flushing with nitrogen for periods of 4, 8, or 24 hr at intervals of 3, 5, 7, 9, 11, or 13 days of storage in air. All experiments were terminated at 30 days. The nitrogen environments were obtained by placing the cultures in a sealed desiccator from which air was evacuated then replaced with nitrogen four times. The nitrogen was changed once (at 12 hr) during the 24 hr period to insure a low level of carbon dioxide and other unwanted gases. After exposure the cultures were stored in an incubator at 27 C. Ten replicates were used for each treatment and the experiments were repeated once. For comparison, treatments of 95% N_2 + 5% O_2, or 98% N_2 + 2% O_2 were substituted for the nitrogen.

A. avenae was harvested by extracting the entire mycelial mat (nematodes, fungus, and PDA) placed top side down on wet-strength tissue paper on a Baermann funnel for 48 hr.

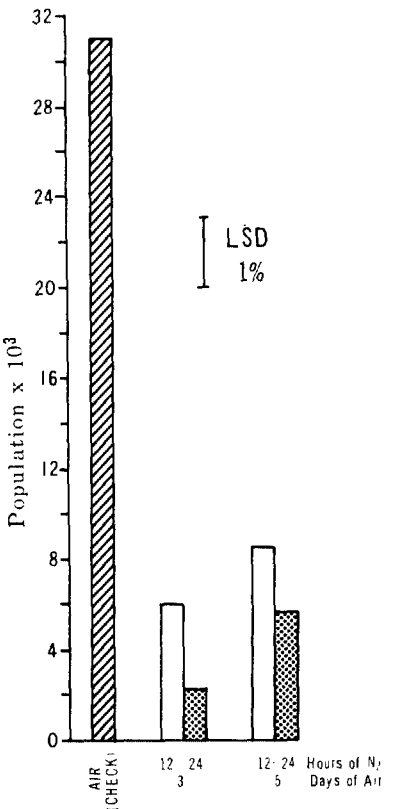

FIG. 1. Average (4 reps.) population of *Hemicycliophora arenaria* grown on tomato roots having access to atmospheric air interrupted with exposures to nitrogen for either 12 or 24 hr every 3 or 5 days.

The harvesting of *Caenorhabditis* sp. was accomplished by flushing the culture with tap water onto a 325-mesh screen and washing for 1 min with tap water. The nematodes and remaining debris were washed into a beaker and allowed to settle for 30 min. The excess water was decanted. The nematodes were placed on wet-strength tissue paper, spread on a concave screen, which was partially submerged in water, and recovered after 48 hr. All counting was done on a Doncaster counting dish at 95× under a dissecting microscope.

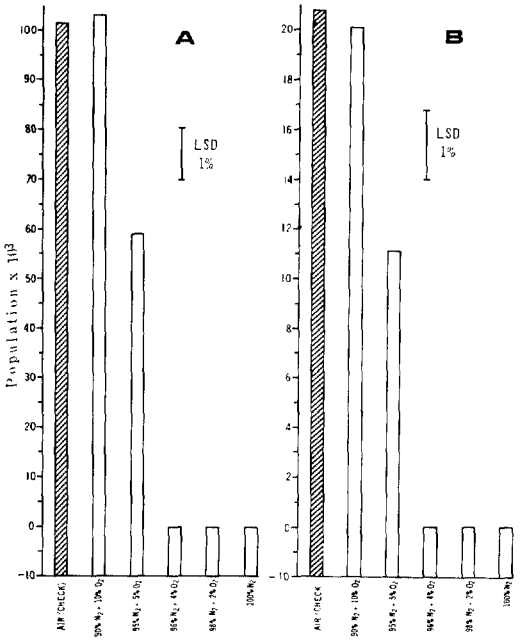

FIG. 2. Average (6 reps) population sizes of two nematode species after continuous days in various N_2/O_2 mixtures at 27 C. **A.** *Aphelenchus avenae* cultured on *Rhizoctonia solani*; **B.** *Caenorhabditis* sp. cultured on mixed bacteria.

RESULTS

The influence of relatively short periods of nitrogen exposure on the reproduction of *H. arenaria* feeding on tomato roots in soil under growth chamber conditions are given in Fig. 1. Nitrogen exposure for 12 hr every 3 days (over a 30 day period) decreased the population 80%, compared to those maintained continuously in air. Nitrogen exposure for 24 hr every 3 days inhibited the population by 94%. Similar exposures every 5 days decreased the population 67% and 80%, respectively. The longer the interval between nitrogen treatments the less the influence on the population level attained. Conversely, the longer the nitrogen exposure, at the same interval, the greater the influence on population numbers.

Effects of continuous lowered oxygen con-

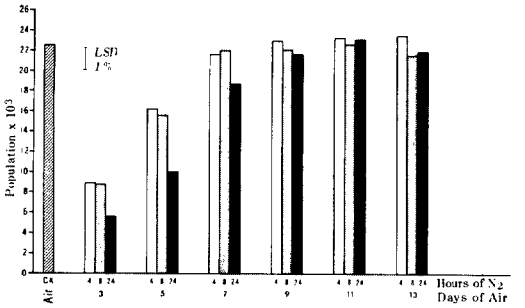

FIG. 3. Total population (average 10 rep. plates) of *Caenorhabditis* sp. grown on bacterial cultures exposed to atmospheric air and subjected to N_2 for 4, 8, or 24 hr every 3, 5, 7, 9, 11, or 13 days (30 days total) at 27°C.

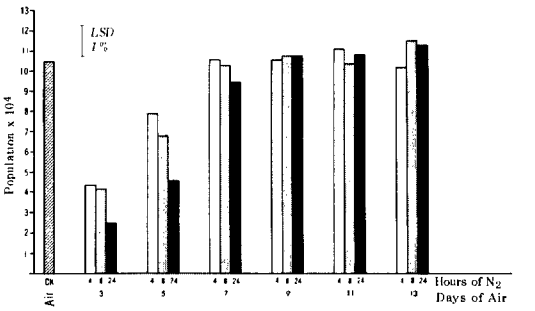

FIG. 4. Total population (average 10 rep. plates) of *Aphelenchus avenae* grown on fungal cultures exposed to atmospheric air and subjected to N_2 for 9, 8, or 24 hr every 3, 5, 7, 9, 11, or 13 days (30 days total) at 27°C.

centration on the population buildup of *Caenorhabditis* sp. and *A. avenae* are given in Fig. 2. It was unaffected down to 10% O_2, reduced at 5%, and inhibited at 4%.

Increased carbon dioxide (5%) combined with 10% oxygen did not significantly alter reproduction from that measured at the same oxygen concentration without carbon dioxide.

The influence of intermittent microaerobic and anaerobic exposure on the population numbers of *Caenorhabditis* sp. and *A. avenae* are given in Fig. 3 and 4. Nitrogen exposures as short as 4 hr every 3 days (for 30 days) to reproducing cultures of *Caenorhabditis* sp. and *A. avenae* slowed the population buildup, compared to those obtained under continuous exposure to air. The longer the exposure to nitrogen (8 and 24 hr) at 3 and 5-day intervals, the greater the population depressing effect. The differences between the 4 and 8-hr nitrogen treatments were, however, not significant. With increased time interval between nitrogen treatments, population effects decreased with no effect observed when the period exceeded 7 days.

DISCUSSION

From an ecological point of view, it is important to study nematodes in controlled environments which approximate, as nearly as possible, major factors in the natural habitats of the species under consideration. Often, however, it is difficult to determine the parameters of environment or to duplicate its variations; therefore, investigators usually employ constant average conditions which can easily be controlled.

The effects of constant reduced aeration on a number of soil-inhabiting nematodes have been explored (2, 4, 5, 10, 11, 12, 20). Most results indicate that this general group of nematodes are facultative anaerobes; growth and reproduction are dependent upon a sufficient supply of oxygen, but as individuals, they can survive anaerobic conditions for varying periods. Soil-inhabiting nematodes appear well adapted to the variations of aeration encountered in soil so it is not generally a critical environmental component. There is growing evidence, however, that aeration may be important for nematode survival in irrigated agricultural soils and greenhouse pot cultures.

Recently the aeration profile of an irrigated agricultural soil was recorded (7, 16). In both studies the oxygen supply dropped to low levels during flood irrigation and progressively rose following application, taking

from 7 to 9 days for normal amounts of oxygen to return to depths of 61 cm. Thus, a large bulk of soil, between 15 and 61 cm, may experience microaerobic conditions for several days. As irrigation frequency increases the period of adequate soil aeration for nematodes is decreased and those in soil at depths from 30 to 61 cm and below may be exposed to continuous low levels of oxygen.

The continuous aeration of the surface soil layers should be sufficient to maintain nematode population. This probably is never obtained in many agricultural soils, however, because the repeated tilling and partial drying of these layers may not be conducive to nematode growth and reproduction (8).

The reproduction of a wide variety of soil-inhabiting nematodes is severely reduced in continuously microaerobic environments (1, 3, 4, 11, 13). This has been substantiated in this study by observations that the rate of reproduction of both *A. avenae* and *Caenorhabditis* sp. was significantly decreased in a continuous environment of 5% oxygen and inhibited at 4% oxygen. The physiological processes essential for reproduction and growth are apparently aerobic.

Many research workers, including the authors, have previously assumed that short interruptions of oxygen supply were of little consequence to the nematode. Our evidence suggests that the oxygen requirement for growth and development is continuous and that short-interval fluctuations between high and low oxygen concentrations may be as disruptive as continuously low oxygen tensions.

In soil, the rate of reproduction of *H. arenaria* was decreased by 80% with an exposure to 12 hr nitrogen every 3 days (for 30 days). Similar controlled environment *in vitro* studies of *Caenorhabditis* sp. and *A. avenae* indicated that 4 hr nitrogen every 3 days (for 30 days) was sufficient to decrease the buildup in their populations by 55% and 60%, respectively. The experimental temperatures were considered near optimum for reproduction. Likewise, an ample food supply was available at all times and it is the opinion of these authors that the influence of fluctuating aeration was directly upon the nematodes and not an indirect effect, such as the lack of sufficient roots on the treated tomatoes. In both the soil and *in vitro* studies the closer the interval between the high and low oxygen concentrations, the greater the influence on population numbers. Conversely, the longer the interval, the less the influence. These results correspond to those found by Van Gundy *et al.* (16), in which the longer the interval between the application of irrigation water (resulting in a lower oxygen supply) the larger the population numbers.

Tests using *Caenorhabditis* sp. and *A. avenae*, substituting a gaseous mixture of 98% N_2 + 2% O_2 for nitrogen alternating with air, produced results similar to those with nitrogen, while alternating 95% N_2 + 5% O_2 with air had no effect on population numbers. Thus, frequent exposures to even microaerobic environments, for short periods, is sufficient to reduce nematode reproduction.

The reasons for, and mechanisms by which, a fluctuating or discontinuous oxygen supply decreases nematode growth and reproduction are at present little understood. At least two soil-inhabiting nematodes (*A. avenae* and *Caenorhabditis* sp.) are capable of performing anaerobic glycolysis in microaerobic and anaerobic environments and could easily survive the relatively short exposures (24 hr) to low oxygen levels (unpublished data). Possibly the shunting back and forth between oxidative and fermentative metabolism prevents the nematodes from developing an adequate capacity for continuous anabolism

and interferes with such processes as lipid metabolism in egg production, egg hatch, etc. Van Gundy and Stolzy (17, 18) observed that females of *H. arenaria*, *M. javanica* and *T. semipenetrans* did not lay as many eggs in continuous microaerobic as in continuous aerobic environments. Le Jambre *et al.* (6) reported similar results for some animal parasitic nematodes. Although egg laying was not specifically observed in these tests, it is speculated that this stage in the life cycle is affected by fluctuating environments and that this may be a behavioral response to a physiological stimulus.

The effects of fluctuating aeration may have wide practical application to nematode ecology, nematode control and greenhouse culturing of nematodes. It has already been shown that the frequency of irrigation in the field had a profound influence on nematode populations and appeared to be more important than temperature (16). The variability of nematode populations in irrigated soils may also be attributed to aeration. In the future, investigators of nematode problems should recognize changes in irrigation practices in field tests to be an important factor to consider. The variability of results in nematode control due to flooding may in part be explained by these results. Flooding may in fact enhance the survival of individuals while the application of frequent regulated irrigations may eventually provide an effective cultural control practice during the cultivation of the host crop, particularly in irrigated desert soils.

Finally, the successful culturing of nematodes, such as *Xiphinema americanum* has been attributed to a narrow range of soil moisture conditions (8). Other nematodes have also been shown to be reduced in wet soil and in greenhouse pot cultures (9, 16). Aeration may well be among the most important environmental factors to be considered in future studies of nematodes, particularly those that have thus far defied culturing.

LITERATURE CITED

1. BRYANT, C., W. L. NICHOLAS AND R. JANTUNEN. 1967. Some aspects of the respiratory metabolism of *Caenorhabditis briggsae* (Rhabditidae). Nematologica 13: 197–209.
2. COOMAN, E. P. 1950. Le metabolisme respiratorie d'Angiullula silusiae de Man. Ann. Soc. Roy. Zool. Belgique 81:5–13.
3. FAIRBAIRN, D. 1960. The physiology and biochemistry of nematodes. *In* J. N. Sasser and W. R. Jenkins (eds.) Nematology. Univ. of N. Carolina Press, Chapel Hill, p. 267–296.
4. FEDER, W. A. AND J. FELDMESSER. 1955. Further studies of plant parasitic nematodes maintained in altered oxygen tension. J. Parasitol. 41:47.
5. FELDMESSER, J., W. A. FEDER, AND R. W. REBOIS. 1958. Further effects of altered gas tensions on plant parasitic nematodes. J. Parasitol. 44:31.
6. LE JAMBRE, L. F. AND J. H. WHITLOCK. 1967. Oxygen influence on egg production by a parasitic nematode. J. Parasitol. 53:887.
7. LETEY, J., L. H. STOLZY, N. VALORAS AND T. E. SZUSZKIEWICZ. 1962. Influence of oxygen diffusion rate on sunflower growth at various soil and air temperatures. Agron. Jour. 54:316–319.
8. LOWNSBERY, B. F. AND J. T. MITCHELL. 1965. Some effects of chemical amendments and cultural conditions on population levels of *Xiphinema americanum*. Pl. Dis. Rep. 49: 994–998.
9. MCELROY, F. D. 1967. Studies on the biology and host-parasite relations of *Hemicycliophora arenaria* Raski, 1958. Ph.D. Thesis. University of California, Riverside. 92pp.
10. NICHOLAS, W. L. AND R. JANTUNEN. 1964. *Caenorhabditis briggsae* (Rhabditidae) under anaerobic conditions. Nematologica 10:409–418.
11. NICHOLAS, W. L. AND R. JANTUNEN. 1966. The effect of different concentrations of oxygen and of carbon dioxide on the growth and reproduction of *Caenorhabditis briggsae* (Rhabditidae). Nematologica 12:328–336.
12. OVERGAARD-NIELSEN, C. 1949. Studies on the soil microfauna. II. Soil inhabiting nematodes. Natura Jutlandica 2:1–131.
13. SAZ, H. J. 1969. Carbohydrate and energy metabolism of nematodes and Acanthocephala. *In* M. Florkin and B. T. Scheer

(eds.). Chemical Zoology III. Academic Press, N. Y. pp. 329–359.

14. STOLZY, L. H., S. D. VAN GUNDY, C. K. LABANAUSKAS, AND T. E. SZUSZKIEWICZ. 1962. Influence of soil-oxygen diffusion rates and temperature on the citrus nematode and sweet orange seedlings. Phytopathology 52: 754.
15. STOLZY, L. H., S. D. VAN GUNDY, C. K. LABANAUSKAS, AND T. E. SZUSZKIEWICZ. 1963. Response of *Tylenchulus semipenetrans* infected citrus seedlings to soil aeration and temperature. Soil Sci. 96:292–298.
16. VAN GUNDY, S. D., F. D. MCELROY, A. F. COOPER AND L. H. STOLZY. 1968. Influence of soil temperature, irrigation and aeration on *Hemicycliophora arenaria*. Soil Sci. 106:270–274.
17. VAN GUNDY, S. D. AND L. H. STOLZY. 1961. Influence of soil oxygen concentration on the development of *Meloidogyne javanica*. Science 134:665–666.
18. VAN GUNDY, S. D. AND L. H. STOLZY. 1963. The relationship of oxygen diffusion rates to the survival, movement and reproduction of *Hemicycliophora arenaria*. Nematologica 9:605–612.
19. VAN GUNDY, S. D., L. H. STOLZY, T. E. SZUSZKIEWICZ AND R. L. RACKHAM. 1962. Influence of oxygen supply on survival of plant-parasitic nematodes in soil. Phytopathology 52:628–632.
20. WALLACE, H. R. 1956. Soil aeration and the emergence of larvae from cysts of the beet eelworm, *Heterodera schachtii* Schm. Ann. Appl. Biol. 44:57–66.