Reports

Influence of Soil Oxygen Concentrations on the Development of Meloidogyne javanica

Abstract. Roots of tomato plants infected with *Meloidogyne javanica* were grown in soil subjected to oxygen concentrations of 21 percent, 5.5 percent, 3.5 percent, 2.0 percent, 0.6 percent, and 0 percent for 28 and 35 days. The lowest oxygen tension which still allowed development of the host and the nematode was 3.5 percent. Below this level the plant root growth, size of developing females, and production of nematode eggs were reduced. Nematode activity as measured by the number of nematode galls on the roots of treated plants was sharply reduced at the 5.5-percent level of oxygen.

Little information is available on the activities of plant-parasitic nematodes under various soil-oxygen tensions. Failure of *Heterodera schachtii* eggs to hatch was thought by Wallace (*1*) to be due to an oxygen shortage in the soil, caused by blockage of the soil pore system by excess water. Stolzy, Van Gundy, and Letey (*2*) have shown that numbers of *Meloidogyne incognita, Trichodorus christiei, Tylenchulus semipenetrans,* and *Xiphinema americanum* surviving in different soils were reduced after exposure to 0 percent oxygen for 10 days. Hatching of *T. semipenetrans* eggs was reduced after 3 days at 0 percent oxygen. The respiration of some free-living and plant-parasitic nematodes has been determined in various artificial atmospheres (*3*). These studies have shown that respiration under these conditions was independent of oxygen tension, and dependent upon temperature and presence of CO_2. It was also observed that the respiration decreased with length of time the nematodes were removed from soil. A method and apparatus for varying the oxygen concentration above the soil surface has been described by Stolzy *et al.* (*4*).

Tomato seedlings were infected by planting in small containers of silica sand infested with 500 larvae of *Meloidogyne javanica.* After 48 hours the seedlings were removed from the infested sand, transplanted in plastic cylinders containing 1 liter of steamed sandy soil, and grown for 3 weeks. The soil bulk density was uniformly adjusted in each cylinder to 1.65. Nematode development on duplicate plants had reached five and ten egg masses per plant, each containing approximately 100 eggs. The containers were sealed with the tops of the plants extended through holes in tightly fitted lids. The soil and roots were then subjected to the following oxygen concentrations derived by mixing air with nitrogen: 21, 5.5, 3.5, 2.0, 0.6, and 0 percent. One experiment ran for 28 days and another for 35 days after the treatments were applied. There were a total of six replicates per treatment. Moisture control was based on the weight-pot technique for keeping the soil between 18 and 30 percent (volume basis). This represents approximately 50 percent and 80 percent, respectively, of the total soil porosity filled with water. At the termination of the experiment the roots were washed from the soil and the nematode galls were counted. Half of the galls on each plant were stained with acid fuchsin, and ten developing females were removed and measured for size as described by Bird (*5*), except that the area was determined with a planimeter. The other half of the galls were placed in a vial for 11 days, after which the total number of larvae were counted.

The effects of the different oxygen tensions on the growth of the tomato plants in two experiments are similar to those already described by Letey *et al.* (*6*). Plant growth (Fig. 1) and water use were reduced with a decrease in oxygen concentration. Plants grown at 0 percent oxygen were barely alive, while those at 21 percent oxygen were vigorous.

The number of nematode galls produced by secondary infection was significantly reduced between 21 and 5.5 percent oxygen (Fig. 1). However, the root growth was not significantly reduced above 3.5 percent oxygen. Possibly the hatching of eggs and the activity and ability of the nematode to infect plant roots in soil were affected by greater oxygen concentrations than required to affect root growth. Oxygen concentrations which significantly reduced the growth of the plant roots also caused a significant reduction in the size of the developing female nematodes.

The area, determined by outline drawings of female nematodes dissected from galls of plants grown at 0.6 percent and 0 percent oxygen tensions, was significantly smaller (127,000 μ^2) than those from plants grown at 21 percent (213,000 μ^2) (Fig. 1). Nematodes measured at the beginning of the oxygen treatments averaged 162,000 μ^2. These measurements indicate that nearly all the nematodes measured at the 0.6 percent and 0 percent levels were of the second generation.

Although there was no significant difference in the size of the nematodes in plants grown at oxygen tensions of 21, 5.5, and 3.5 percent, the average number of larvae hatched per egg mass

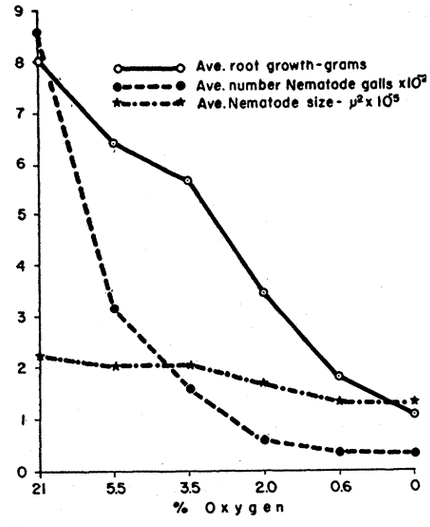

Fig. 1. Average tomato root growth, number of root-knot galls, and size of female nematodes grown at various oxygen concentrations for 28 and 35 days.

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Type manuscripts double-spaced and submit one ribbon copy and one carbon copy.

Limit the report proper to the equivalent of 1200 words. This space includes that occupied by illustrative material as well as by the references and notes.

Limit illustrative material to *one* 2-column figure (that is, a figure whose width equals two columns of text) or to *one* 2-column table or to *two* 1-column illustrations, which may consist of two figures or two tables or one of each.

For further details see "Suggestions to contributors" [*Science* **125**, 16 (1957)].

after 11 days incubation was 235, 130, and 63, respectively. Only a few eggs were found within the host plants grown at 2 percent oxygen.

The large reduction in number of galls at the lower oxygen levels appears to be due to the reduced hatching rate and development of the eggs of the original females and the reduction in infectivity of the larvae in the soil. The possibility that soil aeration affects nematodes has long been recognized; however, it has been difficult to establish direct evidence that inadequate oxygen in the soil limits the activity of nematodes except under rather extreme conditions. These results indicate that the activity of some nematodes in the soil phase is more dependent upon the availability of oxygen than previously thought.

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Simultaneous Appearance of Free Arginine and Deoxyribosidic Compounds during Mitosis

Abstract. Sequential measurements were made of the free amino-acid pool in the premitotic region of microspores of *Lilium longiflorum*. The basic amino acid arginine appears in the pool and then disappears. Arginine appears at the same developmental stage at which it has been reported that the free deoxynucleosides appear.

The mitotic division in the microspores of *Lilium longiflorum* presents a particularly favorable subject for the study of the chemistry of mitosis. It is especially favorable for two reasons. First, a morphological index of development (*1*) orders the sequence of events during the interphase preceding mitosis; second, the microspores undergo this mitosis in synchrony (*1*). Using this material, Foster and Stern (*2*) and Taylor (*3*) have been able to show the precise time, during the interphase preceding mitosis, at which deoxyribonucleic acid (DNA) is synthesized. Nasatir, Bryan, and Rodenberg (*4*) showed changes in the composition of the soluble proteins during interphase and mitosis. The large quantitative change in the free deoxyribosidic compounds (*2*) and the correlation of this peak value with the doubling of one of the soluble proteins (*4*) led us to an examination of the associated changes in the free amino acids.

Table 1. Change in concentration of the free amino acids in the anther, with respect to the length of the flower bud in *Lilium longiflorum*. Mitosis of the microspores occurs at 60 mm.

Bud length (mm)	Change in concentration (μmole/anther)		
	Lysine	Histidine	Arginine
50	4.67	0.32	
52.5	1.25	5.10	2.25
54	2.40	0.31	
55	1.62	2.10	
60	3.40	7.30	

Flower buds of *Lilium longiflorum* Thunb. cv. "Croft" of varying lengths were removed, and the anthers were excised. The anthers were homogenized in 70-percent ethanol and centrifuged. The supernatant fraction was evaporated and the residue was dissolved in 2.5 ml of *p*H 2.2 buffer. The amino acids were separated by column chromatography by the methods of Moore and Stein (*5*). The quantity of each amino acid was determined by the ninhydrin reaction described by Moore and Stein (*6*).

Changes in many amino acids were found, but the most striking were the changes of the basic amino acids, lysine, histidine, and arginine. A measurable amount of arginine was present only at a bud length of 52.2 mm. This is the same bud length at which Foster and Stern (*2*) found free deoxyribosidic compounds and at which Nasatir, Bryan and Rodenberg (*4*) found the doubling of one of the soluble proteins. Histidine and lysine, in contrast to arginine, were present at all the bud lengths measured: 50, 52.5, 54, 55, and 60 mm. At the time of DNA synthesis, arginine appeared and histidine increased over tenfold, but lysine decreased by a factor of four. Later, at the time of mitosis, arginine was absent, but the concentrations of both lysine and histidine tripled. These results are summarized in Table 1.

Because of the small number of buds analyzed, it is difficult to say definitely how the pattern of the free amino-acid pool changes with development. For example, it is not possible to decide whether the high level of arginine is present during all the premitotic fluctuations in free deoxyribosides or whether the concentration follows these fluctuations in detail. If the concentration fluctuates, there may be more than one peak. To demonstrate such a second peak would require the use of a much larger number of buds.

On one point, however, the data are quite unambiguous: Two amino acids, distinctively present in chromosomal protein, undergo marked concentration changes close to the time of DNA doubling. This result leads us to speculate that the pertinent protein synthetic mechanisms undergo a corresponding change related to the requirements of chromosome duplication.

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Virus–Tumor Synergism

Abstract. More than 30 mouse tumors are associated with virus-like agents that may readily be detected by enzymic techniques. Radiation and chemically induced tumors do not ordinarily give evidence of such activity. The present report is of experiments in which a synergistic effect has been observed to occur when animals were inoculated with both the filtrable agent and a virus-free tumor. Synergism was shown by accelerated growth of the tumor and by elevation of a glycolytic enzyme (lactic dehydrogenase) in the host plasma.

Most transplantable mouse tumors and their hosts have been shown recently to have a virus-like agent, or agents, associated with them (*1, 2*). While these tumor-host-virus associations now include over 30 varieties of tumor types as revealed by enzymic techniques, the relationship of these agents to the neoplastic process is still uncertain (*2*).