Effects of Methyl Bromide Dosage on Microorganisms in Soil Before and After Growth of *Nicotiana glutinosa*

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ABSTRACT

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Soil fumigation for various periods with a controlled concentration of methyl bromide (MB) in a moving airstream affected the populations of various microorganisms. In soil fumigated with 27,000 μl of MB per liter of air, soil fungi and *Pseudomonas* spp. were eliminated within 16 hr; *Streptomyces* spp. were greatly reduced within 64 hr and were undetectable in soil fumigated for 128 hr; but aerobic bacteria and *Bacillus* spp. were only slightly affected by treatment for 128 hr (the longest time tested). Soil was treated with 32,000 μl of MB per liter of air for various times, planted with *Nicotiana glutinosa*, and microbial populations also were determined in nonfumigated soil planted with *N. glutinosa*. Microbial populations of soil in which plants had been grown increased as the time after fumigation increased. After 89 days, populations of fungi in soil fumigated for 32 or 128 hours were as great as those initially found in nonfumigated soil. *Penicillium* spp. and *Trichoderma* spp. were the dominant fungi isolated from soil after fumigation for longer than 8 hr. Recolonization of fumigated soil by *Streptomyces* spp. was rapid; after 38 days, numbers were greater in soil fumigated for 32 hr than those in nonfumigated soil Recolonization by *Streptomyces* spp. was slow in soil fumigated for 128 hr and populations after 89 days were still lower than those in nonfumigated soil. Populations of aerobic bacteria and *Bacillus* spp. were greater in soils fumigated for 32 or 128 hr than in nonfumigated soil at all sampling periods. Fluorescent *Pseudomonas* spp. were not isolated from fumigated soils in which *N. glutinosa* was grown, but they were present in planted nonfumigated soils.

The purpose of these experiments was to establish a dosage-response relationship for the effect of methyl bromide (MB) on microbial populations in soil similar to that developed for aerated steam (2). Selective methods were used to assess the effects of fumigation on the fungi, total bacteria, and fluorescent *Pseudomonas* spp., *Bacillus* spp., and *Streptomyces* spp. The effects on microorganism populations of growing plants in fumigated and nonfumigated soil also were studied.

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MATERIALS AND METHODS

Fumigation of soil. Soil was fumigated by continuous exposure to 27,000 or 32,000 μl of MB per liter of air in a previously described continuous-flowing controlled-concentration apparatus (3,6,7). Exposure times were based upon the time (E) for gas concentrations to equilibrate in the fumigation cylinders plus a progression of hourly exposures designated E, E+2, E+4, E+8, E+16, E+32, E+64, and E+128.

Assays of populations of organisms in soil. In one experiment, soil was fumigated with 27,000 μl of MB per liter of air for the periods indicated above. Two 75-g soil samples were taken from each treatment and assayed 1 day after fumigation. This

experiment was repeated four times in 1 mo. Soil obtained from the same source was used and was stored in a covered 120-L plastic can. Data were analyzed from experiments that utilized a randomized nested complete block design. Each experiment was considered to be a block in the design.

In another experiment, soil was assayed after growth of seedlings of *Nicotiana glutinosa* L. Pots 10 cm in diameter containing nonfumigated soil or soil fumigated with 32,000 μl of MB per liter of air for E+32 or E+128 hr were seeded heavily with disinfested (0.5% sodium hypochlorite, 3 min) seeds of *N. glutinosa.* Plants were grown for 14, 38, 63, or 89 days in an environmental chamber with a daylength of 13 hr and a diurnal temperature cycle of 30 C day and 23 C night. Photosynthetically active light (400–700 nm) averaged 4.8 $\mu W/cm^2/nm$ during the light portion of the photoperiod. After each growth period, soil from four pots in each treatment was passed through a 2-mm sieve to remove roots. Five 25-g subsamples from each pot were bulked and mixed in a polyethylene bag. Populations of various organisms in these bulked subsamples were determined by the dilution plate technique, as described below, except that dilute tryptic soy agar (5) was used in place of soil extract agar for the determination of total aerobic bacteria and *Bacillus* spp.

Soil dilutions were made in soil extract agar to avoid osmotic shock (8) and to keep microorganisms suspended (9). The medium was prepared by adding 100 ml of soil extract (1 kg soil plus 1 Liter of tap water, shaken, allowed to settle, decanted, and clarified by slow speed centrifugation) to 900 ml of tap water containing 1.0 g of agar (Bacto). The media were autoclaved for 30 min. Duplicate assays were made by plating out 1.0 ml of each dilution (10^{-1}, 10^{-2} . . . 10^{-8} on a medium specific for the organisms that were being sought. Incubation was at 24 C except as noted. Fungal populations were assayed on rose bengal agar (10) and counted after 3 and 6 days. *Streptomyces spp.* were assayed on casein glycerol agar (4) and counted after 5 days with the aid of a dissecting microscope. Fluorescent *Pseudomonas spp.* were detected by spreading 0.1 ml of a soil suspension evenly over the surface of King's Modified Medium B agar (8) by using a bent glass rod. After 48 hr at 30 C the colonies were counted under ultraviolet light. Aerobic bacteria were grown in soil extract agar (1) and counted after 6 days. *Bacillus spp.* were assayed in the same dilutions as the other groups after heating them for 10 min at 80 C. Soil extract agar (1) was used and colonies were counted after 6 days.

RESULTS

Microbial populations in soil 1 day after fumigation. There were only minor changes in composition of microbial populations treated with 27,000 μl of MB per liter of air for the first 8 hr (Fig. 1). After longer exposures, however, significant ($P = 0.05$) differences in tolerance of the organisms to MB were observed. Fungi and *Pseudomonas* spp. were most sensitive to MB; populations were not detected after exposures as short as E+16 hr. *Streptomyces* spp. were less sensitive to MB than were fungi and the response was more gradual; populations progressively declined as exposures were increased from E+16 to E+64 hr and no *Streptomyces* spp. were detected after exposure for E+128 hr. Aerobic bacteria and *Bacillus* spp. were the most resistant to MB, since their populations were essentially unchanged by fumigations even as long as E+128 hr.

Changes in microbial populations following growth of *N. glutinosa* in fumigated soil. Planting *N. glutinosa* in soil fumigated with MB (32,000 μl of MB per liter of air for E+32 or E+128 hr) affected the subsequent recolonization of microorganisms in various ways (Fig. 2).

Fungi. Fungal populations (Fig. 2A) steadily increased in fumigated soils from zero detectable propagules at planting to populations significantly higher than populations in nonfumigated soils after 89 days. There was a lag period of at least 14 days before growth resumed in soils fumigated for E+128 hr; thereafter, the rates of population increase in soils fumigated E+32 or E+128 hr were essentially similar. There was, as well, a qualitative change in composition of the fungal populations. *Penicillium* spp. and *Trichoderma* spp. predominated in fumigated soils, whereas a more heterogeneous group of fungi predominated in nonfumigated soils.

Streptomyces spp. The response of *Streptomyces* spp. (Fig. 2B) was similar to that of the fungi, except that the rate of recovery of *Streptomyces* spp. was slower in soil fumigated for E+128 hr and by 89 days the populations were still significantly lower than those of nonfumigated soils. Also, colonization after an initial lag period was very rapid and then became much slower as the populations approached their maximums (between 63 and 89 days for soils fumigated for E+32 and E+128 hr, respectively). *Streptomyces* spp. could not be detected in soil fumigated for E+32 or E+128 hr on day 0 or in soil fumigated for E+128 hr on day 14 because the surface of the plates (10^{-2} dilution) were covered by bacteria.

Total aerobic bacteria. Populations of aerobic bacteria (Fig. 2C) were generally 10–100 times greater in fumigated soils than in nonfumigated soils. An exception occurred on day 0 when populations were less in soil fumigated for E+32 hr than in nonfumigated soil. Bacterial populations were maximum on the 63rd day and declined on the 89th day in all three soils. Populations of aerobic bacteria were significantly greater during most of the sampling period in soil fumigated for E+128 hr than populations in soil fumigated for E+32 hr.

Bacillus spp. The responses of *Bacillus* spp. (Fig. 2D) were very similar to those of the other aerobic bacteria; ie, growth was stimulated and bacteria were more numerous in fumigated soil than in nonfumigated soil. The populations reached their maximums on the 63rd day after planting and declined significantly by the 89th day. Populations were consistently highest in soil fumigated for E+128 hr and lowest in nonfumigated soil, except immediately after fumigation.

Fluorescent *Pseudomonas* spp. were not detected in either of the fumigated soils during the growth period, although populations in nonfumigated soil ranged from 2.6×10^5 colony-forming units (cfu) at planting to 8×10^4 cfu 38 days after planting. A bacterium that formed a yellow, nondiffusible pigment was dominant in plates of King's Modified Medium B agar containing dilutions of soil fumigated for E+32 hr, whereas an off-white bacterium predominated in soil assayed at E+128.

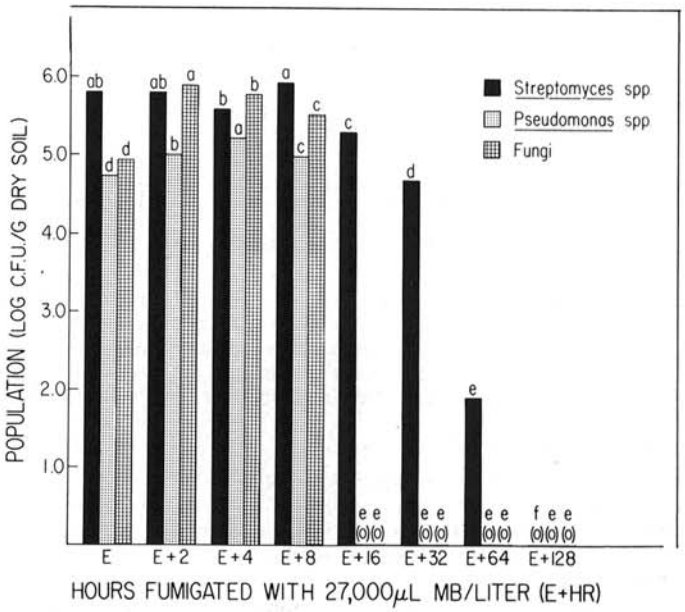

Fig. 1. Populations of *Streptomyces* spp., fluorescent *Pseudomonas* spp., and fungi in soil immediately after fumigation with 27,000 μl MB per liter of air. Values given are the means of eight samples from each treatment. E is the time required for the concentration of methyl bromide to equilibrate in the fumigation vessels. CFU is colony-forming units. Comparable means within each class of organisms indicated by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.

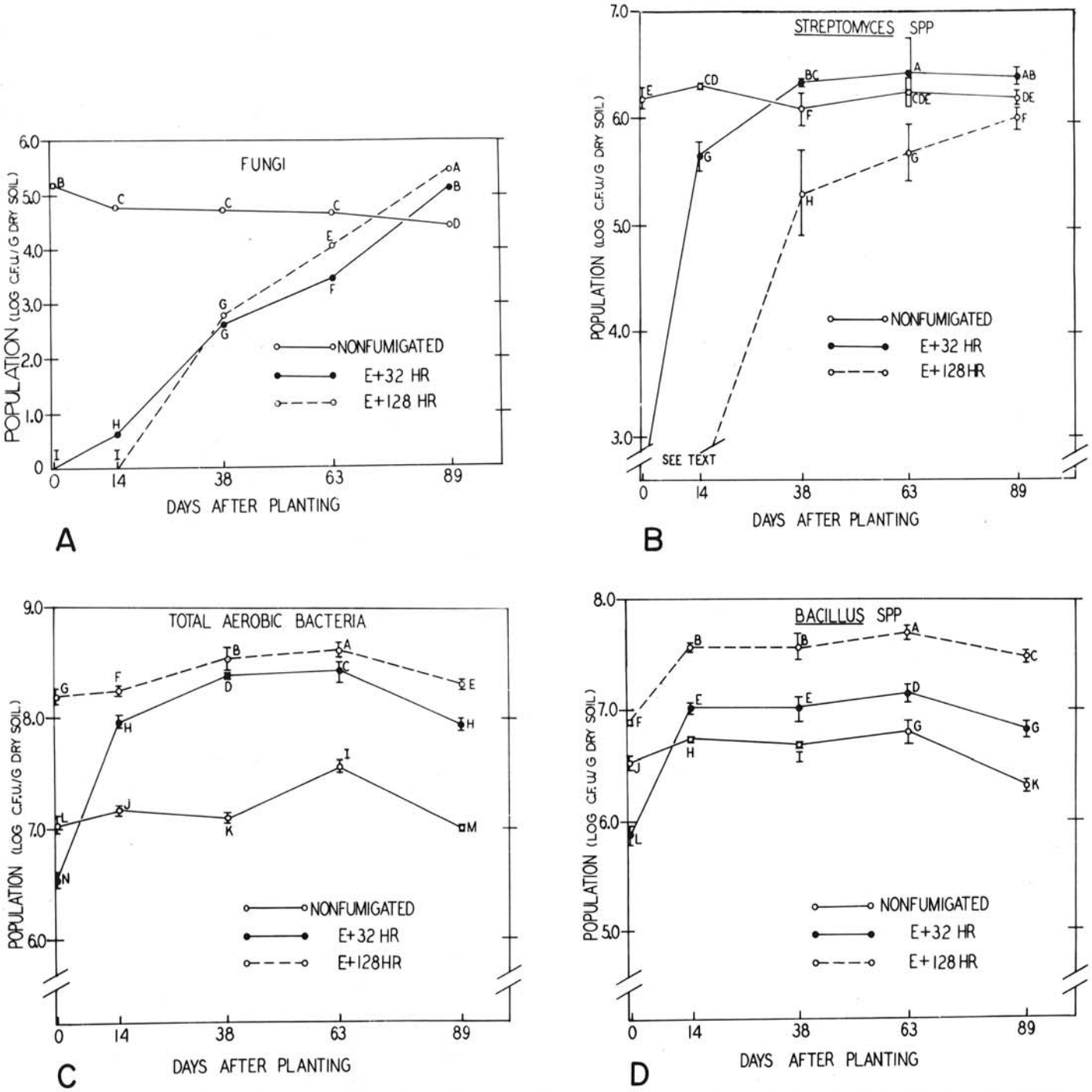

Fig. 2. Populations of **A,** fungi, **B,** *Streptomyces* spp., **C,** aerobic bacteria, and **D,** *Bacillus* spp. defected in nonfumigated soil or soil fumigated for E+32 or E+128 hr with 32,000 μl of methyl bromide per liter of air after *Nicotiana glutinosa* had been grown for various periods. Data are based upon the mean of four replicates. Data points with the same letter are not significantly different ($P = 0.01$) as determined by Duncan's multiple range test. Vertical bars at each data point indicate the range of standard error, except in A, for which none is shown because the range did not exceed the width of the data points. E is the time required for the concentration of methyl bromide to equilibrate in fumigation vessels. CFU is colony forming units.

DISCUSSION

Soil fumigation with MB drastically affected the microflora of the soil. Certain populations initially declined (eg, fungi, fluorescent *Pseudomonas* spp., and *Streptomyces* spp.) while others, (eg, *Bacillus* spp. and other aerobic bacteria) increased. These quantitative changes were related to dosages of MB. In addition to these quantitative changes, the fungal and bacterial composition of the soil microflora was altered after fumigation. Fumigated soil contained fewer species of fungi and presumably fewer species of bacteria.

Besides its use in the study of increased plant growth after soil fumigation, microbial selection by means of the continuous-flow methyl bromide apparatus may prove valuable in other studies such as take-all decline, action of suppressive soils, and disease complexes involving more than one pathogen.

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